# DEVELOPMENT OF SEMOLINA FILM REINFORCED WITH ZINC OXIDE AND KAOLIN NANOPARTICLES

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# DEVELOPMENT OF SEMOLINA FILM REINFORCED WITH ZINC OXIDE AND KAOLIN NANOPARTICLES

**by**

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**Thesis submitted in fulfillment of the requirements
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To:

My Father and My Mother

My Brother

My Nephew

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| $a^*$ | Redness |
| $b^*$ | Blueness |
| $L^*$ | Lightness |
| AFM | Atomic Force Microscopy |
| EB | Elongation Break |
| EDX | Energy-Dispersive X-Ray Spectroscopy |
| FTIR | Fourier Transform Infrared Spectroscopy |
| HM | High Moisture |
| LM | Low Moisture |
| MC | Moisture Content |
| OP | Oxygen Permeability |
| $P_2O_5$ | Phosphorus Pentoxide |
| PDA | Potato Dextrose Agar |
| RH | Relative Humidity |
| ROS | Reactive Oxygen Species |
| SEM | Scanning Electron Microscopy |
| SPI | Soy Protein Isolate |
| TEM | Transmission Electron Microscopy |
| TGA | Thermogravimetric Analysis |
| TS | Tensile Strength |

| | |
|---|---|
| TSA | Trypton Soy Agar |
| TSB | Trypton Soy Broth |
| UV | Ultraviolet |
| WG | Wheat Gluten |
| WPC | Whey Protein Concentrate |
| WPI | Whey Protein Isolate |
| WS | Water Solubility |
| WVP | Water Vapour Permeability |
| XRD X. | Ray diffraction |
| YM | Young Moduls |
| ZnO- | Nr Zinc Oxide Nano Rod |

**PEMBANGUNAN FILEM SEMOLINA YANG DIPERKUATKAN DENGAN NANOPARTIKEL ZINK OKSIDA DAN KAOLIN**

## ABSTRAK

Kajian ini dijalankan bagi melihat kegunaan filem terbiodegrasi berasaskan biopolimer sebagai pembungkus makanan yang bertujuan untuk mengurangkan pencemaran alam sekitar yang disebabkan oleh pembungkus makanan sintetik. Plasticizer (30%, 40%, 50% dan 60%), nano rod zinc oxide (1, 2, 3, 4 dan 5%), nano-kaolin (1, 2, 3, 4 dan 5%) dan kombinasi nano-kaolin dan ZnO-nr (1:4, 2:3, 3:2 dan 4:1) telah digunakan sebagai bahan pembungkus semolina selama 72 hari bagi mengekang kesan kimia-fisiko,mikrobiologikal dan kualiti organoleptic yang terdapat dalam keju Mozarella. Kesan plasticizer dan zarah nano ke atas ketelapan wap air (WVP), ketelapan oksigen (OP), kuantiti pengaliran dan penyerapan ultra ungu, kebolehlekatan haba, fizikal, morfologi, mekanikal dan kesan antimikrob turut dikaji. Kandungan lembapan dan kebolehlarutan filem yang boleh dimakan meningkat, manakala kandungan WVP, OP dan kekuatan regangan filem tersebut akan berkurangan dengan peningkatan plasticizer iaitu dari 30% ke 50% ($p<0.05$). ZnO-nr dan nano-kaolin mempunyai kesan terhadap ciri-ciri filem dan mengurangkan OP, WVP, kelarutan, kandungan lembapan dan proses pemanjangan semasa waku berhenti dengan ketara tetapi meningkatkan kekuatan regangan. Walau bagaimanapun, keputusan yang ketara dapat dilihat dalam ciri-ciri mekanikal filem yang bergabung dengan nano-kaolin. Oleh yang demikian, dengan peningkatan nano kaolin dari 0% hingga 5%, ia menunjukkan 61% peningkatan kekuatan regangan dalam filem komposit nano ($p<0.05$). Penyaliran UV filem semolina dipengaruhi oleh jumlah filem ZnO-nr dan Komposit nano yang dipamerkan oleh 0%

penyaliran UV spektrum inframerah terdekat. Seterusnya, ketelapan oksigen dan WVP filem yang digabungkan dengan campuran nano kaolin dan ZnO-nr berkurangan disebabkan oleh peningkatan dalam peratusan ZnO-nr, kandungan mekanikal bertambah baik dengan peningkatan peratusan nano kaolin dan jumlah tertinggi dapat dilihat dalam nisbah 1: 4 ZnO-nr: nano kaolin. Keputusan ujian mikrobiologi membuktikan bahawa sampel yang mengandungi lebih daripada 3% ZnO-nr mempunyai aktiviti antimikroorganisma yang baik terhadap Staphylococcus aureus, E. coli dan kulat. Begitu juga, dengan sifat-sifat fiziko-kimia seperti pH, keasidan tertitrat, garam, lemak, protein dan kelembapan sampel keju yang dibungkus dengan pembungkusan terpilih telah dikaji semasa penyimpanan pada 4 ° C selama 72 hari. Yis dan kulat, koliform dan Staphylococcus aureus juga telah ditentukan dan kajian sensori keju turut dinilai. Keju yang dibungkus dengan pembungkusan Komposit nano memberikan hasil yang positif berbanding dengan keju yang dibalut dengan filem kemas (tanpa nanoparticle). Tambahan pula, protein dan kandungan lemak keju meningkat. Selain itu, pH dan kandungan kelembapan keju berkurangan. Keasidan dan kandungan garam keju turut meningkat semasa penyimpanan dalam semua keadaan yang dikaji. Di samping itu, sampel keju yang dibungkus dengan ZnO-nr: nano kaolin (3: 2) dan (4: 1) menunjukkan pengurangan kandungan berat pada tahap 12% dan 20 %, mempunyai pH tertinggi, tekstur, skor penerimaan keseluruhan dan ketiadaan pencemaran mikrobiologi selama lebih daripada 57 hari. Ini membuktikan bahawa pembungkusan yang mengandungi partikel (ZnO-nr dan nano-kaolin) lebih berkesan dalam mengurangkan koliform, S.aureus dan kulat dalam keju dan dapat mencegah pengoksidaan dan bau hanyir keju.

# DEVELOPMENT OF SEMOLINA FILM REINFORCED WITH ZINC OXIDE AND KAOLIN NANOPARTICLES


## ABSTRACT

This study was to examine the use of biopolymer-based biodegradable films as food packaging with the goal of reducing environmental pollution produced by synthetic food packaging. Plasticizer (30%, 40%, 50%), nano rod zinc oxide (1, 2, 3, 4 and 5%), nano-kaolin (1, 2, 3, 4 and 5%) and combination of nano-kaolin and ZnO-nr (1:4, 2:3, 3:2 and 4:1) were incorporated into a Semolina packaging to impact on the physico-chemical properties, microbiological features and organoleptic quality of "Mozzarella cheese" throughout a storage period of 72 days. The effects of plasticizer and nanoparticles content on the water vapor permeability (WVP), oxygen permeability (OP), ultraviolet absorbance and transmittance, heat sealability, thermal, physical, morphology, mechanical and antimicrobial properties of the films were investigated. The moisture content and solubility of edible films increased while, the WVP, OP and tensile strenngth of the films decreased with increasing plasticizer from 30% to 50% ($p<0.05$). Both ZnO-nr and nano-kaolin had a marked impact on properties of the films and significantly reduced the OP, WVP, solubility, moisture content and elongation at break, whereas enhanced tensile strength. However, notable results were observed in mechanical properties of those films incorporated with nano-kaolin. therefore, with increased the nano-kaolin from 0% to 5% showed a 61% increased in the nanocomposite film 'tensile strength ($p<0.05$). UV transmittance of the semolina films was greatly influenced by the amount of ZnO-nr and the nanocomposite films exhibited 0% UV transmittance the near infrared spectra. meanwhile the amount of oxygen permeability and WVP of the films incorporated with blend of nano-kaolin and ZnO-nr decreased by the increase in ZnO-nr percentage, their mechanical properties improved by increasing of nano-kaolin percentage and the highest number of them observed in ratio 1:4 ZnO-nr: nano-kaolin. Results of microbial test revealed that samples contain more than 3% Zno-nr exhibited good antimicrobial activity against Staphylococcus aureus, E. coli and fungi. Likewise, such physico-chemical properties as pH, titratable acidity, salt, fat, protein and moisture of cheese samples wrapped by selected packaging were measured during 72 days of storage at 4 °C. Yeast and mold, coliform and Staphylococcus aureus were also determined and sensory properties of cheese were evaluated. Cheese packed with nanocomposite packaging revealed a positive behaviour in comparison to cheese wrapped with neat film (without nanoparticle). Furthermore, protein and fat content of cheeses increased. Besides, while pH and moisture content of cheeses were reduced, the acidity and salt content of cheeses increased during storage in all conditions studied. In addition, the cheese samples packaged with ZnO-nr: nano-kaolin (3:2) and (4:1) exhibited lowest weight loss (12%) and (20%) respectively, highest pH, texture, overall acceptability score and absence of microbiological contamination for more than 57 days. As the results, it can be argued that packaging incorporated with nanoparticles (ZnO-nr and nano-kaolin) might be effective in reducing *coliform*, *S.aureus* and *fungi* numbers in cheese and preventing the oxidation and rancidity of cheese. Thus, bionanocomposite packaging could be used to effectively preserve the Mozzarella cheese without affecting its quality characteristics.

# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

Plastics are one of the most commonly used materials in food packaging. It is significantly more advantageous to use than traditional materials such as tin or glass. Plastics are unrivaled when it comes to versatility and flexibility, able to be specifically designed for the product it intends to contain (López-Rubio et al., 2004). The downside of dependence of plastics is that product it has negative environmental impacts. It contributes to environmental pollution on many different levels. As it is created using petroleum derivatives, the main concerns are that plastic packaging materials are not renewable, and thus would it be difficult to dispose of in the long term. There are numerous problems in disposing plastic food packaging, in the decontamination process, and also methods of disposal such as burning, burying, and recycling processes. Therefore, scientists and researchers started to develop biodegradable food packaging materials that could replace the petroleum derived plastic food packaging materials. It would simultaneously create a boom in the agricultural sector of the economy (Souza et al., 2009; Ghanbarzadeh and Almasi, 2011).

Concerning the use of biodegradable materials in food packaging, this industry has exploited a diverse types of proteins (Khwaldia et al., 2010). Having such favorable properties as renewable, biodegradable, adhesive/cohesive as well as favorable film forming have turned the wheat protein to become among the valuable candidate in food packaging purposes (Tu ̈re et al., 2013). Semolina flour is full of gluten which helps the nutritional features of the material. It is translucent, cheap, extremely hard, and light-colored. It also has the ability to release antioxidants, by suppressing "radical-induced" lipid peroxidation and scavenging radical cations (Souze et al., 2009). One of its weaknesses is that it has high water permeability and hydrophilic properties when compared to conventional films and these properties are difficult to control. (Wilhelm et al., 2003). Hence, nanomaterials, which reinforce biopolymers through the formation of nanocomposites, have been recently employed to overcome these limitations (Petersson and Oksman, 2006).

The nanoparticles used in the biopolymer matrix remarkably strengthen the physicochemical, thermal, optical properties of nanocomposites in comparison with pure biopolymer (Kovacevic et al., 2008). Recent studies have explored the applications of ZnO in the food packaging industry because of ZnO nanoparticles have high surface energy, surface area, and has the capability for interfacial interaction and nano-scale dispersions in a protein matrix. This makes it an ideal nanoparticle (Rouhi et al., 2013; Nafchi et al., 2011 & 2014; Arfat et al., 2014).

In addition, ZnO is a heat stable and generally recorded as safe substance approved by the Food and Drug Administration (FDA, 2011). Regarding such applications as pharmaceutical materials, pigments, coating materials and cosmetics, ZnO has been extensively utilized as useful filler in UV absorbers (Kumar and Singh 2008; Li et al., 2009). As mentioned by Shahrom and Abdullah (2006), the important factors in nanoparticles' basic properties include their morphology, size, composition, shape and the crystallinity of the particles. For example, nano rods zinc oxide can act as needles for easy penetration through the cell wall and has also been proven to be effective against spoilage bacteria and food borne pathogens (Tayel et al., 2011). As well as, there is also an UV absorption ability exhibited by ZnO nanorods (ZnO-nr) (Lin et al., 2009). In addition, due to the clay layer of nano clays, they have also been commonly used as practical and helpful filler in protein films. This layer results in the decrease in water permeability (WVP) as well as the increase of the film's strength (Sothornvit et al., 2009). As a hydrated aluminosilicate, several industries have applied the natural mineral kaolin nanoclay. This is due to their such favorable properties as their low cost, wide range of application, availability, abundance and being environmentally friendly (Ma and Bruckard 2010). Thermoplastic amylose/kaolin composites (Su et al., 2010) given their inherent properties, ZnO-nr and nano-kaolin have been considered as reinforcements in biopolymers.

Mozzarella cheese the best-selling cheese in the U.S. market, originating from Italy. It comes in two varieties, one of them being "low moisture" (LM) and the other being "high moisture" (HM). Low moisture Mozzarella cheese is used mainly as condiments, such as on pizza or baked potatoes. High moisture Mozzarella cheese is used mainly as table cheese (Mastromatteo et al., 2014). Both cheeses, however, are vulnerable to microbiological spoilages. Coliforms and staphylococcus bacteria grow on the cheese surface usually from the water used during manufacturing and heat treatment when the cheese is still in curd form. Therefore, they have short shelf lives (Cantoni et al., 2003; Cantoni, et al., 2003).

Most Mozzarella cheese is packaged in plastic, as it is favorable in terms of avoiding contamination, and maintaining the quality of the cheese. This makes them popular despite their negative environmental impact and low recycling rate (Lagaron and Lopez, 2011; Siracusa et al., 2008). To change this trend, new biodegradable and edible materials that show antibacterial property have been developed. These materials must match plastic in terms of maintaining shelf life and preserving quality (Auras et al., 2006; Salarbashi et al., 2014).

Taking all these information into consideration, Semolina has potential to show good properties for biodegradable food packaging production due to its high gluten content and ZnO-nr and nano-kaolin could be powerful materials in enhancing the physicochemical, thermal, and hurdles properties of semolina films. To the best of our knowledge, ZnO-nr and nano-kaolin reinforced semolina has not been widely understood and there is a lack of scientific research on antimicrobial packaging for dairy products. Therefore, as can see in figure 1.1 we developed a new edible film using semolina due to its biodegradability, renewability, and low price. We also optimized the semolina films by testing three different concentrations of the sorbitol and glycerol blend, then intended to fabricate high-performance semolina-based films reinforced with ZnO-nr, nano-kaolin and combination of them for food packaging applications, and their properties regarding morphology, heat sealability, thermal, physicochemical, barrier and antimicrobial were inspected, as well as the effectiveness of nano-packaging on microbiological, physicochemical and sensory quality of fresh mozzarella cheese was evaluated.  The beneficial effects of biopolymer material have let the researcher to propose fabrication biodegradable packaging for food products from bio-source instead of the synthetic source. With these premises, the problem statements of this research were elaborated in the next section.

## 1.2 Problem statements

The problem statements of present study were:

(i) Synthetic packaging films will contribute to environmental problems and becoming evident that the ecosystem is considerably disturbed and damaged as a result of the non-degradable plastic materials and release of toxic gases.

(ii) Biodegradable packaging has shown some limitations in terms of thermal resistance, water barrier function, mechanical and antimicrobial properties.

(iii) The short shelf life of mozzarella cheese has been attributed to microbiological spoilage (coliform), mostly coming from water used in the manufacturing (heat treatment during curd stretching).

To address the above-mentioned problems, the research objectives were formulated which were listed as follows;

## 1.3 Objectives

(i) To investigate physico-mechanical and microstructural properties of semolina film as influenced by different sorbitol/glycerol concentrations.

(ii) To improve the physical and protective functions of semolina film by embedding nanocomposite (ZnO-nr and nano-kaolin)

(iii) To study the effects of nanocomposite semolina-film on spoilage, sensory and physicochemical properties of mozzarella cheese.

## 1.4 Research plan

The overall view of the research plan of this thesis is illustrated in figure 1.1.

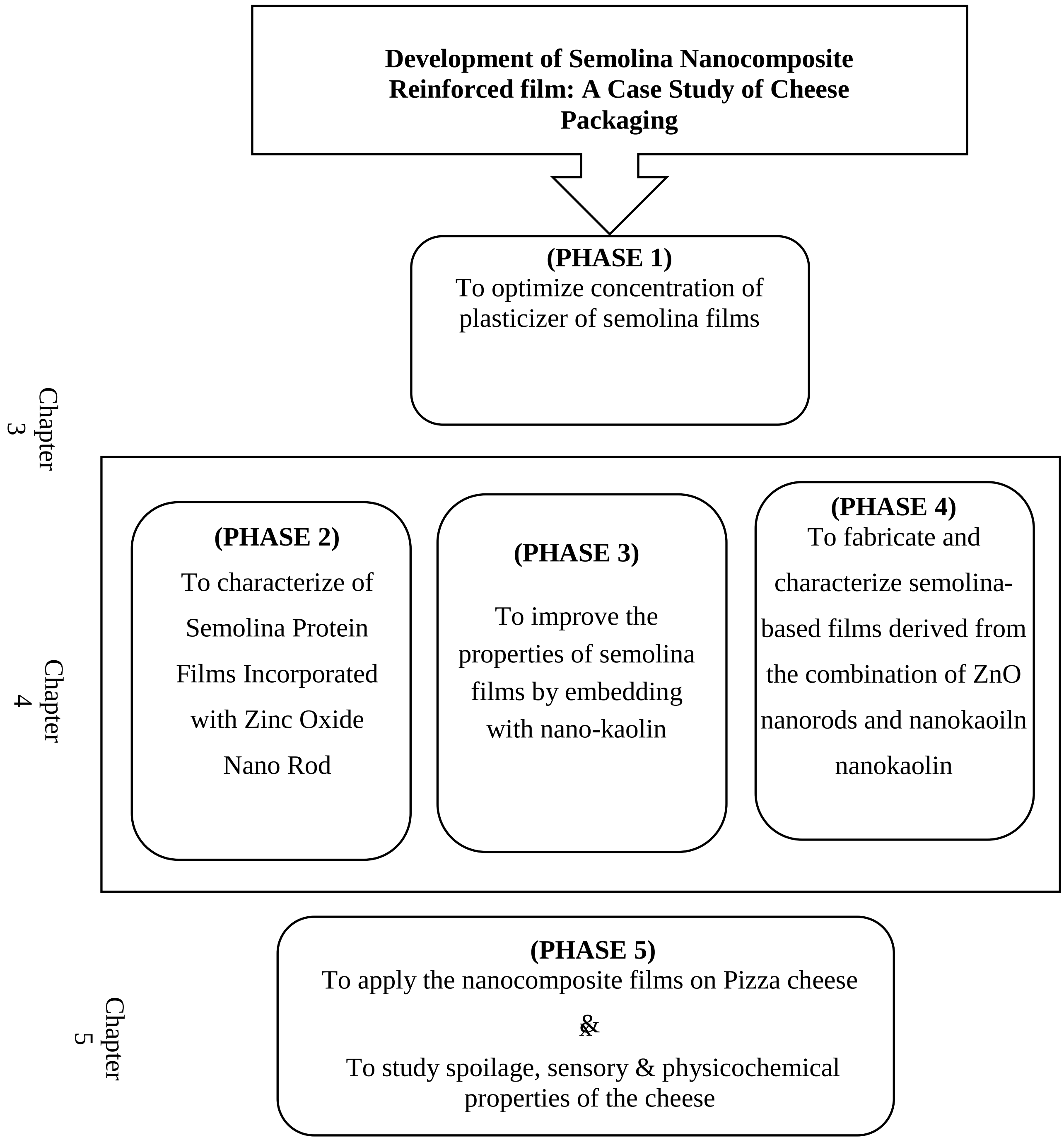


Figure 1. 1 Overview of the thesis research design

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Food Packaging

In the area of polymer, the main application is food packaging. The versatility and facile production of food packaging have made polymers, which are usually in the film form or rigid shape, as an acceptable replacement for conventional matters like metals and glass. The process of food packaging is comprised of preparing food safely for the purpose of transporting, distributing, storing, and retailing. Currently, the consumption of most food products, which are transported from a distant manufacturer, transcends the production date. This makes the issue of preventing the deterioration critical in the domain of food packaging. Storage temperature and water activity are the two main factors affecting the deterioration of food either in raw or processed form. Therefore, a careful technological intervention for longer preservation is required (Shi et al., 2016).

Garcia et al. (2016) argue that the primary purpose of food packaging is preserve the food’s safety and quality from the time of its production to consumption. Foods are considered to be perishable matters vulnerable to chemical, biological, and physical deterioration at the stages of storage and distribution. Oxygen, water vapor, and carbon dioxide are reported to be the most important solutes of transport that cause food deterioration. Food barrier property refers to the materials’ strong resistance to the solutes of transport. Microbial spoilage and chemical and physical alternation at the storage stage are the main reasons for deteriorating sensory qualities, lowering the nutrition level, and damaging food safety. A safe packaging should be able to ameliorate the deteriorating effect of the solutes of transport (Peelman et al., 2013).

Packaging is directly related to food safety; if the material used in packaging does not provide a suitable barrier around the food, food content might be contaminated by microorganisms and become unsafe. However, microbial contamination can also arise if the packaging material does not prevent against moisture or $O_2$. Such situation provides a ground for microorganisms that are innocuous in the absence of moisture or $O_2$ to grow and present a risk to the consumer (Robertson, 2016). As in the case of microbial deterioration, enzymatic reactions are also favored by water content and ease $O_2$ infiltration into a package entailing deteriorations in food (Tartaj, 2011).

Food quality is also affected by chemical and physical reactions within the packaging. There are numbers of possible deteriorative chemical reactions adversely affecting the quality of food especially favored by transferred $O_2$ and water vapor. Lipid oxidation (autoxidation) initiated by molecular $O_2$, non-enzymatic browning, color changes related with the presence or absence of $O_2$, and reactions of vitamins or proteins with $O_2$ decreases the nutritional value of packaged food. Chemical changes of food also result in physical changes. Some physical changes deteriorating the food quality can be listed as softening, caking especially in powder products, toughening, emulsion breakdown, swelling/shrinkage and crushing/breakage that can be altered by controlling water uptake as well as protecting from physical impacts.

Mechanical properties of the packaging materials are important as well as barrier properties to ensure the success of the packaging. A packaging material must be durable itself in order to coat and protect the food product inside. An important function of a packaging is to resist some degree of impact without significant/permanent changes during the distribution of the product. An ideal packaging material must be strong and flexible at the same time in order to protect the product that it contains. Mechanical properties are also important for the other functions of packaging such as barrier properties. Resistance to solutes of the material is also related with the microstructure of the packaging material and durable mechanical properties also guarantee homogeneous barrier to solutes.

Processed and raw food packaging is very important. In addition to packaging technology, materials used in food packaging should have the following features: toughness, low cost, flexibility, lightness, resistance to impacts, inertness, easy fabrication, prevention of water vapor and oxygen transmission, high wet-strength (Tartaj, 2011). Polyethylene and polypropylene are famous polymeric packaging materials for having these properties; they have been widely used in the food industry for a long time around 50 years (Garcia et al., 2016). The resistance of polypropylene to water vapor and chemicals (but not oxygen gas) is very helpful in food packaging. Thus, polyviniylidene chloride or ethylene vinyl alcohol is used in PP's multilayer structure in order to improve the properties of oxygen barrier (Marsh and Bugusu, 2007). Polypropylene had 23% of the thermoplastic used in Western Europe in 2000. Concerning all plastics sales in the world, Polypropylene was known to be the third most important material (Brachet et al., 2008). Overall, it is estimated that over 180 million tons of packaging materials have been globally produced over a year and that the demand for more packaging materials' production is annually increasing (Papadakis, 2015). Regarding the market of plastic packaging, food packaging has the largest proportion. It is estimated that 25% of over 125 million tons of plastics produced annually worldwide is employed in food packaging (Vejdan et al., 2016). Also, it has been shown that food production and beverage contribute 70% of the 100$ billion packaging market in the United States (Hanani et al., 2012).

For the last ten years, it appeared that it was due time to add one more feature to the plastics' desirable properties with 18-month or less a service life (Garcia et al., 2016).  Last decade, environmental issues have been seriously considered in selecting food packaging materials; therefore, in addition to the given properties of an ideal food packaging, environmental effect should be considered in the packaging process. In contrast to economical value and the growing use of polymers, plastic materials are not sustainable and reusable. A large amounts of polymers used in packaging materials are dumped in landfills. This seems to be the cheapest and quickest way to remove the generated wastes. United States according to EPA (2006) statistics, the wastes of packaging contribute 5.7% of all generated waste and only 9.4% of this amount is recycled in the United States. This amount of recycling in the area of food packaging is quite low compared to other types of packaging materials such as paperboard and paper (58.8%), metals (51.3%), and glass (25.3%) (Manoli and Voutsa, 2016). Difficulty in identifying and separating polymeric materials of packaging has caused such a low recovery percentage (Manoli and Voutsa, 2016; Garcia et al., 2016; Papadakis, 2015). EPA (2006) displays identification and separation mechanism via recovery statistics of polymeric resins. The recovery percentage of polyethylene terephthalate (PET) consumed in producing plastic bottles is about 18%; this recovery seems to be easy since the separation of this polymeric material from the waste is not difficult. However, compared to other non-polymer materials, it is a very low percentage. Polyolefins recycling introduces some difficulties. The recycling of polypropylene along with other polymers in the coating applications and multilayer barrier films is only around 0.25% (Manoli and Voutsa, 2016).

Concerning the waste of packaging materials, land filling is still one of the important issues. Besides landfills, incineration or combustion is applied in order to manage waste. In addition to heat and electricity generation, burning wastes of polymeric materials helps decrease waste volume by 70% to 90% (Manoli and Voutsa, 2016). Even in this case, the remaining waste would be dumped in landfills, raising environmental issues due to the emission. In order to avoid this problem, the idea of biobased food packaging idea was presented in the area of waste management. Biobased (biopolymer) packaging refers to a special kind of packaging consisting of reusable biological raw materials which are derived from agricultural origins.

Biopolymers have opportunity to be used as food packaging materials. Plant derived biopolymers are known to be effective barrier to oxygen, most of the biopolymers are thermoplastics that can be shaped into solid articles or processed into films. Recent technological advances also have allowed biopolymers to be processed similarly to petroleum based plastics whether in sheets, by extrusion, spinning, injection molding, or thermoforming (Garcia et al., 2016). In spite of their excellent barrier to oxygen and other gases, biopolymers are poor water vapor barrier and moreover, their barrier and mechanical properties are dependent to moisture which is not desirable especially for the packaging of certain food types. One of the challenges facing the food packaging industry in its efforts to produce biobased primary packaging is to match the durability of the packaging with the product shelf life. The biopolymer packaging material must remain stable without changes of mechanical and/or barrier properties and must function properly during storage until disposal (Ortega-Toro et al., 2016).

There should be new ways to overcome the two weak points of polymers (its dependency on water vapor, and poor barrier and mechanical properties). For the last two decades, using nanotechnology in the science of materials has been one of research topics. Nanoscale manipulations on materials are highly likely to lead to satisfying effective results. Nanocomposite advances are considered to be as an alternative in the polymers of bio-based food packaging. Employing nanocomposite science in biobased food packaging can help overcome disadvantageous features including low water-vapor barrier or weak mechanical properties.

Nanocomposites are considered to be a new classification of composites. They are used in particle-filled polymers which have one aspect of the dispersed particles. This dimension is supposed to be in the nanometer range (Rhim et al., 2013). The widely used method in polymer industry is to use inorganic fillers in composite structures in order to improve the properties of polymers. Relying on nano-fillers instead of conventional ones yields desirable results including mechanical properties improvement, reduction of weight, improvement of technology (e.g. fire resistance), antimicrobial attributes, and the most important improving resistance to water vapor and other gases. Using nanocomposite makes it possible to create stronger and more durable biopolymers which are environment friendly and can be successful replacements for petroleum based polymeric materials used in food packaging.

## 2.2 Edible Food Packaging

Edible films refer to thin layer that both can be consumed and provide a protection against water vapor, $O_2$, and salute movement of food. They could take the form of a food coating or can be easily disposed as a layer among food components (Babuskin et al., 2015). The forms of food coating in edible films and free-standing films could be used as gas aroma barrier in food (Rocca-Smith et al., 2016). Nevertheless, there is growing need for more technological information in order to improve films in food industry (Weng et al., 2014).

Special materials could be used in edible films that have a film forming application. In the manufacturing process, solvents such as alcohol, water, mixture of alcohol and water, or other solvents' mixture could be used to dissolve film materials. In this stage, antimicrobial agents, plasticizers, flavors, or colors could be added. Heating the solutions or adjusting the pH could be used to accelerate the dispersion for the specific polymer. Film solution is then casted and dried at a desired temperature and relative humidity to obtain free- standing films.

Edible films and coatings have received considerable attention in recent years because of their advantages over synthetic films. The main advantage of edible films over traditional synthetics is that they can be consumed with the packaged products. There is no package to dispose even if the films are not consumed they could still contribute to the reduction of environmental pollution. The films are produced exclusively from renewable, edible ingredients and therefore are anticipated to degrade more readily than polymeric materials.

### 2.2.1 Edible films and coatings classification

There are three main categories of components which are used in the production of edible films: protein (proteins of animals: whey, casein, gelatin and collagen, proteins of plants: wheat gluten, pea protein, soy protein, corn zein), and polysaccharide (e.g., chitosan, starch, alginate, and pectin), lipids (e.g. acylglycerol, fatty acids, and waxes).

#### 2.2.1. (a) Protein films

Proteins are known as agro-polymers, which are essential renewable resources derived from plants (e.g. corn zein, wheat gluten, soy protein, and pea protein), animals (casein, gelatin, whey protein). Concerning their original forms, there are two groups of proteins. First is fibrous proteins that appear to be water insoluble. This type of protein mainly make the structure of the materials in the animal tissues. The second groups is globular proteins that are soluble in water and aqueous solutions (acids, salts, and bases). Their main function is limited to living systems (Angor, 2014).

While the fibrous proteins are connected to each other in a parallel form through hydrogen bonding and are fully extended, the globular proteins which are bound together through mixture of ionic, hydrogen, covalent (disulfide), and hydrophobic bonds, have spherical intricate forms (Angor, 2014). The physical and chemical features of these two types of proteins are determined by the placement of amino acid residues along the protein polymer chain and the relative volumes of the amino acid residues.

Different types of globular proteins such as soy protein, corn zein, and whey protein have been examined in order to gain films features. Dispersion or solutions of the protein, are usually used to form protein films. In protein films preparation, ethanol, water, or mixture of both are usually the solvents (Rocca-Smith et al., 2016).

In general, base, acid, heat, or solvent are used to denature proteins in order to prepare extended structure for films formation. As protein chains are extended, they can be bound together through the mixture of ionic, hydrogen, covalent, and hydrophobic bonding. The degree of chain extension and the sequence and nature of amino acid residues affect the chain-to-chain interactions, which create cohesive protein films. These interactions could be increased through unifying the distribution of hydrophobic, polar or thiol groups within the polymer chain. This in turn makes films much stronger with lower flexibility and lower permeability to vapors, gases, and liquids (Rocca-Smith et al., 2016). In fact, hydrogen and ionic bonding in polymers could provide strong oxygen barrier films, which are susceptible to water vapor (Jiménez et al., 2015). Therefore, protein films create great oxygen barriers in lower amounts of humidity. There are a great number of edible protein films such as gelatin, casein, whey protein, wheat gluten, corn zein, soy protein, bean protein, mung, and peanut protein (Wihodo and Moraru, 2013; Bourtoom and Chinnan, 2008).

**2.2.1. (b) Wheat**

In 2013 wheat crop was the firs-ranked agricultural commodity in Europe; it was also the fourth in the world, which has been produced in a great amount (≈ 716 million tons) following sugar cane, maize and rice (Faostat, 2013). This large number signifies the considerable amount of available wheat-based foods that are consumed by humans in different types of forms such as pasta, baked goods, snacks, and meal foods. The most important reason for its success is the presence of gluten-forming proteins (glutenins and gliadins) in wheat endosperm, which improves its functional properties.

Starch content contains the dominant chemical composition of wheat grain (around 70% of the whole dry weight) (Lasztity, 1986) followed by protein content (6-20%) (Simmonds, 1981) Different type of starch has different structures and wheat starch is oval or round. Starch is called a complex carbohydrate because it is made up of many sugar molecules linked together. It has two main part: amylose and amylopectin. Amylose is a straight or linear chain of sugar molecules linked together. Amylopectin is a branched chain of sugars (Figure 2.1). To be more specific, starch only exists in the endosperm, while protein has been relatively distributed all other parts of the grain (Figure 2.2). There is a good interaction between glutenins and Gliadins, which create a protein network called wheat gluten. This network is readily available in dough making with viscoelastic features needed for the production of wheat based foods eaten by humans (Lagrain et al., 2010). The purity, quality, and supplier of wheat play an important role in the final price of the wheat gluten, which usually ranges between 0.5 to 2 dollars per kilogram. The wheat gluten enjoys its functional features, which have industrial significance in both not-food and food uses.

The best idea in valorizing this affordable protein is to use it as raw material in improving food bio-packaging. It has been argued that wheat protein shows good properties of film forming. These properties offer a semipermeable membrane to oxygen, water vapor and $CO_2$ molecules (Li et al., 2014).

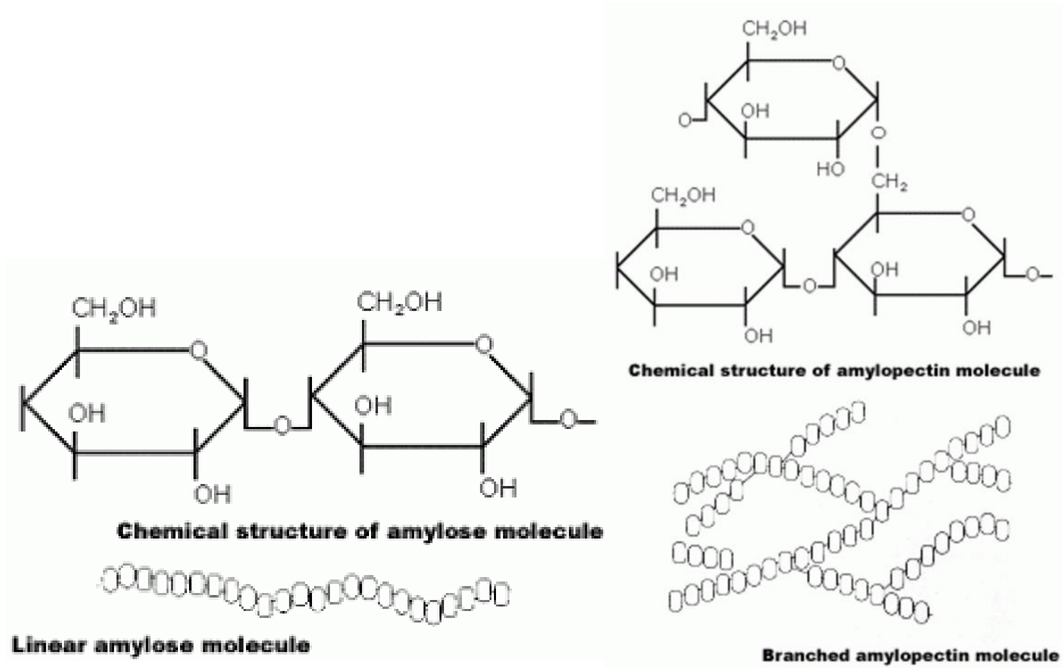


Figure 2. 1 Chemical structure of amylose and amylopectin molecules.

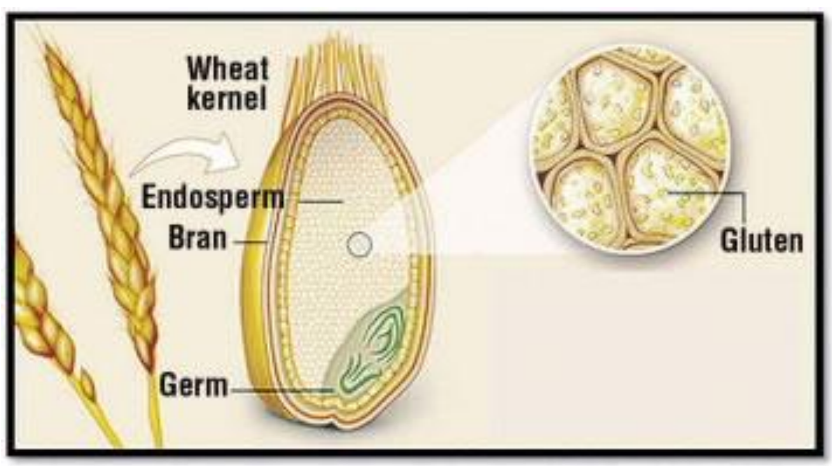


Figure 2. 2 Structure of a Grain of Wheat

Source: Cheng et al. (2010)

#### 2.2.1. (c) Semolina

There are a lot of studies on using plant proteins as renewable bio-based plastics. Of many available biodegradable materials in food packaging (Khwaldia et al., 2010), it seems that wheat offers the best properties for the purpose of food packaging due to its low cost, renewability, biodegradability good quality in film-forming, and cohesive/adhesive features (Türe et al., 2013). Wheat is usually classified in two groups of semi- crystalline granules. The first is a large A-type and the second one is a small B-type (Kim and Huber, 2008). The former has lenticular form with an average diameter of 10–35 mm while the latter, which ranges from 1 to 10 mm in diameter, has spherical shape (Maaran et al., 2014).

It appears that these two types of granules (wheat A- and B-type) present distinct functional properties and chemical compositions. In fact, semolina flour is known as a large A-type containing a significant amounts of gluten content. This improves the nutritional quality of edible coatings (Giannone et al., 2016). Semolina is known to be light-colored, translucent, and rigid with antioxidant functions. The extracts of semolina are used to repress radical cation scavenging activity and radical-induced liposome lipid peroxidation (Do et al., 2014). Gluten is produced by mixing the storage proteins of usual wheat and it is considered as an appealing co-product in starch industry (Blomfeldt et al., 2010; Wretfors et al., 2010; Cho et al., 2011).

Wheat gluten is a complex of protein carbohydrate whose main component is proteins. As a general term for wheat’s water-insoluble proteins, wheat gluten composed of a blending of polypeptide molecules, is known as globular proteins. The elastic and cohesive properties of gluten is very vital in wheat dough and accelerate the formation of film. Wheat gluten carries fractions of the glutelin and prolamine in our proteins, which are named as glutenin and gliadin, respectively. While glutenin is not soluble in ethanol, gliadin is supposed to be highly soluble in 70% ethanol (Li et al., 2014). Wheat gluten is not soluble in natural water; however, aqueous solutions of low or high pH dissolve wheat gluten with a low ionic strength (Figure 2.3) (Paynel et al., 2015).

Drying gluten's aqueous ethanol solutions can form edible films. Besides hydrogen and hydrophobic bonds, the formation of new disulfide bonding at the stage of film drying and the native disulfide bonds' cleavage at the stage of heating of film-shaping solutions are considered to play a critical role in forming wheat gluten films structure (Li et al., 2014).

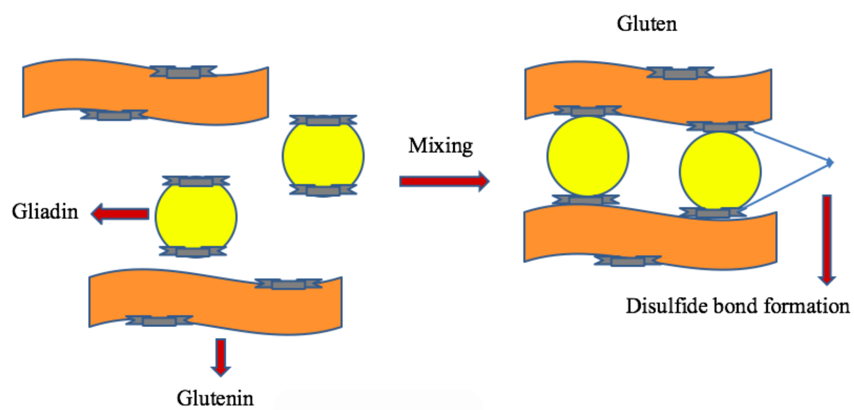


Figure 2. 3 Structure of protein in Semolina

Source: Buehler (2006)

## 2.3 Film Preparation

There are a number of ways to produce films from bio-based polymers. The main and most applicable methods are dry method and wet method. In general, the process of film formation happens in two stages. The first one is concerned with restructuring state and the second one has to do with structure formation.

### 2.3.1 Dry Film Preparation

This method is used when thermoplastic properties such as synthetic polymer are revealed in materials. Some polysaccharides could be considered to be thermoplastic materials in water. In dry film preparation, the same procedures used in synthetic polymers are involved: blow modeling, extrusion, thermoforming and injection molding. (Bordes et al., 2009).

### 2.3.2 Wet Film Preparation

Concerning the wet method, macromolecules are dissolved in an aqueous medium. After that, then the solvent is evaporated in order to gain a solid film. There are a number of methods for making wet films depending on the application field.

Dipping is the most conventional method for creating protective films in food industry (Garcia et al., 2016). This old technique is most useful in making pharmaceutical hard capsules (Fridrun and Brian, 2004). Based on this method, a mould is soaked in a solution. This is repeated for several times in order to reach a desirable thickness.

Spraying method is one of wet techniques, through which polymer solution is sprayed over the surface of the mould. This creates a very thin layer of film. Another technique is panning which is mostly employed in pharmaceutical and confectionery industries. Based on this technique, the product is coated by being placed in a big spinning bowl, which is known as pan. After that, it involves spraying onto the pan. The content of the pan must be tumbled in order to evenly distribute coating solution over the pharmaceutical material's surface or the food. Ambient or elevated temperature along with forced air is used to dry the film in order to dry the coating (Lee et al., 2002; Donhowe and Fennema, 1994).

Spinning technique is another method in forming fibers in textile industry. Based on this method, a polymer solution is placed in a spinneret which has a pin hold under pressure. In a unique study, Garcia et al. (2016) altered the procedure of spinning to create films from casein. This change involves using a special kind of plate instead of the spinneret in order to produce flat films. Accordingly, protein solutions are forced into a coagulating bath under pressure. This method has been represented as an alternative in forming soy protein films (McGill and Chrisey, 2000).

Another method for solvent coating is extrusion. According to this method, shearing and elevating temperature are used to create melten and soft polymer. This action produces cohesive/adhesive film matrix. Compared to the solvent casting method, extruding proteins into films offers more advantages because it consumes less energy and is faster. These two properties are gained through highly-concentrated film solutions that are formed in the extruder. A lot of energy and time are consumed in order to evaporate water and ethanol in solvent casting. This in turn increases the price of producing edible films. However, time and energy are efficiently used in extrusion method (Siemann, 2005).

Overall, solvent casting is still considered as one of the desirable ways in film preparation. It is one of most common ways to produce protein films. The most convenient way to do so is to spread film solution containing plasticizer and protein. Then, using constant temperature to evaporate the solvent under controlled condition. Time and temperature of drying, features, and structure of the plate must be controlled in order to make fine film. There are different types of drying sources for film such as hot air or microwave energy in this new method. In the present study, solvent casting technique was used for making semolina film since it is known to be the most common method in preparing biodegradable film (Bao et al., 2009; Jongjareonrak et al., 2008).

## 2.4 Protein Film Properties

### 2.4.1 Mechanical Properties

Concerning films' tensile testing, a stress-strain experimental is done in a sense that at a constant speed, the film specimen is pulled out until it fractures. Then, the breaking force is accurately recorded. Stress-strain curve is used to obtain elongation at break, tensile strength, and Young's modulus. The distance of deformation before breaking is elongation at break (%)$\varepsilon$. It is defined as $\varepsilon = \Delta L/L$, where L stands for the specimen's initial length and $\Delta L$ is the increase of length made by the applied force. Tensile strength (MPa) $\sigma$ is referred to as $\sigma = F/A$, (F stands for the applied force and A stands for the specimen's cross-section area). The last part is young's modulus (MPa) which is estimated through the slope area under the linear section of the stress-strain curve. The higher the value of tensile strength, the higher resistance the material shows in mechanical tension. Higher values for elongation at break reflect flexibility. Moreover, high values for young modulus show materials' stiffness. The special type of use made of film determines significance of stability of tensile strain. In the area of edible films, these properties are not as much essential as other applications.

It seems that synthetic films have more mechanical properties than protein films (Cuq, 2002; Kanmani and Rhim, 2014). There are a number of factors impacting the biodegradable films' mechanical properties including the length of polymer chain, surface charges, and hydrophobicity (Garcia et al., 2016). Furthermore, films' properties are also affected by the level and type of plasticizer (Cuq, 2002).

One of main characteristics of semolina flour is a great amounts of gluten content, which is cohesive and has a variety of flours (Giannone et al., 2016). This feature is expected to promote the mechanical features of films. In fact, mechanical properties are determined by density and distribution of inter and intramolecular interactions between the chains of the polymer in film's matrix (Liu et al., 2015). Further, the mechanical strength of the semolina-based films is affected by the nature of amino acid sequence and level of chain elongation.

#### 2.4.1(a) The effect of plasticizer on semolina film's mechanical properties

Generally, protein films specially semolina film due to high gluten have high elasticity; however, these films are still brittle for packaging applications (Espitia et al., 2014). The only propose solution for this problem is to add a plasticizer, which is likely to improve the mechanical properties of bio-based films. A plasticizer is known to be a low molecular weight nonvolatile substance and usually extruded into the film in order to lower chain-to-chain interactions of biopolymer. This as a result enhances film's stretch ability as well as flexibility. It is worth mentioning, however, that plasticizers also augment the permeability of a film permeability (Bergo et al., 2008; Furuyama et al., 2007; Galdeano et al., 2009; Rodriguez et al., 2006). In addition, Previous work demonstrated that the unplasticized biodegradable films were relatively brittle and needed to be handled very carefully but increase the plasticizer can improve the flexibility of film and increase elongation at break. The increased flexibility of the films at higher plasticizer concentration can be attributed to interaction of plasticizer–polymer chains which facilitated the sliding of chain and thus help to improve the overall flexibility and the chain mobility. Bourtoom (2006) investigated effect of three types of plasticizers (sorbitol, glycerol and polyethylene glycol) at different concentrations on mechanical properties of blend films from rice starch-chitosan. Their results showed that an increase in the concentration of these plasticizers yielded a decrease in TS and increase in EB. The sorbitol plasticized films had significantly higher TS and lower EB than glycerol and polyethylene glycol plasticized films. These results might be explained by the ring structure of sorbitol, which lead to difficult to interact between efficiently rice starch-chitosan molecules resulting in less effectiveness in disrupting the rice starch-chitosan interruptions (Yang and Paulson, 2000). Furthermore, the low water-attracting