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DEVELOPMENT OF A NEW TOTALLY BIODEGRADABLE FILM BASED ON STARCH AND NANOFIBRILLATED CELLULOSE FOR BARRIER PROPERTIES

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The final project entitled “DEVELOPMENT OF A NEW TOTALLY BIODEGRADABLE FILM BASED ON STARCH AND NANOFIBRILLATED CELLULOSE FOR BARRIER PROPERTIES”, has been carried out and researched in the laboratory of LEPAMAP Group of University of Girona (UdG), Spain, under the supervision of Dr. José Alberto Méndez González and Dr. Gerard Arbat Pujolràs, Professors of UdG, and Dr. Borarin Buntong, Professor of Royal University of Agriculture, Cambodia.

Girona, 17th of June of 2015

Dr. José Alberto Méndez González   Dr. Gerard Arbat Pujolràs   Dr. Borarin Buntong

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Summary

Starch is a natural, renewable, sustainable, inexpensive, and degradable material which has many advantages and plays important roles in human life. Starch is also a natural polymer available everywhere in the world. Nanofibrillated cellulose (NFC) is a main material to reinforce and increase the properties of composites such as paper and packaging materials. Both of starch and NFC are degradable materials and environmental friendly. In this study, starch was used as a raw material to produce a flexible, transparent and biodegradable film which was compounded by glycerol, plasticizer, and reinforced by NFC.

There were three steps which must be practiced to create such kind of film included pre–blends, blends, and films preparation. Starch, glycerol, glycerol/NFC, and added water were mixed together to obtain the pre–blends. The appropriate proportions of these compositions were found to acquire good pre–blends. For this step, there were 7 formulations to prepare the pre-blends. After that, the ready prepared blends were then passed though Brabender mixing to get the blends. Optimum temperature, rotation speed, and time of mixing were investigated and observed carefully in this step in order to achieve transparent and flexible blends. The process to obtain the blends was named as compounding. Later on, transparent, flexible, and thin thickness films were processed by those prepared blends through thermoforming. To complete these properties of films successfully, the suitable temperature, applied forces/pressures, and times of thermoforming must be rightly determined.

After the films were completely obtained, they were then characterized some of their properties. Mechanical characterization, water absorption, water vapor permeability and biodegradability were the properties of films which must be determined. The effects of NFC with different amount of pulp oxidation (5, 10, and 15 mmol) and higher or lower content of fibers added in the created films were studied on these properties. There were 2 conditions, 23^{o}C and 50% RH and 7^{o}C and 50% RH, of mechanical characterization which would be tested as water absorption determination had 3 conditions contained 23^{o}C and 50% RH, 23^{o}C and 75% RH, and 7^{o}C and 50% RH. The samples were taken to a laboratory in Valencia, Spain, to experiment and study the capacity of water vapor barrier. Finally, biodegradability property of films under enzymatic activity was analyzed and evaluated.

List of Abbreviations and Symbols

AFM	:	Atomic force microscopy
ASTM	:	American Society for Testing and Material
BOPP	:	Bi-oriented polypropylene
CH_3COOH	:	Acetic acid
CMS	:	Carboxymethyl starch
CPP	:	Cast polypropylene
CTE	:	Coefficient of thermal expansion
DIP	:	Deinked recycled pulp
DMA	:	Dynamic mechanical analysis
DP	:	Degree of polymerization
DS	:	Degree of substitution
EVOH	:	Ethylene vinyl alcohol
MAP	:	Modified atmosphere packaging
MFC	:	Microfibrillated cellulose
MMT	:	Montmorillonite
NaBr	:	Sodium bromide
NaClO (NaOCl)	:	Sodium hypochlorite
NaOH	:	Sodium hydroxide
NFC	:	Nanofibrillated cellulose
OMG	:	Old magazines
OML	:	Overall migration limits
ONP	:	Old newspapers
OPP	:	Mono-oriented polypropylene
OPS	:	Oriented polystyrene
PA	:	Polyamide
PC	:	Polycarbonate
PE	:	Polyethylene
PE-HD	:	High-density polyethylene
PE-LD	:	Low-density polyethylene
PE-LLD	:	Linear low-density polyethylene
PET	:	Polyethylene terephthalate
PP	:	Polypropylene
PS	:	Polystyrene

PVA (PVOH)	:	Polyvinyl alcohol
PVC	:	Polyvinyl chloride
PVDC	:	Polyvinylidene chloride
RH	:	Relative humidity
SML	:	Specific migration limits
SNC	:	Starch nanocrystal
SSA	:	Specific surface area
StNP	:	Starch nanoparticles
TEM	:	Transmission electron microscopy
TEMPO	:	(2,2,6,6-tetramethylpiperidine-1-oxyl radical)-mediated oxidation
USDA	:	The U.S. Department of Agriculture
WRV	:	Water retention value
σ	:	Stress
ε	:	Strain
A	:	Cross-section area
E	:	Young’s modulus

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1. Introduction

1.1 Overview of food packaging

Packaging is a major role of application for plastic materials and plays a variety of important fields in the food industry [1]. Protection the foods from spoilage by microbial contamination, physical damages, or biochemical reactions is one of the main roles of packaging. The conveniences in handling, efficiency of storage, attractiveness, and the information of product for food are also derived from packaging. The ideal food packaging material serves all of these purposes and is also cost–efficient. The development of self-service stores with their large variety of products is a creative thing. Preservation the quality of packed products is another important function of a packaging material. Among these goods, according to their principal chemical instability, foods hold a place of special importance [2].

Food packaging is an essential sector of modern food processing and will play a significant function in the food industry as the increasing of use of new and food processing operations. Food packaging is defined as a coordinated industrial and marketing system for enclosing products in a container to meet the 7 needs: containment, protection, preservation, distribution, identification, communication, and convenience [3, 4].

Historically, developments in food packages have been a corporation with developments in society. The increased amount of products production and urban society during the industrial revolution in the 1700s created the need for packaging distribution to transport large quantities of products out of factories and bring the large quantities of foods into the cities. In the late 1800s, developments in supermarkets and refrigeration increased products distribution, and brand marks were introduced to package labeling. In the 1950s, the development of fast-food restaurants created the demand for disposable single-service packages. An increasingly urban, international society with consumer demands for convenience and a wider variety of food products has increased the need for packaging to extend the shelf life of foods. Single events, such as the need for tamper-evident packages created after the Tylenol tampering incident in 1982, also may change the dynamics of the packaging industry [4]. The role of packaging in the food industry will continue to evolve to complete the demands of users created as new societal and consumer expectations development.

1.2 Definitions and basic functions of packaging

There are many ways to give the definitions of packaging reflecting different emphases. For an example, packaging is a means of assuring the safe delivery to the consumers in a sound condition at optimum cost. It is referred to a coordinated system of preparing goods for transport, distribution, storage, retailing and end–use. Packaging is also a techno–commercial function aimed to optimize the costs of delivery while maximizing sales in order to get more profits [5].

1.3 The value of packaging to society

The value of food packaging to society has never been greater and attracted so much adverse media publicity and political attention. In response, stakeholders in the food industries need to fully appreciate and actively promote the positive contributions that their packaging makes to the quality of life. Food packaging is controlled by a mass of laws, regulations, codes of practice and guidelines [5]. The benefits of packaging to society may be included:

- prevents or reduces product damage and food spoilage, thereby saving energy and vital nutrients, and protecting the health of the consumer
- requires less municipal solid waste disposal since it promotes processed food residue recycling for use as animal feed or compost
- lowers the cost of many foods through economies of scale in mass production and efficiency in bulk distribution. Savings are also derived from reduced product damage
- reduces or eliminates the risk of tampering and adulteration presents food in an hygienic and often aesthetically attractive way
- communicates important information about the food and helps consumers make informed purchases
- provides functional convenience in use or preparation, freeing up more time
- promotes goods in a competitive marketplace and increases consumer choice
- facilitates the development of modern retail formats that offer consumers the convenience of the one-stop shop and availability of food from around the world throughout the year
- extends the shelf life with the benefit of prolonged product use, thereby reducing wastage
- saves energy through the use of ambient packs that do not require refrigeration or frozen distribution and storage.

1.4 Functions of packaging

The general functions of food packaging has been described in the overview of food packaging: containment, protection, preservation, distribution (transportation), identification, communication, and convenience [3, 4]. An ideal package will enable a safe and quality food product to reach the consumer at minimum cost. The importance of each function of packaging will depend on the type of food product, the location of the packaged product in the distribution chain, and the intended destination end point. It is important to note that if a package fails to function properly, much of the expense and energy put into the production and processing of the food product will be wasted. The basic functions of packaging are more specifically stated:

- **Containment:** depends on the product's physical form and nature. For example, a hygroscopic free–flowing powder or a viscous and acidic tomato concentrate
- **Protection:** prevention of mechanical damage due to the hazards of distribution
- **Preservation:** prevention or inhibition of chemical changes, biochemical changes and microbiological spoilage
- **Information about the product:** legal requirements, product ingredients, use etc.
- **Convenience:** for the pack handlers and user(s) throughout the packaging chain
- **Presentation:** material type, shape, size, color, merchandising display units etc.
- **Brand communication:** by the use of typography, symbols, illustrations, advertising and color, thereby creating visual impact
- **Promotion (Selling):** free extra product, new product, money off etc.
- **Economy:** for example, efficiency in distribution, production and storage
- **Environmental responsibility:** in manufacturing or production, use, reuse, or recycling and final disposal.

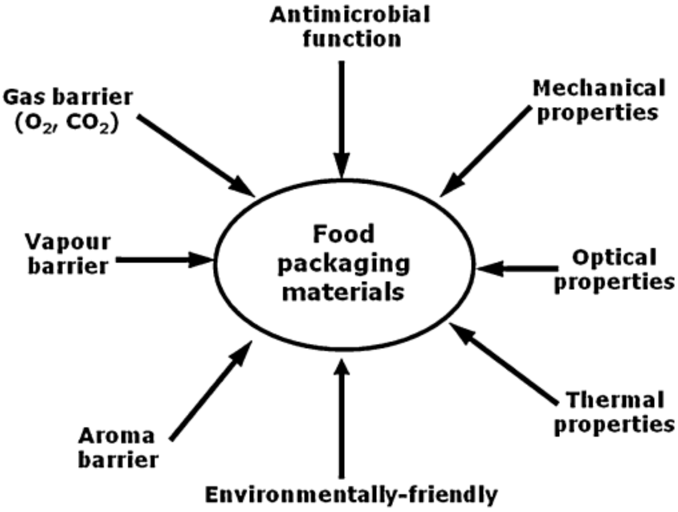


Figure 1.4: General properties required for food packaging materials [6]

1.5 Levels of packaging

Four basic levels of packaging are classified which contained primary package, secondary package, distribution or tertiary package, and unit load or quaternary package. Two types of package are defined by the destinations (consumer package and industrial package).

A primary package is in direct contact with the food product and is responsible for many of the general packaging functions. A secondary package contains the primary package and often provides physical protection for the food product and the primary package. A distribution package contains secondary packages and functions to protect their contents and enable handling. Finally, a unit load is comprised of distribution packages that are bound together to facilitate handling and storage throughout the distribution chain [7].

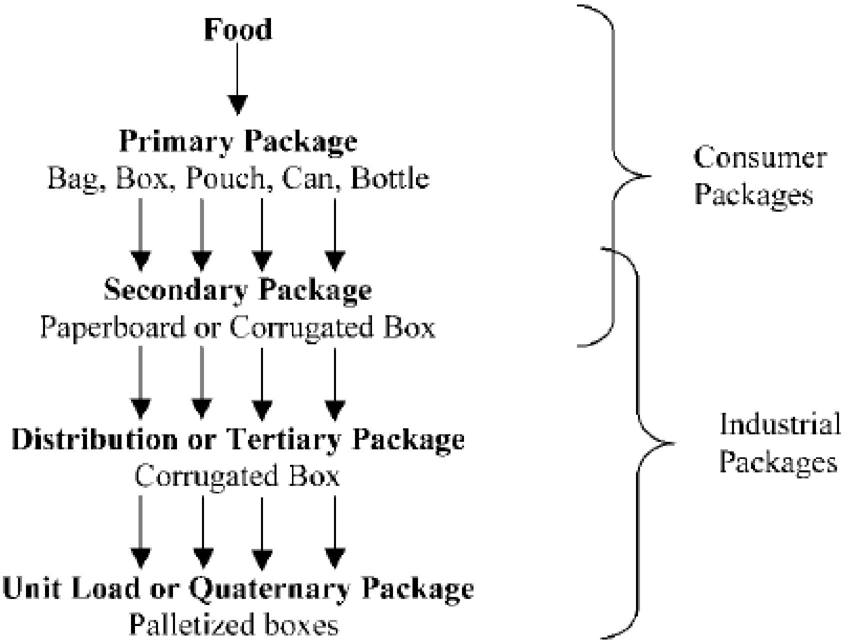


Figure 1.5: The four basic level of packaging [7]

1.6 Food contact applications

Plastic materials were widely used for food packaging and it was directly contacted to foods. Plastic materials are not inert and some interaction with the food substance can occur. Additives, monomers, catalyst residues, polymer degradation products, or residual polymerization solvents can migrate into the food in response to a concentration gradient; concentration of the substance in the food is lower than that in the packaging material. Interaction of food products with packaging can occur during preparation or storage and is affected by environmental conditions such as temperature and storage time. Migration can result in product toxicity or the formation of undesirable flavors or odors [8].

1.6.1 European food packaging regulations

In the European Union, Directives have been established to ensure that food contact materials do not transfer any constituents to foods in quantities that could endanger human health. A positive list approach is used, in which all substances permitted in food contact materials must be listed, along with the identity of the material and any restrictions. For new food contact materials to be included in the list, migration testing studies must be completed. Materials must adhere to both overall migration limits (OML) and one or more specific migration limits (SML); testing must be performed in all food simulants, unless food contact use will be restricted. The overall migration limit is the total amount of migration allowed from the packaging material.

1.7 Flexible materials

There are often long, and pointless, discussions about the various definitions and meanings of films and sheet, and the not consistent line between flexible, semi-flexible, semi-rigid and rigid. In common use, films are planar forms of plastics, which may be thick enough to be self–supporting, but thin enough to be flexed, folded and/or creased without cracking [9].

In German and French traditions, film means a quite flexible, continuous, material that can be put on reels; and sheet means a material that can only be made and used in cut formats. The difference in definitions is not clear enough, since many materials can be put on continuous reels, even those with thickness up to 1.5 mm, or 1500 μm, a truly rigid sheet material for thermoforming. The dividing lines between film and sheet are sometimes given as 2 to 200 μm, flexible; 200 to 400 μm, semi–rigid or semi–flexible; and over 400 μm, rigid [9].

The definition between sheet and film has become quite blurred. This shift in the thickness preference for thinner sheet has opened up many thermoformed container markets to coextruded film producers, thus enhancing barrier films at the expense of barrier sheet. Probably, the best criterion is the thickness up to which the various fabricating and converting processes can be used [9]. Films can be up to 400 μm thick by lamination. In blown co–extrusion, the maximum thickness is rarely more than 150 μm, because the film is cooled with air after extrusion. Cast co-extrusion can go up to 500 μm, sometimes well over 1 mm. The cast material is cooled with water and chill rolls. However, when polyamide (PA) is a component in a cast coextruded material, the thickness should not be above 500 μm, and the resulting material is still considered as a film. Sheet ranges from 400 to 2000 μm, and co-extrusion is the only possible process – lamination is not used [9].

1.7.1 Types of films and materials

The processing of the plastic into a film calls for the plastic to be melted, fed into a shaping at a metered rate, cooled and fed to a wind-up station. Many variants and complexities can be added to this simple concept. All flexible packaging–whether films, laminates, paper, foil, cartons, cases, bags, pouches or labels–starts as material on a reel. A wide variety of plastics materials are used for films such as PE, PE-HD, PE-LD, PE-LLD, PP, OPP, BOPP, CPP, PVC, PS, OPS, PET, EVOH, PVA or PVOH, PVDC, PA, PC, cellulosic, cellophane, and combinations of these films as multilayers [9].

1.7.2 Consumption of PE films

Plastics are defined as processed materials based on polymers. These materials can be transformed into finished products, such as bottles, containers, films, hoses, coatings, lacquers, etc. As a result of today’s multitude of plastic applications, there is a corresponding enormous variety of plastic materials. The polymer matrix as well as the incorporated plastic additives can be made to differ in such a variety of ways with respect to their chemical composition and structure that one finds or can develop a tailor made product for every application [2].

The total consumption of plastics in the world was estimated at 165 million tons in 2003. Films were a very large segment of this total, representing about 40 million tons. The sector was dominated by commodity plastics, especially polyethylene (PE) and polypropylene (PP) were combined together about 34 million tons [9]. In Europe, the PE film producing companies were located in Italy, Germany, UK, France, Benelux, Scandinavia, and Spain in the number of 275, 150, 185, 120, 70, 80, and 100 companies, respectively.

Polyethylene films have been the most widely used, representing some 75–80% of the total tonnage of plastic films. PE films represented the largest tonnage of all plastic films. Out of about 60 million tons of polyethylene consumed in the world in 2003, almost half or 30 million tons were for PE films, an estimated 10 million tons were PE-LD, 12 million tons were PE-LLD, and other 8 million tons were PE-HD.

Europe and USA showed very comparable tonnage figures, for PE consumption, as for most other plastics consumption. Together, the USA and European PE film consumption amounts to 40% of the world total, or 12 million tons out of 30 million tons of PE films. China uses another 5 million tons of PE films, with the particular feature of large exports of PE bags to Europe and the USA, and of agricultural films to neighboring Asian countries.

Table 1.7.2: Estimated consumption of PE for films (in thousands of tons, 2003)

World consumption of PE, grand total	60,000
World consumption of PE for films, total	30,000
of which, world consumption of PE-LD films	10,000
world consumption of PE-LLD films	12,000
world consumption of PE-HD films	8,000
European consumption of PE, total	15,000
European consumption of PE for films	6,000
US consumption of PE, total	15,000
US consumption of PE for films	6,000

Source: Plastic films – Situation and Outlooks [9]

1.8 Thermoplastics

1.8.1 Polyethylene (PE)

PE is the most widely used mass–produced plastic. The worldwide production of PE at the early 1990s was $40 \times l0^6$ metric tons per year. Of this amount $16 \times l0^6$ tons were LDPE, $8 \times l0^6$ were LLDPE, and the remainder was HDPE [2]. PE is a wax like thermoplastic which softens at temperatures between 80–130°C and possesses good chemical stability. The mechanical properties are dependent on the molecular weight and degree of chain branching. PE can be easily heat sealed, is tough and has high elasticity. It has good cold resistance properties and is a good water vapor barrier. LDPE has low barrier properties to gases, aromas and fats. With increasing density, all the barrier properties increase as well as the stiffness, hardness and strength, as a result of the higher crystallinity. At the same time there is a decrease in the impact resistance, toughness, resistance to stress cracking, cold resistance and transparency [2].

The processing of PE is normally carried out at temperatures between 150–210^{o}C. However temperatures can be reached as high as 300^{o}C during paper coating. PE is stable at these high temperatures under inert atmospheres and, when being processed under these conditions, the oxygen concentration in and around the plastic should be kept as low as possible [2].

1.8.2 Polypropylene

Since approximately 1986 PP has ranked third in the bulk plastic production after PE and PVC, with an estimated annual production of 21 × $l0^6$ tons. PP has three different classes: homopolymer PP; copolymers containing primarily PP; and PP elastomer mixtures. PP is composed of linear hydrocarbon chains and therefore its properties quite closely resemble those of PE. The properties of isotactic PP are particularly useful. The stereo regularity of the macromolecule chain construction and the related high crystallinity give PP its outstanding characteristics. Large scale commercially produced PP is up to 95% isotactic in nature [2]. PP possesses good water vapor barrier and fat resistance properties. Normal PP films have limited food packaging applications because of their low cold temperature resistance. Copolymer mixtures with ethylene are used to improve cold resistance and heat seal ability as well as material strength and seal strength [2].

1.8.3 Polystyrene

With an annual production of over 6 × $l0^6$ tons (excluding eastern European countries) polystyrene (PS) is the fourth rank behind PE, PVC and PP on the bulk polymer list. PS has been commercially produced since 1930. As a thermoplastic plastic, it can be processed between temperatures of 150 to 300°C. At higher temperatures depolymerization takes place by splitting out the styrene. Products formed from PS are hard and transparent, with high brilliance and resistance to many chemicals. Its disadvantages are its brittleness and sensitivity to stress cracking [2].

1.8.4 Polyvinyl chloride

The ability of vinyl chloride to polymerize was first observed over 150 years ago. Polyvinyl chloride has been industrially manufactured since approximately 1930. Even though pure PVC is fairly unstable, it is ranked second after PE for bulk plastic production. The manifold applications of PVC are made possible by the discovery of effective stabilizers and other additives for the polymer. PVC can be blended with numerous other polymers to give it better the feasibility of processes and impact resistance [2].

1.9 Biopolymer from renewable resources

1.9.1 Introduction and functions of biopolymers

A wide range of polymers derived from renewable resources is available for material applications and those polymers are naturally occurred. Cellulose and starch are the examples of them which are widely used in products today, while many others remain underutilized. These biopolymers are derived from a diverse set of polysaccharides, proteins, lipids, polyphenols, and specialty polymers produced by bacteria, fungi, plants and animals [10]. Polymers occurred in the nature perform different functions in their native setting. Many of the material functions desired in applications for these types of polymers are already part of their natural function such as barriers, connectors or supporting structures, membranes, coatings, emulsifiers, detergents, and biodegradability [10].

Worldwide annual plastics production is estimated to over 300 million tons by 2015, representing trillions of dollars in terms of global economic returns [11]. Petroleum resources are extensively used in the productions of these polymers, which leads to concerns in terms of both economic and environmental sustainability. Nowadays, the largest fields of materials used in packaging industries are produced from fossil fuels and practically non-degradable. From this, packaging materials for foodstuff represent a serious global environmental problem [12]. A big effort to extend the shelf life and enhance food quality while reducing the packaging waste has encouraged the exploration and new discovery of new bio–based packaging materials, such as biodegradable films from renewable resources [13].

Currently, there is growing of interest in developing bio–based polymers and innovative process technologies that can reduce the dependence on fossil fuel and move to a sustainable materials basis. Biodegradable nanocomposites are the next generations of materials for the future [6]. Biological polymers include polysaccharides, polypeptides, and polynucleotides which are commonly found in food products, protein sources, and bio–organisms [14, 15]. Synthetic polymers have been developed for various industrial applications[16, 17].

1.9.2 Starch

Starchy foods have always been contributed as an important part of the human diet. It shouldn’t be surprised that other applications for this abundant natural material were developed early in the history of man. Nowadays, depending on new knowledge and modern priorities or technologies, many new products are being developed such as healthier, low fat diets and more efficient and environmentally friendly technologies included fat substitutes, thickeners, paper coatings, absorbents, binders and biodegradable plastics. Because of its abundant supply, low cost, renewability, biodegradability and convenience of chemical modification, starch continues to be an attractive material for study and research [18, 19]. Moreover, starch is an interesting biopolymer material because of its inexpensive cost, its availability in agricultural field as a surplus raw material and its thermal process ability using conventional plastics processing equipment.

a) Sources of starch

Starch is a polysaccharide produced by many plants and derived from natural resources. It is intracellularly stored in the form of spherical granules 2–100 µm in diameter [20]. Most commercially available starches are isolated from grains such as corn and wheat and from tubers such as potato and tapioca which contain 60–90% on a dry weight basis [20]. Tapioca and rice are typically grown in tropical climates while corn, wheat and potatoes are grown in colder climates. Cereal and tuber starches vary significantly in their physical properties and suitability for specific applications.

b) Structure and composition

Starch is synthesized by plants and composed by two primary polymers contained amylose which is linear and lightly polymer comprised of α–1,4–linked D–glucose and molecular weight 1×10^5 to 2×10^6 and amylopectin which is tree–like and comprised of $\alpha - 1,4$–linked D–glucose with α–1,6–linked D–glucose branches and molecular weight 4×10^7 to 4×10^8 [21]. Amylose and amylopectin occur in varying ratios depending on the plant species (about 25 to 30% amylose in corn starch) [10].

Amylopectin is partially crystalline while amylose is in an amorphous (non-crystalline) state. The length of the amylopectin side chains determines the type of crystal structure. A–type crystal structure is for cereal starches which have the shortest average amylopectin side chain length (26 units) and. B structure is referred to root starches such as potato as well as high amylose starches have longer side chains (46 units). The C structure is associated with intermediate length side chains (28 units) and occurs in arrowroot and tapioca starches. The amylopectin side chains are oriented within the granule and the overall crystallinity of starches ranges from 20 to 40%. The crystallites serve to strengthen the granule and condense the starch into a small area for storage [20].

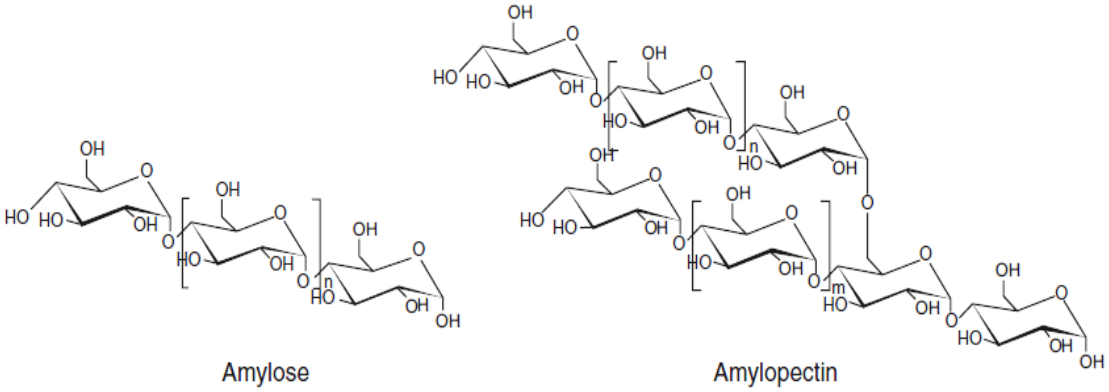


Figure 1.9.2 (a): Chemical structure of amylose and amylopectin [1]

The structure of starch has been investigated and studied for years and an universally accepted model of its structure has been lacking because of its complexity [22]. Starch consists in a multi–scale structure as presented in figure 1.9.2 (b). The granule of starch (a, 2–100 μm) consists in alternating amorphous and semi–crystalline growth rings (b, 120–400 nm) which is an onion–like structure when observed under a scanning electron microscope. These growth rings consist respectively of smaller and larger blocklets (d, 10–100 nm) made of crystalline and amorphous lamellae (c, 9 nm) containing (g) amylopectin structure, and (h) amylose chains (0.1–1 nm) [23].

On the surface of starch granules, pores can be observed. They are believed to be channels going through the growth rings to the center of the granule which was called hilum. The refracted “Maltese cross” corresponding to the crystalline region is characteristic of a radial orientation of the macromolecules [22]. Starch granules consist of concentric alternating amorphous and semi–crystalline growth rings growing by apposition from the hilum. The shape and particle size of granules, as well as the number and thickness of the growth rings, is strongly dependent on botanic origin. Starch is used as extracted from the plant and is called native starch, or it undergoes one or more chemical modifications to reach specific properties and is then called modified starch [1].

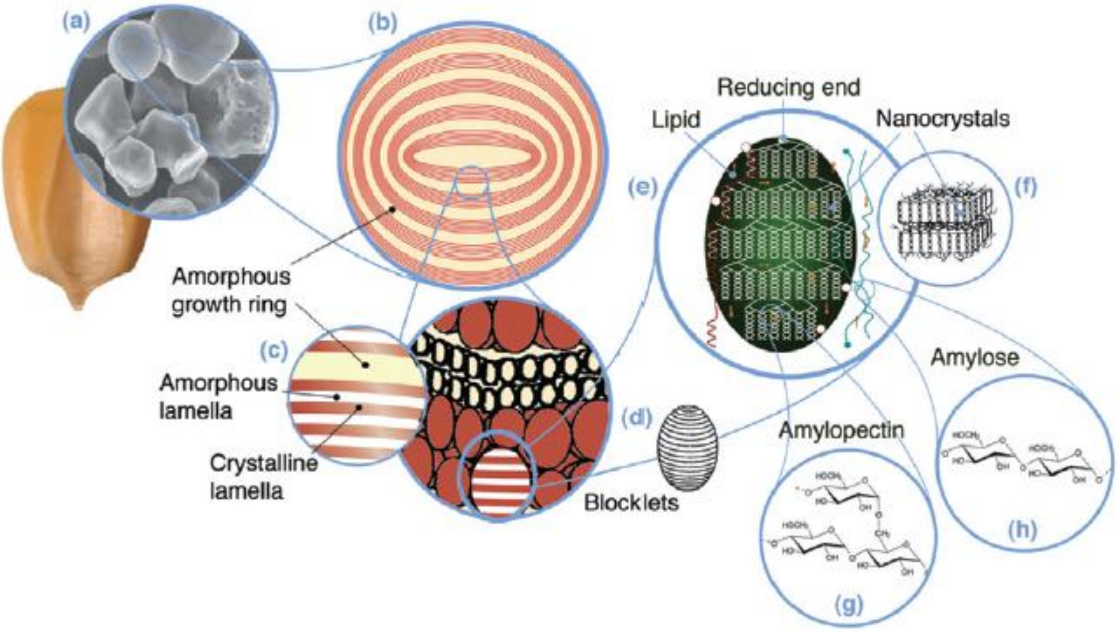
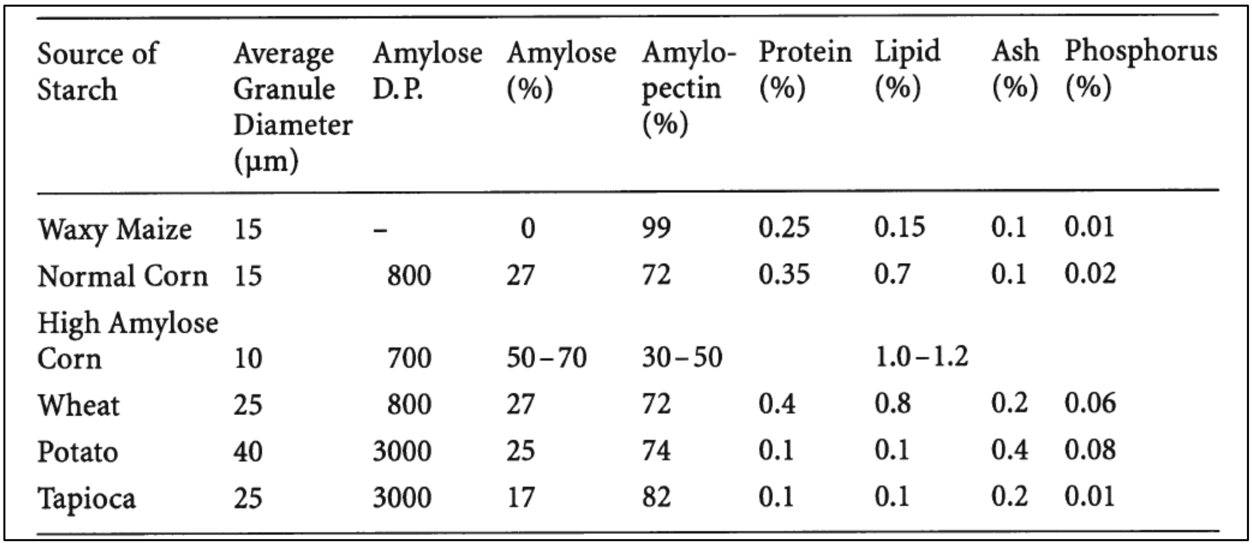


Figure 1.9.2 (b): Starch multi–scale structure. (a) Starch granules from normal maize (30 μm); (b) amorphous and semi-crystalline growth rings (120–500 nm); (c) amorphous and crystalline lamellae (9 nm): magnified details of the semi-crystalline growth ring; (d) blocklets (20–50 nm): constituting unit of the growth rings; (e) amylopectin double helices forming the crystalline lamellae of the blocklets; (f) nanocrystals: other representation of the crystalline lamellae called starch nanocrystal (SNC) when separated by acid hydrolysis; (g) amylopectin’s molecular structure; (h) amylose’s molecular structure (0.1–1 nm), [23]

In most common types of starch, the weight percentages of amylopectin ranges between 72% and 82% and the amylose content ranges from 18% to 28%. But some types of starch have very high amylose content (up to 70% and more for amylomaize) and some have very low amylose content (0–2% for waxy maize). Minor components of starch granules are of three types: (1) cell–wall fragments, (2) surface components, and (3) internal components. The main constituents of surface components are proteins, enzymes, amino acid, and nucleic acid, whereas internal components are composed mainly of lipids. So the minor components of starch are often described as proteins, lipids, and minerals [22, 24]. The proportion of these components depends on the botanical origin of starch as well as species and varieties. Several different minerals has been found in starch in small amount and phosphorus is the most important mineral [22].

Table 1.9.2 Structure and composition of common commercial starches



Source of Starch	Average Granule Diameter (μm)	Amylose D.P.	Amylose (%)	Amylo-pectin (%)	Protein (%)	Lipid (%)	Ash (%)	Phosphorus (%)
Waxy Maize	15	–	0	99	0.25	0.15	0.1	0.01
Normal Corn	15	800	27	72	0.35	0.7	0.1	0.02
High Amylose Corn	10	700	50–70	30–50		1.0–1.2		
Wheat	25	800	27	72	0.4	0.8	0.2	0.06
Potato	40	3000	25	74	0.1	0.1	0.4	0.08
Tapioca	25	3000	17	82	0.1	0.1	0.2	0.01

D. P.: average degree of polymerization.

Note: Compositions given are based on a percentage of starch dry weight; starch normally contains 10–18% of water [10, 20]

c) Properties of starch

The rigidity of starch and the tendency of the hydroxyl groups of starch to from the intermolecular hydrogen bonds are referred to the physical properties of starch. However, starch molecules are not highly extended because of the α linkage. Since water is also a poor solvent for starch (x>0), so starch–water interactions are not expected to increase significantly starch chain dimensions in aqueous solution [20]. Because starch chains are relatively rigid and highly hydrogen bonded, melting and glass transition temperatures are high, solubility in water is low, cohesive energy density and surface energy are high, and film strength is high. The melting (T_m) and glass transition (T_g) temperatures of dry starch (~ 230°C) are higher than the temperature at which degradation becomes rapid (~220^{o}C). Removal of lipids from starch by alcohol extraction increases the degradation temperature because of the reduction of hydro–peroxide catalyzed degradation reactions [10].

d) Starch films

Starch has been used as a filler in some materials included blends such as starch–urethanes, starch–polyethylene with ethylene–acrylic acid copolymer, graft copolymers with vinyl or acrylic monomers using a free radical initiating process, crosslinking reactions, and in a variety of other material technologies [10]. To take the advantage of the biodegradable nature of starch, starch blends have been developed but most of the early blends were compounded with non–

biodegradable components such as polyethylene as the starch content was usually below 10%. Thermal gelatinization starch–based technology for blends with polyethylene, acrylic acids, and other polymers have been developed by the U.S. Department of Agriculture (USDA) [10]. Blown films containing up to 60% starch were successfully processed.

Starch films, prepared by casting from aqueous solution, tend to be rather stiff and brittle which reflect the highly hydrogen bonded nature of starch chains. Starch films having higher amylose contents tend to have higher strength and flexibility because the long linear amylose chains can form more effective entanglements than the short amylopectin branches [25]. Strength and flexibility also increase with increasing amylose molecular weight. Starch can be used as an energy source by many microorganisms and higher forms of life. It is biodegradable in a variety of environments according to a number of recent studies on the biodegradation of starch based materials [21].

The mechanical properties of starch films are also dependent on a number of factors including method of preparation, amylose content, molecular weight, crystallinity, humidity, added plasticizers, and time [10]. They normally have an amorphous or slightly crystalline structure since long times are required for crystallization [18]. Since starch is a hydrophilic polymer, the properties of starch films are very sensitive to moisture content. Water acts as a good plasticizer for starch and hence at high humidity starch films absorb water and become soft and flexible. If a starch film is immersed in water, over 95% of its strength is quickly lost. They are very permeable to water but have low permeability to oxygen and organics when dry. Many non-volatile, hydrophilic plasticizers such as glycerol, glycols and amides have been added to starch films in order to decrease the brittleness of starch [10].

Flexibility at low humidity is improved by these plasticizers but water absorption at high humidity is enhanced leading to loss of strength [21]. Addition of polymeric plasticizers such as poly (vinyl alcohol) or grafting of flexible polymers onto the starch have been shown to be effective ways of reducing the brittleness of starch films [26]. The current researches have been focusing on the improving starch film flexibility and water resistance. The adhesive or binding strength of starch films to various substrates is a property of commercial interest but few basic studies of them are available. Cationic starches are known to adhere well to negatively charged cellulose fibers in paper [18].

e) Applications of starch

Currently, starch produced in the U.S. approximately 15% is used in foods, 65% for paper and paperboard, and 20% for other uses including textiles, adhesives, binders, absorbents, flocculent, and building materials [27]. Starch has many functions in foods including thickener, gelling aid, texturizer, emulsifier, appearance modifier, bulking agent, coating, adhesive, water–binding, and fat substitute [27]. Modified normal and high amylose starches are often used as gelling agents in candies and jellies as well as to achieve good film formation in batter coated products [10].

For thousands of years, starch has been used as a binder in making paper and it continues to be an important use. Starches derivatized with cationic substituents are used to bind together negatively charged cellulose fibers into a continuous sheet [18]. Later in the papermaking process, more starch is added to give the paper with a smooth surface, increase strength, and bind pigment particles to that paper [28]. To improve the wet strength, additives such as cross-linking agents and polymer latexes has been given. However, in order to bind together the different layers of paper, starch is similarly used in the manufacture of corrugated paperboard [18].

In the textile industry, most starch is used for warp sizing. Different modified starches are coated onto yarn to prevent abrasion during weaving and are removed later by washing the finished fabric. Poly (vinyl alcohol) is sometimes blended with starch because of its superior flexibility and abrasion resistance [26, 27]. Starch and dextrin pastes have long been used as adhesives for envelopes, stamps, bags, bottle labels, tape, and wallpaper [18].

There are many current interests about the biodegradable polymers based on renewable resources in replacing the plastic derived from petroleum [29, 30]. Plastics intended for single use items such as food packaging are difficult to recycle and constitute 8×10^9 kg/year market in the U.S. alone. Use of biodegradables will help preserve diminishing oil supplies, reduce the need for disposal by landfilling, and reduce hazards of non-degradable plastic waste to wildlife. In this way, starch is a biodegradable material which can degrade rapidly under the environmental conditions such as in compost, soil, or waterways [10].

1.9.3 Cellulose

Cellulose is the most abundant, renewable, and high polymer resource. It has been estimated that photosynthesis of biomass is 170 billion tons yearly and 40% is polysaccharides; mainly cellulose and starch. But only about 3% of the available polysaccharides are utilized yearly [10]. Cellulose is not soluble in water or most organic solvents and is mineralized by a variety of cellulose-producing microorganisms. Due to the hydrogen bonding, cellulose can't be thermally processed; undergoing thermal decomposition before melt, but can be solvent cast [10].

Cellulose naturally occurred and is the main constituent of many plants including wood, cotton, flax, kemp, jute, bagasse, ramie, cereal straws, etc. The main sources of cellulose for industrial processes are wood, cotton fiber and cotton linters. It constitutes 40–50% by weight in wood, while in cotton it is nearly 90%. Cellulose is also produced by a type of acetic acid-producing bacterium [31]. Cellulose was one of the first polymers which was studied by Anselm Payen's investigations [32].

The use of cellulose and its derivatives in different ways of applications, such as fibers, films, plastics, coatings, suspension agents, composites, wood and paper products, continue to grow on a worldwide basis. Relatively newer uses of cellulose and cellulose derivatives include cellulosic membranes for hemodialysis and hemafiltration, chiral isomer separations, and calorie-free fat substitutes [31]. Some kinds of products such as paper and paper products, membranes and films, fabrics, food and medicine as wound dressings and other related application are derived from cellulose. Bacterially derived cellulose is used in the production of speaker diaphragms in headphones due to the higher crystallinity and finer diameter fibrils in comparison to plant–derived cellulose. Because cellulose is insoluble so it is difficult to process into films or coatings. Water solubility is increased through derivatization reactions to form carboxy methyl cellulose, methyl cellulose, hydroxy propyl cellulose, and other structures. Chemically substituted celluloses are not always biodegradable, and usually the type and degree of substitution will impact degradation kinetics [10].

a) Chemical composition and structure

Cellulose is a linear polymer composed of β–l,4–linked D–glucose synthesized by plants and many bacteria [33]. Payen first determined the elemental composition of cellulose to be $C_6H_{10}O_5$ classifying it as a carbohydrate [32]. Hydrolysis with 72% sulfuric acid gives a high yield (91%) of α–and β–D–glucopyranose. Yields are increased to 95.5% if chlorine and sulfur dioxide are used as catalysts.

The basis for the chemical and physical properties of cellulose in its various polymorphic states is the understanding of chemical composition, conformation and intra–and intermolecular interactions. Although cellulose was first investigated over 150 years ago by Payen but it has been the interests of extensive research and there are still a number of unanswered aspects regarding this naturally occurring polymer [34].

Cellulose is a homopolysaccharide with a degree of polymerization (DP) of approximately 10,000 but for native cellulose in cotton is reported in a value of 15,000. The chemical structure of cellulose is shown in figure 1.9.3 (a). The defining feature of the cellulose structure is the availability of three hydroxyl groups per anhydroglucose unit. These groups participate in extensive hydrogen bonding, which contributes to the multi–scale nature of cellulose, its crystalline and amorphous structure, and its highly cohesive nature [35].

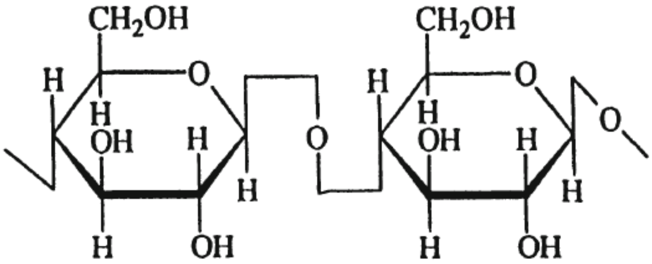


Figure 1.9.3 (a): Structure of cellulose

b) Fibrillar structure of cellulose

Both native and regenerated cellulose fibers are composed of microfibrils which are crystalline in nature. Depending on species they vary in breadth from 10–30 nm, and up to 150 nm in length as shown by light microscopy [10]. Transmission electron microscopy (TEM) shows the microfibrils composed of elementary fibrils, 3.5 × 3.5 nm in cross–section, which aggregate into the larger microfibrils. The elementary fibrils contain 36 cellulose chains and they are composed of sub–elementary fibrils, 1.5 nm in width [10].

The organization of native cellulose microfibrils has generally been described in terms of the fringed micellar theory in term of the microfibrils are being regarded as an aggregation of crystalline and amorphous regions and these regions are formed by the transition of the cellulose chain from an orderly arrangement in the direction of the microfibrils in the crystalline regions to a less ordered orientation in the amorphous areas [36].

1.10 Biopolymer nanocomposites

Currently, biobased materials which have various applications in different fields have been developed and modified by many researchers because of fossil depletion and growing environmental concerns. Engineered nanoscaled materials are used globally in numerous industrial applications and the list of proposed commercial applications continues to grow. Actually, these nanomaterials exhibit the outstandingly enhanced properties even when used at very low content [1]. Ecological concerns are the main reasons behind this renewed interest in natural and compostable materials. Natural polymers are renewable, biocompatible, and biodegradable if compared with their synthetic materials. Productions of nanocomposites from natural polymers, such as starch, chitin, and cellulose, and specific research aim to increase the properties of the products and develop the newer techniques. The abundant polysaccharide polymers in nature are increasingly being used for nanocomposites preparation [1].

Biopolymers are the biodegradable polymers designed to degrade through the action of living organisms. Biopolymers derived from renewable resources are more important than others for obvious reasons [10]. Plant–based biopolymers usually come from agricultural nonfood crops so the use of biopolymers would create a sustainable industry. Biopolymers have also been reported to be close to carbon-neutral. When a biodegradable material is obtained completely from renewable resources, then it can be called as a green polymeric material [1]. Biodegradable polymers can be classified in 3 types according to their sources. First, polymers directly extracted or removed from biomass such as polysaccharides, proteins, polypeptides or polynucleotides. Second, polymers produced by classical chemical synthesis using renewable bio–based monomers or mixed sources of biomass and petroleum including polylactic acid or bio–polyester. Third, polymers produced by microorganism or genetically modified bacteria such as bacterial cellulose and xanthan [37].

Proteins, starch and cellulose are examples of natural polymers. Polymer nanocomposites represent new and conventional polymers and they are the materials in which nanoscopic inorganic or organic particles, typically 10–1000 Å in at least one dimension, are dispersed in an organic polymer matrix in order to improve the properties of the polymer dramatically. They have increased modulus and strength, outstanding barrier properties, improved solvency, heat resistance, and lower flammability and they have no detrimental effects on ductility [1].

Vilaseca, F., et al (2007) studied the biodegradable composite materials form starch–based biopolymer and hemp strands by focusing on the formulation of hemp strand/starch–based composite and their characterization [38]. The addition of hemp strand reinforcement to the starch biopolymer produced at strengthening and stiffening of the composites. The improvement was explained in terms of the quality of interface, because of the morphology and chemical structure of composite constituents, and good dispersion of the reinforcement induced by the shear mixing process applied. The obtained hemp strand/starch composite materials behaved satisfactorily when submitted to controlled moisture and temperature conditions [39].

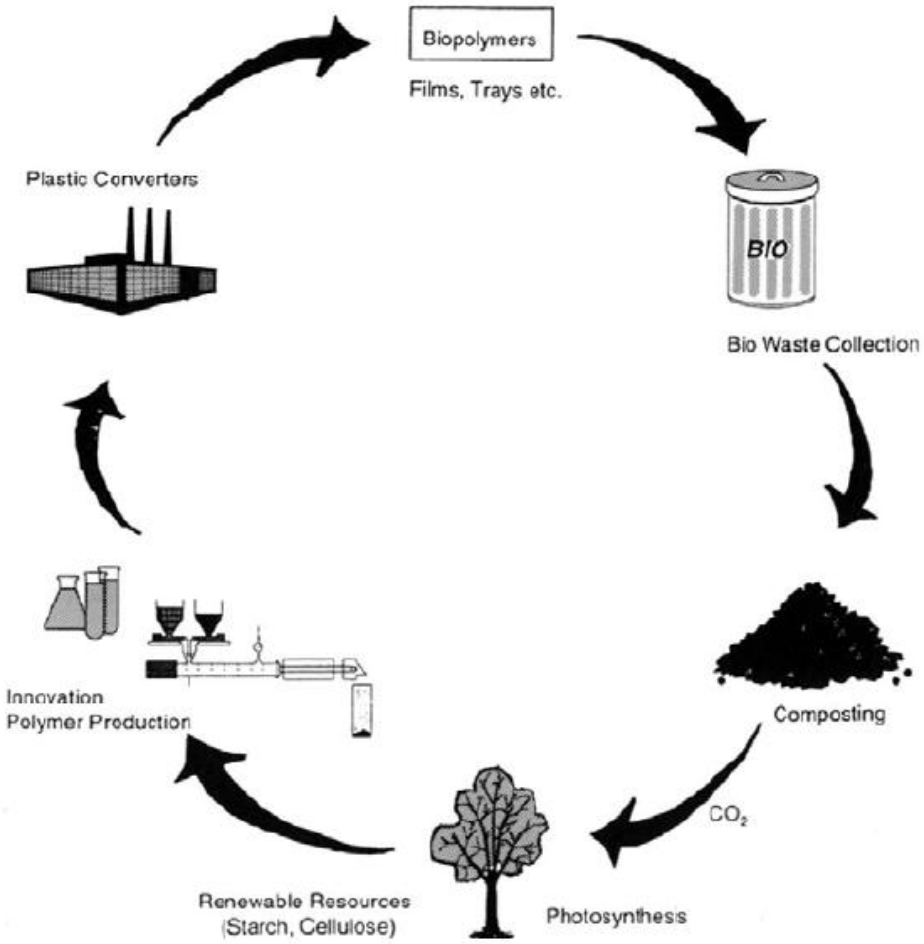


Figure 1.10: Life cycle of biodegradable polymers [40]

1.10.1 Starch nanoparticles

Polysaccharides (especially nanocrystals/nanoparticles) have been more attracted and interesting due to the development of biodegradable materials. Starch is a good candidate for production of nanoparticles because of its benefit as a biodegradable natural polymer and it has a partly crystalline structure that confers interesting properties. Many interesting and detailed reviews and articles on the preparation and application of starch nanoparticles (StNP) have been published [23, 41, 42]. There are many potential applications of StNP in various products such as food, medicine, binders, implant materials, and plastics. The most common application is the addition of StNP to a matrix, for example starch or protein, as reinforcement in the preparation of degradable bionanocomposites [43-52]. StNP are formed through the application of alkali, acid and/or enzymes which open and disperse the starch structure, and allow the formation of hydrogen bonds [53]. The StNP added to degradable bionanocomposites may enhance the mechanical properties of the nanocomposites. Because StNP contains reactive surfaces covered with hydroxyl groups, so the chemical modification of surface is possible in order to obtain specific functions, increase the compatibility between matrix and nanoparticles, and expand the application of StNP and types of polymers which can be used as matrices [1].

Acid hydrolysis is the typical method used for generating starch nanocrystals. Practical application of an acidic method is difficult due to its low yield, long treatment period, and use of concentrated acid [54], and the recent development has turned attention to use the enzymes [55] such as α–amylase for the pretreatment to enhance the efficiency of preparation. Both high pressure homogenization and extrusion are simple, environmentally, friendly, and mechanical methods which can also be used to efficiently prepare nanoparticles. Starch particles may also vary in degree of substitution (DS) after chemical modification. Nanosized (100–400 nm), smooth, hexagonal, flaked carboxymethyl starches (CMS) with a DS of 1.15 have been synthesized by reacting starch with sodium hydroxide under ultrasonic irradiation [56].

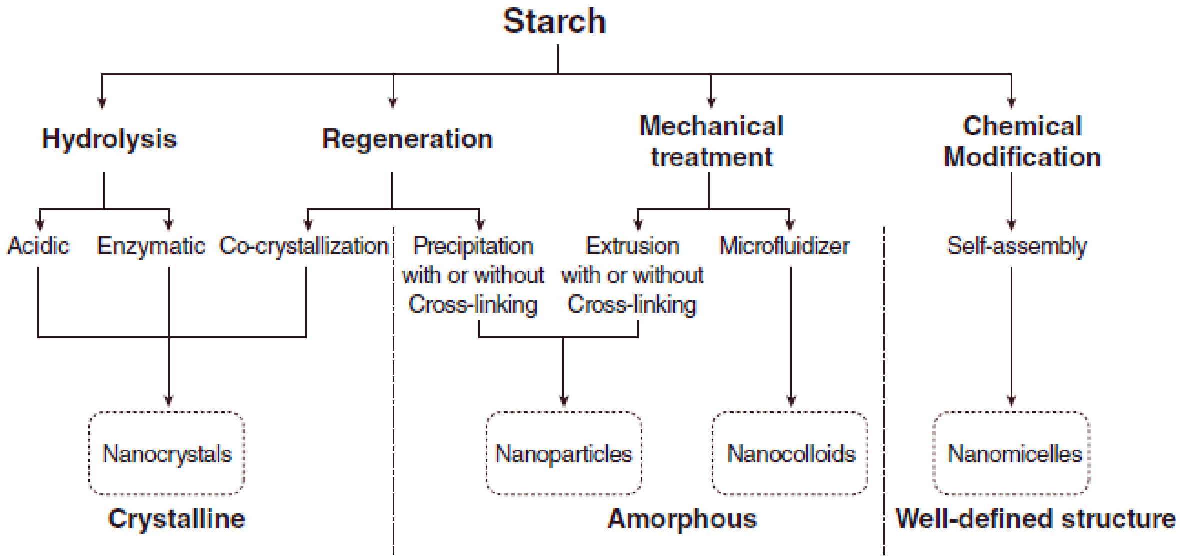


Figure 1.10.1: Different methods for preparation of starch nanoparticles

1.10.2 Starch–based bionanocomposite

For bioplastic, starch–based biodegradable polymers have great commercial potential but some of properties including brittleness, low heat distortion temperature, high gas permeability, and low melt viscosity restrict their uses in a wide range of applications so the modification of biodegradable polymers through innovative technology is a challenge for material scientists [57]. Addition of nano–reinforcement to the polymers for preparing the nanocomposite has already proven to be an effective way to improve these properties concurrently [58]. Hence, nanocomposites are being considered as the next–generation materials. A number of researchers have presented work in the field of starch–based bionanocomposites, which can be obtained by filling a thermoplastic starch (TPS) matrix with nanofillers such as layer silicates, carbon nanotubes, carbon black, cellulose, and starch nanocrystals. Montmorillonite (MMT) [59, 60] and kaolinite [61] are the usual layer silicates used in starch–based bionanocomposites. Cellulose nanocrystals or whiskers have been used to reinforce starchy material [58, 62, 63]. Starch can also be used as a filler to produce reinforced plastics [64, 65]. A small amount of starch (6–30%) is usually compounded with synthetic polymer, increasing the biodegradability of the product.

1.10.3 Applications of starch nanoparticles and starch–based bionanocomposites

Due to the abundant availability of starch and its low cost, renewability, biocompatibility, biodegradability, and nontoxicity, starch nanoparticles are having the great attention. Natural fillers, synthetic polymeric matrices and reinforcing agents have been created by starch nanocrystals obtained by acid hydrolysis of starch [66]. Nanoparticles (fillers) can enhance the mechanical and physical properties such as permeability or fire retardancy. Their properties depend on the nature and effectiveness of interactions at the interfacial region, on both surface areas and the dispersion of the particles [67]. Starch nonofillers can be used in natural polymer matrices and synthetic polymer matrix [1].

Bionanocomposites are biodegradable materials with a biological origin. Bionanocomposites serve a number of important functions, such as extending food shelf life and enhancing food quality as barriers to moisture, water vapor, gases, and solutes and as carriers of some active substances, such as antioxidants and antimicrobials [1]. The main types of plastics currently used in all applications are derived from nonrenewable petroleum resources which result in non-biodegradable plastic materials. Because the volume of plastics discarded annually creates a substantial waste disposal issue so methods to reduce the quantity of persistent plastic wastes are required. One of methods is to make materials from biodegradable materials able to degrade through composting or feedstock recycling. A number of biodegradable materials have been investigated for use as plastics [68].

Nanotechnology has great potential applications in all aspects of the food industry including storage, quality monitoring, food processing, and food packaging. It ranges from intelligent packaging to creation of on–demand interactive food that allows consumers to modify food, depending on tastes and nutritional needs. Different types of materials, including plastics, cardboard, and metal, are used for food packaging depending on specific needs. The use of plastics in food packaging, including films, is common and increasing because of low cost and functional advantages over other materials [69-71]. Bionanocomposites are considered as hybrid nanostructured materials with improved mechanical, thermal, and gas barrier properties and they are used for food packaging in order to protect the food and increase its shelf life as well as become a more environmentally friendly solution because it reduces the requirement of using the plastics as packaging materials. Most of the traditional packaging materials are made from nondegradable materials which increase environmental pollution [72, 73].

Cassava starch has been extensively used to produce biodegradable films which are the promising materials. Films developed from starch are described as isotropic, odorless, tasteless, colorless, nontoxic and biologically degradable [74, 75]. The association of cassava starch with plasticizers as glycerol, sucrose, and inverted sugar can promote alterations in the films by justifying the study of these additives to develop a potential and ecological change to the synthetic packaging of several food products. The results show that films based on plasticized cassava starch reinforced with clay nanoparticles can be considered as an interesting biodegradable packaging material [75]. As a natural biopolymer, besides its biodegradable character, starch would be a promising material for the development of new food packaging materials because of its attractive combination of availability and price [75].

According to the recent studies demonstrated that biodegradable starch-based polymers have a range of properties that make them suitable for use in several biomedical applications which ranging from bone plates and screws to drug delivery carriers and tissue engineering scaffolds with biofunctionality and biocompatibility [76]. The world consumption of plastic materials in agriculture amounts yearly to 6.5 million tons has been used to improve crop cultivation and protect agricultural products after harvesting, in the form of greenhouses, tunnels, mulch, silage films, and bale wraps. There is a huge amount of plastics discarded into the environment, buried in the soil, or burnt by farmers, releasing harmful substances with the associated negative consequences to the environment. A solution to this problem can be the introduction in agriculture of biodegradable films disposed directly into the soil or a composting system at the end of their lifetime [68].

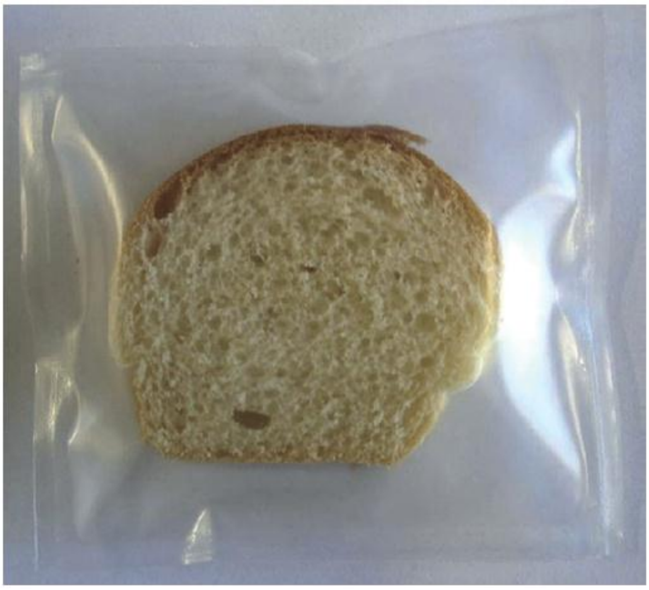


Figure 1.10.3: Toast packaged with a biodegradable film [75]

1.10.4 Nanofibrillated cellulose

Cellulose is the main structural component in the cell walls of all plants. It is a fibrous, tough, and water–insoluble substance which is widely considered to be the most abundant bioderived polymer on the earth. From an industrial sector, cellulose has been mainly contributed to the wood products and pulp and paper industries for past 100 years. The amount between 10^{10} and 10^{11} tons of cellulose are produced each year but only about 6×10^9 tons of this amount are used annually by industry to form pulp, paper, textiles, chemicals, and other materials [77].

a) Nanocellulose

Depending on sources, the microfibrils of cellulose formed during biosynthesis are 2–20 nm in diameter and can be several micrometers in length as each microfibril consists of crystalline domains interspersed with disordered amorphous regions [78]. Nanofibrillated cellulose (NFC) is obtained by mechanical treatment of the materials in the general category of nanocellulose as cellulose whiskers known as cellulose nanocrystals, nanorods, or nanowhiskers are obtained by acid hydrolysis in order to remove the amorphous segments and leave highly crystalline [79-81]. The unique nanofibrillar structure of bacterial cellulose confers excellent physical and mechanical properties such as high porosity, high elastic modulus, and high crystallinity [82-84]. Nanocellulose has long been recognized for its potential in a wide variety of applications including paper and paperboard, composites, hygiene and absorbent products, food additives, emulsifiers and dispersants, oil recovery, cosmetic, medical, and pharmaceutical uses [81, 85].

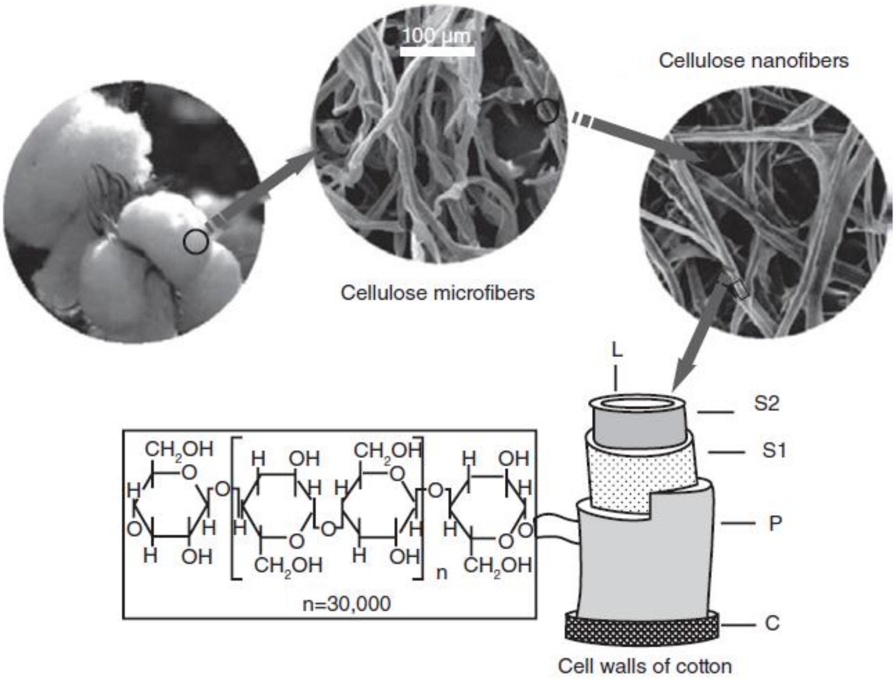


Figure 1.10.4 (a): The multi–scale nature of cellulose as illustrated for cotton [35]

b) Nanofibrillated cellulose

NFC referred to as microfibrillated cellulose (MFC) was first prepared by Turbak and colleagues at the ITT Rayonier laboratories in the United States in the late 1970s. A Gaulin–type milk homogenizer was used in this early work to process wood pulp at high temperatures and pressures into a gel–like material obtained after ejection from the homogenizer against a hard surface. The Gaulin homogenizer was first demonstrated at a dairy exhibition in Paris in 1900 and the equivalent is now manufactured by SPX Corporation in Germany and the United States. The first publications on the preparation of MFC were done in the early 1980s by Turbak and coworkers at ITT Rayonier when term of microfibrillated cellulose first came into use [86, 87].

NFC is obtained by a homogenization process and composed of expanded high–volume cellulose, moderately degraded, and greatly expanded in surface area. NFC consists of aggregates of cellulose microfibrils and has fibril diameters in the range of 20–60 nm. Since cellulose microfibrils are 2–10 nm in diameter and are several tens of micrometers in length, the individual elements of NFC is comprised consist of between 10 and 50 microfibrils. NFC exhibits gel–like properties in water with pseudoplastic and thixotropic properties and can be cast into transparent films shown in figure 1.10.4 (b_1) [88]. The figure 1.10.4 (b_2) shows the NFC gel created in the laboratory of LEPAMAP Group, University of Girona (UdG), and NFC film obtained from pure NFC 10 mmol of bleached pine pulp by drying in rapid köthen about 15 min.

Figure 1.10.4 (b_1): NFC film and 0.4% consistency NFC gel produced at the Paper and Fiber Research Institute (PFI), Trondheim, Norway, being handled by Kristin Syverud (left) and Ingebjorg Leirset (right) [88]

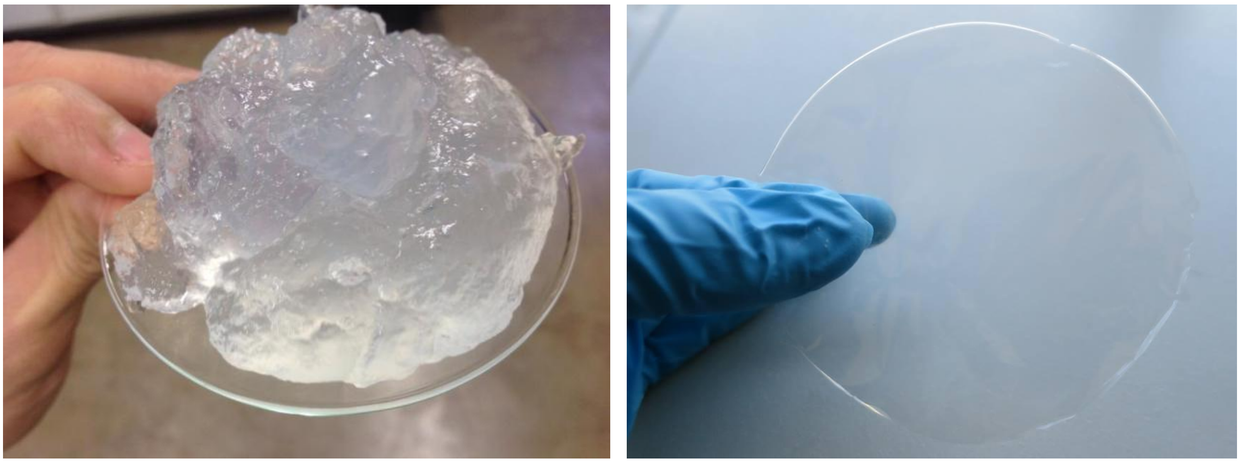


Figure 1.10.4 (b_2): NFC gel 10 mmol from bleached pine pulp (left) and NFC film obtained by drying in rapid köthen about 15 min (right)

1.10.5 Preparation of nanofibrillated cellulose

a) Mechanical Treatments

The Gaulin homogenizer first used to generate NFC gels by Turbak and coworkers is still in use today to manufacture various forms of NFC. In this process, cellulose slurries are pumped at high pressure and fed through a spring–loaded valve. The valve opens and closes in rapid succession and the fibers are subjected to large changes in pressure with high shearing forces. The results of this procedure provide a high degree of cellulose fiber fibrillation [1]. The same researchers noted that the grinding process resulted in a decrease in fibril length and would be undesirable for some applications. According to a number of authors, the physical nature of NFC gels with intertwined fibrils makes accurate measurement of fibril length a significant challenge. The outer layers of fibers are defibrillated, with of them passing to a fibrous suspension in the form of fines. Defibrillating allows the swelling of fibers by hydration [89]. Beating also decreases the fiber’s length due to the cutting effect and superficial fibrillation. Beaten fibers exhibit more flexibility. The bonding ability between fibers is enhanced in the last staged of drainage when the distance between them becomes short enough to improve the paper sheet cohesion [89]. A number of properties of NFC from bleached and unbleached hardwood pulp samples and the energy consumption during production as a function of the manufacturing process are examined by Spence and colleagues [90]. These researchers discovered that microfluidization with refining pretreatment or micro–grinding processes used less energy than homogenization and also produced cast NFC films with better physical, optical, and water interaction properties [90].

b) Pretreatments

In order to reduce energy consumption in NFC production, a number of methods have been suggested for pretreatment of plant or wood fibers. The methods generally involve one of three different principles: (1) interfering with hydrogen bonding between fibrils, (2) adding electrostatic charges, or (3) decreasing the DP or amorphous linkages between fibrils. The use of combined refining and enzymatic pretreatments for NFC production has proven effective as a means of reducing energy consumption. Researchers have produced high aspect ratio NFC with good nanofibril diameter uniformity through a combination of enzymatic hydrolysis with mechanical shearing and high pressure homogenization [91-93].

The use of TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical)-mediated oxidation as a method for fiber pretreatment has been broadly reported. This procedure allows carboxylate and aldehyde groups to be introduced into solid native cellulose under mild aqueous conditions. TEMPO–mediated oxidation significantly decreases the required energy consumption if compared with repeated high pressure homogenization. The principle behind TEMPO oxidation is the addition of NaOCl to aqueous cellulose suspensions in the presence of catalytic quantities of TEMPO and NaBr at pH 10–11 and room temperature. The outcome is the selective conversion of C_6 primary hydroxyl groups on cellulose to aldehyde and then carboxylate groups. As a consequence, the presence of ionized carboxylate groups on the fibril surfaces leads to repulsive forces, which cause improved separation. In general, increase in concentration of NaOCl leads to higher levels of carboxylation, but also to a requirement for longer oxidation times [94].

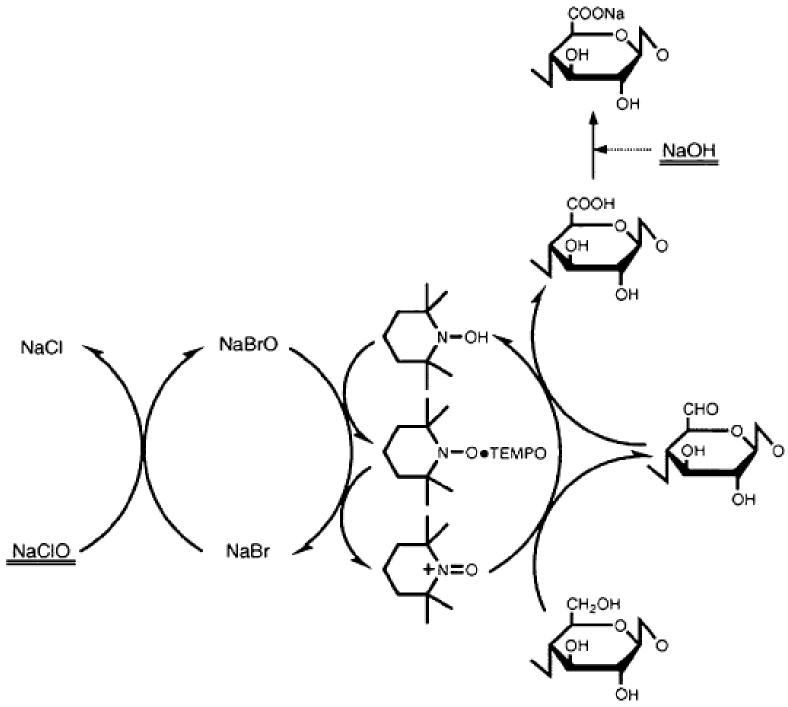


Figure 1.10.5 (b$_1$): Schematic of TEMPO oxidation of cellulose [94]

Application of TEMPO oxidation to nanocellulose production was primarily developed by Isogai and coworkers and has been used to process various different forms of cellulosic biomass [95, 96]. Oxidation efficiencies vary from 62 to 96% depending on the type of starting plant material. An approach to TEMPO oxidation involves the use of NaOCl in combination with NaO_2Cl instead of NaBr at pH 5–7 [97-99]. In contrast to the first method, in which some residual aldehyde groups are presented on the cellulose nanofibrils after pretreatment, the combined TEMPO/NaOCl/NaO_2Cl method at pH 5–7 leaves no residual aldehyde groups. However, the carboxylate content is lower in this case and the optimum reaction time and temperature are increased [94]. A representative process for producing TEMPO-oxidized cellulose is shown schematically in the figure 1.10.5 (b$_2$).

Pretreatment of fibers through carboxymethylation is also an established method for NFC production. In the case with TEMPO oxidation, the formation of carboxylate groups on the surface of the NFC introduces repulsive charges, which assists in the process of nanofibril separation [100-103]. Treatment using sodium salts ensures that the incoming wood pulp swells as much as possible. Swollen pulps have reduced fiber cell wall cohesion and therefore should be easier to delaminate.

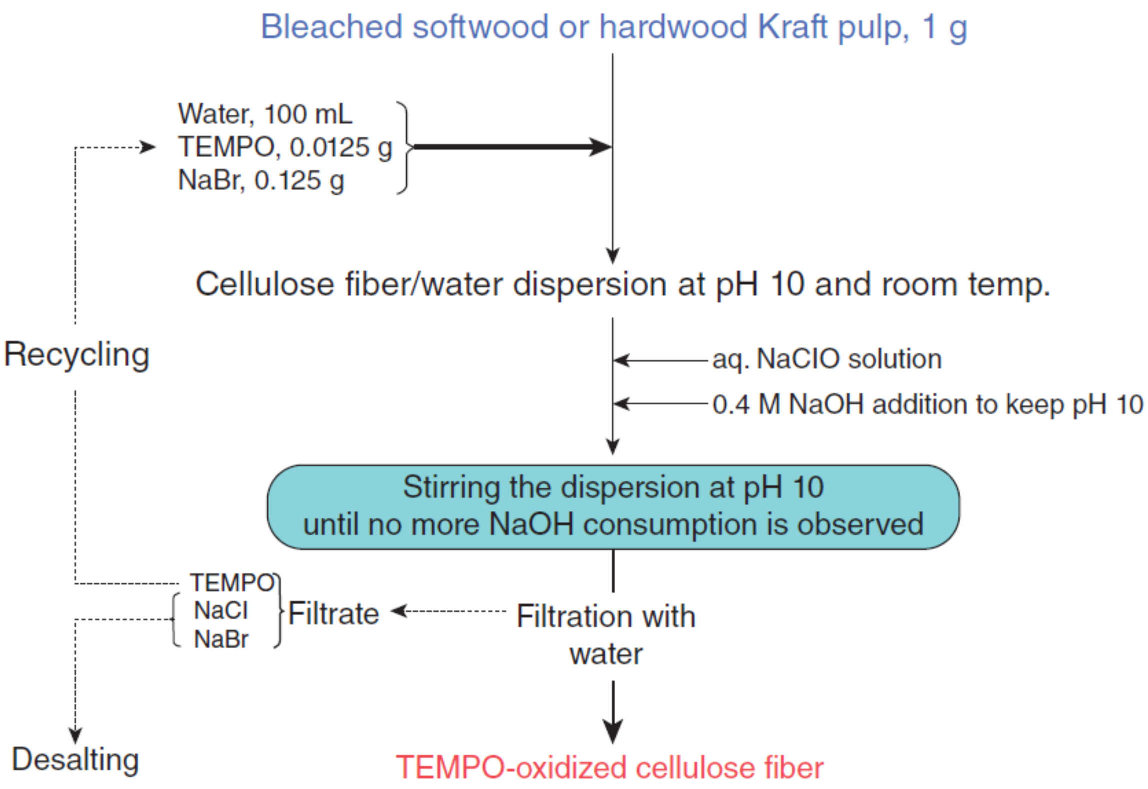


Figure 1.10.5 (b$_2$): A representative process for preparation of TEMPO–oxidized cellulose by the TEMPO/NaBr/NaOCl system at pH 10 [94]

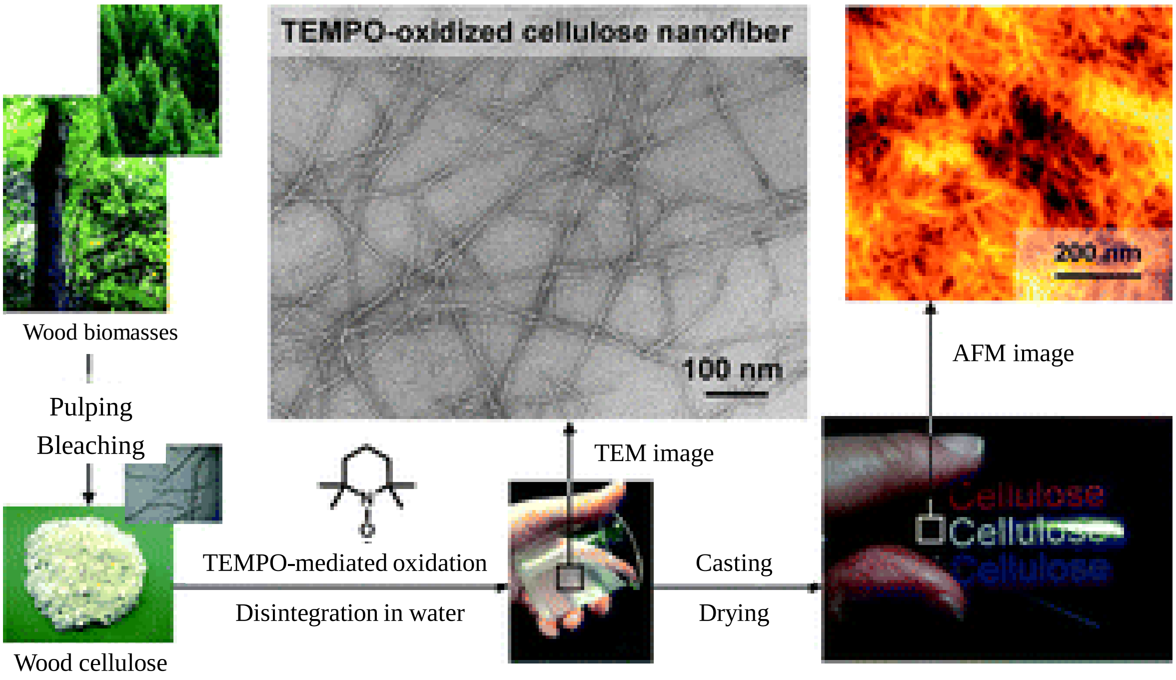


Figure 1.10.5 (b$_3$): Microscopic structure of TEMPO–oxidized cellulose [94]

1.10.6 Implementations of nanofibrillated cellulose

What is clear is that the incorporation of NFC into lignocellulosic fibrous slurries should result in an increase of the physical–mechanical properties of the biocomposites produced from the mixture. In recent years, the use of NFC as a reinforcing additive to improve physical and mechanical properties of paper has been studied [104-106]. NFC presents some very interesting properties for papermaking such as a large specific surface area and high aspect ratio; moreover, it enhances tensile strength and reduces the porosity of the final paper sheet [106-110]. The enhancement of tensile strength and Young's modulus with respect to NFC content was linear. This improvement is given by several factors: (1) the presence of nanofibrils with high intrinsic mechanical properties; (2) a high specific surface area in the slurry that boosts the number of hydrogen bounds; (3) a homogeneous distribution of all the fibers in the paper structure that guarantees that stress is uniformly distributed across the paper; (4) a reduction of void spaces between fibers that lessens the number of failure-starting areas [111]. The presence of NFC generates a propitious interface without the need for any prior beating [112]. The use of nanofibrillated cellulose (NFC) contributes significantly to increasing the lignocellulosic fibrous network's specific surface area (SSA) as a consequence of NFC's nanometric size [110].

González, I., et al (2014) studied the evolution of physical and mechanical properties from papers with 100% content of bleached eucalyptus fibers to 100% tetramethylpiperidine-1-oxyl radical (TEMPO)–NFC from the same bleached pulp, with intermediates of 25, 50 and 75% content of NFC [113]. In addition to the properties of NFC including their low weight, abundance, biodegradability and renewability, high strength and rigidity, and reinforcement agent, NFC presents the ability to form networks of great strength within the matrix through hydrogen-bonding [114, 115]. The resulting nanocomposites present improved mechanical strength, barrier properties, transparency and biodegradability. Its addition on papermaking slurries improves strength, increases density and reduces porosity of the ensuing paper [106]. The films or sheets made completely of NFC (nanopaper) have been produced either by casting of a diluted NFC water suspension and evaporation of water at room temperature or in an oven [116-118], or by vacuum filtering of the same dilution [119-124]. Nanopapers become denser, bulkier and smoother as more NFC is added and opacity was greatly reduced in accordance to the amount of NFC [113]. As results of this study, the evolution of mechanical properties is also in relation to the amount of NFC in the nanopaper as tensile strength and Young's modulus increased linearly after more NFC was added to the paper. NFC also induced an improvement in the elastic limit [113]. The dependence of paper strength on the amount of NFC indicates that the ultimate tensile strength relies on nanofibril breakage [113].

Alcalá, M., et al (2013) fabricated and characterized NFC obtained from bleached eucalyptus pulp through TEMPO–mediated oxidation at pH 7 as well as studied the physical and intrinsic mechanical properties of the ensuing NFC. The NFC was used as reinforcement in unbleached eucalyptus pulp–based biocomposites, which were later characterized from their mechanical and thermal properties [110]. The results of this study showed that tensile strength and Young's modulus improved linearly, increasing up to 150 and 60%, respectively, in biocomposites reinforced with 9 wt% of NFC. Moreover, biocomposites became denser as more reinforcement was added, showing a good correlation with the evolution of tensile strength and Young's modulus. As a consequence of increasing density, the porosity decreased. The results also permitted calculating the coupling and efficiency factors which presented values very similar to those reported for composites with polymeric matrices. Finally, it was also demonstrated that dispersion of NFC significantly improves the properties in biocomposites as well as both coupling and efficiency factors [110].

Deinked fibers are of particular interest and numerous efforts have been made to fully exploit them [125-127]. The use of nanofibrillated cellulose (NFC) as an alternative to mechanical beating to improve the strength properties of paper produced from a deinked recycled pulp (DIP) obtained by disintegration and flotation of a mixture of old newspapers (ONP) and old magazines (OMG) is studied [111]. Paper containing 1.5% of NFC presented a higher tensile strength and stiffness than paper from beaten pulp with similar freeness and water retention values (WRV). A higher amount of NFC further improved the mechanical properties, up to double the tensile index, which opens new possibilities of use for recycled paper [111]. The effects of NFC were compared with soft mechanical beating. The increase in paper's strength was significantly higher compared to beaten DIP with similar density. This allowed the recovering and even improvement of the original strength of recycled papers. The tensile index and tensile strength also improved with respect to non–reinforced, beaten DIP. Papers also became stiffer, denser and less porous with the presence of NFC [111]. A significant advantage of CNF over beaten is that no further structural damage is applied to the fibers, which allows a longer lifetime of them. The lower structural damage allows working with lower basis weights or even the addition of mineral fillers. Higher percentages of NFC require the optimization of physicochemical formulations in order to keep the runnability of the fibrous suspension [111].

Nanocomposites

Light, yet strong automotive parts can be made by mixing cellulose nanofibers with resins and rubbers.

Filter

Filters made from nanofibers with a large specific surface area can collect small dust particles.

Gas Barrier Film

Films through which air cannot easily pass are effective in maintaining freshness in food.

Cosmetics, Food, Coating Mixtures, etc.

Imparts viscosity in water, and can distribute particles

Transparent Sheet

Can be used as a material such as for a transparent display

Figure 1.10.6: Applications of nanofibrillated cellulose (NFC) [128]

1.11 Plasticization and plasticizers

1.11.1 Plasticization

Starch is most often plasticized with water and polyols such as glycerol. For example, a glycerol content of 25% allows decreasing the glass transition temperature of potato starch–based materials stored at 57% RH from 285°C to –10°C [129]. Depending on the plasticizer content, two mechanical behaviors are evidenced whatever the starch origin. For a low plasticizer content, mechanical properties present similarities to the antiplasticization effect generally observed in synthetic polymers [130, 131]. For higher amounts of plasticizer, mechanical properties are controlled by the glass transition, which involves the molecular motions.

The plasticization effect is a phenomenon that governs the mechanical properties and characterized by an increase in the strain at break and a decrease in both the strength and Young's modulus [132]. Plasticizers (water and glycerol) play the role of internal lubricants by preventing the rigidification of noncrystallized macromolecular starch chains at ambient temperature. Currently, all formulations of thermoplastic starch materials require the use of very large amounts of plasticizer (roughly 20 wt%) to avoid antiplasticization events. As a result, starch–based materials have globally poor mechanical properties due to the relaxation of the polymer network in the presence of plasticizer.

In the case of plasticized starch/glycerol systems, dynamic mechanical analysis (DMA) shows the presence of two phenomena associated with a two-step storage modulus drop. It is characterized by two peaks on loss modulus and tan δ curves, around −40°C and 40°C (25 wt% of glycerol and 15 wt% of water), revealing the heterogeneity of the system and a phase separation. The first peak is associated with the relaxation of glycerol–rich domains, and the second one with starch-rich domains [133-135].

1.11.2 Plasticizers

Additives can gel the polymer and improve processibility and flexibility of plastics by decreasing their melt viscosity, glass transition temperature and modulus of elasticity without alteration of the chemical character of the polymer. Some extents of them are extractable into the environment. Although their toxicity is very low, aspects of toxicology during oral, dermal or intraperitonal exposures have been regularly tested. PVC and other chlorinated polymers are the principal application fields for plasticizers [2]. Plasticizers are generally differentiated as external and internal. The former are bound to the plastics macromolecule as a solvent, by physical (van der Waals) forces. The amount of adsorbed plasticizers is almost unlimited. However, even small amounts of most plasticizers have noticeable effects in plastics. The advantages of external plasticizers consist in the wide variations of both amounts which can be used and the properties of the bound substances [2]. The role of diffusion and volatility of external plasticizers increases rapidly with temperature. Some thermo–oxidation and thermolysis of plasticizers may also result. High-molecular-weight external plasticizers with limited migration rates are always preferred. The plasticizer content in the plastics plays a key role in the evaluation of food contact materials and articles. As long as the danger exists that the plasticizer can transfer to food, the application in plastics is fundamentally undesirable. Soft PVC films with high oxygen permeability for packing fresh meat may not contain phthalates and phosphates and the total amount of plasticizers may not exceed 22%. Some plasticizers used at low levels (up to 15%) can increase brittleness, as a consequence of increased orientation of the plastic and formation of crystalline regions [2].

1.12 Thermoplastic starch (TPS)

Thermoplastic starch (TPS) is appeared to be one of the most useful and promising materials for packaging purpose and it is considered as a fully biodegradable biopolymer. Thermal and mechanical processing should disrupt semi–crystalline starch granules to obtain TPS. As the melting temperature of pure starch is substantially higher than its decomposition temperature, there is a necessity to use plasticizers such as glycerol [136]. TPS can be obtained by the proper treatment with temperature and pressure in the presence of a plasticizer, like water, glycerol or sorbitol [137]. The plasticizer penetrates into the starch granules and disrupts the initial crystallographic structure. The material undergoes a melting process and forms a continuous amorphous mass which does not exhibit diffraction anymore due to temperature and shear forces [138-143].

Starch is known to be completely degradable in soil and water and can promote the biodegradability of a non-biodegradable plastic when blended [12]. Starch is a blend of amylose and amylopectin, both of which are polysaccharides composed of α–D–glucopyranosyl units, $(C_6H_{10}O_5)n$. Native starch granules swell when they absorb water through hydrogen bonding with their free hydroxyl group, but they still retain their order and crystallinity. However, when these swollen starch granules are heated, hydrogen bonding between adjacent glucose units is disrupted. The crystallinity is progressively destroyed. This process is called gelatinization [144]. As a packaging material, starch alone does not form films with appropriate mechanical properties unless it is first plasticized, or chemically modified. Common plasticizers for hydrophilic polymers, such as starch, are glycerol and other low molecular weight polyhydroxy compounds, polyethers, urea and water [12]. TPS is the preferred starch derivative, as it represents a reactive intermediate product with complete film forming properties. The newly developed manufacturing procedures are based on the technologies of compounding extrusion and reaction compounding extrusion, and lead to optimize the compostable plastic raw materials that can be further processed in the plastics processing industry to packaging materials, textiles and consumption articles using existing production lines. Many biodegradable TPS which are either already on the market or at an advanced development stage are reported [145, 146].

TPS alone often cannot meet all these requirements. The hydrophilicity performance of the starch changes during and after processing due to the water content changes [12]. A first insight of TPS and kaolin clay interaction was reported by Curvelo and De Carvalho [147]. Starch, plasticizer (w/w 30%) and clay were mixed in a polyethylene bag till the formation of powder. During thermogravimetric analysis the residual weight was proportional to the filler content in the matrix. In the same direction biodegradable thermoplastic hybrid was prepared by melt intercalation method [148]. Wilhelm and Sierakowski prepared nanocomposites of starch and concluded that there is no significant effect of clay on the thermal degradation of starch, whereas a significant increase in thermal stability was observed when nanocomposites of TPS and unmodified MMT was prepared by melt intercalation method even at 5% filler content [135]. The plasticization effect has been studied in starch layered silicate nanocomposites [149]. The direct degradation studies of starch nanocomposites have not been done so far and thus there is no experimental explanation about the effect of clay on the microbial consumption of starch [37].

Recently, starch/clay nanocomposite films were obtained by dispersing MMT nanoparticles via polymer melt processing techniques. Mechanical characterization results show an increase of modulus and tensile strength. Moreover, the conformity of the resulting material samples with actual (2005) regulations and European directives on biodegradable materials was verified by migration tests [150]. Several applications of bio-based as well as edible films and coatings are reported in literature. However, producers of packaging materials and manufacturers of food products haven't yet demonstrated their interest because of the problems related with the application of these materials [151]. The problems associated with biodegradable polymers are threefold such as performance, processing, and cost. Although these factors are somewhat interrelated, problems due to performance and processing are common to all biodegradable polymers [37, 152]. In particular, brittleness, low heat distortion temperature, high gas and vapor permeability, poor resistance to protracted processing operations have strongly limited their applications [151, 153].

1.13 Composite materials

Composite materials are formed of two or more materials with very different properties, which act synergistically to create new properties that cannot be achieved by each single material alone. Typically, one of the materials of the composite acts as a matrix, whereas the other materials act as reinforcing phases. Composite materials can be classified as metal–matrix, ceramic–matrix, or polymer–matrix. For each of these composite materials, the reinforcing phases can be a metal, a ceramic, or a polymer, depending on the targeted applications.

As reinforcements in composite matrices, natural fibers attracted a particular attention and interest in recent years as substitutes to synthetic fibers such as glass and carbon fibers. They improve the mechanical properties, allow a gain in weight and in cost, and can be recycled [154]. Many researches have been done to study the mechanical properties and the interfacial adherence performances based on natural fiber composites especially between the hydrophilic natural fibers and the hydrophobic polymer matrices [155, 156]. The adhesion between the fibers hydrophilic surface and the hydrophobic polymer used as the matrix is affected by the humidity adsorption capacity and the presence of non–cellulosic components (waxy material, lignin and hemicelluloses). The fibers in these cases undergo significantly damage and reduce the material strength [157, 158]. Many focused studies on different methods and means of treatment of natural fibers surfaces as a reinforcement of composite materials were conducted to improve inter facial link properties and to increase their resistance to humidity [159-161].

Mutjé, P., et al (2006) studied the effect of maleated polypropylene as coupling agent for polypropylene composites reinforced with hemp strands [162]. The improvements in the static mechanical properties are obtained in spite of the fact that the interface between the hemp strands and the matrix does not present the best conditions to be an efficient reinforcement. It is well known that the high polarity of the lignocellulose strands is due to its chemical structure, and the low polarity of the thermoplastic matrices is derived from petroleum [163]. Hemp strands show an irregular surface and considerable porosity. Thus, the surface morphology of the strands also affects the interface allowing mechanical anchoring between the fiber and the PP. This effect is due to the coating and the inter–diffusion of the polymer on the surface and in the pores of the reinforcement [163-165]. The effect of a refining process of the strands on the mechanical properties of the final composite is also studied and developed [89, 165]. The refining process increases the specific surface leading a rise in the mechanical properties values because of a surface fibrillation, an increase in the flexibility and a slightly decrease in the strand length. The aim of this kind of modifications is the reduction of the polarity of the surface of the fibers or the branching of structures to simulate the nature of the polymer matrices improving a better compatibility in the interface. Another methodology is the establishment of a covalent bond between the fiber and the matrix [166].

Chronologically, inorganic minerals such as glass fiber, talc, calcium carbonate, etc. were initially used as fillers in polymer composites. This is closely followed by the natural organic filler [167]. Calcium carbonate is the most commonly type of mineral fillers that are incorporated into plastic, specifically polypropylene (PP) due to its lower price and its filler loading that can be manipulated to high filler loading. Flaky mineral filler such as mica, kaolin and talc on the other hand could bring a good advantage to improve rigidity of composites. It is also important for polymer composites to have exceptionally low coefficient of thermal expansion (CTE) because it can reduce fracture when there is a sudden change in temperature. With the incorporation of mineral fillers mentioned above including silica, this can help in reducing the value of CTE in polymer composites [168]. Apart from that, incorporation of inorganic mineral fillers can help to enhance the performance of polymer composites by improving the stiffness, heat distortion temperature, toughness, dimensional stability and hardness. It is worth to note that the particle shape, loading, size and dispersion of fillers affect the properties of polymer composites [169].

1.14 Barrier properties and packaging

Barrier materials are coatings or multilayer combinations of plastics designed to reduce water and gas diffusion into and/or out of the rigid or flexible package. Barriers to oxygen are necessary to preserve food freshness since oxygen can cause a chemical change in the product and rancidity in fat products. Water vapor can change the physical appearance of a product by either caking a dry material or by causing it to become soggy. Carbon dioxide does not cause food spoilage and is used to protect some foods against spoilage. Barriers to other gases such as nitrogen are used in modified atmosphere packaging to extend the shelf life of fresh foods without the use of preservatives [170]. Modified atmosphere packaging (MAP) is used for fresh products such as meat, fish, poultry, salads, and vegetables, in order to satisfy an increasing demand for fresh, additive and preservative foods. In these packages, the composition of the natural air atmosphere inside the container is replaced by a gaseous mixture [170].

Barrier properties of the materials form an important physical property of the system and very important to be understood and optimized for the potential use of the materials in a number of different applications. Polymer nanocomposites have been generally synthesized for the improvement in the mechanical performance thus allowing their use as engineering materials. Other properties have also been studied in these nanocomposites but barrier properties have mostly been neglected. It is generally assumed that improvement in the mechanical properties leads to automatic improvements in the other properties [171]. The barrier films are used mainly in packaging, as they are able to provide a barrier to gases, essentially oxygen, as PVDC and EVOH are the largest tonnage barrier materials. Yet there are many other barrier materials and solutions. Other plastics offer a number of properties required for many functional applications, such as exceptional strength, temperature resistance, stiffness, printable surface, and as a barrier medium. PET and nylon are the main films that contain these functions, but there are also a number of very specialized films, more used in applications other than packaging [9].

1.15 Permeation of packaging materials

Generally, permeability of a penetrant through a polymer depends on many factors, including the nature of the polymer, thickness of the film, size and shape of the penetrant, pressure, and temperature. The structural properties which can influence the permeability of polymers include polarity, unsaturation, symmetry, lateral chains, steric hindrance, degree of cross–linking, hydrogen bonding, intermolecular forces, presence of comonomers, crystallinity, glass transition temperature, and orientation [172].

Table 1.15: Permeability of oxygen, carbon dioxide, and water vapor of some plastic films

Permeability to	$O_2 \times 10^{11}$ mL cm cm^{-2} s^{-1} (cm Hg)$^{-1}$ at 23°C, 0% RH	$CO_2 \times 10^{11}$ mL cm cm^{-2} s^{-1} (cm Hg)$^{-1}$ at 23°C, 0% RH	$H_2O \times 10^{11}$ g cm cm^{-2} s^{-1} at 23°C, 100% RH
LDPE	15–30	60–160	5–10
HDPE	5–17	150	1.8–3.5
EVA (15%VA)	30–40	—	21–25
Ethylene acid copolymer (ionomer)	20–35	—	5–11
PP	9–16	30–50	4–10
PET	0.14	1.2	4–6[a]
PS	18–25	60–90	9–46
PVC plasticized	1.7–100[b]	6–180	—
PVC rigid	0.3–1.2[b]	1.2–3	14
PA6	0.09–0.11	0.6–0.8	46[a]
PA66	0.2	—	86
PVdC	0.006[b]	—	0.7[a]
EVOH (32% C_2H_4)	0.0015	0.018	17.5[a]

Source: Massey (2003) [173]

a: 40°C and 90% RH

b: 23°C and 50% RH

1.16 Factors affecting product quality and shelf life

For many foods, the product shelf life is limited by specific or key attributes that can be predicted at the time of product development. There are 2 factors, intrinsic and extrinsic factors, which can determine the shelf life of products [5]. Selection of raw materials is important for controlling intrinsic factors, since subsequent processing can rarely compensate for poor-quality raw materials. Intrinsic factors are the properties resulting from the make–up of the final product. They include water activity (aw), pH/total acidity, natural microflora and surviving microbiological counts in final product, availability of oxygen, redox potential (Eh), natural biochemistry/chemistry of the product, added preservatives, product formulation, and packaging interactions [5]. Whereas, extrinsic factors are a result of the environment that the product encounters during life and include time–temperature profile during processing, temperature control during storage and distribution, relative humidity (RH) during storage and distribution, exposure to light (UV and IR) during storage and distribution, composition of gas atmosphere within packaging and consumer handling [5].

1.17 Chemical interaction between food and packaging materials

There are four chemical interactions between food and packaging materials including migration, scalping, egress permeation, and ingress permeation. Migration is referred to the transferring of package components to the products as scalping is meant the transport of product components to the package. Egress permeation is defined as the transmission of components of through the package to the environment but ingress permeation is explained as the transport of environmental components through the package to the product. Several package–food interactions can reduce the quality of packaged foods and provide the undesirable effects including alteration of aroma, changes of package performance, transmission or addition of toxicants, moisture gain/loss, oxidation, gas loss, nutrient loss, favor deterioration, etc. However, it is possible to design packaging which utilizes package–food interactions to maintain or improve the quality of product and can be able to create the desire effects including oxidation and microbial growth inhibition, alteration of gas atmosphere, removing of undesirable aromas/flavors, desirable aromas addition, etc. [174].

2. Objectives

The objectives of the study "development of a new totally biodegradable film based on starch and nanofibrillated cellulose for barrier properties" are summarized as follows:

- To study the feasibility of the creation of a new film totally biodegradable and environmental friendly which is made by starch and nanofibrillated cellulose (NFC) compounding, considered as a biodegradable and good reinforcement and barrier agent.
- To determine the optimum conditions and parameters of pre-blend, blend, and transparent and flexible film preparations.
- To characterize films' properties: mechanical characterization (tensile testing), water absorption, biodegradability, and water vapor permeability capacity determination.

3. Research methodologies

3.1 Experimental locations

The experiment, development of a new totally biodegradable film based on starch and nanofibrillated cellulose for barrier properties, was conducted in laboratories of LEPAMAP Group of Escola Politècnica Superior (EPS) and Parc Científic i Tecnològic of University of Girona (UdG), Spain. The testing of properties and quality of samples were also done in the laboratories of these research locations. All facilities for this research were also available and found in those laboratories.

3.2 Materials

3.2.1 Materials for films preparation

a) Potato starch

Potato starch, shown in figure 3.2.1 (a), was used as a raw material in this experiment. It was made in France and it can be edible and used in foods. This material was completely graded according to European standard and supported by AVEBE Company, Veendam Holland. Indeed, it was considered as a kind of biodegradable materials used to produce food packaging materials with high biodegradability and sustainability.

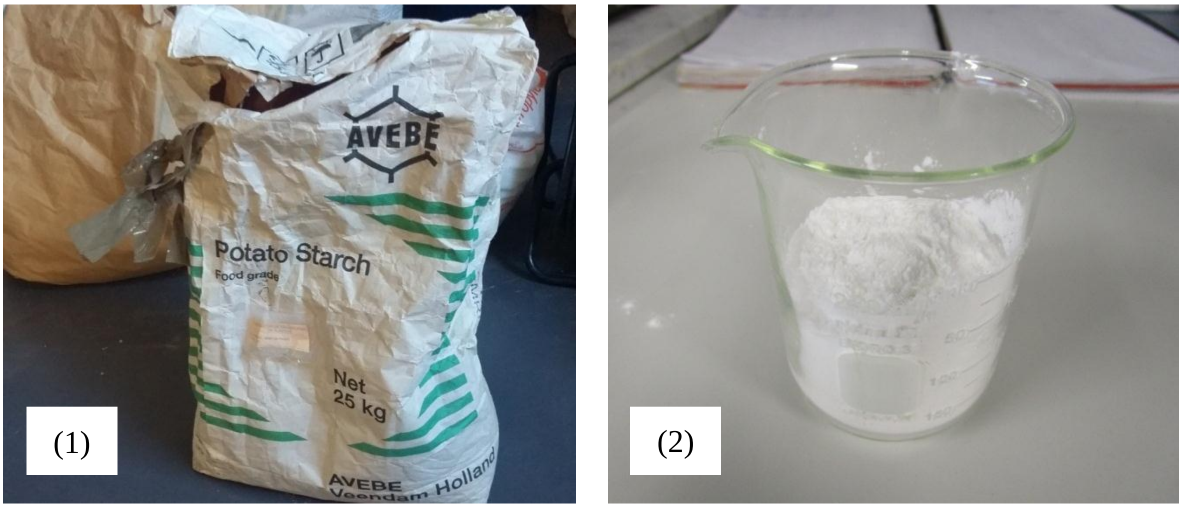


Figure 3.2.1 (a): (1) and (2) potato starch for using and producing the biodegradable films

b) Glycerol

Glycerol, played role as plasticizer, was used as a chemical compound to link starch molecules to become a new material. It has an important role to change the properties of starch and break down crystallization of amylopectin. This chemical compound was brought from Sigma-Aldrich Company. Figure 3.2.1 (b) shows about the used glycerol.

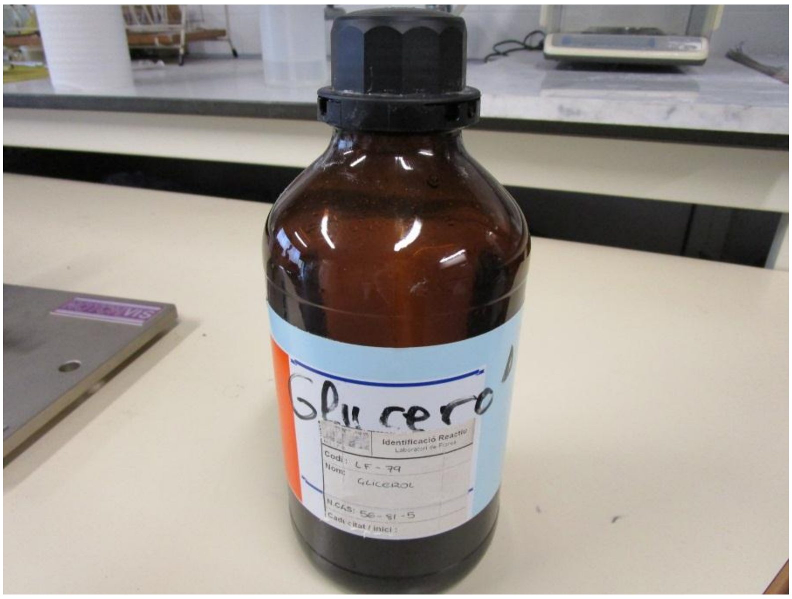


Figure 3.2.1 (b): The used pure glycerol in the experiment

c) Nanofibrillated cellulose (NFC)

Nanofibrillated cellulose (NFC) has been made in laboratory of LEPAMAP Group. It was made in different amount of oxidation such as 5, 10, and 15 mmol. Bleached eucalyptus pulp and glycerol were considered as the compositions of creating NFC. NFC was formed by several methods which would be described in the next steps. Those three different types of oxidation include 5, 10, and 15 mmol of NFC which are proved in figure 3.2.1 (c).

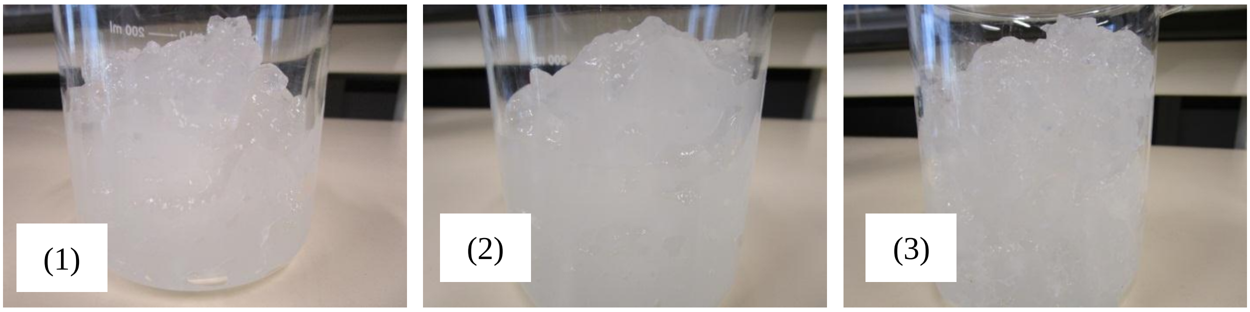


Figure 3.2.1 (c): (1) NFC gel 5 mmol, (2) NFC gel 10 mmol, and (3) NFC gel 15 mmol

d) Distillated water

Distillated water was used and added into the pre-blends in order to improve starch and glycerol or NFC combination.

3.2.2 Materials for preparation of pulp oxidation

In this case, bleached eucalyptus pulp with specifically determined concentration, sodium bromide (NaBr), TEMPO, sodium hypochlorite (NaClO), sodium hydroxide (NaOH), and distillated water were considered as the raw material and chemical elements to produce oxidized pulp. These materials are described in the following figure, figure 3.2.2. Buffer 7 and 4 were used to calibrate pH meter.

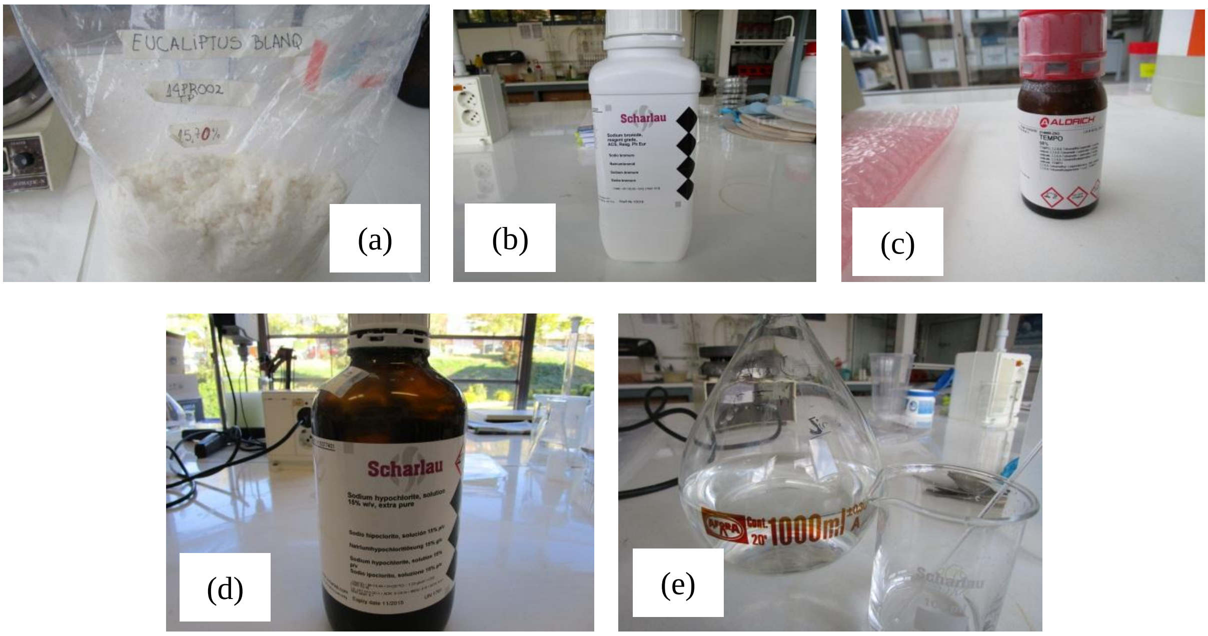


Figure 3.2.2: Materials for preparation of pulp oxidation (a) bleached eucalyptus pulp with 15.70% of concentration, (b) Sodium bromide (NaBr), (c) TEMPO, (d) 12% Sodium hypochlorite solution (NaClO), and (e) Sodium hydroxide (NaOH) 0.5 M

3.2.3 Materials for nanofibrillated cellulose (NFC) preparation

Nanofibrillated cellulose (NFC) was produced by ready prepared oxidized pulp with known dry mass content, shown in the figure 3.2.3, and added glycerol through homogenization process. In this case, NFC 5, 10, and 15 mmol of oxidation were needed to use in films preparation.

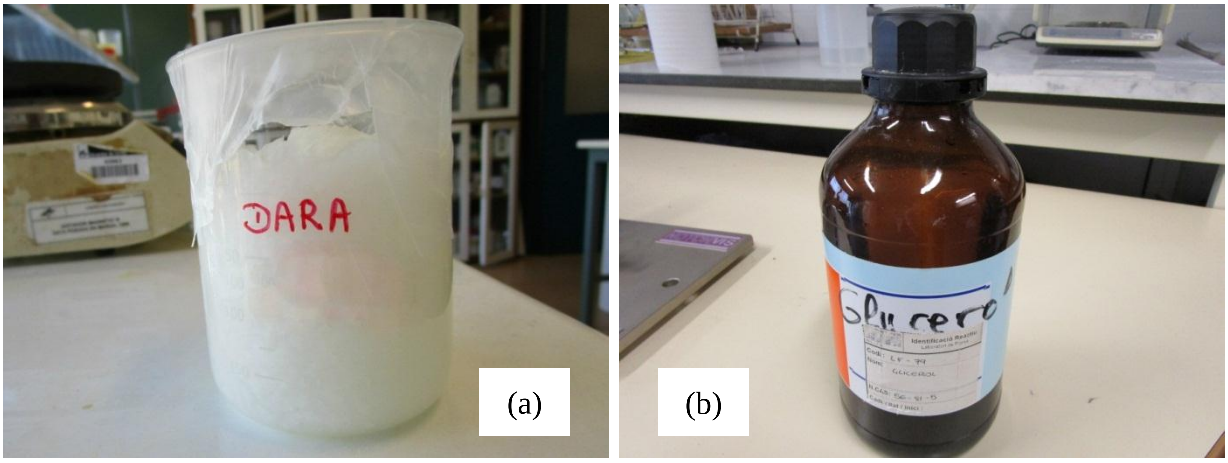


Figure 3.2.3: Materials for NFC preparation (a) oxidized pulp with known dry mass content and (b) glycerol for addition

3.2.4 Enzyme solution for using in biodegradability testing

$\alpha-$Amylase was a type of enzyme which was used to test the biodegradability property of the transparent and degradable films. It was supported by Novozymes Company, located in Denmark. Such kind of enzyme solution would be able to degrade the samples which were made by starch during different periods of time. The used enzyme solution is shown in figure 3.2.4.

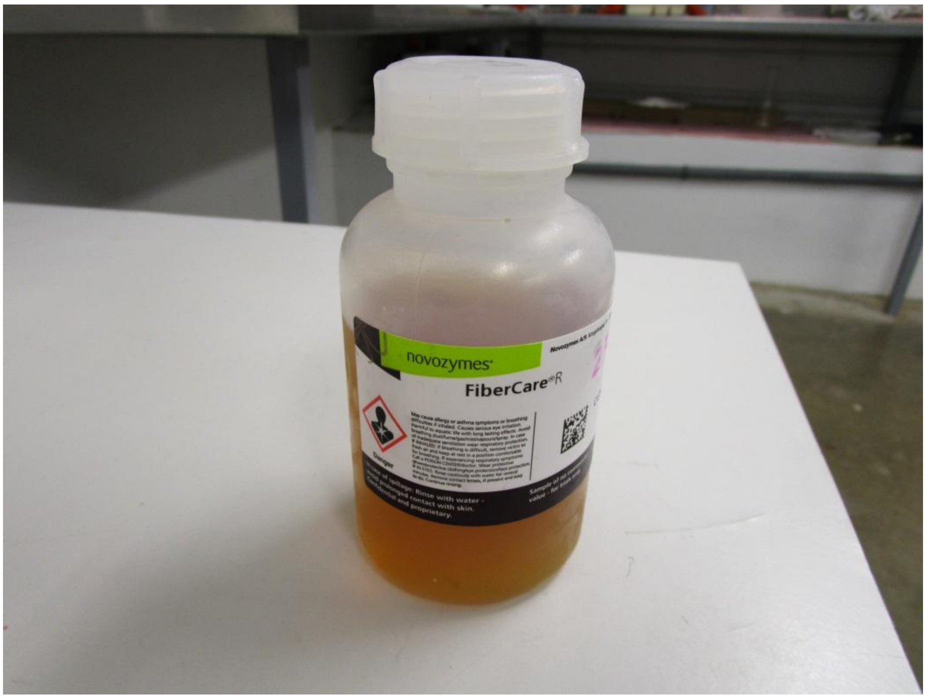


Figure 3.2.4: $\alpha-$Amylase solution (Aquazym 480 L) for biodegradability testing of degradable films

3.2.5 Acetic acid solution for stopping the biodegradability of samples

In order to stop biodegradability of the films, acetic acid (CH_3COOH) was used and added into the enzyme solution. It must be diluted first from high to low concentration before adding in that solution. Figure 3.2.5 shows the acetic acid with reliable specification, 99.8% of concentration, M = 60.05, and D = 1.05 g/cm^3. This acetic acid solution was made by Scharlab S.L., based in Spain.

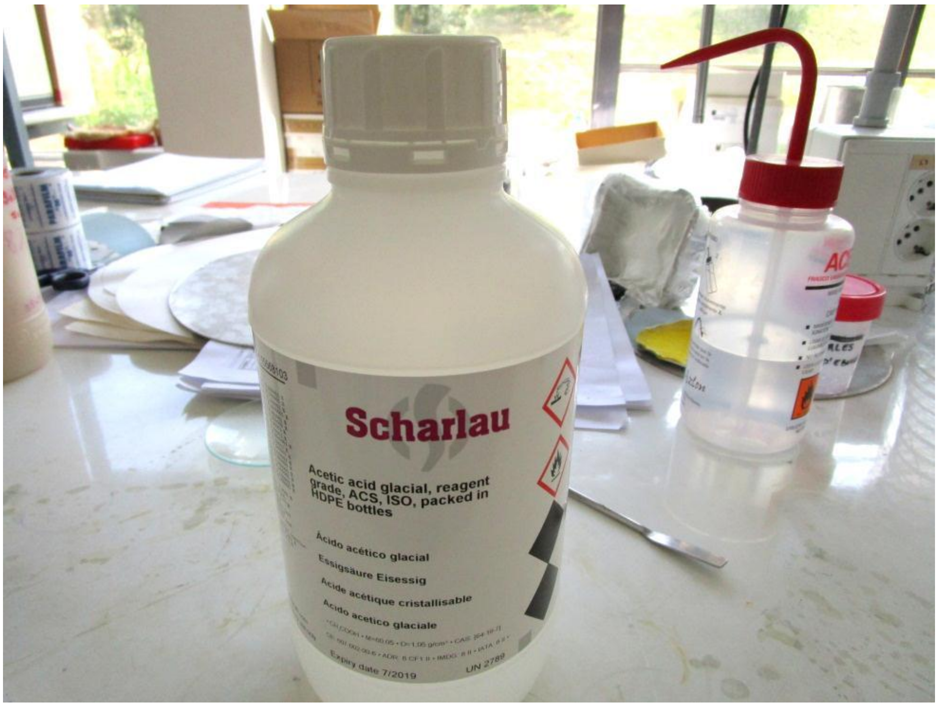


Figure 3.2.5: Acetic acid with 99.8% of concentration

3.3 Equipment

3.3.1 Analytical balances for using in the experiment

Four kinds of balances were used to weigh the samples included pre–blends, blends, films preparations and water absorption and biodegradability testing. They are available in laboratories of LEPAMAP Group at Parc Científic i Tecnològic and EPS of UdG. The following figure, figure 3.3.1, illustrates the balances used in the experiment.

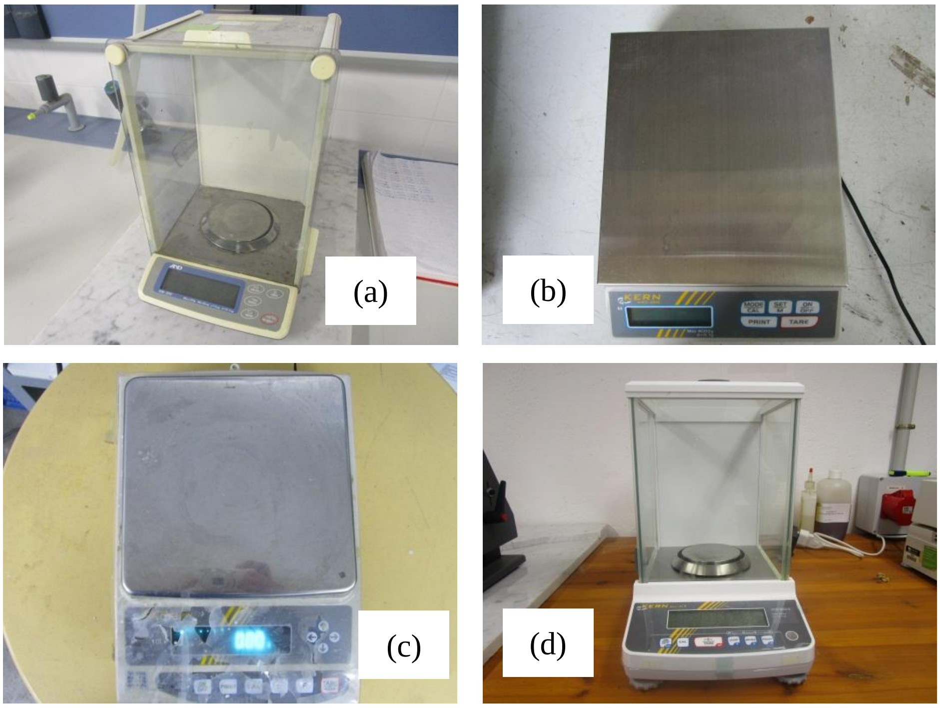


Figure 3.3.1: Analytical balances for using in the experiment (a) pre–blends preparation, max: 310 g and min: 10 mg, (b) blends preparation, max: 4000 g, (c) films preparation, max: 4200 g and min: 0.5 g, and (d) water absorption and biodegradability testing, max 220 g.

3.3.2 Brabender mixer

Brabender mixer, figure 3.3.2, is a kind of internal mixing machine which can be used to mix and melt some types of materials into different shape and properties. Temperature, rotation speed, and shear force are the main parameters of this machine to melt compositions completely. In this case, such kind of machine was used to melt pre–blends created by potato starch and glycerol or glycerol/NFC. Brabender mixer has been equipped in LEPAMAP Group laboratory at Parc Científic i Tecnològic of UdG.

Figure 3.3.2: Brabender mixer, mixing machine

3.3.3 Thermoforming

Figure 3.3.3 shows about thermoforming machine (hot presses) which has been used to produce films and other products in different shapes based on forming box or molds. It can be called as compression machine. In case of films, under an appropriate temperature condition, the blends were melted and according to the optimum applied forces and duration of compression, the blends were also extended and had thin thickness with transparency of surface. This machine was made in Italy and equipped with water cooling system, temperature and force controller, security door, and time controller. It has been installed in LEPAMAP Group laboratory of EPS. The maximum force of this machine is 300 kN and minimum is 60 kN.

Figure 3.3.3: Thermoforming, compression machine

3.3.4 Iron sheets for using in thermoforming process

In this case, iron sheets were used to cover both transparent plastic sheets during thermoforming process. They can be performed as the molds to produce a shape of film with flexible and transparent characteristics. Specific thickness, length, and width of those iron sheets were measured and shown in the below figure. Figure 3.3.4 shows the iron sheets for using in the experiment.

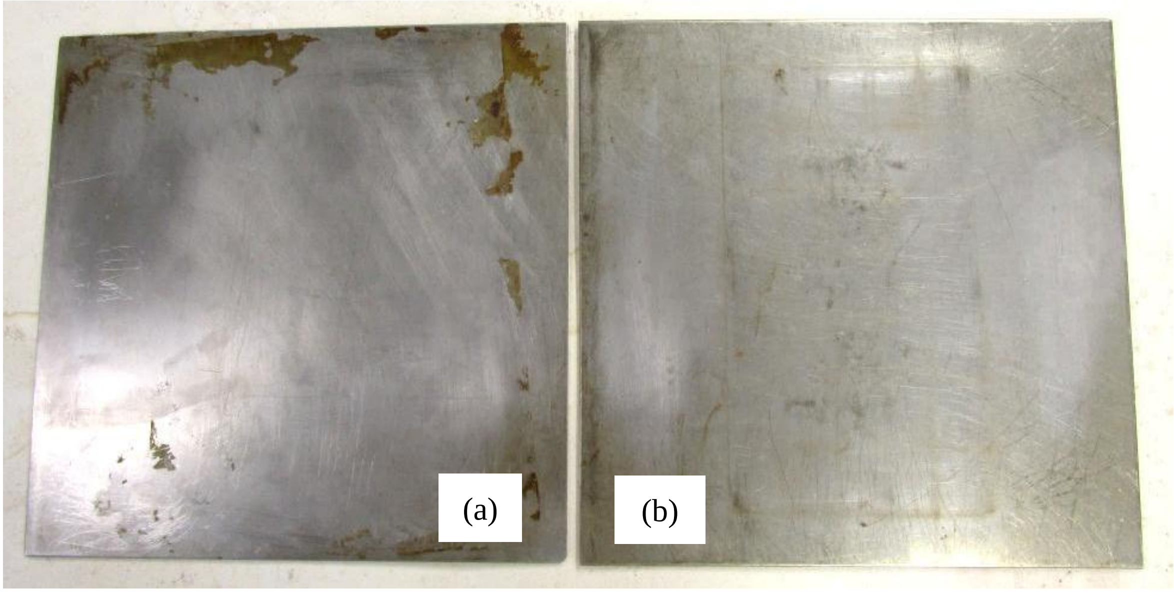


Figure 3.3.4: Iron sheets for using in thermoforming process (a) width and length 300 mm and thickness 2 mm and (b) width and length 300 mm and thickness 3 mm

3.3.5 Transparent plastic sheets for covering blends in thermoforming process

These materials, transparent plastic sheets, were used to cover the prepared blends in order to create the transparent and flexible films by thermoforming. Blend was covered by those plastic sheets during compression process and then films can be obtained and removed from the plastic sheets after that process has been finished. Because they are tolerant to the high temperature so they aren't melted at that condition. The transparent plastic sheets are shown in the below figure, figure 3.3.5.

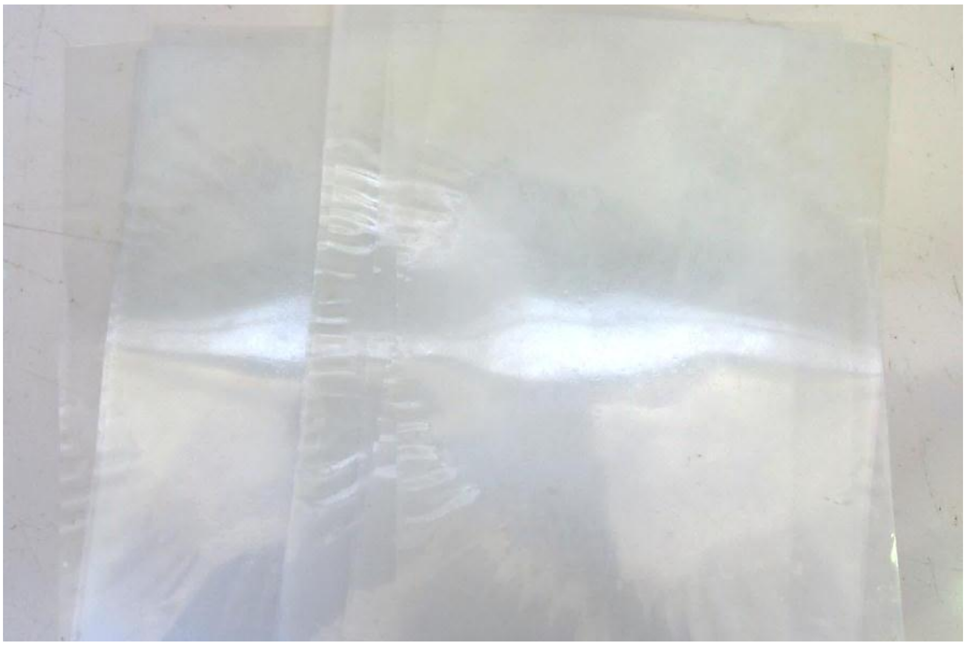


Figure 3.3.5: Transparent plastic sheets for blends covering during thermoforming

3.3.6 Blade for films cutting

After films have been obtained, they were cut by blade in 15 mm of width. One type of blades was used to cut the films in appropriate shape before passing the mechanical testing. This blade can be changed the dimension of desired width since iron sheets were responsible for the changing. All films were cut into specimens with uniformity of width, 15 mm. Figure 3.3.6 shows about the blade for cutting the formed films.

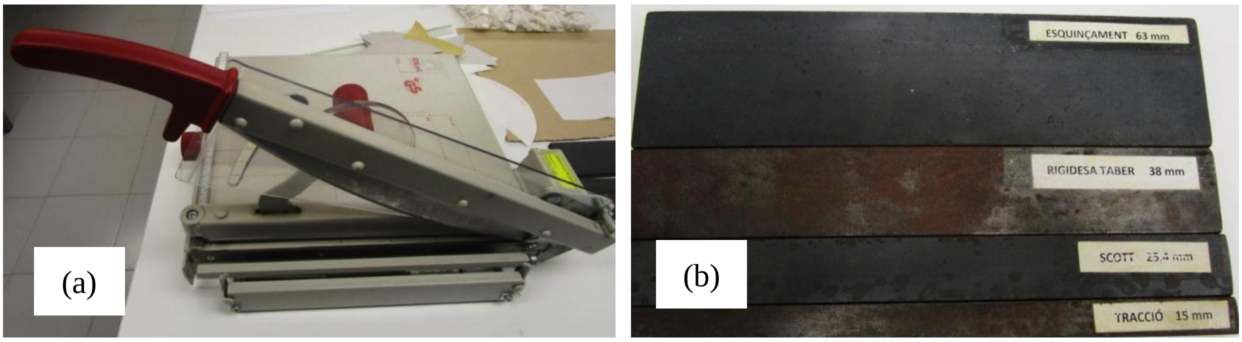


Figure 3.3.6: (a) blade for films cutting into specimens (b) desired width of iron sheets

3.3.7 Films' thicknesses measurement

One kind of machine has been used to measure the thicknesses of films. Films' thicknesses were considered in micrometers and their thicknesses were found less than 250 μm. Anyway, each specimen of films has been measured the thickness for 3 to 5 times. The measurements of thickness have been put in a table and the averages of thickness dimensions were then calculated. This machine is shown in the following figure, figure 3.3.7.

Figure 3.3.7: Machine for films' thicknesses measurement

3.3.8 Climatic chamber for conditioning

The climatic chamber was used to acclimatize the samples after they have been ready cut and prepared before analyzing the mechanical properties. Temperature and relative humidity (RH) have been considered as the main parameters for conditioning and they could be changed according to the samples testing requirements. The range of RH of climatic chamber is between 30 to 98% and temperature can be set from 0 to 70°C. Figure 3.3.8 shows about the climatic chamber for samples conditioning.

Figure 3.3.8: Climatic chamber for samples conditioning

3.3.9 Tensile testing machine

Following figure, figure 3.3.9, illustrates about a type of machine for tensile or mechanical properties testing of materials or samples called universal testing machine. It was used to analyze the mechanical properties of films included tensile strength, Young modulus or modulus of elasticity, maximum forced applied to the sample at break point, and percentage of elongation at break. Tensile testing machine was equipped with specific load cell 2.5 kN, computer, and gas system for clamp closing. All desired parameters have been shown in the computer system.

Figure 3.3.9: Tensile testing machine with 2.5kN of load cell

3.3.10 Equipment for oxidized pulp preparation

For oxidized pulp preparation, several equipment are needed which are described in the figure 3.3.10. Among those equipment, mixer, pH meter, and vacuum filtration were used to prepare such kind of pulp. Mixer was used to combine TEMPO and NaClO and pH meter was utilized to control pH of solution. Vacuum filtration was practiced to filter the solution to obtain oxidized pulp after pH of that solution was constant.

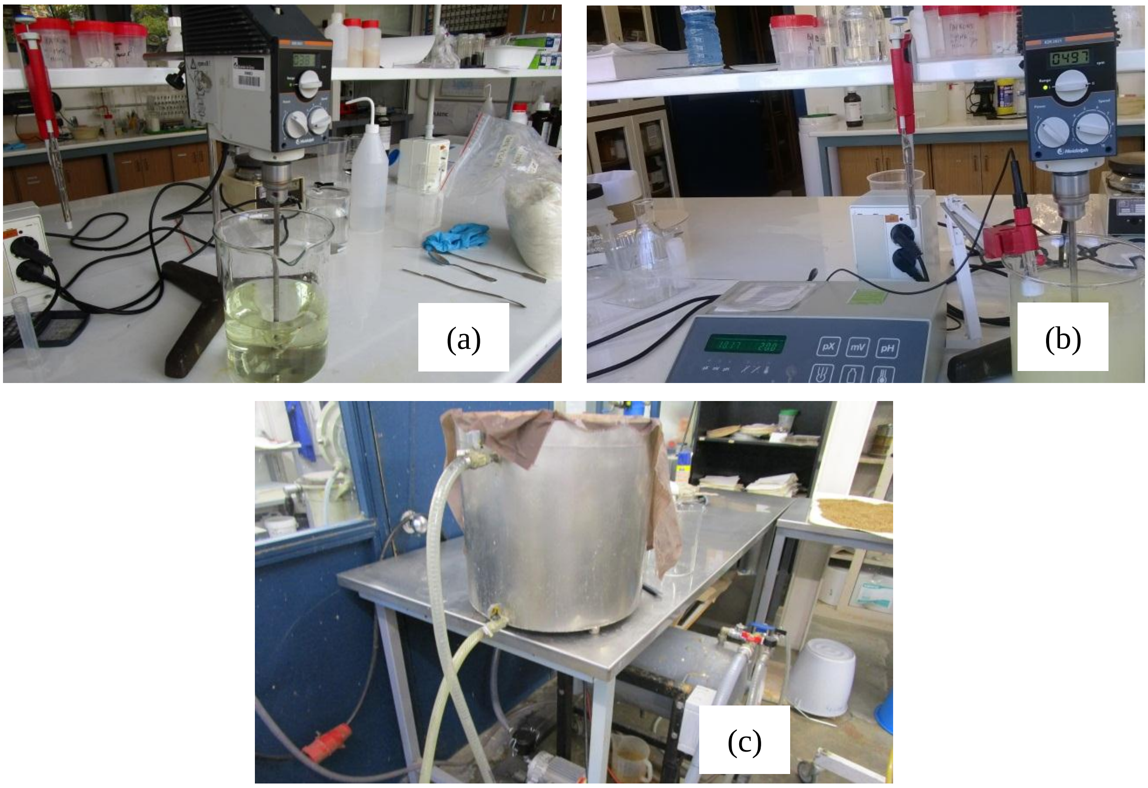


Figure 3.3.10: Equipment for oxidized pulp preparation (a) mixer, (b) pH meter, and (c) vacuum filtration machine

3.3.11 Drying oven for dry mass content determination in oxidized pulp

Figure 3.3.11 (a) shows about the drying oven which is used to dry ready prepared oxidized pulp in order to determine the dry mass content or water content of that pulp. 105^{o}C of temperature is a condition for this process. The samples were put in the oven until the constant masses were obtained. Desiccator, shown in the figure 3.3.11 (b), is used to keep samples after drying before reweighing.

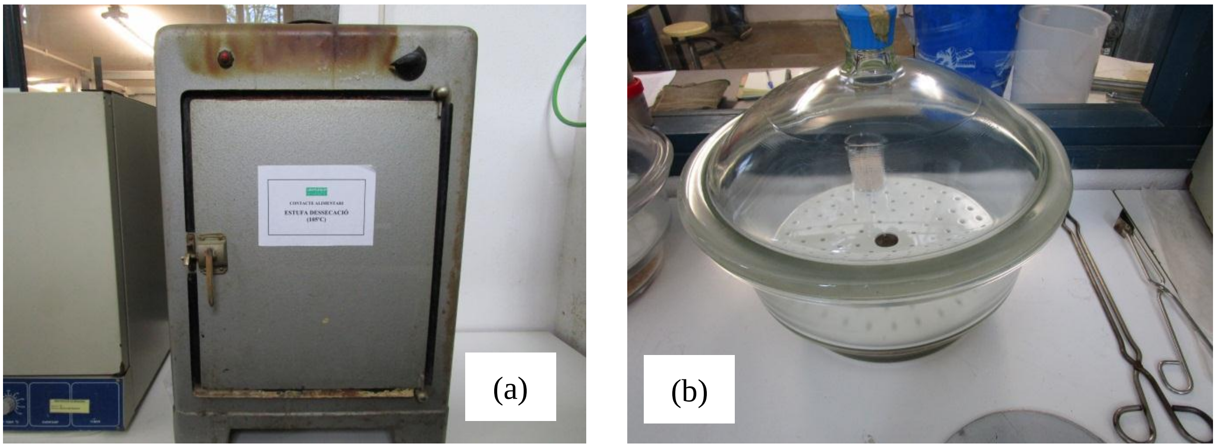


Figure 3.3.11: (a) drying oven and (b) desiccator

3.3.12 Homogenizer for NFC preparation

To obtain NFC in gel property, homogenizer was used to homogenize the solution, which was made by oxidized pulp and added glycerol combination, by passing solution through this machine under the pressure condition. It was made in Italy by GEA Mechanical Equipment Italia S.p.A. Such kind of machine is shown in the figure 3.3.12.

Figure 3.3.12: Homogenizer for NFC preparation

3.3.13 Refrigerators for keeping NFC

Obtained NFC was put in the refrigerator in the direction of keeping the NFC quality and properties, besides putting outside or at room temperature. The refrigerators are shown in the below figure, figure 3.3.13.

Figure 3.3.13: Refrigerators for keeping NFC gel

3.3.14 Drying oven for water uptake and biodegradability determination of films

Another drying oven for water uptake and biodegradability testing, instead of the above oven, was used to remove water content of samples at lower temperature. The temperature 50^{o}C was set to evaporate water from samples until constant masses were gotten. If higher temperature was used, it could affect to samples properties such as samples could be fragile or breakable. This drying oven was made by BINDER Company located in Germany. Figure 3.3.14 represents such type of drying oven.

Figure 3.3.14: Drying oven for water uptake and biodegradability testing of films

3.3.15 Autoclave

Figure 3.3.15 depicts the sterilization and pasteurization machine, autoclave. In this case, autoclave was used to sterile the bottles and Erlenmeyer flasks for putting enzyme solution and samples in biodegradability testing. This process was practiced to make sure that the microorganisms in the bottles or Erlenmeyer flasks were completely died and didn’t affect to the determination of biodegradability.

Figure 3.3.15: Autoclave for bottles sterilization

3.3.16 Equipment for filtration in biodegradability testing

To filter the enzyme solution in order to know about the percentages of biodegradability of the prepared films, another vacuum filtration machine and specific filtration tools were used. Filter was assured that it wasn't affected by $\alpha -$amylase activity during filtration. Vacuum filtration machine and filtration glasses are shown in figure 3.3.15 (a) and (b) respectively. This machine was made in Italy by Lovato Company. The filtration process is done under the pressure of this machine which is between 0 to –1 bar.

Figure 3.3.16: (a) vacuum filtration machine and (b) filtration glasses

3.4 Methodologies

3.4.1 TEMPO – mediated oxidation of fiber

One gram of dry mass of pulp needs NaBr 0.1 g, TEMPO 0.016 g, and NaClO 5 mmol per gram of dry mass of pulp. So to oxidize 15 g of dry mass of pulp, NaBr 1.5 g, TEMPO 0.24 g, NaClO (46.525 ml for 5 mmol, 93.05 ml for 10 mmol, and 139.575 ml for 15 mmol) are required. 95.54 grams of total mass of pulp, included wet and dry mass, is needed and 1500 ml of additional distillated water is the requirement.

There are several steps to prepare pulp oxidation or treated pulp. NaBr and TEMPO, 1.5 and 0.24 g respectively, were weighed and put in a plate. Then less amount of distillated water was added into that plate in order to mix TEMPO and NaBr. This mixture was put in a beaker and more water was added into that container. Later on, mixer was used to combine that blend. Mixing time and speed were depended on TEMPO or mixture. Total amount of pulp 95.54 g was weighed and added into the beaker after TEMPO was completely dissolved. Then more distillated water was added until 1500 ml and mixing process was continued. NaClO (e.g 46.525 ml for 5 mmol of oxidation) was dropped wise to the mixture. pH must be kept constant between 10.15–10.40. In this case, when pH level went down, it meant oxidation of pulp was happened. If pH was decreased to more or less than 10.15, NaClO was more added to make pH stable. pH was not allowed to increase over 10.40. NaClO was added until it was totally finished. After completing adding NaClO, NaOH 0.5 M was continued counting by dropping wise to keep pH constant which was ranged between 9.9 – 10.20. Mixer and pH meter was stopped operating after pH level was regular, not decreased anymore. After that, the obtained solution was completely filtered. Distillated water was then added into solution again and it was filtered again. This process was repeated 3 times. Finally, oxidized pulp or pulp oxidation was successfully obtained with different oxidation degree according to the added amount of NaClO.

3.4.2 Dry mass content of treated pulp determination

Drying oven was used to determine the dry mass content of oxidized pulp. First of all, small plates were dried in the oven at 105°C for 10 min to make sure that those plates were completely dried. Then they were taken from the oven and continued putting in a desiccator for 5 min. Those plates were weighed. After that, oxidized pulp was also weighed by adding in the dried plates. Oxidized pulp should be weighed more or less than 2 grams. Both masses of plates and the pulp must be recorded. After finishing weighing, the samples were dried in the oven at 105°C until masses of them were constant. The samples were taken out from the oven and put in the desiccator. Later on, they were reweighed and masses of them were noted again. Finally, dry mass content of oxidized fiber was calculated by the following formula:

$$Dry\ mass\ content\ (\%) = \frac{m_2 - m_1}{m_0} \times 100$$

- m_0 : mass of pulp before drying (g)
- m_1 : mass of plate (g)
- m_2 : mass of plate and dry pulp (g)

3.4.3 NFC preparation from oxidized/treated pulp

In this process, oxidized pulp with known dry mass content in percentage was considered as the raw materials to make NFC. Homogenizer was used to create NFC by passing the solution, contained treated pulp and glycerol, through that machine. There were some procedures to obtain NFC. First, treated pulp was weighed and put in a beaker. Total mass of that pulp was recorded. Then amount of dry mass in gram was calculated by below equation.

$$Dry\ mass\ content\ (g) = \frac{percentage\ of\ dry\ mass}{100} \times total\ mass\ of\ pulp$$

After that, total mass, derived from total treated pulp and added glycerol, was computed to obtain 1.04% solution of dry mass content.

$$Total\ mass\ content\ (g) = \frac{100}{1.04} \times dry\ mass\ content\ (g)$$

Glycerol was added into treated pulp to obtain the desired total mass content which was already calculated. Then that solution was homogenized by passing through homogenizer. First homogenization process was done without pressure. But the second homogenizing step was applied with 600 or higher bars of pressure to obtain NFC gel. That process was continued doing, 3 to 5 times by passing through homogenizer, until NFC in gel attitude was gotten. It can be passed through homogenizer easily or difficultly depending on amount of treated pulp oxidation. Finally, NFC was contained in a plastic bottle and kept in the refrigerator.

3.4.4 Films preparation

a) Pre–blends preparation

Potato starch, glycerol, and nanofibrillated cellulose (NFC) were the materials for producing the transparent and flexible films. Glycerol has an important role to break crystallization of starch and it can’t be evaporated during Brabender mixing and thermoforming process because the boiling point of this chemical element is 290°C as the temperature of mixing and thermoforming are 110°C and 135°C respectively. Less or excess amount of added glycerol could be affected to the mixture properties. NFC was added into starch to increase the mechanical properties of samples and other characteristics. For distillated water, it was counted to mixture in order to improve the combination between starch and glycerol or NFC. To obtain pre–blends, several steps must be practiced. Glycerol or glycerol/NFC (5, 10, and 15 mmol) were weighed and then put in different plastic bags respectively according to the below table, table 3.4.4 (a). Later on, potato starch was also weighed and added into glycerol or glycerol/NFC. Manual mixing was done until starch and glycerol or glycerol/NFC were mixed together. After that, distillated water was weighed/measured and added into the mixture in a specific content. That was noted that added water will be evaporated during mixing and thermoforming process. Manual mixing was repeated again until the compositions were seemed to be combined each other. All prepared pre–blends were kept for a day before mixing in Brabender, mixing machine. That procedure was aimed to enhance combination or reaction between the compositions of pre–blends. Finally, pre–blends were completely achieved. Figure 3.4.4 (a) shows the ready prepared pre–blends.

Table 3.4.4 (a): Pre–blends preparation

Experiment	**Treatment**	**Potato starch**	**Glycerol**	**Glycerol/NFC**	**H_2O**
Control	T_0	60%	40%	–	20% of total amount of starch
Experiment 1 (NFC 5 mmol)	T_1		–	40%	
	T_2		20%	20%	
Experiment 2 (NFC 10 mmol)	T_3		–	40%	
	T_4		20%	20%	
Experiment 3 (NFC 15 mmol)	T_5		–	40%	
	T_6		20%	20%	

Figure 3.4.4 (a): Ready prepared pre-blends

b) Blend preparation by Brabender mixer

After pre–blends were ready prepared, they were continued mixing to obtain the composites. That process can be named as compounding. In this case, Brabender was responsible for combining and melting the pre–blends contained starch and glycerol or glycerol/NFC. 50 g of mass of samples is the limited amount for each mixing operation. 110^{o}C of temperature, 100 rpm of rotation speed, and 12 min of mixing times have been considered as the optimum conditions of this study which were described in the table 3.4.4 (b). Less or over used temperature and rotation speed could be affected to blends properties because starch couldn't be melted and the obtained blends could be rigid, respectively.

Table 3.4.4 (b): Conditions of Brabender mixing

Experiment	**Mass of pre-blend (g)**	**Temperature of mixing (^{o}C)**	**Speed of Mixing (rpm)**	**Time of mixing (min)**
T_0	50	110	100	12
T_1				
T_2				
T_3				
T_4				
T_5				
T_6				

Some procedures must be practiced to get the blends. Ready prepared pre–blends were weighed in 50 g for the first step. Then Brabender and computer connected to the Brabender were turned on and operated. After that, equipment/tools of Brabender such as screws and key were installed. One program in computer system called WinMix was chosen to run mixing process. Next step, parameters of mixing were set and depicted as following.

- Order : Brabender
- Operator : Name of operator
- Sample : Name of Sample
- Sample mass : 50 g
- Speed (1/min) : 100 rpm
- Mixer temperature : 110^{o}C
- Test time : 12 min
- Measure range : 100
- Damping : 1
- Mixer : W 50 EHT. 3 Zones
- Drive unit : Plastograph
- Loading chute : Manual + 5 kg

After finish setting the parameters of mixing, Start Test side was then selected. There would be some messages appeared on computer screens. Start button of Brabender was pressed to run machine. Calibration process would be started after pressing the Start button. Another step, pre–blends were ready added into the mixer after calibration was completed according to a message emerged on screen of computer. Then it must be spent for 12 min for mixing the materials. Curve of torque would be shown on the computer screen and also another message contained time, torque, speed, temperature stock, and energy. Stop Test section was selected when 12 min of mixing time was arrived. Equipment/tools and key of Brabender were removed quickly to collect the blends. Blends were collected from the screws and machine. Because the mixer was still hot so carefulness should be practiced. Screws and other tools were then put into the water to clean for each operation or a new blend after ending the blends collection. Later on, data talked about mixing results was saved in the computer. Finally, blends were successfully obtained and waited for thermoforming process to produce films.

The obtained blends had acceptable properties because they were flexible and transparent and starch was completely melted. Starch and glycerol or glycerol/NFC were totally combined each other to form a new shape and kind of matter which was useful to create a final shape of material. The following figure, figure 3.4.4 (b), shows the blend after passing through Brabender mixing process.

Figure 3.4.4 (b): Obtained blend after Brabender mixing

c) Thermoforming to produce films

In this case, thermoforming process was practiced to conduct transparent and flexible films. The ready prepared blends were the material used to prepare the films. The blends were completely melted depending on an appropriate temperature condition and they were also extended according to the optimum applied forces and duration of compression. Because of those conditions, a new shape of material, film, would be conducted. The suitable temperature, forces, and times of pressing were selected as 135^{o}C, 100 kN for 10 min, and 150kN for 5 more min, respectively. These conditions were applied to all blends with different formulas.

There would be some steps of thermoforming which must be practiced to make that kind of films. First of all, blends were cut in a suitable shape and then weighed. They are shown in figure 3.4.4 (c). Thermoforming machine was then operated. Temperature 135^{o}C of upper and lower plates of thermoforming was set. Force 100 kN for the first step and 150 kN for the second step of pressing were selected. Both of iron sheets, two transparent plastic sheets, and weighed sample were taken into the machine together at 60^{o}C of temperature. It would be spent 6 min to grow up from 60^{o}C to 110^{o}C. Then it would be taken 4 more min to increase the temperature from 110^{o}C to 135^{o}C. Later on, 10 min of time was taken into account for the early step of thermoforming at 100 kN and counted down after temperature was already reached to 135^{o}C. After 10 min at 100 kN were arrived, 5 more min was set for the next step of pressing at 150 kN and started counting down. Then the temperatures of both plates were decreased to 60^{o}C after 5 min at 150 kN of compression was finished. Cold water system was used and opened to push temperatures felt down quickly. To decline the temperature from 135^{o}C to 60^{o}C, it would be spent a few minutes. Cold water system and thermoforming machine were then turned off after temperature was fallen to 60^{o}C. Transparent plastic sheets were taken out from those iron sheets and kept to be cold to remove films from them easily. Cold air could be used to make the films more convenient to take it out from the plastic sheets. The last step, films were removed from those plastic sheets and waited for mechanical and other characterizations.

Figure 3.3.4 (c): Shape of blend for film preparation

Table 3.4.4 (c): Film preparation by thermoforming

T	R	m_1 (g)	Temperature of thermoforming	Force: 100 kN	Force: 150 kN
T0	R1	13.61	135°C	10 min	5 min
	R2	12.61			
	R3	16.19			
T1	R1	15.60			
	R2	16.49			
	R3	13.17			
T2	R1	19.11			
	R2	13.32			
	R3	15.81			
T3	R1	19.22			
	R2	13.61			
	R3	16.79			
T4	R1	16.74			
	R2	12.09			
	R3	12.30			
T5	R1	14.95			
	R2	10.97			
	R3	16.04			
T6	R1	13.90			
	R2	11.88			
	R3	8.95			

3.4.5 Figure of film conducting

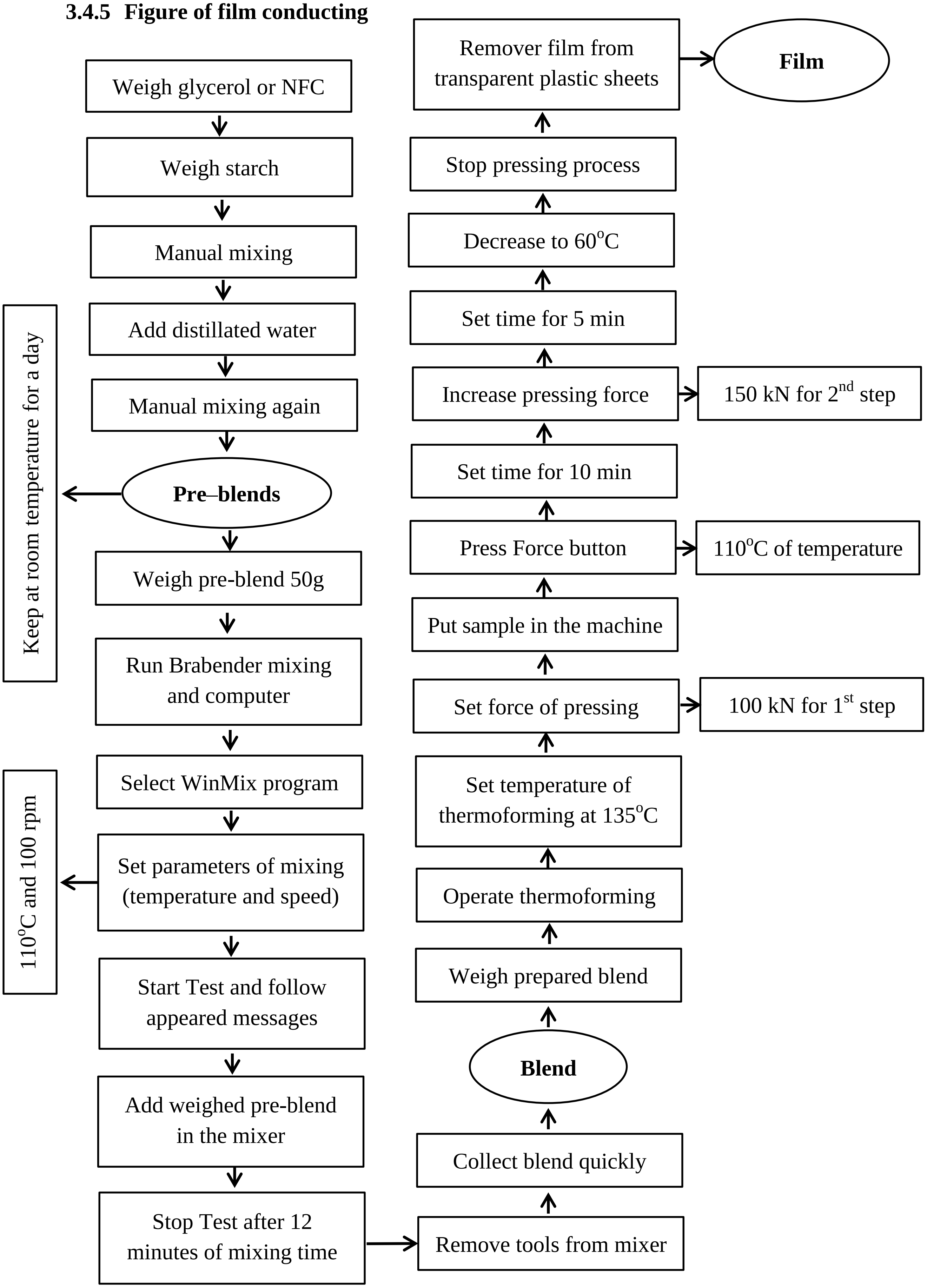


Figure 3.4.5: Film making based on starch and NFC

3.4.6 Film cutting by blade

After films were ready made, they were then cut into specimens in 15 mm of width by one type of blades. 15 mm of width was selected, based on American Society for Testing and Materials (ASTM D 882–02). The purpose of that cutting was to obtain an appropriate shape of films for mechanical characterizations. The uniformity of width must be done to all specimens. The length of films would be determined for tensile testing and being depended on ASTM D 882–02. The specimens are shown in the figure 3.4.6.

Figure 3.4.6: Specimens of films after cutting

3.4.7 Samples conditioning preparation

After all films were already cut into specimens, they were prepared to put in a climatic chamber at 23±1^{o}C of temperature and 50% RH prior at least 40 hours before passing through mechanical testing according to ASTM D 882–02. Figure 3.4.7 illustrates the samples conditioning in the climatic chamber.

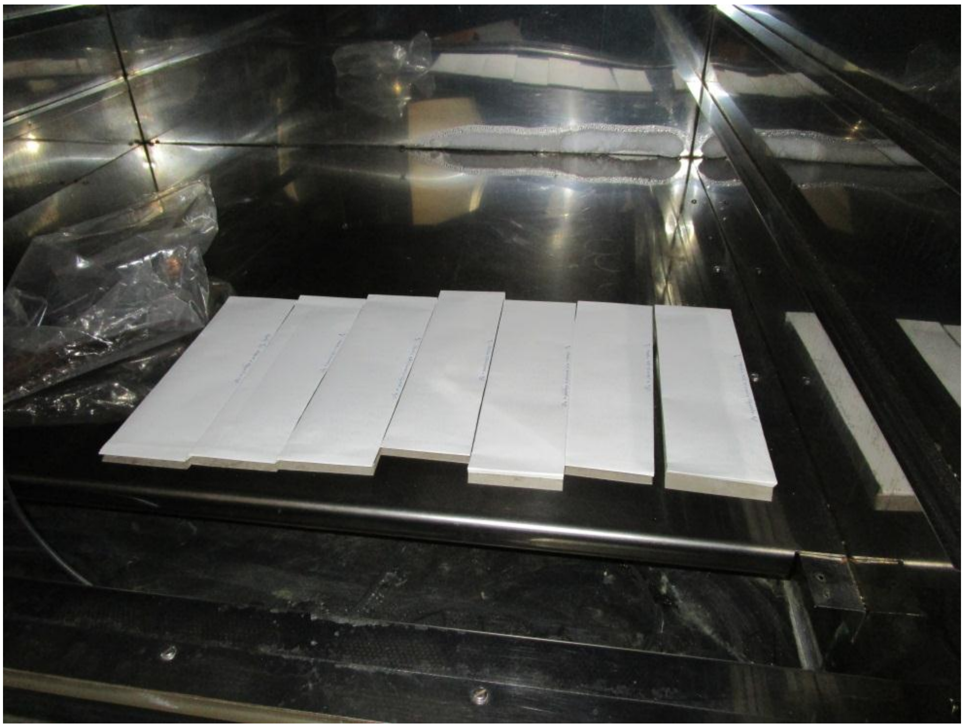


Figure 3.4.7: Sample preparation for conditioning

3.4.8 Films' thicknesses measurement

Films' thicknesses were measured 3 to 5 times per specimen and then calculated the average dimension. As the result, the thicknesses dimensions of every specimen were less than 1 mm and all data were put into a table. The thickness measurement procedure is shown in figure 3.4.8.

Figure 3.4.8: Films' thicknesses measurement procedure

3.5 Characterizations

There would be four characterizations on the samples which were included tensile testing, water absorption, water vapor barrier capacity, and biodegradability.

3.5.1 Tensile testing (ASTM D 882–02)

After all specimens of films were acclimatized for at least 40 hours at 23 ± 1^{o}C of temperature and 50% RH in the climatic chamber, mechanical properties under tensile stresses were then determined. In this case, tensile testing was contained maximum applied force at break, percent elongation at break, tensile strength at break, and modulus of elasticity or Young's modulus. Universal testing machine connected with a computer system was responsible for this characterization.

Tensile testing was practiced according to the international standard on materials testing, The American Society for Testing and Materials (ASTM D 882–02). Based on ASTM D 882 – 02 standard, if the percentage of elongation at break was less than 20%, the length 125 mm, initial strain rate 0.1 mm/mm·min and speed 12.50 mm/min were chosen. Specimens' thicknesses were totally depended on specific measurement and calculation. The load cell 2.5kN was used to experiment the samples for this characterization. After finishing setting the width, length, thickness dimension, and speed of testing or rate of grip separation, specimen was then put in the both grips. Testing was started and tensile process was begun. All measurements were performed at ambient conditions (23±1°C and 50% RH) and five specimens/replications were tested for each composite formulation. Later on, a curve would be emerged on the computer screen. At the end of testing, some results were successfully obtained. Tensile testing is shown in figure 3.5.1.

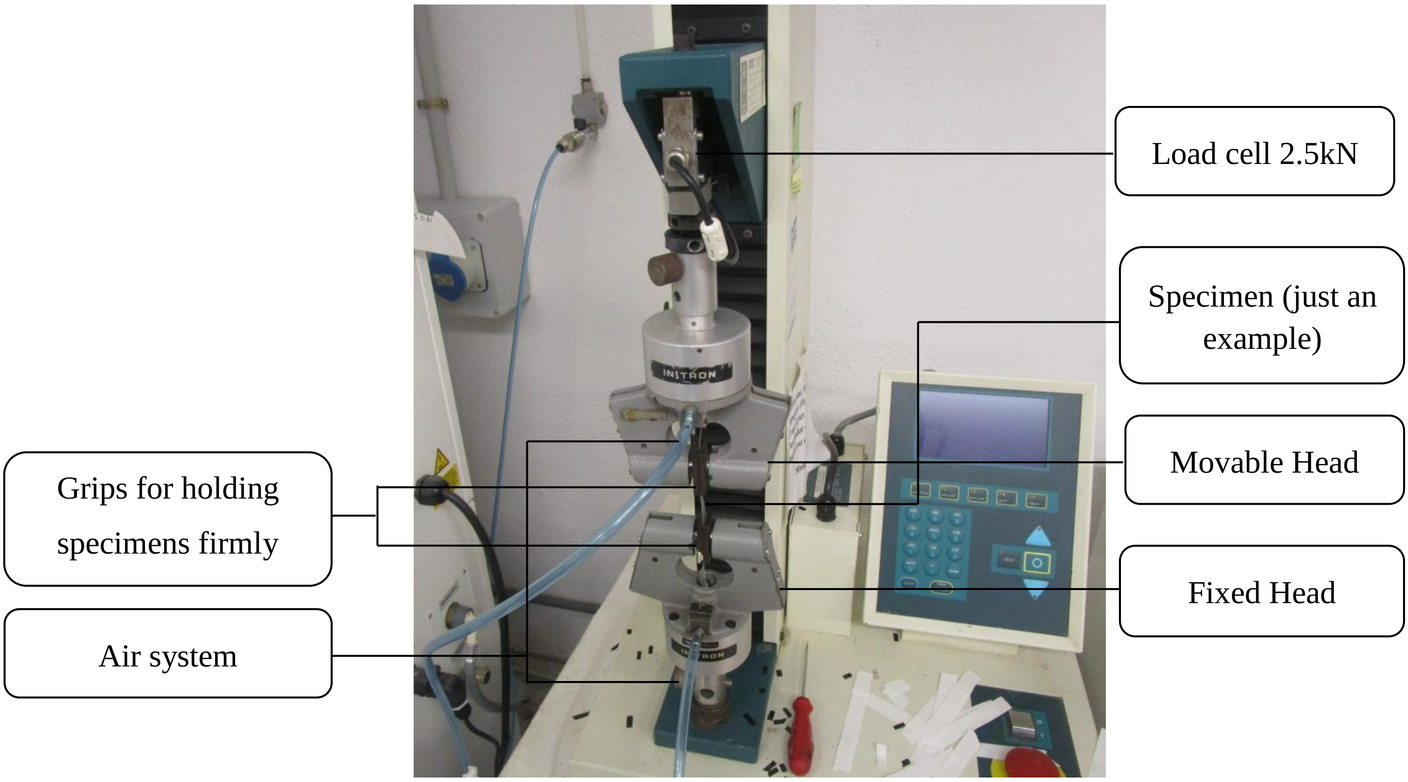


Figure 3.5.1: Tensile testing

a) Stress calculation

Stress is a ratio between applied force (F) and cross section area (A), which was derived from width multiply thickness dimension. It was represented by σ and its’ unit was considered as Pa, MPa, or GPa based on unit of force and cross section area. Stress was calculated by following equation.

$$\sigma = \frac{F}{A}$$

F : Applied force to the samples (N)

A : Cross section area (mm^2)

σ : Stress (MPa)

b) Strain calculation

Strain is considered as a ratio between variation of lenght (ΔL) and initail length (L_o) . ε is represented the strain. It was computed by the below equation.

$$\varepsilon = \frac{\Delta L}{L_o}$$

ΔL : Variation of specimen’s length (mm)

L_o : Initial specimen length (mm)

ε : Strain

c) Young's modulus or modulus of elasticity determination

Young's modulus or modulus of elasticity is referred to a coefficient of stiffness of materials which have elasticity attitudes and it is determined to evaluate the mechanical properties of those materials. Pa, MPa, or GPa were considered as the units of Young's modulus (E). It was figured by a ratio between stress and strain as shown in below equation.

$$E = \frac{Stress}{Strain} = \frac{\sigma}{\varepsilon} = \frac{F/A}{\Delta L/L_0} = \frac{FL_0}{A\Delta L}$$

E : Young's modulus or modulus of elasticity

F : Applied force to the samples (N)

A : Cross section area (mm^2)

ΔL : Variation of specimen length (mm)

L_o : Initial specimen length (mm)

3.5.2 Water absorption determination

Water absorption testing was made in order to compute the percentages of water that would be absorbed by films. This characterization required some equipment containing drying oven, climatic chamber, and analytical balance. There were three conditions of such type of testing, included 23°C and 50%, 23°C and 75%, and 7°C and 50% RH, would be depicted in the table in appendix section. First, all pieces of films of 7 formulations were dried in an oven at 50°C until constant masses were obtained. After that, they were put in desiccator and weighed. Their initial masses (W_0) were taken into account. Then those pieces of films were kept in a climatic chamber at three different conditions as shown in the below table. They were taken out from climatic chamber and weighed again (W_1) after every 30 min, 1, 2, 3, 4, or 5 hours, etc. Later on, they were placed in that climatic chamber and reweighed again after determined times. These processes must be continued practicing until constant masses of samples were obtained. Finally, percentages of water absorption of samples were calculated by following equation.

$$Water\ absorption\ (\%) = \frac{W_1 - W_0}{W_0} \times 100$$

W_0 : Initial mass of sample before putting in climatic chamber (dry film) (g)

W_1 : Mass of sample after putting in climatic chamber for a specific time (g)

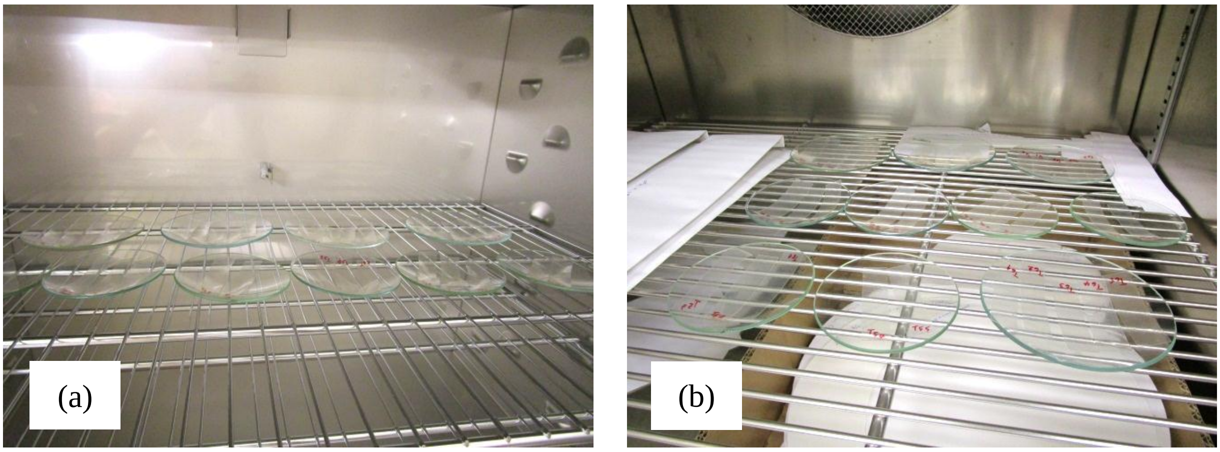


Figure 3.5.2: (a) dried samples in oven at 50^{o}C and (b) samples were placed in climatic chamber

3.5.3 Biodegradability determination

a) α −Amylase solution preparation

First of all, α–amylase solution was diluted in order to determine the degradability of films at different periods of time since quick degradability can be happened because of high concentration of enzyme and this characterization at varied times wasn't completely determined. There are two types of enzyme solution which must be prepared such as 0.02 g of α–amylase per liter of distillated water and 6 ml from 0.02g/L and added distillated water to supplement 1 liter. Erlenmeyer flasks for putting enzyme solutions were sanitized and sterilized in autoclave at 120^{o}C for 30 min to make sure that there were no microorganisms in them. Then α −amylase (Aquazym 480 L) was weighed in amount of 0.02 g and added in the flask. Distillated water was added into that flask until 1 liter. The solution was manually stirred and 0.02 g/L enzyme solution was obtained. After that, 6 ml from 0.02 g/L solution were pumped and added in another Erlenmeyer flask. Distillated water was then added in that flask to complete 1 liter. The solution was well mixed and the second enzyme solution, 0.12 mg/L or 0.06 KNU/L, was successfully gotten. The ready prepared enzyme solutions were finally stored in the fridge to keep the quality and property of enzyme. The figure 3.5.3 (a) shows about the both prepared enzyme solutions.

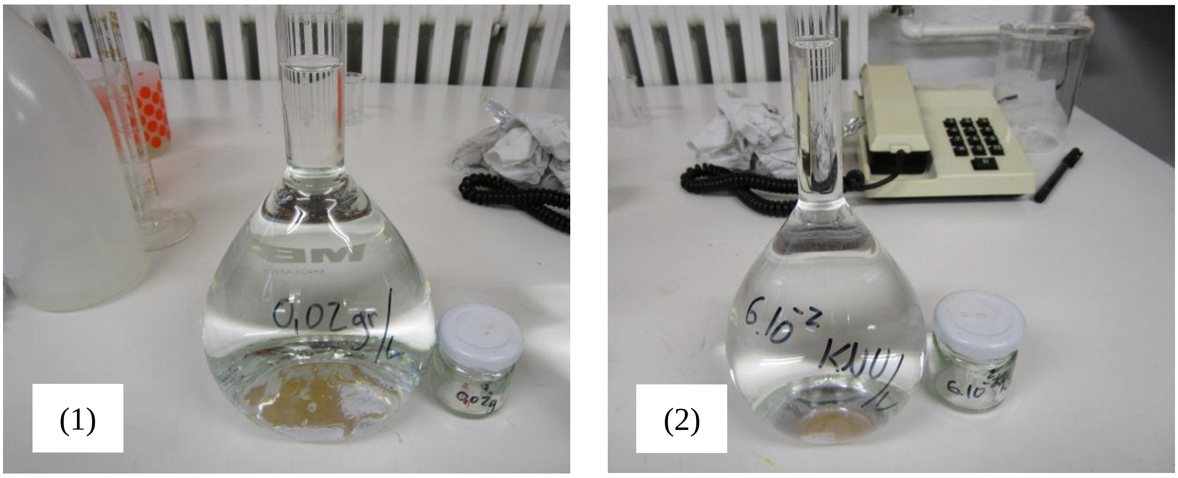


Figure 3.5.3 (a): Ready prepared enzyme solutions, (1) 0.02 g/L and (2) 0.06 KNU/L

b) Acetic acid (CH3COOH) 2M preparation

Acetic acid (CH_3COOH) 2M was prepared and used to quit the $\alpha -$Amylase activity, aimed to determine the biodegradability of films in separable periods of time. First, acetic acid with high concentration 99.8% was diluted to get a lower concentration, 2M. To obtain 2M of concentration, 114.38 ml of acetic acid with 99.8% was required. Acetic acid 114.38 ml was measured and added into an Erlenmeyer flask. Distillated water was then totaled to 1 liter. The acid solution was completely mixed and finally acetic acid solution 2M was ready prepared. Acetic acid 2M is illustrated in figure 3.5.3 (b).

Figure 3.5.3 (b): Acetic acid (CH_3COOH) 2M

c) Biodegradability testing processes

Biodegradability testing was conducted to determine the percentages of degradability of films at various periods of time under enzyme activity. α −Amylase solution 0.06 KNU/L was considered as an appropriate enzyme solution to use in this characterization. Acetic acid 2M was also used as the solution to discontinue the enzyme action at different determined times. 50^{o}C of temperature was selected as a condition for this testing. In this case, there were totally 96 hours of biodegradability characterization which were separated in 9 critical points such as 1, 2, 4, 8, 24, 30, 48, 72, and 96 hours. Three replications of each sample were practiced for these critical points.

There were some procedures required in the biodegradability characterization. First of all, the pieces of films of all formulations were made sure that they were dried in an oven at 50^{o}C until regular masses were gotten. After that, they were kept in a desiccator. The small glasses for putting the pieces of dried films were weighed and their masses (W_1) were noted. The dried films were then weighed and put in those glasses. Their initial masses (W_0) were taken into account. Later on, 20 ml of 0.06 KNU/L of α −amylase solution were added into each glass. For the next step, those small glasses were covered and kept in the oven at 50^{o}C. After 1, 2, 4, or 8 hours, etc. of storing in the oven, they were taken out and each sample was added by 1 ml of CH_3COOH 2M to cease biodegradability testing. After CH_3COOH 2M addition, the solutions were then filtered by vacuum filtration machine. 3 times of filtration per each sample were practiced. Later on, the samples were taken from filtration glasses and put in the same small glasses. Those samples were kept in the oven at 50^{o}C again in order to dry them until constant masses were obtained. Their constant masses after drying (W_2) were reweighed and recorded. Finally, percentages of biodegradability of films were calculated by below equation:

$$Biodegradability\ (\%) = \frac{W_0 - (W_2 - W_1)}{W_0} \times 100$$

W_0 : Initial mass of sample (dry film) (g)

W_1 : Mass of small glass for putting sample (g)

W_2 : Mass of small glass and dry sample (g)

Figure 3.5.3 (c): Biodegradability testing (1) samples were put in the oven at 50°C and (2) samples were dried in the oven at 50°C after filtration

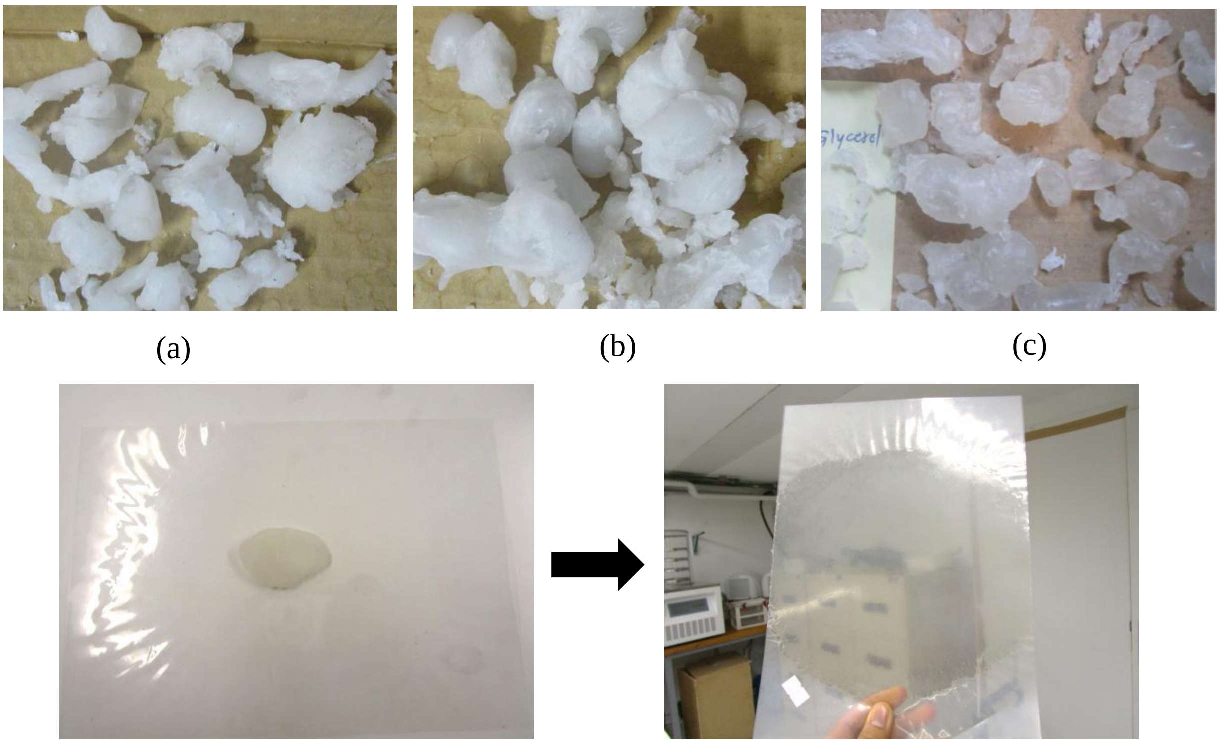
3.5.4 Water vapor permeability determination

Direct permeability to water was determined from the slope of weight loss versus time curves at 24^{o}C. The films were sandwiched between the aluminum top (open O-ring) and bottom (deposit for the permeant) parts of a specifically designed permeability cell with screws containing deionized water as the permeant. A Viton rubber O-ring was placed between the film and the top part of the cell to enhance sealability. The cells were placed inside a desiccator at 0 % RH, and the water weight loss through a film area of 0.001 m^2 was monitored and plotted as a function of time. In order to estimate the permeability values of the films, only the linear part of the weight loss data was used to ensure sample steady-state conditions. Cells with aluminum films were used as control samples to estimate solvent loss through the sealing. Water weight loss was calculated as the total cell weight loss minus the loss through the sealing. Tests were done in duplicate [175].

4. Results and Discussion

4.1 Results

4.1.1 Films' properties



(a) (b) (c)

(d) (e)

Figure 4.1.1: (a) blend at 100^{o}C and 80 rpm, (b) blend at 100^{o}C and 100 rpm, (c), blend at 110^{o}C and 100 rpm, (e) prepared blend for thermoforming and (e) obtained film after thermoforming

According to the figure 4.1.1 (a) and (b) showed that the blends produced at 100^{o}C and 80 rpm and 100^{o}C and 100 rpm of Brabender mixing, respectively, weren't completely transparent because starch was seemed not to be melted at both conditions. In contrast, the blend showed in figure 4.1.1 (c) was much different from the previous blends. It was made under a suitable condition, 110^{o}C and 100 rpm of Brabender mixing, which provided a totally melting of starch. The blend was transparent since starch was successfully melted at this condition. Furthermore, the blend had flexibility which was a good parameter for creating a transparent and flexible film. Figure 4.1.1 (e) shows an example of films obtained from thermoforming. As the results, the obtained films were totally flexible and transparent. The dimensions of their thicknesses were acceptable, between 150-250 μm. Their shapes were still kept in good properties and weren't broken after removing from transparent plastic sheets. Such kind of films had transparency of surfaces which seemed to be similar to some types of film produced by polymer matrix. In this case, the films were considered to be appropriate for mechanical and other characterizations.

4.1.2 Tensile testing (23°C and 50% RH)

At this condition, 23°C of temperature and 50% RH, tensile testing characterization of samples included maximum forces, percentages of elongation at break point, tensile strength, and Young's modulus were determined. The results of these parameters are shown in the figure 4.1.2.

Base on figure 4.1.2 (a), the force applied of the sample without fiber addition, T0, was lower than other samples with added fiber, from T1 to T6. The maximum forces at break point such as 13.42, 19.97, 16.05, 23.70, 14.78, 16.68, and 13.63 kN were referred to T0, T1, T2, T3, T4, T5, and T6, respectively. The forces were significantly changed by different oxidation of pulps, 5, 10, and 15 mmol. As results shown in the figure 4.1.2 (a), the highest applied force at break point, 23.70kN, was obtained from T3 with 10 mmol of pulp oxidation, followed by T1 with 5 mmol of oxidation of pulp, 19.97 kN, and 16.68 kN for T5 from 15 mmol of eucalyptus oxidation and T0 had the lowest applied force, 13.42 kN. The forces were declined if added fibers were also decreased as shown in T2, T4, and T6 if they were compared to samples made from higher amount of fiber. T2, T4, and T6 added by 5, 10, and 15 mmol of pulp oxidation were investigated that they were needed forces to break less than T1, T3, and T5, respectively.

Figure 4.1.2 (b) describes about the percentages of elongation at break of the samples. As the results, percentages of elongation at break of samples with fiber addition included T1, T3, T4, T5, and T6 were higher than sample without fiber addition, T0, except T2 which is similar to T0. The percentages of elongation at break were 19.79, 25.96, 19.64, 33.66, 27.17, 21.16, and 22.3% which were referred to T0, T1, T2, T3, T4, T5, and T6, respectively. Due to these values, T3 was investigated that it had the largest percentage of elongation, 33.66%, among all samples, and was followed by T4, 27.17%, and T1, 25.96%. The percentages of elongation of T0, T2, T5, and T6 were seen that they were not much different from each other but samples with fiber containing were still better than sample with no fiber containing.

Tensile strength of samples at 23°C of temperature and 50% RH is measured and shown in the figure 4.1.2 (c). According to this figure, the results of tensile strength were indicated that T0, T1, T2, T3, T4, T5, and T6 had 3.55, 5.48, 4.67, 6.08, 4.99, 5.82, and 4.54 MPa, separately. Based on these results, T3 with 10 mmol of pulp oxidation addition had the highest tensile strength, 6.08 MPa, if it was compared to other samples, chased by T5 with 15 mmol, 5.82 MPa, and T1 with 5 mmol of oxidized pulp, 5.48 MPa. T0 with no fiber addition had the lowest tensile strength, 3.55 MPa, if it was matched to the other 6 samples with fiber containing. The tensile strengths of T2, T4, and T6 with decreased fiber content were seemed to be similar to each other but they were different from T1, T3, and T5 with more amount of fiber. Due to the fiber addition and values of pulp oxidation, tensile strength of samples would be improved if compared to the samples without any fiber additions.

Young’s modulus or modulus of elasticity characterization is depicted in figure 4.1.2 (d). Due to this figure, different Young’s modulus values of all samples were obtained. As the results demonstrated that the Young’s modulus such as 134.90, 264.12, 228.95, 284.81, 212.24, 351.10, and 216.99 MPa, respectively, were belonged to T0, T1, T2, T3, T4, T5, and T6. In this case, T5 added by 15 mmol of oxidized pulp and full amount of fiber was found that it had the highest Young’s modulus value, 351.10 MPa. It was followed by T3 included by 10 mmol of treated pulp and T1 added by 5 mmol of pulp oxidation which were 284.81 and 264.12 MPa, separately. The lowest Young’s modulus value, 134.90 MPa, was referred to the sample without any fibers inclusion, T0. As results shown in the figure 4.1.2 (d), T2, T4, and T6 diminished amount of added fiber were seen that their modulus of elasticity values weren’t much different from each other but they were dissimilar from other 3 samples, T1, T3, and T5, which made formed by higher content of added fibers. However, Young’s modulus of films would be enhanced depending on fibers inclusion and amount of oxidized pulp and such kind of characterization would be better than if compared to the samples with no fibers addition. By this way, added fibers could play an essential role to develop and enrich the properties of samples.

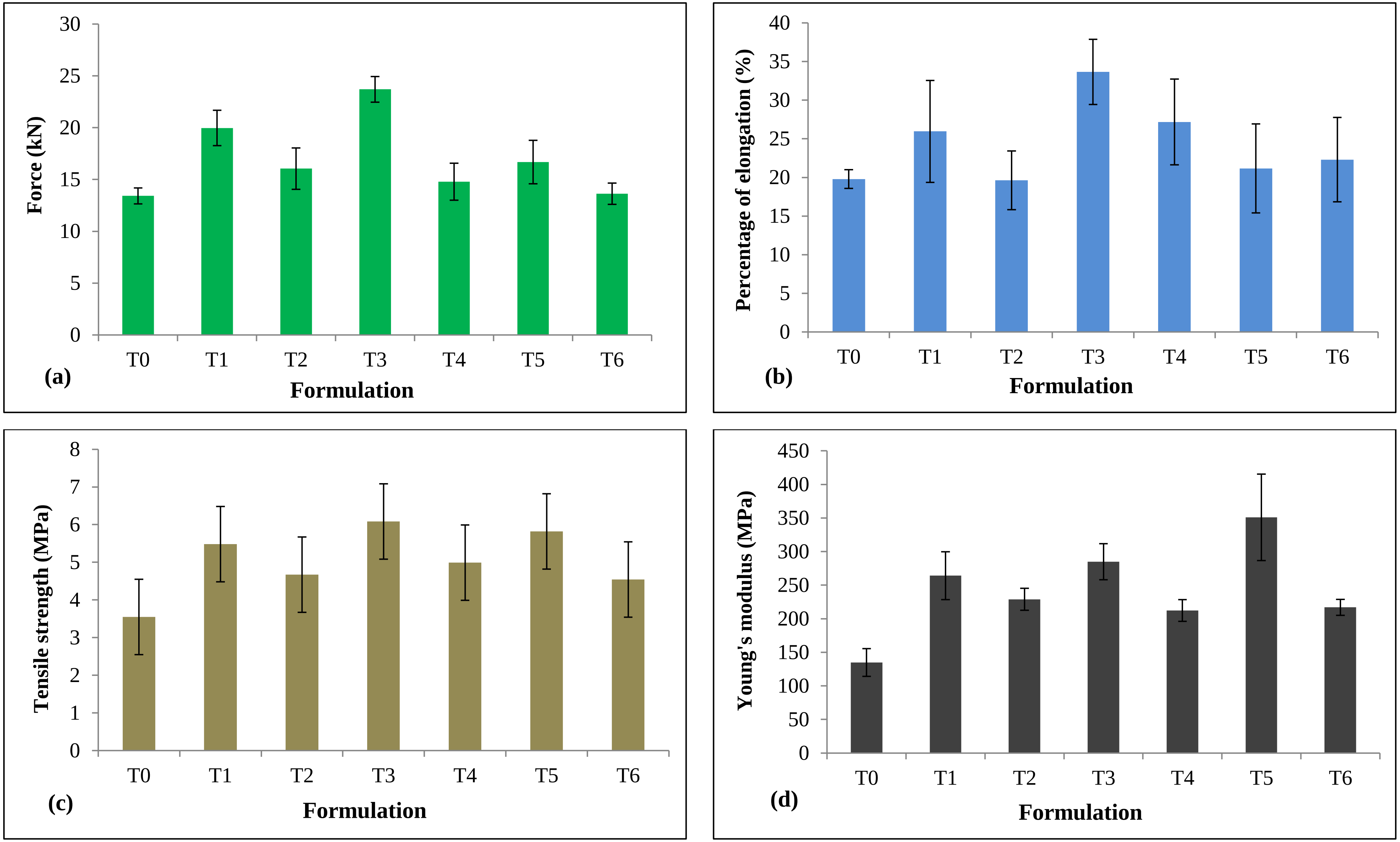


Figure 4.1.2: Tensile testing (23°C & 50% RH), (a) applied force, (b) percentage of elongation, (c) tensile strength, and (d) Young's modulus at break

4.1.3 Tensile testing (7°C and 50% RH)

Another condition, 7°C of temperature and 50% RH, was determined to characterize the tensile. The four parameters contained maximum forces, percentages of elongation at break, tensile strength, and Young's modulus are shown in the figure 4.1.3 (a), (b), (c), and (d), respectively.

Due to figure 4.1.3 (a), the sample with no fiber addition, T0, was seen that the applied force of it at break point was lower than other 6 samples, T1 to T6, with fiber containing. The values of 10.85, 24.47, 16.08, 22.73, 14.38, 17.21and 14.52 kN was belonged to T0 to T6. It was found the applied force of T1 added by 5 mmol of oxidized pulp was the highest, 24.47 kN, among all samples and it was followed by T3 with 10 mmol and T5 with 15 mmol of pulp oxidation, 22.73 and 17.21 kN respectively, as the lowest applied force was referred to T0, 10.85 kN. The applied forces of samples with lower amount of fiber addition, T2, T4, and T5, were investigated that they weren't as high as the samples with more amount of fiber containing, T1, T3, and T5. This parameter would be decreased if added fibers were also diminished so it was significantly changed by the values of different oxidation of fiber and content of added fibers.

The percentages of elongation at break of the samples are described in figure 4.1.3 (b). As the results shown in this figure, the values of percentages of elongation 20.36, 26.96, 33.59, 36.73, 27.28, 33.10, and 22.83% were respectively referred to T0 to T6. It was seen that the samples with fiber inclusion, T1 to T6, had higher percentages of elongation than the sample without any fibers addition, T0. Depending on these values, T3 had the biggest percentage of elongation, 36.73%, and it was followed by T2 and T5 which were 33.59 and 33.10% as T0 was the lowest. The values of samples of T1 and T4 were similar and they were a bit different from each other. T6 was also noted that it had similar value to T0 but it was still better than.

The figure 4.1.3 (c) represents the results of tensile strength of the samples which have been acclimatized at 7°C and 50% RH. According to these results, the samples of T0 until T6 had 3.67, 5.81, 4.50, 5.85, 4.47, 5.33, and 3.94 MPa of tensile strength respectively. T3 added by 10 mmol of oxidized pulp had the highest tensile strength, 5.85 MPa, and it was chased by T1, 5.81 MPa, and T5, 5.33 MPa, which were included by 5 and 15 mmol of pulp oxidation, separately. In this case, T3 and T1 were seemed to be similar to each other. T0 was observed that the tensile strength of it was still the lowest among all tested samples which was 3.67 MPa. The value of tensile strength of T2 and T4 were quite similar but they were higher than T6 which was close to T0. Overall, the tensile strengths of samples with pulp addition were higher than the sample without any pulps addition and the samples decreased fibers content had tensile strength lower than those samples with more fibers content.

Another characterization, Young’s modulus, is depicted in the figure 4.1.2 (d). Base on this figure, different values of Young’s modulus of all tested samples were gotten. The results illustrated that the values of 158.56, 304.50, 186.47, 275.51, 178.08, 234.25, and 161.09 MPa of Young’s modulus were separately referred to the samples of T0 to T6. It was remarked T1, composed by 5 mmol of the oxidized pulp and full fiber content, had the highest Young’s modulus value, 304.50 MPa. It was pursued by T3, 275.51 MPa, and T5, 234.25 MPa, which were compounded by 10 mmol and 15 mmol of oxidation of pulp. The lowest value of Young’s modulus was belonged to the sample without any fibers addition, T0, which was 158.56 MPa. Between T0 and T6 compounded by 15 mmol of oxidized fiber and less content of fiber, they were found that their Young’s modulus values were close to each other but T6 was a bit higher than T0. T2 and T4 also had similarity in values of Young’s modulus but T4 was a bit smaller than T2. Due to these results, the samples of T2, T4, and T6 diminished amount of added fiber had modulus of elasticity values lower than other 3 samples, T1, T3, and T5, which were formed by more content of oxidized fiber. Depending on fiber addition and content of oxidized pulp, Young’s modulus of films would be improved and better than the films without any fibers intercalation.

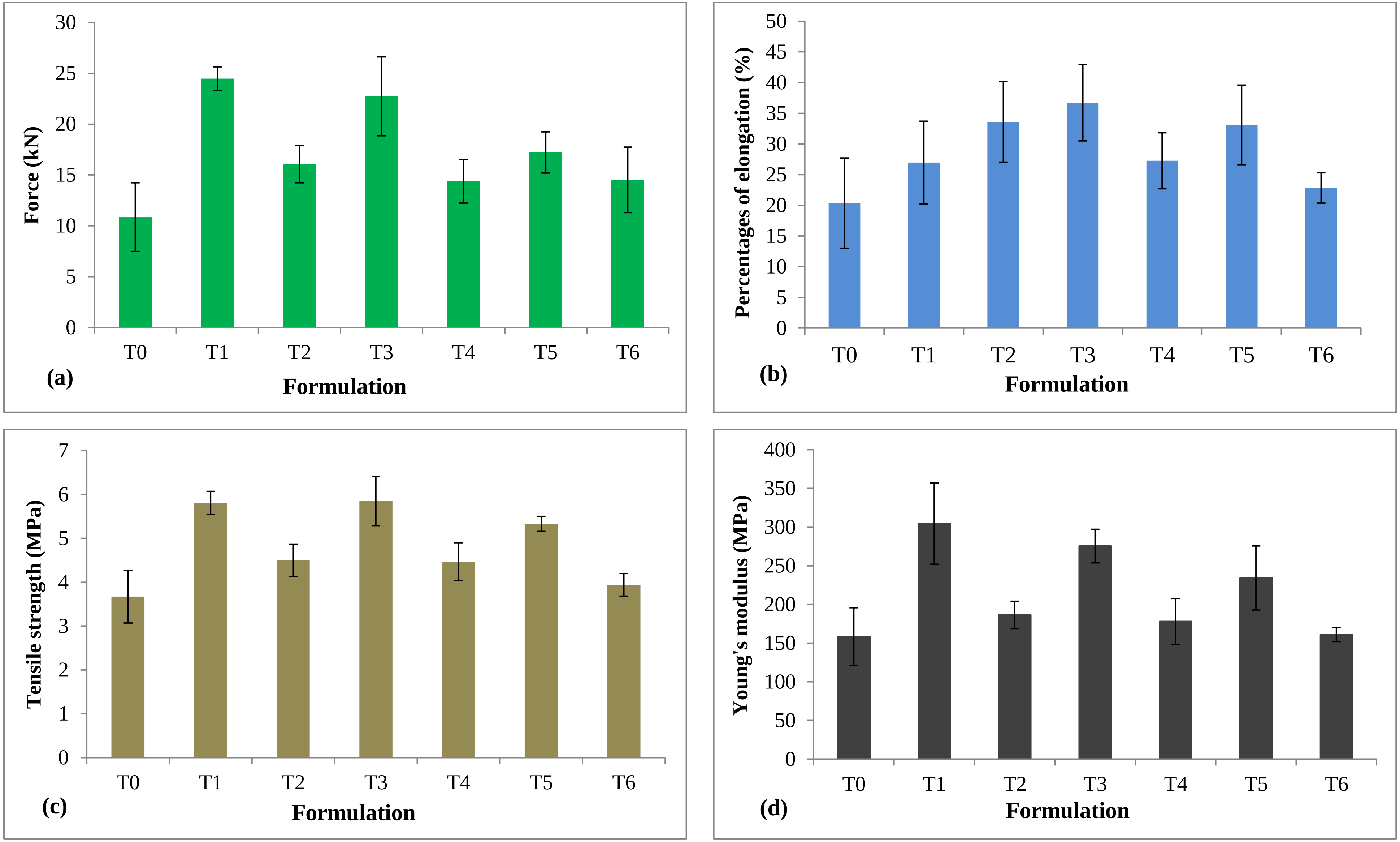


Figure 4.1.3: Tensile testing (7^{o}C & 50% RH), (a) applied force, (b) percentage of elongation, (c) tensile strength, and (d) Young’s modulus at break

4.1.4 Water absorption determination

For water absorption characterization, three different conditions were determined and investigated which included 23°C & 50% RH, 23°C & 75% RH, and 7°C & 50% RH. The results of such kind of testing are shown in the figures 4.1.4.

Figure 4.1.4 (a) illustrates the determination of water absorption at 23°C and 50% RH. In this case, there were 9 different critical control points, included 1, 2, 3, 5, 7, 24, 48, 72, and 96 hours, which would be observed. For the first hour, the samples of T0 to T6 absorbed the water respectively 4.20, 3.99, 3.99, 3.83, 3.86, 3.94, and 5.34%. Later on, the percentages of water absorption of all tested samples, T0 until T6, continued increasing from these values to 8.61, 6.85, 7.92, 6.81, 7.48, 5.93, and 8.32 after keeping in this condition for 7 hours. According to the figure, the percentages of water absorption of the samples were considered to be constant at 7 hours. It meant that the samples were saturated and equilibrium to the environment surrounding them. Even that, the experiment was still carried on until the last critical control point, 96 hours. The results showed that they were a bit fluctuated during 24, 48, 72, and 96 hours. Due to the results obtained at 7 hours critical point, the samples contained fiber, T1 to T6, absorbed the water lower than the sample without fiber containing, T0. The percentage of water absorption of T5 compounded by 15 mmol of oxidized pulp and high content of fiber was the lowest, 5.93%, among those samples asT0 with no fiber content had the highest water absorption value, 8.61%. It was followed by the samples added by 10 and 5 mmol of pulp oxidation which were T3, 6.81%, and T1, 6.85%, respectively. Water absorption percentages were increased and higher to the samples decreased the content of oxidized fiber as shown in T2, T4, and T6 but they were still smaller than T0. All percentages of water absorption at 9 critical points of those samples are shown in table in appendix section.

The second condition of water absorption determination, 23°C and 75% RH, is shown in figure 4.1.4 (b). There were also 9 different critical control points which would be investigated. Those disparate critical points were referred to varied periods of time included 0.5, 1, 1.5, 2, 3, 5, 7, 24, and 48 hours. The percentages of water absorption at the early hour of testing were 17.58, 17.87, 16.59, 17.18, 16.30, 16.70, and 16.66% which were belonged to T0 until T6, respectively. It was remarked the water absorption percentages of the tested samples were kept on growth from the previous values at 0.5 hour to 24.42, 22.07, 22.42, 21.96, 22.69, 20.79, and 21.72% at 2 hours, separately. Base on values of water absorption and the figure, the critical control point at 2 hours was thought as a stable point of this condition. It signed that the samples didn’t attract water vapor anymore because they were equivalent to the conditions of environment around them. The characterization was still continued practicing until 48 hours of final critical control point was finished. According to the results at 2 hours showed that T0, the sample without oxidized fiber addition, had larger percentage of water absorption than other samples, T1 till T6, which were compounded by fibers. It had the highest value of water uptake, 24.42%, as T5 had the lowest percentage of water absorption, 20.79%. The percentages of water absorption of the remains samples included T1, T2, T3, T4, and T6 weren't much different which were a bit more or less than 22%. The water absorption percentages of the left critical control points of all samples after 2 hours were investigated they weren't fluctuate highly and shown in the table of appendix section.

7^{o}C and 50% RH was the last condition of water absorption testing for this experiment. It is shown in figure 4.1.4 (c). Different from other 2 conditions, 11 critical control points were remarked for this condition covered by 1, 2, 3, 4, 5, 7, 9, 24, 48, 72, and 96 hours. The results showed that at the first hour, the percentages of water absorption of every tested sample increased slowly from 0 to 2.21, 1.91, 1.79, 1.54, 1.61, 1.49, and 1.71%. These numbers were separately belonged to T0 until T6. But it was found the values of water uptake were continued rising until 24 hours of critical points which were 9.86, 9.07, 9.25, 8.83, 8.94, 8.41, and 8.59%. Depending on the results and figure, the eighth critical control point, 24 hours, was seen to be a regular part of water absorption of this testing because all tested samples were seemed to be equivalent to the conditions of climatic chamber. It was the equilibrium between humidity and samples. The remains critical control points after 24 hours, contained 48, 72, and 96 hours, were also experimented in order to determine the constant point of water absorption of samples and observe the fluctuation. Due to the results obtained at 24 hours, the percentage of water uptake of T0 was the highest, 9.86%, if it was compared to other 6 samples as T5 had the lowest value of water absorption, 8.41%. It was pursued by T2 and T1 which were 9.25 and 9.07% respectively. In this case, the samples of T1 and T2, T3 and T4, and T5 and T6 had similarity in percentages of water absorption which were 9.07 and 9.25%, 8.83 and 8.94% and 8.41 and 8.59%. The left results of water absorption of the samples, which weren't described in this section, would be shown in the following figure and table in appendix section.

In short, the percentages of water absorption of sample without any fibers addition were found they were always higher than those samples which were compounded by oxidized fibers and different oxidations of pulp for both less and more content of the fibers inclusion.

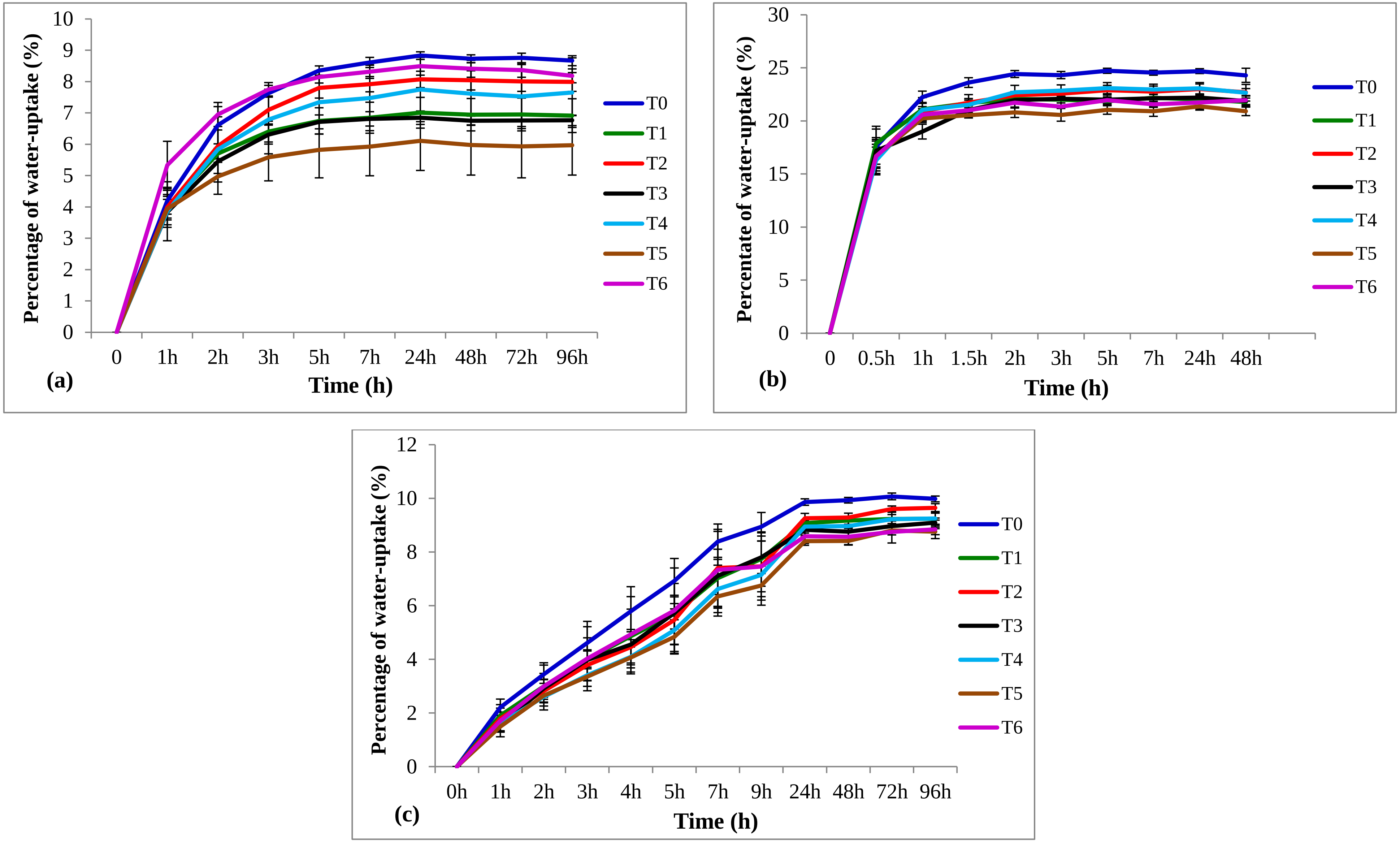


Figure 4.1.4: Water absorption determination, (a) 23^{o}C and 50% RH, (b) 23^{o}C and 75% RH, and (c) 7^{o}C and 50% RH

4.1.5 Water vapor permeability of biodegradable films

The results of water vapor permeability (WVP) determination are shown in the following figure, figure 4.1.5. According to these results and figure, the values of water vapor permeability were 6.05, 2.84, and $6.14.10^{13}$($Kg.m.Pa^{-1}.s^{-1}.m^{-2}$) which were belonged to the samples of T0, T3, and T4, respectively. It was found that T3 compounded by 10 mmol of pulp oxidation and high content of fibers had the lowest water vapor permeability among the tested samples as other 2 samples, which were T4 also composed by 10 mmol of oxidized pulp but low content of fibers and T0 without any fibers addition, had higher results of water vapor permeability and their values were similar to each other.

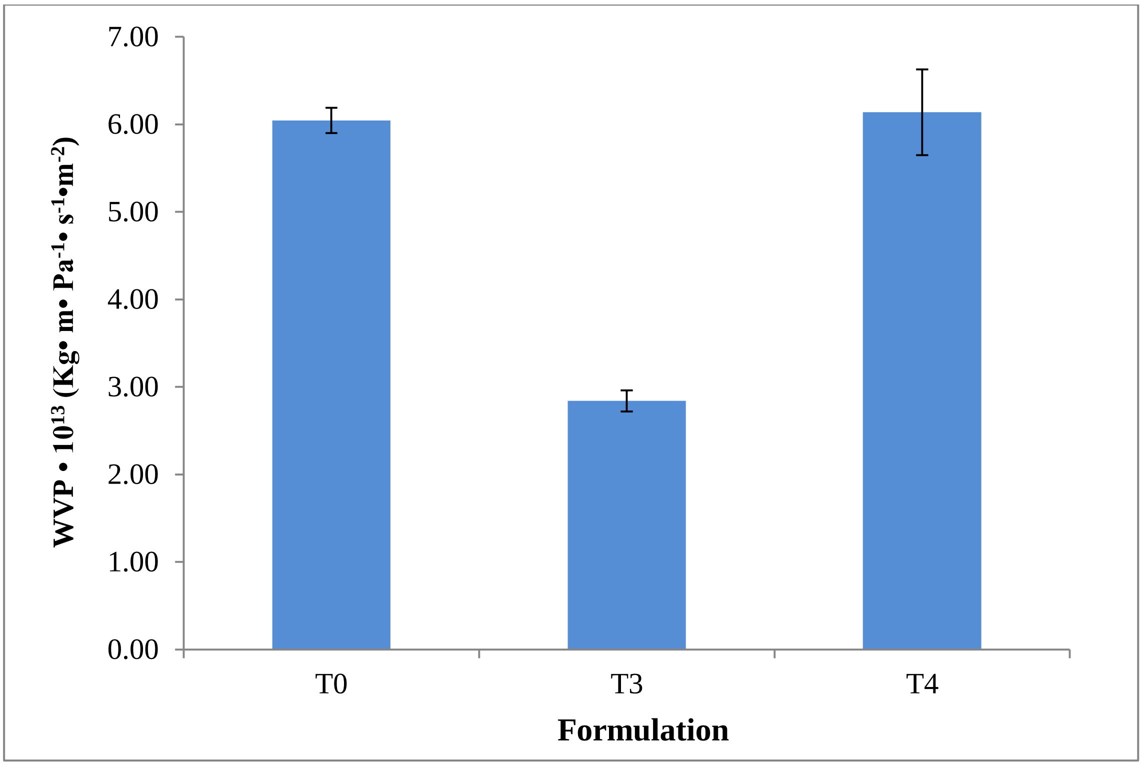


Figure 4.1.5: Water vapor permeability capacity of biodegradable films

4.1.6 Biodegradability determination and comparison of degradation between enzyme solution and water addition

Figure 4.1.6 (a) and (b) shows separately about the biodegradability determination of films by enzyme solution (α–amylase) and the comparison of degradation between the enzyme solution and water addition. There were the same 9 critical control points of these 2 experiments which would be observed and tested. These 9 critical control points were referred to the different periods of time of experiments and they were 1, 2, 4, 8, 24, 30, 48, 72, and 96 hours shown in the figure 4.1.6 (a) and (b).

According to the figure 4.1.6 (a), the percentages of loss of weight of all tested samples increased to 28.15, 21.81, 22.80, 23.88, 26.57, 22.37, and 23.45% at the first hour of testing for T0 until T6, respectively. It was investigated that these values were kept on rising due to the increased times of determination. The samples incorporated by oxidized fibers, T1 to T6, didn't have much different percentages of weight loss at that time. The results were seen that at 30 hours of experiment, the tested samples had similar loss of weight percentages contained 34.24, 31.32, 31.82, 34.05, 30.85, 31.95, and 33.15% which were respectively referred to T0, T1, T2, T3, T4, T5, and T6. At that time, the percentages of loss of weight of fiber contained samples were noted that they increased quickly than the sample with no fiber containing, except T4 which was lower than. From the last critical control point of such kind of testing, the percentages of loss of weight of T0 till T6 were remarked that they were risen highly from the early hours to 50.08, 66.11, 52.11, 55.23, 48.95, 53.40, and 51.60% at 96 hours of immersion times. Due to these results obtained at 24 hours, the sample of T1 had the highest value of weight loss, 66.11%, if it was matched to other 6 samples and followed by T3 and T5, which were 55.23 and 53.40%, as T4 had the lowest percentage, 48.95%. It was found the samples of T0 and T4, T2 and T6, and T3 and T5 didn't have quite difference in values of loss of weight which were 50.08 and 48.95%, 52.11 and 51.60%, and 55.23 and 53.40%. The remains results of this characterization, which weren't depicted, would be illustrated in the figure 4.1.6 (a).

Depending on the figure 4.1.6 (b) showed the percentages of degradation of the samples without any fibers compounding, T0, rose to 28.15 and 25.30% at the early hour of experiment. These numbers were referred to the samples keep in the enzyme solution (T0: Enzyme) and distillated water (T0: H_2O), respectively. Due to the results indicated T0: Enzyme had the loss of weight value higher than T0: H_2O. The percentages of weight loss of T0: Enzyme were noted that they were carried on increasing based on the growth of determination times. But in contrast, T0: H_2O was very different from T0: Enzyme since it was found the percentages of weight loss seemed to be constant at all critical control points even the determination times grew up. From 96 hours of the last critical control points, T0: Enzyme had 50.08% and T0: H_2O had 29.22% of weight loss. Therefore, the value of weight loss of T0: Enzyme was so dissimilar to the first hour of testing and still higher than T0: H_2O which was nearly as same as the early hour of experimentation. More details about this testing are shown in the following figure.

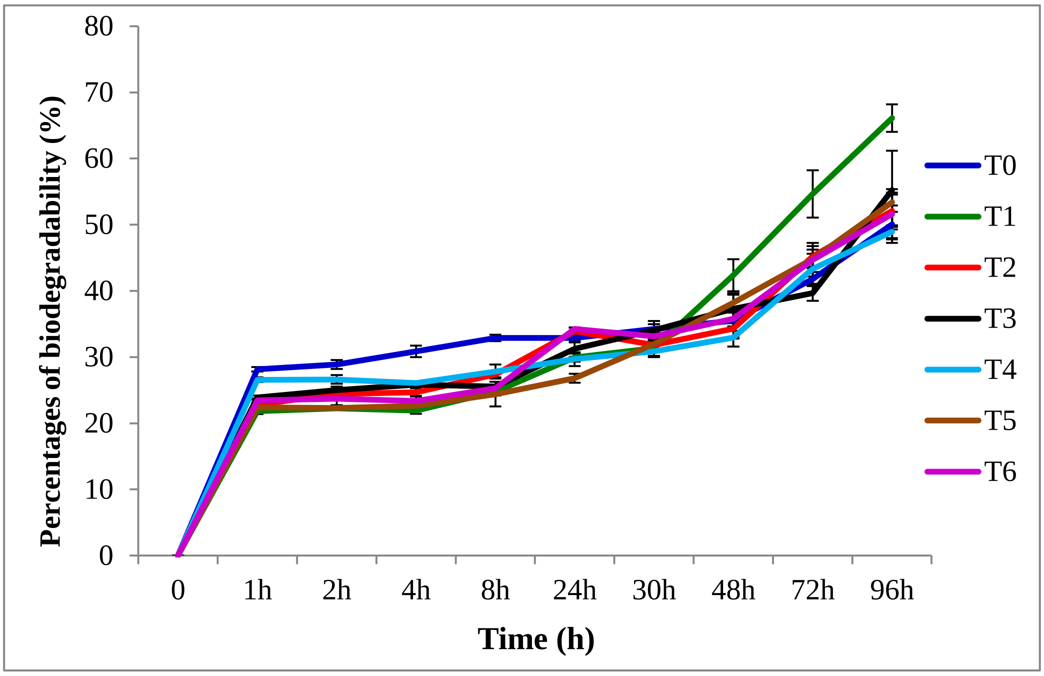


Figure 4.1.6 (a): Biodegradability determination of films

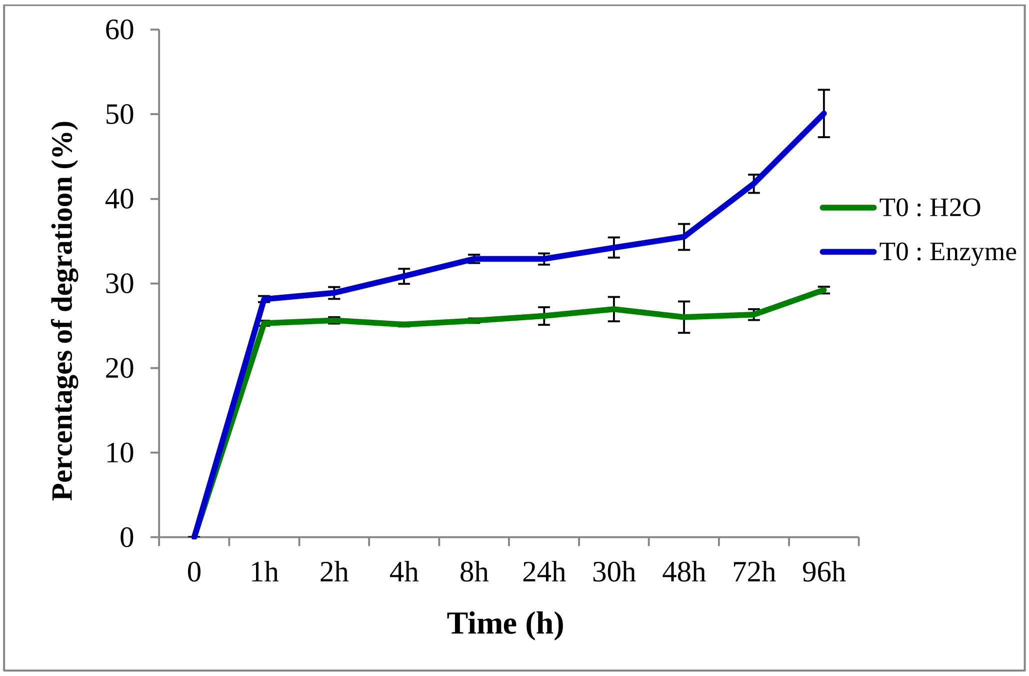


Figure 4.1.6 (b): Degradation by distillated water and enzyme solution (α–amylase)

4.2 Discussion

According to the obtained results, flexible and transparent films were promising achieved by thermoforming process. They had thin thicknesses which were between 150 to 250 μm. Even their thicknesses were still higher than thickness of some kinds of films derived from plastic materials but they were adequate for mechanical and other 3 characterizations. The surfaces of them were completely transparent and their textures were certainly flexible. The conducted films were found they had biodegradable properties which would be good to the environment. The biodegradability of films were done under the enzyme (α–amylase) activity since they were compounded by starch, natural polymer and renewable and degradable material. All obtained films were totally depended on the 2 factors included the pre–blends and blends preparation.

Pre–blends were made by potato starch, glycerol, glycerol/NFC and added distillated water. Starch is a polysaccharide composed by amylose, linear chain and non–crystalline, and amylopectin which is branched chain and tree–like and also has crystallinity. Starch is a biopolymer which plays an important role in biodegradable films production. Glycerol is a non-toxic materials and it is used as a plasticizer to break down the crystallization of amylopectin. Both glycerol and starch has polarities which are able to connect each other. Another composition of pre–blends was NFC gel. It was used to reinforce and improve the properties of samples if compared to the sample without NFC compounding. Water was also a composition of pre-blends which was added in order to improve characteristics of pre–blends. In this case, added water also played an important role as a breaker of crytallinity of amylopectin and linked to glycerol and NFC. It was able to increase the properties of blends and films during Brabender mixing and thermoforming respectively. The water content was evaporated and removed in these both steps but not at all; it still presented in the ready prepared films which could be kept the flexibility. For pre-blends preparation, 60, 40, and 20% of starch, glycerol or glycerol/NFC, and added water based on total amount of starch respectively were considered as the optimum contents in this step. Pre–blends were more viscous and liquid because of the inappropriate amounts of composition of it. In this case, if more content of starch was added, the pre-blend then could be more viscous but it could be more liquid if more amount of glycerol was intercalated.

Blends were then created by ready prepared pre–blends through Brabender mixing. The temperature, rotation speed, and time of mixing were found they were the important parameters in this step. Temperature is a parameter to melt the pre–blends as mixing process is performed by rotation speed (rpm). Determination of appropriate temperature, rotation speed, mixing time would give a blend with good and high property. For an example, lower or over set temperature, rotation speed, and time of mixing could cause the blends attitudes because starch wouldn't be melted or the obtained blend would be rigid, not flexible, separately. It was found that starch would be breakable at high temperature, 165^{o}C. For this step, 110^{o}C of temperature, 100 rpm of rotation speed, and 12 min of mixing time have been considered as the suitable parameters since the obtained blends were seemed to be transparent and flexible because of starch melting and present of water in it. Starch wouldn't be breakable at low temperature, 110^{o}C, and some of water contents were still kept in the blends without evaporation.

The ready prepared blends were continued experimented to create the composites, films, through thermoforming. In this last step to obtain films, temperature, pressures/forces, and times of thermoforming were thought as the main parameters. It was remarked the blends were melted and had transparency of surface under an appropriate tested temperature and they were also extended and had thin thicknesses because of the optimum applied forces or pressures. The blends wouldn't be dissolved and the films would be breakable if the lower and higher temperatures were separately used. Base on higher or lower applied forces, the films would have thinner or larger thicknesses. But the films with quite thin thickness, they would be difficult to remove from transparent plastic sheets and usually breakable during removing. For this case, the temperature 135^{o}C, and the forces 100 kN for 10 min and 150 kN for 5 min were found as the suitable parameters of thermoforming since the ready prepared films had transparency of surfaces, flexibility, and thin thicknesses. It was observed the decreased masses of samples after thermoforming was related to the evaporated water contents of blends but some amounts of water were still presented in the films.

After flexible and transparent films were successfully obtained, they were then determined some characterizations. First of all, it was referred to mechanical testing which contained 2 conditions had 23^{o}C and 50% RH, based on ASTM D882–02, and 7^{o}C and 50% RH, aimed to understand and investigate the properties of films during keeping in the refrigerator conditions as well as to obtain the packaging material for fresh foods in these conditions. As the results showed in the figure 4.1.2 and 4.1.3, the sample of T3 compounded by 10 mmol of oxidized pulp and high content of fiber addition were found it was the best formula if it was compared to other 6 samples since it had tensile strengths and percentages of elongation higher than those samples for these both conditions, 6.08 MPa and 33.66% for 23^{o}C and 50% RH and 5.85 MPa and 36.73% for 7^{o}C and 50% RH, as the tensile strengths and percentages of elongation of T0, composed by empty oxidized fibers addition, were the lowest which were 3.55 MPa and 19.79% and 3.67 MPa and 20.36% at 23^{o}C and 50% RH and 7^{o}C and 50% RH, respectively. Tensile strength and percentage of elongation were considered as the significant parameters for composite materials. It was seen that the Young’s modulus of T5 for 23°C and 50% RH was the highest, 351.10 MPa, as T1was the highest, 304.50 MPa, for the condition of 7°C and 50% RH. Overall, because T3 had two parameters which were better than T1 and T5 so it was the desired formula of this experiment. Due to the fibers compounding and different of pulp oxidations, the mechanical characterization would be improved if compare to the samples without any fibers addition since added fibers could play an essential role to develop and improve this characterization of the samples. Moreover, higher or lower content of added fibers and amount of fiber oxidations would also influence to these properties.

Another characterization was referred to water absorption of films contained 3 conditions which would be experimented and investigated, 23°C & 50% RH, 23°C & 75% RH, and 7°C & 50% RH. Different periods of time were determined and checked for this testing. According to the obtained results of water absorption in 4.1.4 section, it was remarked the sample of T0 without any fibers containing always had the highest percentage of water absorption for these 3 conditions if it was matched to other samples, T1 until T6, which were compounded by oxidized fiber. It meant because of presence of fibers content, the percentages of water uptake of T1 to T6 were always lower than T0. Another reason was based on a good chemical compatibility of starch and oxidized fiber/cellulose so the water molecules couldn’t pass easily through the materials. The figures of the results of this experiment were found that they were both exponential profiles for 2 conditions, 23°C & 50% RH and 23°C & 75% RH, but they were linear for another condition, 7°C & 50% RH. To study the effects of temperature and humidity to the samples, the sample T0 was discussed in this section. T0 was observed it was equilibrium at 7, 2, and 24 hours for 23°C & 50% RH, 23°C & 75% RH, and 7°C & 50% RH, respectively, which were around 9, 25, and 10% of water absorption. If the first and second conditions were compared each other with same temperature but different RH, they were investigated the relative humidity (RH) had high influence to the water uptake of samples since the rate of water absorption at 23°C & 75% RH was both faster and higher than at 23°C & 50% RH because of the increased RH. Another thing, if the first and last conditions were matched each other with the same RH but different temperatures, they were noted that the temperature also had influence to the rate of water absorption but not as high as humidity. In this case, the rate of water uptake of T0 at 23°C & 50% RH was reached to equilibrium at shorter periods of time, 7 hours, than 7°C & 50% RH, 24 hours. It meant the rate of water absorption at first condition was faster than last condition. In short, the rates/percentages of water absorption of films were depended on the temperature, humidity, content of fibers addition and amount of pulp oxidation.

The next characterization of films was water vapor permeability determination. Due to the results achieved in the section 4.1.5, there were 3 samples include T0, T3, and T4 which were tested in this experiment. T3 compounded by 10 mmol of oxidized pulp and high content of fibers was found it had mechanical and water absorption properties better than other samples so it was continued analyzing the capacity of water vapor barrier by comparing to T0 composed by only starch and glycerol and T4 contained lower content of fibers. Depending on the more fiber contents and optimum amount of pulp oxidation added, T3 had the lowest rate of water vapor permeability, $2.84.10^{13}(Kg.m.Pa^{-1}.s^{-1}.m^{-2})$, among the tested samples. This result showed that the content of water vapor passed through T3 was lower than other 2 samples so T3 was still the best formulation of this testing. Water vapor permeability and water absorption were thought they were related each other and according to the results of them, T3 had higher capacity of water vapor barrier as T4 was similar to T0.

The last characterization of the experiment was focused on biodegradability of films. The samples were observed they were degradable under α–amylase activity because of the present of starch in those composites. The rate of biodegradability was partially relied on the concentration of enzyme solution. In the same condition of substrate content, pH, and temperature, the biodegradability rates would be faster or slower than if higher or lower concentration of enzyme was separately used. In this case, $\alpha -$amylase was first diluted to a lower concentration in order to determine and observe the rate of biodegradability at different periods of time. 0.12 mg/L or 0.06 KNU/L of $\alpha -$amylase solution was found as an appropriate concentration of this testing. From the figure of biodegradability, it was investigated that there were 2 interesting and remarkable parts; glycerol delivery and degradation of starch. Glycerol was delivered from starch from 0 to 30 hours of determination times and then from 30 to 96 hours, starch was degraded and hydrolyzed by enzyme activity. At 96 hours of tested times, all samples were seen they were mostly degradable and the percentages of biodegradability were between 50 to 66%.

Comparison of degradation of T0 between enzyme solution and water addition showed that it was quite different between both conditions as T0: Enzyme always had the rate of degradation higher than T0: H_2O. The values of degradation of T0: H_2O were seemed to be stable at every critical control points from 1 to 96 hours as T0: Enzyme kept on growth during the increasing of experimental times. T0: H_2O also delivered glycerol from starch but starch couldn’t be able to degrade in this condition. Due to the results of biodegradability, enzyme activity absolutely had effects to the degradation of films as added water didn’t have. Glycerol was released from starch and starch was then degradable which was controlled by enzyme condition.

5. Conclusions and Recommendations

5.1 Conclusions

After the experiment and sample characterizations have been completely finished, the results contained materials processing and preparation, mechanical testing, water absorption, water vapor permeability, and biodegradability are possible to conclude as follows:

- Starch is a polysaccharide and natural polymer able to produce a film with flexibility, transparency, biodegradability, and thin thickness, which was compounded by plasticizer, glycerol, and reinforced by nanofibrillated cellulose (NFC).
- 60, 40, and 20% of starch, glycerol or glycerol NFC, and added water depended on total content of starch, respectively, have been considered as a suitable proportion to prepare an appropriate pre-blend.
- For blend preparation by Brabender mixing, it has been found 110^{o}C, 100 rpm, and 12 min of temperature, rotation speed, and mixing time were good parameters in this procedure.
- The films were produced by thermoforming. The temperature 135^{o}C and the forces and times of thermoforming 100 kN for 10 min and 150 kN for 5 min have been remarked they were the optimum parameters in this step.
- The obtained films were transparent and flexible and their thicknesses were between 150 to 250 μm acceptable and suitable for characterization.
- Addition of NFC, fiber contents, reinforced and increased the mechanical properties of the composite materials, films.
- The sample of T3 added by 10 mmol of pulp oxidation and more content of fibers had tensile strength and percentage of elongation higher than other 6 samples for both conditions, 23^{o}C and 50% RH and 7^{o}C and 50% RH, as the Young's modulus of T5 and T1 were 351.10 MPa and 304.50 MPa for 23^{o}C and 50% RH and 7^{o}C and 50% RH, respectively.
- Three conditions of water absorption were conducted which contained 23^{o}C and 50% RH, 23^{o}C and 75% RH, and 7^{o}C and 50% RH. Depending on the results of this characterization showed the percentages of water uptake of T0 without any fibers addition were always higher than the other samples which were composed by different contents of oxidized fibers and amount of pulp oxidation (5, 10, and 15 mmol).

- The water vapor permeability determination was taken T0, T3, and T4 as the samples of this testing. As results, T3 added by 10 mmol of oxidized pulp and high content of fibers had rate of water vapor permeability lower than other 2 samples which was $2.84.10^{13}(\mathrm{Kg.m.Pa^{-1}.s^{-1}.m^{-2}})$ as T0 and T4 were quite similar for this assay. T3 has been considered as a good barrier formulation according to the lower rate of water absorption and water vapor permeability.
- There was a condition of biodegradability testing included 50^{o}C of temperature and 20 ml of 0.12 mg/L or 0.06 KNU/L of α–amylase solution. This enzyme concentration has been found as an appropriate condition for this experiment at different periods of time. Under the enzymatic activity, all formulations of samples were almost degraded and the rates of biodegradability grew up based on the increase of determination times. But the sample tested with distillated water addition wasn't mostly degraded since the rates of degradation were similar at every critical point. Glycerol delivery and degradation of starch were the 2 noticeable and interesting parts of this characterization.

5.2 Recommendations

According to the results, discussions, and conclusions of experiment, some recommendations to improve and develop this work or the next research in the future would be given as follows:

- More fibers content should be added in the films in order to enrich their mechanical properties but it should be studied the feasibility first.
- Other methods better than thermoforming should be found in order to obtain a better film.
- Solubility of films should be determined.
- Oxygen or carbon dioxide barrier properties of films should be tested.
- Microscopic structure of films shall be analyzed.

Experimental Budgets

Nº	Items		Cost (€)
1	Pre–blends preparation	Potato starch	2.00
		Glycerol	2.00
		Analytical balance	5.50
2	Blends preparation	Brabender mixing	200.00
3	Films preparation	Thermoforming	17.00
		Transparent plastic sheets	3.00
4	TEMPO–mediated oxidation of fiber	Eucalyptus pulp	0.50
		Sodium bromide (NaBr)	5.00
		TEMPO	7.00
		Sodium hypochlorite (NaClO)	2.00
		Sodium hydroxide (NaOH)	3.00
		Mixer	1.00
		pH meter	1.00
		Vacuum filtration machine	26.00
		Drying oven (105°C)	5.00
5	NFC preparation	Homogenizer	2.00
		Refrigerator	0.50
6	Mechanical characterization	Blade for films cutting	2.00
		Universal testing machine	1.50
		Climatic chamber	50.00
		Thickness measurement machine	0.50
7	Water absorption determination	Drying oven (50°C)	5.00
		Desiccator	1.00
8	Biodegradability testing	Enzyme solution (α–amylase)	0.00
		Acetic acid (CH_3COOH)	0.50
9	Water vapor permeability	External characterization	200.00
10	Energy	Water	50.00
		Electricity	80.00
11	Salary of engineer	-	15,000.00
		Total	15673.00

* The cost of equipment: amortization

**α–Amylase was kindly given by Novozymes Company.

Appendix

Table 1: Percentages of water absorption (23^{o}C & 50% RH)

T	0	1h	2h	3h	5h	7h	24h	48h	72h	96h
T0	0	4.20	6.61	7.63	8.35	8.61	8.83	8.73	8.75	8.67
T1	0	3.99	5.70	6.40	6.74	6.85	7.01	6.94	6.95	6.91
T2	0	3.99	5.94	7.10	7.80	7.92	8.07	8.04	8.01	7.99
T3	0	3.83	5.45	6.31	6.72	6.81	6.85	6.75	6.76	6.76
T4	0	3.86	5.84	6.79	7.34	7.48	7.75	7.61	7.52	7.65
T5	0	3.94	4.97	5.58	5.82	5.93	6.11	5.97	5.93	5.97
T6	0	5.34	6.95	7.74	8.15	8.32	8.49	8.41	8.36	8.18

Table 2: Percentages of water absorption (23^{o}C & 75% RH)

T	0	0.5h	1h	1.5h	2h	3h	5h	7h	24h	48h
T0	0	17.58	22.24	23.61	24.42	24.32	24.73	24.54	24.68	24.29
T1	0	17.87	21.09	21.67	22.07	22.01	21.85	22.18	22.03	21.79
T2	0	16.59	20.89	21.72	22.42	22.56	22.89	22.76	22.99	22.70
T3	0	17.18	18.99	21.02	21.96	22.10	22.00	22.13	22.19	21.87
T4	0	16.30	21.04	21.51	22.69	22.85	23.08	22.97	23.07	22.66
T5	0	16.70	20.24	20.54	20.79	20.57	21.05	20.91	21.37	20.90
T6	0	16.66	20.59	20.99	21.72	21.35	21.96	21.56	21.73	21.96

Table 3: Percentages of water absorption (7^{o}C & 50% RH)

T	0h	1h	2h	3h	4h	5h	7h	9h	24h	48h	72h	96h
T0	0	2.21	3.44	4.62	5.79	6.92	8.39	8.94	9.86	9.93	10.07	9.98
T1	0	1.91	2.99	4.00	4.87	5.69	7.03	7.74	9.07	9.17	9.23	9.24
T2	0	1.79	2.82	3.79	4.46	5.47	7.41	7.47	9.25	9.28	9.60	9.64
T3	0	1.54	2.92	3.98	4.55	5.73	7.11	7.80	8.83	8.76	8.96	9.10
T4	0	1.61	2.60	3.40	4.10	5.09	6.62	7.14	8.94	8.96	9.22	9.25
T5	0	1.49	2.65	3.35	4.07	4.84	6.34	6.75	8.41	8.41	8.80	8.76
T6	0	1.71	3.00	4.02	4.93	5.82	7.34	7.46	8.59	8.57	8.75	8.84

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