Preparation and Application of Starch Nanocrystals for Reinforcing in Rice Starch Film

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ชื่อวิทยานิพนธ์ การเตรียมและการประยุกต์ใช้ starch nanocrystals เพื่อเสริมแรงในฟิล์มสตาร์ชข้าวเจ้า
ผู้เขียน นางสาวปิยะดา แก้วพูล
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บทคัดย่อ

จากการศึกษาวิธีการเตรียมสตาร์ชนาโนคริสตัลด้วยวิธีการย่อยด้วยกรดซัลฟูริกที่ระดับความเข้มข้นต่างๆ (2, 3, 4 และ 5 โมลาร์) ที่อุณหภูมิ 40 องศาเซลเซียสเป็นเวลา 5 วัน พบว่าการย่อยด้วยกรดซัลฟูริกที่ระดับความเข้มข้น 3 โมลาร์มีร้อยละของผลผลิตสูงที่สุดและเกิดการรวมตัวกันของอนุภาคของสตาร์ชนาโคริสตัลซึ่งมีขนาดของกลุ่มอนุภาคประมาณ 3.97 ไมโครเมตร และเมื่อทำการวิเคราะห์ความเป็นผลึกด้วยเครื่อง X-Ray diffraction พบว่าสภาวะดังกล่าวมีความเป็นผลึกสูงกว่าสภาวะอื่น ๆ ที่ทำการศึกษา เมื่อทำการศึกษาผลของปริมาณสตาร์ชนาโนคริสตัลต่อสมบัติของฟิล์มจากสตาร์ชข้าวเจ้า พบว่าเมื่อทำการเติมสตาร์ชนาโนคริสตัลสามารถปรับปรุงสมบัติทางกลและสมบัติการต้านทานไอน้ำของฟิล์มจากสตาร์ชข้าวเจ้า เนื่องจากเมื่อเติมสตาร์ชนาโนคริสตัลส่งผลให้สมบัติทางกลของฟิล์มจากสตาร์ชข้าวเจ้าดีขึ้น เนื่องมาจากการเติมสตาร์ชนาโนคริสตัลจะไปเพิ่มปริมาณความเป็นผลึกของฟิล์มจากสตาร์ชข้าวเจ้า นอกจากนี้เมื่อปริมาณการเติมสตาร์ชนาโนคริสตัลเพิ่มขึ้นยังส่งผลให้อุณหภูมิการเปลี่ยนสถานะจากของแข็งคล้ายแก้ว (Glass transition temperature, Tg) ของฟิล์มจากสตาร์ชข้าวเจ้าเพิ่มสูงขึ้นและปริมาณเถ้า (Char yield) หลังจากการเผาเพิ่มสูงขึ้น ดังนั้นสามารถกล่าวได้ว่าการเติมสตาร์ชนาโนคริสตัลลงในฟิล์มจากสตาร์ชข้าวเจ้าสามารถปรับปรุงสมบัติทางความร้อนของแผ่นฟิล์มได้ จากการศึกษาพบว่าปริมาณที่เหมาะสมสำหรับการเติมสตาร์ชนาโคริสตัล โดยให้สมบัติทางกล สมบัติทางความร้อนและการต้านทานการซึมผ่านไอน้ำของฟิล์มจากสตาร์ชข้าวเจ้าที่ดีที่สุดเท่ากับร้อยละ 15

เมื่อทำการศึกษาผลของชนิดของไขมัน (น้ำมันปาล์ม มาการีน และน้ำมันรำข้าว) และระดับความเข้มข้น (ร้อยละ 3, 6, 9, 12 และ 15) ต่อสมบัติการต้านทานไอน้ำ สมบัติทางกายภาพและสมบัติการดูดซับน้ำของฟิล์มจากสตาร์ชข้าวเจ้าที่เสริมแรงด้วยสตาร์ชนาโนคริสตัลที่ร้อยละ 15 พบว่าเมื่อทำการเติมไขมันสามารถปรับปรุงสมบัติการต้านทานไอน้ำและการดูดซับน้ำของฟิล์มจากสตาร์ชข้าวเจ้า เมื่อเติมไขมันในปริมาณที่เพิ่มขึ้นส่งผลให้ความสามารถในการซึมผ่าน

ไอน้ำของแผ่นฟิล์มจากสตาร์ชข้าวเจ้าลดลง เนื่องจากการเติมไขมันเป็นการเพิ่มส่วนที่ไม่มีขั้วลงในแผ่นฟิล์ม แต่อย่างไรก็ตามการเติมไขมันในปริมาณที่เพิ่มขึ้นส่งผลให้สมบัติทางกลและความสามารถในการยืดตัวของแผ่นฟิล์มลดลงด้วย พบว่าการเติมน้ำมันปาล์มที่ร้อยละ 3 ให้สมบัติของฟิล์มจากสตาร์ชข้าวเจ้าที่ดีที่สุด ทั้งสมบัติทางกลและการต้านทานการซึมผ่านไอน้ำ สำหรับการศึกษาผลการดูดซับความชื้นที่อุณหภูมิคงที่ (Equilibrium Moisture Content) พบว่าฟิล์มจากสตาร์ชข้าวเจ้ามีการดูดซับไอน้ำอย่างรวดเร็วในช่วงเริ่มต้นและปริมาณการดูดซับไอน้ำจะลดลงเมื่อเวลาในการดูดซับไอน้ำเพิ่มขึ้น การเติมน้ำมันปาล์มร้อยละ 3 ในฟิล์มจากสตาร์ชข้าวเจ้าที่เสริมแรงด้วยสตาร์ชนาโนคริสตัลร้อยละ 15 มีอัตราการดูดซับไอน้ำลดลงอย่างแตกต่างทางสถิติที่ระดับความชื้นสัมพัทธ์ต่ำ นอกจากนี้พบว่าที่ระดับความชื้นสัมพัทธ์สูงขึ้นส่งผลให้ฟิล์มจากสตาร์ชข้าวเจ้ามีค่าความต้านทานแรงดึงลดลง เมื่อทำการศึกษาการประยุกต์ใช้แผ่นฟิล์มจากสตาร์ชข้าวเจ้าในผลิตภัณฑ์แครกเกอร์ โดยนำแผ่นฟิล์มจากสตาร์ชข้าวเจ้ามาห่อรอบแครกเกอร์และเก็บรักษาในเดสซิเคเตอร์ที่บรรจุสารละลายโซเดียมคลอไรด์อิ่มตัว ที่อุณหภูมิห้อง (27±3 °C) ความชื้นสัมพัทธ์ร้อยละ 75 พบว่าเมื่อระยะเวลาในการเก็บรักษาเพิ่มขึ้นส่งผลให้แรงกด (Compression load) ที่กระทำต่อแครกเกอร์เพิ่มสูงขึ้น เนื่องจากแครกเกอร์สูญเสียความกรอบจากการดูดซับไอน้ำ แครกเกอร์ที่ห่อด้วยฟิล์มจากสตาร์ชข้าวเจ้าเสริมแรงด้วยสตาร์ชนาโนคริสตัลร้อยละ 15 และเติมน้ำมันปาล์มร้อยละ 3 สามารถเก็บรักษาแครกเกอร์ได้นานกว่าแครกเกอร์ที่ห่อด้วยฟิล์มจากสตาร์ชข้าวเจ้า ดังนั้นแครกเกอร์ที่ห่อด้วยฟิล์มจากสตาร์ชข้าวเจ้าเสริมแรงด้วยสตาร์ชนาโนคริสตัลร้อยละ 15 และเติมน้ำมันปาล์มร้อยละ 3 สามารถเก็บรักษาแครกเกอร์ได้ (9 วัน) นานกว่าแครกเกอร์ที่ห่อด้วยฟิล์มจากสตาร์ชข้าวเจ้า (6 วัน) นอกจากนี้ปริมาณความชื้นและค่าวอเตอร์แอกติวิตี้ (a_w) ของแครกเกอร์ที่ห่อด้วยฟิล์มทุกชนิดเพิ่มขึ้นเมื่อระยะเวลาในการเก็บรักษาเพิ่มขึ้น แต่อย่างไรก็ตามปริมาณความชื้นและค่าวอเตอร์แอกติวิตี้ (a_w) ของแครกเกอร์ที่ห่อด้วยฟิล์มจากสตาร์ชข้าวเจ้าเสริมแรงด้วยสตาร์ชนาโนคริสตัลร้อยละ 15 และเติมน้ำมันปาล์มร้อยละ 3 ต่ำกว่าแครกเกอร์ที่ห่อด้วยฟิล์มจากสตาร์ชข้าวเจ้า

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ABSTRACT

Rice starch nanocrystals were prepared by acid hydrolysis of rice starch with sulfuric acid at various concentration (2, 3, 4 and 5 M) at 40 °C for 5 days. The optimal conditions of sulfuric acid hydrolysis to be obtained in the highest yield and smallest median size particle of starch nanocrystals was obtained when 3 M sulfuric and was used. Starch nanocrystals are generally observed in the form of aggregates having an average size around 3.97 μm and higher crystallinity value in structure of the starch nanocrystals obtained from X-ray diffraction. Starch nanocrystals was used as a reinforced for rice starch film. The properties of the rice starch films were improved through the addition of starch nanocrystals. The result showed that the mechanical properties of the rice starch film were enhanced by the addition of starch nanocrystals indicated that introduction of starch nanocrystals increased the crystalline peak structure of rice starch film. Furthermore, increasing of starch nanocrystals content, the water barrier properties of the rice starch films was also improved. The glass transition temperature (*Tg*) shifted towards higher temperature with increasing amount of nanocrystals and the char yield of rice starch films was enhanced as starch nanocrystals increased. Therefore, addition of starch nanocrystals into the rice starch films can be improved the thermal stability of rice starch films. The results demonstrated that addition of starch nanocrystals 15% showed better properties including mechanical, water barrier and thermal properties.

The influence of types (Palm oil, Margarine and Rice bran oil) and content (3, 6, 9, 12 and 15%) of lipids on water vapor permeability, mechanical properties and water uptake of rice starch films reinforced with starch nanocrystals at 15% were investigated. The barrier properties and water uptake of the rice starch films were improved through the incorporation of lipid. The results showed that the addition and increasing concentration of lipid resulted in a reduction in water vapor permeability of the rice starch films, due to the fact that, the water vapor permeability decreases with increasing hydrophobicity of the added lipid materials. However, increasing of lipid content, the mechanical properties were lower and reduced its ability to stretch. The incorporation of palm oil 3% showed better properties including water barrier and mechanical properties than other lipids. Moisture adsorption was more rapidly in the initial stage of moisture adsorption and a little of moisture was adsorbed when adsorption time increased. The incorporation of palm oil 3% showed significantly reduces the moisture sorption of rice starch film reinforced with starch nanocrystals 15% and the lowest value was observed when lower relative humidity and applied. Moreover, the lowest tensile strength of rice starch film was obtained when a high relative humidity was used. For application of rice starch films by wrapped around crackers and conditioned at 75% RH and room temperature (27±3°C) by placing them in desiccators over a saturated solution of sodium chloride. The results showed that when the storage time increased resulted in an increase of compression load of crackers in all cases. The grow is associated with a loss of crispness due to the hydration. Cracker wrapped with rice starch films reinforced with starch nanocrystals 15% and containing palm oil 3% showed lower reducing of crispness than rice starch film. Hence, cracker wrapped with rice starch film reinforced with starch nanocrystal 15% and containing palm oil 3% exhibited higher shelf-life (9 days) than cracker wrapped with rice starch films (6 days). Moisture content and water activity of cracker wrapped with all films increased when the storage time increased. However, the result demonstrate that moisture content and water activity of cracker wrapped with rice starch film reinforced with starch nanocrystals 15% and containing palm oil 3% were significantly ($p<0.05$) lower than cracker wrapped with rice starch film all storage time.

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CHAPTER 1

INTRODUCTION

Nowadays, the claim of environmental protection and the lack of petroleum resources provide a new opportunity for developing materials derived from biomass resources (Chiellini *et al.*, 2004) and starch is a biopolymer that is an attractive alternative for packaging material (Bengsson *et al.*, 2003). Several studies have been done to analyze the properties of starch-based films (Arvanitoyannis *et al.*, 1998; Mali and Grossmann, 2003; Mali *et al.*, 2005). Edible and/or biodegradable films are not meant to totally replace synthetic packaging films, however, they do have potential to replace the conventional packaging in some applications. The use of a biopolymer such as starch can be an interesting solution because this polymer is quite cheap, abundant, biodegradable and edible. Starch consists primarily of branched and linear chains of glucose molecules, named as amylopectin and amylose, respectively. Amylose is essentially a linear molecule with a few branches, whereas amylopectin is a highly branched molecule. Preponderance of amylose in starches gives stringer films. Branched structure of amylopectin generally leads to films with different mechanical properties, such as decreased tensile stress (Tharanathan, 2003).

Rice is the most widely consumed basic food in the world. Each year over 500 million tons of rice are harvested, providing sustenance to many countries and people throughout the world. The unique properties of rice starches are found in its many varieties. Due to different climates, soil characteristics and cultures, over 240,000 registered varieties of rice exist in the world. These varieties lead to a wide range of rice starches with many different characteristics including: different onset gelatinization temperatures, textures, processing stabilities and viscosities. Rice starch and its major components, amylose and amylopectin, are biopolymers, which are attractive raw materials for use as barriers in packaging materials. They have been used to produce biodegradable films to partially or entirely replace plastic polymers because of its low cost and renewability, as well as possessing good mechanical properties (Xu *et al.*, 2005). However, wide application of starch film is limited by its mechanical properties and efficient barrier against low polarity compounds

(Kester and Fennema, 1986). This constraint has led to the development of the improved properties of rice-based films by modifying its starch properties and/or incorporating other materials. Jagannath *et al.* (2003) blended starch with different proteins to decrease the water vapor permeability of the films and to increase their tensile strength. However, these films still did not perform well compared to synthetic polymer based films.

Nanocomposites are a relatively new class of composites consisting of polymers filled with particles that at least one dimension in the nanometer range (1-100 nm). Because of their very high surface area to volume ratio, nanoparticle incorporation into polymer matrices leads to composite with unique outstanding properties in comparison to their conventional microcomposite counterparts (Smith *et al.,* 2003). Bionanocomposite or econanocomposites are novel materials born out of the growing interest in nanomaterials and in the development of materials derived from renewable source (Thielemans *et. al.,* 2006). The renewable component can be matrix phase, the nanoparticle or both. Reported examples of renewable polymer nanocomposites can be found in the use of carbon nanotubes in polymers from renewable materials (Labet *et. al.,* 2007). Polysaccharides such as cellulose, starch and chitin are potential renewable source of nanosized reinforcement. They are naturally found in a semicrystalline state and aqueous acides can be employed to hydrolyze the amorphous section of the polymer. Consequentially, the crystalline sections found in these naturally occurring polysaccharides are released, resulting in individual monocrystalline nanoparticle. The obtained particles display different shapes depending on the polysaccharide source: rigid rodlike particles for cellulose and chitin and platelet for starch (Samir *et al.,* 2005).

The preparation and morphological characterization of platelet-like nanoparticles by acid hydrolysis of starch granules was reported (Putaux *et al.,* 2003). They consist of crystalline nanoplatelets about 5-7 nm thick lamellae with a length of 20-40 nm and width of 15-30 nm were seen using transmission electron microscopy. The main advantages of such fillers are their renewable nature, availability, high specific strength, non-abrasive nature that allows easier processing even at high filling levels, biodegradability as well as the relatively reactive surface which can be modified accordingly (Angles *et al.,* 1999). Thus, nanocomposites obtained by incorporation of cellulose, chitin or starch nanocrystals as nanofillers in both synthetic polymeric matrixes (Chazeau *et al.,* 1999) and biopolymers (Angles and Dufresne, 2000) showed desirable improvement of different properties e.g., those related with mechanical reinforcement and/or water resistance. Starch nanocrystals have also been found to be excellent reinforcements (Angllier *et al.,* 2005).

Thus, the objective of this work were to prepare and investigate the effect of starch nanocrystals on barrier and mechanical properties of rice starch films.

Review of Literature

1. Edible Films and Coatings

Edible films are defined as thin layer of material which can be eaten by consumer and provide a barrier to moisture, oxygen and solute movement for the food. The material can complete food coating or can be disposed as a continuous layer between food components (Guilbert, 1986). Edible films can be formed as food coating and free-standing films, and have potential to be used with food as gas aroma barrier (Kester and Fennema, 1986). However, the technical information is still needed to develop films for food application (Donhowe and Fennema, 1993). The edible films and coatings have received a consideration attention in the recent years because of their advantage over the synthetic films. The advantages of edible films over other traditional synthetic films are summarized below:

1. They can be consumed with the package products. This is obviously of critical importance since it represents the environmentally ideal package.
2. There is no package to dispose of even if the film are not consumed they could still contribute to the reduction of environmental pollution.
3. The films are produced exclusively from renewable, edible ingredients and therefore are anticipated to degrade more readily than polymeric materials.
4. The films can enhance the organoleptic properties of packaged foods provided that various components (flavorings, colorings, sweeteners).

5. The films can supplement the nutrition value of foods. This is particular true for films made from proteins.

6. The films can be used for individual packaging of small portion of food, particularly products that currently are not individually packaged for practical reasons such as pears, beans, nuts and strawberries.

7. The films can be applied inside heterogeneous foods at the interfaces between different layers of components. They can be tailored to prevent deteriorative intercomponent moisture and solute migration in foods such as pizzas, pies and candies.

8. The films can function as carriers for antimicrobial and antioxidant agent. In similar application they also can be used at the surface of food to control diffusion rate of preservative substances from the surface to the interior of the food.

9. The films can be vary conveniently used for micro encapsulation of food favoring and leavening agents to efficiently control their addition and release into the interior of foods.

10. Another possible application for edible films could be their use in multilayer food flavoring and leavening agents to efficiently control their addition and release into the interior of foods.

Production of edible films causes less waste and pollution, however, their permeability and mechanical properties are generally poorer than synthetic films (Kester and Fennema, 1986). Extensive research is needed on the development of new materials, methods of films formation, methods to improve film properties and the potential applications.

2. Formulation of Edible Films and Coatings

The formulations of edible films or coatings require the use of at least one constituent capable of forming a matrix with adequate continuity and cohesion. Generally, this is composed of polymers or compounds which, under the preparation conditions used, form continuous crystalline or amorphous structures (hydrophilic polymer, fats and sugars). In the case of coatings for which the films system can be applied directly to the product, two forces are relevant: that between the molecules of the coating material (cohesion) and that between the coating and the support structure (adhesion). The degree of cohesion produces the barrier and mechanical properties of the film. High structural cohesion is manifested by a reduction in flexibility, porosity, and permeability to gases and solutes (Banker, 1966). The degree of cohesion depends on the chemical structure of the film material, the presence of plasticizing and cross-linking agent, the nature of the solvent used and its dilution, the method of application, the procedure of removal of the solvent, and the final thickness of the film. The highest cohesion is generally obtained for ordered polar polymers with long chains which were precipitated in crystalline form. The preparation of average dilution which constitutes a compromise between salvation and extension of polymer molecules and good initial viscosity is preferable (Baker, 1966). The cohesion of films generally increases in proportion in their thickness up to a threshold beyond which it remains constant. The speed of evaporation of the solvent and/or excessive temperature mat be manifested by inadequate cohesion on account of the premature immobilization of the polymer molecular. Regarding the adhesion of the coatings to the foodstuff, this is generally facilitated by hot application.

The formulation of films or coatings base on hydrocolloids requires one of the following mechanisms (Kester and Fennema, 1986):

- Simple coacervation, where a hydrocolloid in a aqueous dispersion is precipitated or undergoes a change of phase by evaporation of the solvent, addition of hydrosoluble nonelectrolyte in which the hydrocolloid is insoluble (e.g., ethanol), addition of an electrolyte which causes “salting out” or bridging or even modification of the pH.

- Complex coacervation, where two hydrocolloid solutions of opposite charge are combined, causing the reaction and precipitation of polymer complexes.

- Gelification or heat coagulation, in which heating the macromolecule causes its denaturation followed by gelification (in the case of certain proteins such as ovalbumin) or precipitation, or cooling of a dispersion of the hydrocolloid causing a sol-gel transition (in the case of gelatin or agar-agar).

Insolubility (if this is sought) and the barrier properties and the retention of solutes can occasionally be increased, thanks to the incorporation of bridging agents, bi-or trivalent ions or by the use of denaturing condition (heat, irradiation, ect.).

For insoluble film materials of lipidic nature such as fat or waxes, these can be formed by application as a hot liquid followed by cooling with direct solidification in the form of an emulsion or microemulsion with water, or in solution in an appropriate organic solvent followed by evaporation of the water or the solvent.

3. Classification of Edible Films and Coatings

Edible films can be produced from materials with film forming ability. During manufacturing, film materials must be dispersed and dissolved in the solvent such as water, alcohol or mixture of water and alcohol or mixture other solvents. Plasticizer, antimicrobial agent, colors or flavor can be added in this process. Adjusting pH and/or heating the solution may be done for the specific polymer to facilitate the dispersion. Film solution is then casted and dried at desired temperature and relative humidity to obtain free-standing films. In the food application, film solutions could be applied to food by several methods such as dipping, spraying, brushing and panning followed by drying step. Kester and Fennema (1986) classified the edible films based on the nature of material as polysaccharide, protein, lipid and composite films.

3.1 Polysaccharides

Polysaccharides use for edible films or coatings include cellulose, starch derivatives, pectin derivatives, seaweed extracts, exudates gums, microbial fermentation gum and chitosan (Krochta and Mulder-Johnson, 1997). Polysaccharides are generally very hydrophilic resulting in poor water vapor and gas barrier properties. Although coatings by polysaccharide polymers may not provide a good water vapor barrier, these coatings can act as sacrificing agents resulting moisture loss from food products (Kester and Fennema, 1986).

3.1.1 Cellulose and Derivatives

Cellulose is composed of repeating D-Glucose units linked through ß-1,4 glycosidic bonds. In its native state, the hydroxymethyl groups of anhydroglucose residues are alternatively located above and below the plane of the polymer backbone. This results in very tight packing of polymer chains and a highly crystalline structure that resists solvation in aqueous media. Water solubility can be increased by treating cellulose with alkali to swell the structure, followed by reaction with chloroacetic acid, methyl chloride or propylene oxide to yield carboxymethyl cellulose (CMC), methyl cellulose (MC), hydroxypropyl cellulose (HPMC) or hydroxypropyl cellulose (HPC). MC, HPMC, HPC and CMC film possess good film-forming characteristic; films are generally odorless and taseless, flexible and are of moderate strength, transparent, resistance to oil and fats, water-soluble, moderate to moisture and oxygen transmission (Krochta and Mulder-Johnson, 1997). MC is the most resistant to water and it is the lowest hydrophilic cellulose derivatives (Kester and Fennema, 1986); however, the water vapor permeability of cellulose ether film is still relatively high. MC and HPMC have the ability to form thermally induced gelatinous coating; they have been used to retard oil absorption in deep frying food products (Kester and Fennema, 1986). MC could be applied as coating on confectionery products as barrier to lipid migration (Nelson and Fennema, 1991). A number of researchers have investigated composite films composed of MC or HPMC and various kinds of solids, such as beeswax and fatty acids (Kester and Fennema, 1986). Many of these have water vapor permeability comparable to low density polyethylene (LDPE). These composite films were all polymer-lipid bilayer formed from aqueous ethanolic solutions of cellulose ether fatty acids.

3.1.2 Chitin and Chitosan

Chitin is the second most abundant naturally occurring biopolymer (after cellulose) and is found in the exoskeleton of crustaceans, in fungal cell walls and other biological materials (Andrady and Xu, 1997). It is mainly poly (ß-(1-4)-2-acetamide-D-glucose), which is structurally identical to cellulose except that secondary hydroxyl on the second carbon atom of the hexose repeat unit is replaced by an acetamide group. Chitosan is derived from chitin by deacetylation in the presence of alkali. Therefore, chitosan is a copolymer consisting of (ß-(1-4)-2-acetamido-D-glucose) and (ß-(1-4)-2-acetamind-D-glucose) units with the latter usually exceeding 80%. Chitosans are described in terms of the degree of deacetylation and average molecular weight and their importance resides in their antimicrobial properties in conjuction with their cationicity and their forming properties (Muzzarelli, 1996). Chitosan can form semi-permeable coatings, which can modify the internal atmosphere, thereby delaying ripening and decreasing transpiration rate in fruits and vegetables. Films from aqueous chitosan are clear, tough, flexible and good oxygen barriers (Sanford, 1989). Carbon dioxide permeability could be improved by methylation of polymers. Butler *et al*. (1996) observed that films from chitosan were rather stable and their mechanical and barrier properties changed only slightly during storage. Chitosan coatings are usually used on fruit and vegetable products such as strawberries, cucumbers, bell peppers as antimicrobial coating (El Ghaouth *et al.,* 1991), and on apples, pears, peaches and plums as gas barrier (Elson and Hayes, 1985).

3.1.3 Starch

Starch is a polymeric carbohydrate composed of anhydroglucose units. This is not a uniform material and most starches contain two types of glucose polymers: a linear chain molecule termed amylose and branched polymer of glucose termed amylopectin (Rodriguez *et al*., 2006). Starches are often used in industrial foods. They have been used to produce biodegradable films to partially or entirely replace plastic polymers because of its low cost and renewability, and it has good mechanical properties (Xu *et al*., 2005). High amylose starch such as corn starch is good source for films formation, free-standing films can be produced from aqueous solution of gelatinized amylose and drying. Normal corn starch consists of approximately 25% amylose and 75% amylopectin. Mutant varies of corn are produced which contain starch with up to 85% amylose (Whistler and Daniel, 1985). Mark *et al*. (1966) reported that films produced from high amylose corn starch (71% amylose) had no detectable oxygen permeability at RH levels less than 100%. This was true for both unplasticized and plasticized (16% glycerol) films. This result is surprising in light of the fact that addition of plasticizers and absorption of water molecules by hydrophilic polymers increase polymer chain mobility and generally lead to increased gas permeability (Banker *et al.,* 2000). Partial etherification of high amylose starch with propylene oxide, to yield the hydroxypropylated derivative, improves water solubility.

3.2 Lipid Films

Lipid compounds utilized as protective coating consist of acetylated monoglycerides, natural wax and surfactants. The most effective lipid substances are paraffin wax and beeswax. The primarily function of a lipid coating is to block transport of moisture due to their relative low polarity. In contrast, the hydrophobic characteristic of lipid forms thicker and more brittle films. Consequently, they must be associate with film forming agents such as proteins or cellulose derivatives (Debeaufort *et al*., 1993). Generally, water vapor permeability decrease when the concentration of hydrophobicity phase increases. Lipid-based films are often supported on polymer structure matrix, usually a polysaccharide, to provide mechanical strength.

3.2.1 Waxes and Paraffin

Paraffin wax is derived from distillate fraction of crude petroleum and consists of a mixture of solid hydrocarbon resulting from ethylene catalytic polymerization. Paraffin wax is permitted for use on raw fruit and vegetable and cheese. Carnauba wax is an exudates from palm tree leaves (*Copoernica cerifera*). Beeswax (white wax) is produced from honey comb. Candellila is obtained from candellila plant. Mineral oil consists of a mixture of liquid paraffin and naphtheric hydrocarbon (Hernandez, 1994). Waxes are used as barrier films to gas and moisture (skin on fresh fruits) and to improved the surface appearance of various foods (e.g., the sheen on sweet). If applied as a thick layer, they must be removed before consumption (certain cheese); when used in thin layers, they are considered edible. Waxes (notably paraffin, carnauba, candellila and beeswax) are the most efficient edible compounds providing a humidity barrier.

3.2.2 Acetoglyceride

Acetylation of glycerol monosterate by its reaction with acetic anhydride yield 1-stearodiacetin. This acetylated monoglyceride displays the unique characteristic of solidifying from the molten state into a flexible, wax-like solid. Most lipids in the solid state can be stretched to only about 102% of their original length before fracturing. Acetylated glycerol monostearate, however, can be stretched up to 800% of its original length (Jackson and Lutton, 1952), water vapor permeability of this film is much less than that of polysaccharide films with the exception of methyl cellulose or ethyl cellulose. Acetylated monoglyceride coating have been used on poultry and meat cuts to retard moisture loss during storage (Kester and Fennema, 1986).

3.2.3 Shellac Resins

Shellac resins are a secretion by the insect *Laccifer lacca* and are composed of a complex mixture of aliphatic alicyclic hydroxyl acid polymers. This resin is soluble in alcohols and in alkaline solutions. Shellac is not a GRAS substance; it is only permitted as an indirect food additive in food coatings and adhesives. It is mostly used in coatings for the pharmaceutical industry and only few studies have been reported on foods (Hernandez, 1994). Rosins which are obtained from the oleoresins of the pine tree are residues left after distillation of volatiles from the crude resin. Resin and its derivatives are widely used in coating for citrus and other fruits (Hagenmaier and Baker, 1993). These coatings were designed primarily to impart high-gloss at the time of in section by the buyer, usually after application of the coating. When coatings are applied to fruit, they form an additional barrier through which gases must pass. Because coatings differ in gas permeance and ability to block openings in the peel, they have different effects on gas exchange. Citrus fruits with shellac and wood resin-based coatings generally have lower internal O_2, higher internal CO_2 and higher ethanol content than fruits with wax coating (Hagenmaier and Baker, 1993). High ethanol content, in turn, is an indication of off-flavor. Shellac and wood resin-based coating also tend to increase prevalence of post harvest pitting (Petracek *et al*., 1997).

3.3 Protein Films

In their native states, proteins generally exist as either fibrous proteins, which are water insoluble and serve as the main structural material of animal tissues, or globular proteins, which are soluble in water or aqueous solutions of acids, bases or salts and function widely in living systems (Scope, 1994). Fibrous proteins are fully extended and associated closely with each other in parallel structures, generally through hydrogen bonding, to form fibers. The globular proteins fold into complicated spherical structures held together by a combination of hydrogen, ionic, hydrophobic and covalent (disulfide) bonds (Scope, 1994). The chemical and physical properties of these proteins depend on the relative amounts of the component amino acid residues and their placement along the protein polymer chain. Several globular proteins, including wheat gluten, corn zein, soy protein, and whey protein, have been investigated for their film properties. Protein films are generally formed from solutions or dispersions of the protein as the solvent/carrier evaporates. The solvent/carrier is generally limited to water, ethanol or ethanol-water mixtures (Kester and Fennema, 1986). Generally, proteins must be denatured by heat, acid, base, and/or solvent in order to form the more extended structures that are require for film formation. Once extended, protein chains can associate through hydrogen, ionic, hydrophobic and covalent bonding. The chain-to-chain interaction that produces cohesive films is affected by the degree produces cohesive films is affected by degree of chain extension and the nature and sequence of amino acid residues. Uniform distribution of polar, hydrophobic, and/or thiol groups along the polymer chain increase the likelihood of the respective interactions result in films that are stronger but less flexible and less permeable to gases, vapors and liquids (Kester and Fennema, 1986). Polymer containing groups that can associate through hydrogen or ionic bonding result in films that are excellent oxygen barriers but are susceptible to moisture (Salame, 1986) at low relative humidities. Various types of protein have been used as edible films. These including gelatin, casein, whey protein, corn zein, wheat gluten, soy protein, mung bean protein, and peanut protein (Gennadois *et al.*, 1994; Bourtoom, 2008)

3.3.1 Gelatin Films

Gelatin is obtained by controlled hydrolysis from the fibrous insoluble protein, collagen, which is widely found in nature as the major constituent of skin, bones and connective tissue. Gelatin is composed of a unique sequence of amino acids. The characteristic features of gelatin are high content of the amino acids glycine, proline and hydroxyproline. Gelatin also has a mixture of single and double unfolded chains of hydrophilic character (Ross, 1987). At approximately 40 °C, gelatin aqueous solutions are in the sol state and form physical, thermoreversible gels on cooling. During gelation, the chains undergo a conformational disorder-order transition and tend to recover the collagen triple-helix structure (Ross-Murphy, 1992). Gelatin films could be formed from 20-30% gelatin, 10-30% plasticizer (glycerin or sorbitol) and 40-70% water followed by drying the gelatin gel (Guilbert, 1986). Gelatin is used to encapsulate low moisture or oil phase food ingredients and pharmaceuticals. Such encapsulation provides protection against oxygen and light, as well as defining ingredient amount or drug dosage. In addition, gelatin films have been formed as coatings on meats to reduce oxygen, moisture and oil transport (Gennadios *et al*., 1994).

3.3.2 Corn Zein Films

Zein is the most important protein in corn. It is a prolamin protein and therefore dissolves in 70-80% ethanol (Dickey and Parris, 2002). Zein is relatively hydrophobic and thermoplastic material. The hydrophobic nature of zein is related to its high content of non-polar amino acids (Shukla and Cheryan, 2001). Zein has excellent film forming properties and can be used for fabrication of biodegradable films. The zein bio-film is formed through the development of hydrophobic, hydrogen and limited disulfide bonds between zein chains (Guilbert, 1986). Edible films can be formed by drying aqueous ethanol solution of zein (Gennadios and Weller, 1990). Formation of films is believed to involve development of hydrophobic, hydrogen and limited disulfide bonds between zein chains in the film matrix (Gennadios *et al*., 1994). The resulting films are brittle and therefore require plasticizer addition for increasing flexibility (Park, 1991). Zein films are relatively good water vapor barriers compared to other edible films (Guilbert, 1986). Water vapor barrier properties can be improved by adding fatty acids or by using a cross-linking reagent. But cross-linking agents are used, the edibility of those films is of concern (Alikornis, 1979). Zein coating have also shown an ability to reduce moisture and firmness loss and delay color change (reduce oxygen and carbon dioxide transmission) in fresh tomatoes (Park *et al*., 1994).

3.3.3 Wheat Gluten Films

Wheat gluten is a general term for water-insoluble proteins of wheat flour which is composed of a mixture of polypeptide molecules, considered to be globular proteins. Cohesiveness and elasticity of gluten give integrity to wheat dough and facilitate film formation. Wheat gluten contains the prolamin and glutelin fractions of wheat flour proteins, typically referred to as gliadin and glutenin, respectively. While gliadin is soluble in 70% ethanol, glutenin is not (Gennadios and Weller, 1990). Although insoluble in natural water, wheat gluten dissolves in aqueous solutions of high or low pH at low ionic strength (Krull and Inglett, 1971). Edible films can be formed by drying aqueous ethanol solution of wheat gluten. Cleavage of native disulfide bonds during heating of film-forming solutions and then formation of new disulfide bonds during film drying are believed to be important to the formation of wheat gluten film structure, along with hydrogen and hydrophobic bonds (Gennadios and Weller, 1990). Addition of plasticizer such as glycerin in gluten films is necessary to improve film flexibility (Gennadios *et al*., 1994). However, increasing film flexibility by increasing sorbitol content reduces film strength, elasticity and water vapor barrier properties (Gontard *et al*., 1992). A review on gluten film was done by Gennadios and Weller (1990). Gennadios and Weller (1992) confirmed the effect of wheat gluten purity on film appearance and mechanical properties, i.e., a greater purity gluten results in a stronger and clearer films. Herald *et al*. (1995) investigated the effect of plasticizer size of wheat gluten; films prepared from spray-dried wheat gluten were stronger than films from flash-dried which had larger sized particles. When used as a coating on grade A-quality shell eggs. The egg quality was maintained for 30 days. Tensile strength of gluten films can be improved by using a cross-linking agent such as glutaraldehyde, or heat curing at 80 °C (Gennadios and Weller, 1992). Determined the influence of relative humidity on carbon dioxide sorption in wheat gluten films. The results showed that the permeability was based on the rise of CO_2 solubility and diffusivity with the increase in RH. The increase in the water content of wheat gluten improves the affinity between carbon dioxide and the protein matrix, leading to outstanding sorption values for high RH.

3.3.4 Soy Protein Films

The protein content of soybeans (38-44%) is much higher than the protein content of cereal grain (8-15%). Most of the protein in soybeans is insoluble in water but soluble in dilute neutral salt solutions. Thus, soy protein belongs to the globulin classification (Kinsella, 1979). Soy protein is globular in nature and is further classified into 2S, 7S, 11S and 15S fraction according to relative sedimentation rate (Gennadios *et al*., 1994). The principal components are the 7S (conglycinin) and 11S (glycinin) fractions, both of which have a quaternary (subunit) structure (Kinsella *et al.,* 1985). Soy protein is high in asparagines and glutamine residues. Both conglycinin and glycinin are tightly folded proteins. While the extent of disulfide cross-linking of conglycinin is limited due to only two to three cysteine groups per molecule, glycinin contains 20 intramolecular disulfide bonds (Kinsella, 1979). Edible films based on soy protein can be produced in either of two ways: surface film formation on heated soymilk or film formation from solutions of soy protein isolate (SPI) (Gennadios and Weller, 1992). Soymilk is produced by grinding soybeans with water followed by separation of milk from extracted soybeans. To form films from both soymilk and SPI, (a) heating of film solutions to disrupt the protein structure, cleave native disulfide bonds and expose sulfhydryl groups and hydrophobic groups, and then (b) formation of new disulfide, hydrophobic and hydrogen bonds during film drying are believed to be important to the formation of soy protein film structure. The use of soy protein in the formation of films or coating on food products has been investigated (Gennadios *et al*., 1994).

3.4 Composite Films

Edible films and coatings may be heterogeneous in nature, consisting of blend of polysaccharides, protein, and/or lipids. This approach enables one to utilize the distinct functional characteristics of each class of film former (Kester and Fennema, 1986). The combination between polymers to form films could be from proteins and carbohydrates and lipids or synthetic polymers and natural polymers. The main objective of producing composite films is to improve the permeability or mechanical properties as dictated by the need of a specific application. These heterogeneous films are applied either in the form of an emulsion, suspension, or dispersion of the non-miscible constituents, or in the form of a solution in a common solvent. The method of application affects the barrier properties of the films obtained (Guilbert, 1986). Kamper and Fennema (1984a) introduced the emulsion films from methyl cellulose (Haggenmaier and Shaw, 1990), methyl cellulose (MC) and lipid (Greener and Fennema, 1989a), MC and fatty acid (Sapru and Labuza, 1994), whey isolate and lipids (McHung *et al.*, 1994), casein and lipids (Avena-Bistillos, 1993), gelatin and soluble starch (Arvanitoyannis *et al.*, 1997), corn zein and corn starch (Ryu *et al.*, 2002), gelatin and fatty acid (Bertana *et al.*, 2005), soy protein isolate and gelatin (Cao *et al.*, 2002), soy protein isolate and polylactic acid (Rhim *et al.*, 2007).

4. Rice Starch

Rice (*Oryza sativa* L.) which belongs to the *Porceae Gramineae* or grass family has been consumed by humans for at least 5000 years. Rice contains two major sub-species : short grained *japonica* or *sinica* variety and the non-sticky, long grained *indica* variety. *Japonica* are usually cultivated in dry fields, in temperature East Asia, upland areas of southeast Asia and high elevations in South Asia, while *indica* are mainly lowland rices, grow mostly submerged, throughout tropical Asia. Rice is known to come in a variety of colors, including: white, brown, black, purple and red (Kennedy and Burlingame, 2003). Rice starch occurs naturally in rice and is the storage polysaccharide of rice. Starch is polymeric carbohydrate compose of anhydroglucose units. This is not a uniform material and starches contain two types of glucose polymer: a linear chain molecule termed amylose and branched polymer of glucose term amylopectin (Rodriguez *et al.,* 2006). Starch is often used in industrial foods. They have been use to produce biodegradable films to partially or entirely replace plastic polymer because of its low cost and renew ability, and it has good mechanical properties (Xu *et al.,* 2005).

4.1 Chemical Composition

Starch is composed of two basic molecular components: amylose and amylopectin. These are identical in their constituent basic units (glucose), but differ in their structural organization (linkages). These variations in linkages in turn affect their functionality in applications. Amylose is a straight chain molecule, while amylopectin is a branched molecule. The ratios of amylose to amylopectin vary among starch sources and play a considerable role in determining reactions and physicochemical properties of starch in processing and applications (Bertoft, 2004).

4.1.1 Amylose is compose of D-glucose molecules, which are linked in an α-1,4 conformation. The glucose monomers therefore form a linear straight chain polymer. Amylose is less predominant (about 20%) and typically constitutes about 20-40% in proportion. Amylose contains α-1,4 bond and is slightly soluble in water. Amylose molecules are arranged in a helical conformation. Amylose forms a blue complex with iodine, which can be read about 650 nm. Amylose is the key component involved in water absorption, swelling and gelation of starch in food processing. It is more susceptible to gelatinization and retrogradation, and hence is most commonly involved in resistant starch formation (Hoseney, 1986).

4.1.2 Amylopectin the major component of most starch, consist of a large number of shorter chains that are bound together at their reducing end side by a α-1,6 linkage (Hoseney, 1986). Amylopectin is therefore highly branched as the α-1,4 linear chains are punctuated with the α-1,6 linkages. The α-1,6 constitute about 5% of the structure of amylopectin and gives rise to branching. The amylopectin molecule therefore is much larger than the amylose molecule. Amylopectin has short branched chains and branch linkages and thus cannot form the helical complex with iodine. The branched dextrin of amylopectin, however, gives a purple color with the iodine complex, identifiable at about 550 nm (Bertoft, 2004).

4.1.3 Other Constituents of Rice Starch

- Protein: The difference between rice flour and starch is that most of the native proteins and lipids have been removed from starch. The protein content of milled rice in a germplasm collection reportedly range from 4.5 to 15.9% (Kennedy and Burlingame, 2003). Therefore, isolation of starch from rice mainly involves techniques to remove proteins. The majority of rice protein is alkaline soluble, thus alkaline steeping methods are commonly used in industry and research to produce rice starch with good recovery and lower residual protein content. The goal for the protein content of isolated rice starch is generally 0.5% or less (Lumdubwong and Seib, 2000).

- Minor constituents: Beside protein, other minor constituents including lipids, phosphorus and trace elements are commonly found in isolate starch. Non-waxy rice contains 0.3-0.4% bound lipid; waxy rice starch reportedly contains less of this fraction (0.03%) (Champagne, 1996). Phosphorus plays an extremely important role in starch functional properties, such as paste clarity, viscosity consistency and paste stability (Jane *et al.*, 1996).

4.2 Structure of Starch Granule

The basic physical structural unit of starch granule. These granules are simple or compound and consist of concentric or eccentric layers of varying density. They are of varying size (2-150 µm), size distribution and shape on the basis of X-ray diffraction experiment, starch granule are said to have a semicrystalline character. About 70% of the mass of a starch granule is regarded as amorphous and 30% as crystalline. The amorphous regions contain the main amount of amylose but also a considerable part of the amylopectin. The crystalline regions consist of primary of amylopectin. Native starches can be divided into type A, B and C. While type A and B are real crystalline modifications, the C-type is a mixed form. The A-type is largely present in cereal starches, and the B-type in potatoes. The C type is not only observed in mixtures of corn and potato starches (Belitz and Grosch, 1992).

4.3 Functionality of Rice Starch

4.3.1 Swelling Power and Solubility

Starch is not water soluble, because granules are too large to form a solution. Starch has a relative high density, about 1.45-1.64 g/cm^3, depending on its source, its prior treatment, and the measurement method. Starch granules absorb some water in suspension at room temperature, but the amount of swelling is limited in intact granules. When heat in water suspension to progressively higher temperature, very little happens, until a critical temperature is reached. At this point the granule begins to swelling rapidly, losing the polarization crosses, a process termed gelatinization.

4.3.2 Gelatinization and Pasting

Starch gelatinization is the collapse (disruption) of the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization. The point of initial gelatinization and range over which it occurs is governed by starch concentration, method of observation, granular type and heterogeneity within the granule population under observation.

Pasting is the phenomenon following gelatinization in the dissolution of starch. It involves granule swelling, exudation of molecular components from the granule and eventually, total disruption of the granule (Atwell *et al.*, 1998).

4.3.3 Retrogradation

Under low energy input, as in freezing and chilling, further hydrogen bonding may occur, resulting in further tightening of structure with loss of water-holding capacity, known as retrogradation. Starch retrogradation is a process which occurs when the molecules comprising gelatinized starch begin to reassociate in an ordered structure. In its initial phases, two or more starch chains may form a simple juncture point that may then develop into more extensively ordered regions. Ultimately, under favorable conditions, a crystalline order appears (Atwell *et al.,* 1998).

5. Nanotechnology

“Nano-” denotes nanometer (10^{-9} m) the concept of nanotechnology was introduced by Richard Feynman in 1959 at meeting of American Physical Society (Khademhosseini and Lager, 2006). Since then, nanotechnology has developed into a multidisciplinary field of applied science and technology. Nanotechnology is the ability to work on a scale of about 1-100 nm in order to understand, create, characterize and use material structures, devices and systems with new properties derived from their nanostructures (Roco, 2003). Because of their size, nanoparticles have proportionally larger surface area and consequently more surface atoms than their microscale counterpart. In the nanoscale range, materials may present different electronic properties, which in turn affect its optical, catalytic and other reactive properties (Boccuni *et al.*, 2008).

Nowadays, most materials used for food packaging are practically undegradable, representing a serious global environmental problem. New bio-based materials have been exploited to develop edible and biodegradable films as a big effort to extend shelf life and improve quality of food while reducing packaging waste (Tharanathan, 2003). However, the use of edible and biodegradable polymers has been limited because of problems related to performance (such as brittleness, poor gas and moisture barrier), processing (such as low heat distortion temperature), and cost. Starch, as an example, has received considerable attention as a biodegradable thermoplastic polymer. However, it has a poor performance by itself because of its water sensitivity and limited mechanical properties (Vaidya and Bhattacharya, 1994) with high brittleness, which is related to the anarchical growth of amylase crystals with time (Dufresne and Vignon, 1998). The application of nanotechnology to these polymers may open new possibilities for improving not only the properties but also the cost-price-efficiency (Sorrentino, *et al.*, 2007).

Several composites have been developed by adding reinforcing compounds to polymers to enhance their thermal, mechanical and barrier properties. Most of these reinforced materials present poor interactions at the interface of both components. Macroscopic reinforcing components usually contain defects, which become less important as the particles of the reinforcing component are smaller (Luduena, *et al.*, 2007).

Polymer composites are mixtures of polymers with inorganic or organic fillers with certain geometries (fibers, flakes, spheres, particulates). The use of fillers which have at least one dimension in the nanometric range (nanoparticles) produces polymer nanocomposites (Alexandre and Dubois, 2000). Three types of fillers can be distinguished, depending on how many dimensions are in the nanometric range. Isodimensional nanoparticles, such as spherical silica nanoparticles or semiconductor nanoclusters, have three nanometric dimensions. Nanotubes or whiskers are elongated structures in which two dimensions are in the nanometer scale and the polymer (or a monomer subsequently polymerized) inside the galleries of layered host crystals (Alexandre and Dubois, 2000).

A uniform dispersion of nanoparticles leads to a very large matrix/filler interfacial area, which changes the molecular mobility, the relaxation behavior and the consequent thermal and mechanical properties of the material. Fillers with a high ratio of the largest to the smallest dimension (i.e., aspect ratio) are particularly interesting because of their high specific surface area, providing better reinforcing effects (Azizi Samir *et al*., 2005; Dubief *et al*., 1999). In addition to the effects of the nanoreinforcements themselves, an interphase region of altered mobility surrounding each nanoparticle is reduced by well dispersed nanoparticles, resulting in a percolating interphase network in the composite and playing an important role in improving the nanocomposites properties (Qiao and Brinson, 2009). According to Jordan *et al*. (2005) for constant filler content, a reduction in particles size increases the number of filler particles, bringing them closer to one another; thus, the interface layers from adjacent particles overlap, altering the bulk properties significantly.

Besides reinforcing nanoparticles, whose main role is to improve mechanical and barrier properties of the packaging materials, there are several types of nanostructures responsible for other functions, sometimes providing active or “smart” properties to the packaging systems. Some particles can have multiple applications, and sometimes the applications can overlap, such as some immobilized enzymes which can act as antimicrobial components, oxygen scavengers and/or biosensors.

5.1 Nanoreinforcements

5.1.1 Clay and Silicates

Although several nanoparticles have been recognized as possible additives to enhance polymer performance, the packaging industry has focused its attention mainly on layered inorganic solids like clays and silicates, due it their availability, low cost, significant enhancements and relative simple process ability.

The layered silicates commonly used in nanocomposites consist of two-dimensional layers, which are 1 nm thick and several microns long depending on the particular silicate. Its presence in polymer formulations increases the tortuosity of the diffusive path for a penetrant molecule (Figure 1), providing excellent barrier properties (Bharadwaj *et al.*, 2002; Mirzadeh and Kokabi, 2007).

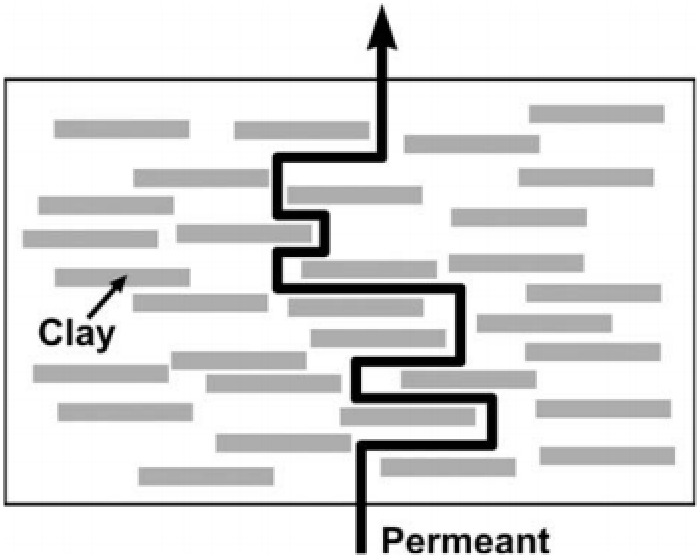

Figure 1. Tortuous path of a permeant in a clay nanocomposite.
Source : Adame and Beall (2009)

In contrast with the tactoid structure predominating in microcomposites (conventional composites), in which the polymer and the clay tactoids remain immiscible, resulting in agglomeration of the clay in the matrix and poor macroscopic properties of the material (Alexandre *et al.*, 2009), the interaction between layered silicates and polymer chains may produce two types of ideal nanoscale composites (Figure 2). The intercalated nanocomposites result from the penetration of polymers chains into the interlayer region of the clay, resulting in an ordered multilayer structure with alternating polymer/inorganic layer at a repeated distance of a few nanometers (Luduena *et al.*, 2007). Exfoliated nanocomposites have been reported to exhibit the best properties due to the optimal interaction between clay and polymer (Adame and Beall, 2009; Alexandre *et al.*, 2009).

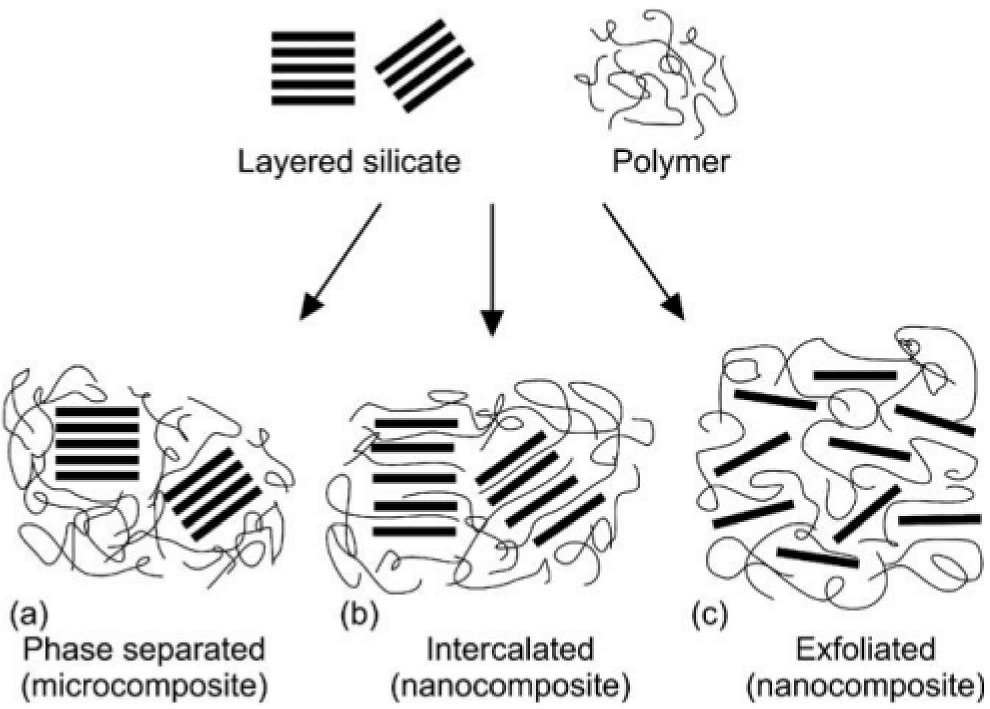

Figure 2. Types of composite derived from interaction between clays and polymer: (a) phase-separated microcomposite; (b) interacalated nanocomposite and (c) exfoliated nanocomposite.

Source : Alexandre and Dubois (2000)

Clays have been also reported to improve the mechanical strength of biopolymers, making their use feasible (Weiss *et al*., 2006). Park *et al*. 2003 generated thermoplastic starch (TPS)/clay nanocomposites with improved mechanical properties and decreased water vapor permeability by using only 5% (w/w) of clays. Reports by Dean *et al*. (2007) indicated that optimum levels of both plasticizer and nanoclay exist for each clay to produce a gelatinized starch film with the highest exfoliation and best improvement in mechanical properties, and those optimum levels have some dependency on the cation exchange capacity (CEC) of the clay. Petersson and Oksman (2006) reported that bentonite was able to improve strength and modulus of a polylactic acid (PLA) matrix, but it drastically decreased elongation of the material. Similar results were reported by Lotti *et al*. (2008) for low-density polyethylene (LDPE) treated with an organoclay, and also by Xu *et al*. (2006) for chitosan films with montmorillonite (MMT). On the other hand, Marras *et al*. (2008) reported that the elongation of poly(ε-caprolactone) was not impaired by MMT. Other authors observed improved mechanical properties of several polymers by addition of nanoclays (Avella *et al.,* 2005; Chen and Evans, 2005; Cyras *et al.,* 2008; Russo *et al*., 2007)

5.1.2 Cellulose-Based Nanoreinforcements

Cellulose, the building material of long fibrous cells, is a highly strong natural polymer. Cellulose nanofibers are inherently a low cost and widely available material. Moreover, they are environmentally friendly and easy of recycling by combustion, and require low energy consumption in manufacturing. All of this makes cellulose nanofibers an attractive class of nanomaterials for elaboration of low cost, lightweight and high-strength nanocomposites (Helbert *et al*., 1996).

Basically two types of nanoreinforcements can be obtained from cellulose-microfibrils and whiskers. In plants or animals the cellulose chains are synthesized to from microfibrils (or nanofibers), which are bundles of molecules that are elongated and stabilized through hydrogen bonding (Azizi Samir *et al*., 2005). The microfibrils have nanosized diameters (2-20 nm, depending on the origin) and lengths in the micrometer range (Oksman *et al*., 2006). Each microfibril is formed by aggregation of elementary fibrils, which are made up of crystalline and amorphous parts. The crystalline parts, which can be isolated by several treatments, are whiskers, also known as nanocrystals, nanorods or rodlike cellulose microcrystals (Azizi Samir *et al*., 2005), with lengths ranging from 500 nm up to 1-2 μm and about 8-20 nm or less in diameter (Lima and Borsali, 2004), resulting in high aspect ratios. Each microfibril can be considered a string of whiskers, liked along it by amorphous domains (which act as structural defects) and having a modulus close to that of crystal of native cellulose (about 150 GPa) and a strength of about 10 GPa (Helbert *et al.,* 1996) values which are only about seven times lower than those of single-walled carbon nanotubes (Podsiadlo *et al.,* 2005).

5.1.3 Carbon Nanotubes

Carbon nanotubes (CNTs) may consist of a one-atom thick single-wall nanotubes (SWNT), or a number of concentric tubes called multiwalled nanotubes

(MWNT), having extraordinarity aspect ratios and elastic modulus (Zhou *et al.,* 2004). Lau and Hui (2002) reported CNTs to have theoretical elastic modulus and tensile strength values as high as 1 TPa and 200 GPa, respectively.

Kim *et al.* (2008) modified CNTs by introducing carboxylic acid groups on their surfaces in order to enhance their intermolecular interactions with the poly(ethylene-2,6-naphtalene) (PEN) matrix. CNTs, even in concentrations as low as 0.1 wt%, greatly improved thermal stability as well as tensile strength and modulus of PEN. Other polymers have been found to have their tensile strength/modulus improved by addition of CNTs, such as PVOH (Bin *et al.,* 2006; Chen *et al.,* 2005), polypropylene (Lopez *et al.,* 2005) and polyamine (Zeng *et al.,* 2006). According to Brody (2006), researches from Nitick indicated that a CNT nanocomposite with PLA exhibited a 200% better water vapor transmission rate than pure PLA, plus increased modulus and toughness.

5.1.4 Silica (SiO_2)

Silica nanoparticles ($nSiO_2$) have been reported to improve mechanical and/or barrier properties of several polymer matrices. Wu *et al.* (2002) observed that the addition of $nSiO_2$ into a polypropylene (PP) matrix improved tensile properties of the material-not only strength and modulus, but also elongation. Improvements in tensile properties-again including elongation-were also reported for a starch matrix as resulting from $nSiO_2$ addition (Xiong *et al.,* 2008). Those authors (Xiong *et al.,* 2008) observed also that $nSiO_2$ addition decreased water absorption by starch. Vladimiriov *et al.,* (2006) incorporated $nSiO_2$ to an isotactic polypropylene (iPP) matrix, using maleic anhydride grafted polypropylene (PP-g-MA) as a compatibilizer. $nSiO_2$ increased storage modulus of iPP, making the material stiffer, and improved the oxygen barrier of the matrix. Jia *et al.* (2007) prepared nanocomposites of PVOH with $nSiO_2$ by radical copolymerization of vinyl silica nanoparticles and vinylacetate. The nanocomposites had improved thermal and mechanical properties when compared to the pure PVOH, due to strong interactions between $nSiO_2$ and the polymer matrix via covalent bonding. Tang *et al.* (2008) prepared starch/PVOH/$nSiO_2$ biodegradable films. With the increase in $nSiO_2$ content, the tensile properties and water resistance of the films were improved. There was also an increase in the intermolecular hydrogen bonds, as well as formation of C-O-Si groups, between $nSiO_2$ and starch, or $nSiO_2$ and PVOH, which improved the miscibility and compatibility between film components. Some authors (Wu *et al.,* 2002; Zhang and Rong, 2003) observed that the presence of grafting polymers on the surface of $nSiO_2$ improved the tailor ability of the composites, that is to say, different species of grafting monomers result in different interfacial interactions and tensile properties.

5.1.5 Starch Nanocrystals

By submitting native starch granules to an extended time hydrolysis at temperature below the gelatinization temperature, when the amorphous regions are hydrolyzed allowing separation of crystalline lamellae, which are more resistant to hydrolysis. The starch crystalline particles show platelet morphology with thicknesses of 6-8 nm (Kristo and Billiaderis, 2007). The use of starch nanoparticles is receiving a significant amount of attention because of the abundant availability of starch, low cost, renewability, biocompatibility, biodegradability and non-toxicity. The latter properties make them excellent candidates for implant materials and drug carriers. Starch nanocrystals have also been found to be excellent reinforcement (Angllier *et al.,* 2005).

Putaux *et al.* (2003) prepared the colloidal aqueous suspensions of starch nanocrystals by submitting native granules from A-type amylopectin rich waxy maize to a hydrochloric acid hydrolysis. The insoluble residue contains poly disperse and more or less individualized platelet nanocrystals corresponding to the lamellae formed by the association of amylopectin side branches into parallel arrays of double helices. After 2 weeks of hydrolysis, 5-7 nm thick lamellae still connected by α (1-6) linkages were seen edge-on using transmission electron microscopy. As the hydrolysis progressed up to 6 weeks, more α (1-6) branching points located in the inter-lamellar areas were severed and the platelets were thus observed in planar view. Despite a variety of shapes, constituting parallel epipedal blocks with a length of 20-40 nm and a width of 15-30 nm. X-ray and electron diffraction showed that these nanoplatelets retain the crystalline A-type of the parent granules.

Angllier *et al.* (2004) investigated the effect of five selected factors on the selective H_2SO_4 hydrolysis of waxy maize starch granules by using Respond

Surface Methodology (RSM). These predictors were temperature, acid concentration, starch concentration, hydrolysis duration, and stirring speed. The goal of this study was to optimize the preparation of aqueous suspensions of starch nanocrystals, i.e., to determine the operative conditions leading to the smallest size of insoluble hydrolyzed residue within the shortest time and with the highest yield. Therefore empirical models were elaborated for the hydrolysis yield and the size of the insoluble residues using a central composite face design involving 31 trials. The authors reported that it was possible to obtain starch nanocrystals after only 5 days of H_2SO_4 hydrolysis with a yield of 15 wt % and having the same shape as those obtained from the classical procedure after 40 days of HCl treatment, with a yield of 0.5 wt %.

Kristo and Biliaderis (2007) studied the nanocomposite materials from sorbitol-plasticized pullulan as the amorphous matrix and an aqueous suspension of starch nanocrystals (prepared by submitting native granules from waxy maize starch to acid hydrolysis at 35 °C) as the reinforcing phase. Wide-angle X-ray diffraction analysis showed an increase of the crystallinity of the composite biopolymer films with increasing of starch nanocrystals content. The water absorption isotherms and kinetics as well as the water barrier properties of nanocomposite films filled with 0-40% (w/w) starch nanocrystals (starch nanocrystals/pullulan + sorbitol) were investigated. The water uptake of pullulan-starch nanocomposites decreased with increasing filler content whereas water vapor permeability (measured at 25 °C and 53/100 relative humidity (RH) gradient) remained constant up to 20% (w/w) and, then decreased significantly with further addition of nanocrystals. The thermomechanical behavior of nanocomposite films was also investigated by means of dynamic mechanical thermal analysis (DMTA) and large deformation mechanical tests (tensile mode). The glass transition temperature (Tg) shifted towards higher temperatures with increasing amount of nanocrystals, which can be attributed to a restriction of the mobility of pullulan chains due to the establishment of strong interactions not only between starch nanocrystals but also between the filler and the matrix. Moreover, the addition of nanocrystals caused strong enhancement of the Young modulus and the tensile strength, but led to a drastic decrease of the strain at break in samples conditioned at deferent environments (from 43% to 75% RH).

Chen *et al.* (2007) prepared pea starch nanocrystals (PSN) dispersion containing nanocrystals in a range of 30-80 nm from native pea starch (NPS) granules by acid hydrolysis. Two series of films were prepared by blending poly(vinyl alcohol) (PVA) with NPS and PSN, respectively. The effects of NPS and PSN on the structure and properties of the resulting films were comparatively investigated by FTIR, XRD, SEM, and testing of light transmittance, tensile, and moisture uptake. The light transmittance (Tr), tensile strength (σb), and elongation at break (εb) of the PVA/NPS films were lower than that of PVA film and decreased with an increase in NPS content. However, the PVA/PSN nanocomposite films containing 5 and 10 wt% of PSN content exhibited improved physical properties over the PVA film. The PVA/PSN films showed higher Tr, σb, and εb, and lower moisture uptake (Mu) than the corresponding PVA/NPS films with the same component ration. For example, the values of Tr, σb, εb, and Mu of the PVA/PSN film containing 10 wt% of PSN content were 91%, 40 MPa, 734% and 71%, respectively; while those of the corresponding PVA/NPS film were 69%, 35 MPa, 579% and 73%, respectively. The results revealed that PSN, comparing with NPS, had much smaller sizes and dispersed more homogeneously in PVA matrix, resulting in stronger interactions with PVA. New applications of native pea starch and its nanocrystals as low-cost fillers were explored in this work, and PSN exhibited greater potential than NPS to improve the properties of PVA-based composites.

Song *et al.* (2007) prepared amphiphilic starch nanocrystals by graft copolymerization of starch nanocrystals with styrene in aqueous emulsion system. The starch nanocrystals of size around 50 nm were used, which were prepared by acid hydrolysis of corn starch. The structure of starch-g-polystyrene nanocrystals was characterized by Fourier transform infrared and ^{1}H nuclear magnetic resonance (^{1}H NMR). The crystalline structure as well as its particle morphology was studied by X-ray diffraction and scanning electron microscopy, respectively. The results indicated that the amphiphilic starch nanocrystals obtained exhibit the size around 80-100 nm. Its crystalline structure is basically not changed after grafting polystyrene suggested that the polystyrene was essentially grafted on the surface of starch nanocrystals.

6. Factor Affecting Edible Films Properties

6.1 Type and Concentration of Plasticizers

A plasticizer is substance that is incorporated into a rigid plastic to increase its flexibility, workability, and distensibility. By reducing the grass transition temperature and increasing chain lubricity, plasticizers also improve processing and extrusion characteristics, reduce the minimum required processing temperature, reduce the plastic’s hardness and improve low temperature flexibility. Generally, two types of plasticizers are distinguished. Internal plasticization is a result of modifications to the chemical structure of polymer, for example, by copolymerization or selected hydrogenation or transesterification in the case of edible fats or similar; external plastification is obtained by adding an agent which modifies the structure and energy within the tree-dimensional arrangement of the film polymer (Banker, 1996). It is the second method which, on the basis of the type of materials and the technology, is mainly used for edible packaging and coatings. A plasticizer may be defined as a compound, which when added to another material and under given conditions, modifies certain physical and mechanical properties of the material. The addition of a plasticizer to a film produces a film which is less likely to break and is more flexible and stronger. The reduction of the intermolecular bonds between the polymer chains, and thus the overall cohesion, facilitates elongation of the film and reduces its glass transition temperature. This is manifested by a reduction in the barrier properties to gases, vapors, and film solutes (Banker, 1996; Kumins, 1965).

A plasticizing agent must be compatible with the film-forming polymer and be permeability present within the solvent-polymer system and under the conditions used. To be compatible, it must be miscible with the polymer, which implies the use of molecular reactions of similar nature. It is important to remember that the formulation of the whole film system (polymer, solvent, plasticizer, other additives) has a direct effect on the nature and characteristics of the film produced. As a result, the polymer and the plasticizer must not only be compatible, but must also have similar solubility in the solvent used. A soluble plasticizer will be generally sought for the development of soluble coating and an insoluble plasticizer (or dispersible) for an insoluble coating or for a slow solubilization.

The permanence of a plasticizer is also of prime importance since this influence the physical and mechanical stability of the film. The plasticizer should not be volatile (or not only very slightly volatile) and its degree of retention by the film should be high. Other properties, such as its chemical stability, hygroscopicity, color, flavor, and so on, are also more or less important depending on the type of film under consideration. In addition, the concentration of plasticizer necessarily varies from 10-60% (dry basis) according to the nature and type of film and the method of application.

The plasticizers that are most often used in the field of edible coatings and films are the following:

- mono-, di-, and oligosaccharides (generally glucose syrups or glucose fructose honey)
- polyols (principally glycerol and its derivatives, polyethylene glycols, sorbitol)
-.lipids and its derivatives (fatty acids, monoglycerides and their esters, acetoglycerides, phospholipids, and other emulsifiers). Plasticizing of a hydrophilic polymer-based film will generally be achieved by the addition of a compound belonging to one of the first two groups and that of wax- or fat-based film by a compound from the third group.

The efficiency, stability, compatibility and permeability and permanence of a plasticizing agent can be evaluated by various semi-empire tests. The final method of plasticization consists of adding to the film system relatively inert solids (fillers which reduce the molecular reactions and cohesion of the final film). The size of these particles and their dispersion are of prime importance. Microcrystalline cellulose, various proteic isolates, and cocoa have been used as plasticizers, particularly, in fat-based films (Kester and Fennema, 1986).

Maria *et al.* (2000) studied the effect of different starch sources for film formulations : corn starch with 250 g/kg amylose and high-amylose corn starch, i.e. amylomaize with 650 g/kg amylose. Starch suspensions were cold gelatinized with NaOH; either glycerol or sorbitol were used as plasticizer. For all tested formulations, film crystallinity increased while gas permeability decreased during storage. Films containing glycerol or sorbitol showed a lower crystalline/amorphous ratio than films without plasticizer. Plasticizers favored chain mobility allowing the development of the more stable structure at shorter storage times. However, plasticizer limited crystalline growth because they interfere with polymer chain alignment due to stearic hindrances. SEM analysis showed that plasticizer addition avoids the formation of pores and cracks. Starch film including sorbitol as plasticizer showed lower gaseous permeability than films with glycerol.

Krogars *et al.* (2002) studied the effects of a combination of sorbitol and glycerol used as plasticizers on mechanical, moisture permeability and solid-state properties of rubbery amylose maize starch films. The starch films plasticized with combination of sorbitol and glycerol (1:1) at equal amount to the polymer weight, were shown to be the most stable alternative of the studied films during the 9 months storage period. The water vapor transmission (WVTR) of the films did not change during the period of storage and neither did the elongation at break, but the tensile strength increased. The combination of sorbitol and glycerol prevented the migration of the plasticizer molecule out of the film.

Mali *et al.* (2005) studied the effects of plasticizers (glycerol, sorbitol, and 1:1 mixture of glycerol and sorbitol) on moisture sorption characteristics of cassava starch films. The combined effects of relative humidity and plasticizer on mechanical properties of starch films were also examined. Water affinities of cassava starch films were affected by hydrophilicity of the plasticizer and its concentration. Films plasticized with glycerol, under all RH conditions, adsorbed faster and more water during its storage comparing to sorbitol films. The presence of plasticizers resulted in lower stress and Young's modulus values; glycerol films were more affected in its mechanical properties, indicating that glycerol exerted a more effective plasticization. Appropriate selection of plasticizer type and concentration will be helpful in controlling moisture content and moisture adsorption rate of a film, thereby improving the film stability under varying RH conditions.

Alves *et al.* (2007) studied the effect of glycerol and amylose enrichment on cassava starch films properties. The effects of different amylose quantities (6.3, 15.6 and 25.0 g/100g of starch) and glycerol contents (20.0, 32.5 and 45 g/100g of starch) on film organic solution were evaluate in barrier and mechanical properties of films. Mechanical and barrier properties of cassava starch films were influenced by glycerol and amylose contents. Glycerol behaved as a typical plasticizer in starch films; with increasing glycerol concentration, water vapor permeability, strain at break and puncture deformation increased, and stress at break, Young's modulus and puncture strength decreased. These effects of glycerol can explanation; when glycerol was incorporated, some structural modifications occurred in starch network, the film matrix became less dense and under stress, movements of polymer chains were facilitated, improving film flexibility.

Muller *et al.* (2007) studies the effects of glycerol and sorbitol concentrations and the relative air humidity on the water vapor permeability, water diffusion coefficient and the solubility coefficient of water in cassava starch films prepared by casting. In all cases, an increase in the water vapor permeability values were observed with increasing plasticizer concentration and RH. The diffusion coefficient, solubility coefficient of water and water vapor permeability values of films prepared with glycerol were greater than those of films prepare with sorbitol. For high RH, the solubility coefficient of water values increases 6-fold for film with glycerol and 7-fold for films with sorbitol, while the diffusion coefficient of water values did not change significantly. These results indicated that the water vapor permeability values are dependent on the solubility coefficient of water in the film, which is not consistent with the proposal that an opening of the polymer chains results in an increase in the diffusion coefficient. The hydrophilic groups of plasticizers could even reduce water molecule mobility and as a result reduce the diffusion coefficient. Thus, it is possible to conclude that water vapor permeability is controlled by the solubility coefficient of water in the film.

6.2 Amylose and Amylopectin Content

Starch consists of two polysaccarides, the essentially linear amylose, and the branched amylopectin (Manners, 1989). Pure amylose films are stronger than amylopectin films (Rindlav-Westling *et al.,* 1998) and they have a higher elasticity (Lourdin *et al*., 1995). The ability of amylose to produce self supporting films has been known for a long time (Wolff *et al*., 1951) and which is attributed to the ability of its linear chains to interact by hydrogen bonds to a higher extent than the branched amylopectin chains. Amylopectin films, on the other hand, are rather frail due to the higher degree of entanglement caused by the extensive branching and the short average chains length (Rindlav-Westling *et al*., 1998). Lourdin *et al.* (1995) has been found that linear amylose chains confer better mechanical properties compared to branched amylopectin molecules.

Maria *et al.* (2000) developed edible film by using different starch sources (corn starch and amylomaize). Starch suspensions were cold gelatinized with NaOH; either glycerol or sorbitol were used as plasticizer. Films were characterized by Differential Scanning Calorimetry (DSC), X-ray diffraction, Scanning Electron Microscopy (SEM) and gas (CO_2 and O_2) permeabilities. SEM observations showed that plasticizer addition was necessary for film integrity. The evaluation of film formation by DSC indicated that cold gelatinization was the main factor of thermal transitions. Film crystallinity was analyzed by DSC and X-ray diffraction during storage. For all tested formulations, film crystallinity increased while gas permeability decreased during storage. Films containing glycerol or sorbitol showed a lower crystalline/amorphous ratio by X-ray diffraction and DSC than unplasticized films. Amylomaize films with higher crystalline/amorphous ratio gave lower gas permeabilities than the corresponding corn starch films; films containing sorbitol showed lower permeability values than those containing glycerol.

Rindlav- Westling *et al.* (2002) were prepared films from potato starch, amylose, and amylopectin and blends by solution casting and examined using X-ray diffraction, light microscopy, transmission electron microscopy, and differential scanning calorimetry. Amylose films had a relative crystallinity of about 30% whereas amylopectin films were entirely amorphous. Blending of amylose and amylopectin resulted in films with a considerably higher degree of crystallinity than could be predicted. This is explained by cocrystallization between amylose and amylopectin and possibly by crystallization of amylopectin. The crystallized material gave rise to an endotherm detected with differential scanning calorimetry. The enthalpy and peak temperature of the transition also increased as the water content decreased. When the amylose proportion in the blends was low, separate phases of amylose and amylopectin were observed by light microscopy. At higher amylose proportions, however, the phase separation was apparently prevented by amylose gelation and the formation of continuous amylose network. The amylose network in the films, observed with transmission electron microscopy, consisted of stiff strands and open pores and became less visible as the amylose proportion decreased. The water content of the films was dependent on the microstructure and crystallinity.

Alves *et al.* (2007) studied the effect of glycerol and amylose enrichment on cassava starch films properties. This study showed the mechanical and barrier properties of cassava films were influenced by glycerol and amylose contents. The amylose enrichment originated stronger films and this could be explained because during drying of film-forming solutions, water evaporates, allowing the formation of starch network, and during this stage, the proximity of starch chains induced by higher amylose contents could facilitate the formation of matrix with more polymer content per area. Amylose network structure is very stable, with strong molecular orientation, forming film denser than amylopectin films. However, cassava starch films exhibited increased water vapor permeability values at increased amylose addition what could be related with a higher number of free hydroxyl groups, that might enhanced interactions with water, favoring water vapor transmission through the films.

Yun and Yoon (2009) were prepared starch/PVA-blended films from commercial starches with the different amylose contents, PVA, and additives by using a simple mixing process and casting method. Glycerol (GL), sorbitol (SO), tartaric acid (TA) and citric acid (CA) were used as additives. The physical properties such as tensile strength (TS), elongation at break (%E), degree of swelling (DS), and solubility (S) with amylose contents of starches were investigated. The amylose content of starches was analyzed by the colorimetric method. Thermal analysis of films was measured by using a differential scanning calorimeter (DSC). Finally, biodegradability of the films was evaluated in a 6-month soil burial test. The examination of the physical properties of biodegradable films indicates that with the higher amylose contents of starch used in preparing the film, TS and DS of films increased, whereas %E and S decreased. The additives containing both carboxyl and hydroxyl groups, i.e. TA and CA, improved the physical properties of films. A thermal analysis of films revealed that the glass transition temperature (Tg) rose because of the increased crystallization of films with the increasing the amylose contents. Also, films degraded rapidly at the beginning and slow degradation took place until the experiment was completed. The films showed 50-80% degradation.

6.3 Type and Content of Lipids

A large number of lipidic compounds such as oils and animal and vegetable fats, acetoglycerides, natural waxes, or surfactants have been used to produce edible films or coatings. They are used basically on account of their excellent humidity barrier properties, either as a protective surface layer, or protective barrier between two parts of a heterogeneous foodstuff. They may also fulfill other functions, such as acting as supports for liposoluble additives, protecting the surface from abrasion or scorching of fruits during transport or storage the surface from abrasion or scorching of fruits during transport or storage (Hardenburg, 1967).

Edible films are generally prepared using biological polymers such as proteins, polysaccharides, lipids and resins. Natural biopolymers have the advantage over synthetic polymers in that they are biodegradable and renewable, as well as edible. Each group of materials has certain advantages and disadvantages. Protein and polysaccharide films generally provide a good barrier against oxygen at low and intermediate relative humidity, and have good mechanical properties, but their barrier against water vapor is poor due to their hydrophilic nature (Guilbert, 1986; Kester and Fennema, 1986). In contrast, films prepared with lipid materials have good water vapor barrier properties, but are usually opaque and relatively inflexible. Lipid compounds commonly used for the preparation of lipid-based edible films and coatings include neutral lipids, fatty acids, waxes, and resins (Kester and Fennema, 1986; Hernandez, 1994). One way to achieve a better water vapor barrier is to produce a composite film by adding hydrophobic components such as lipid and wax materials. A composite hydrocolloid-lipid film or coating is particularly desirable, since it has acceptable structural integrity imparted by the hydrocolloid materials and good water vapor barrier properties contributed by the lipid materials (Greener and Fennema, 1989b). The efficiency of the lipid materials in composite films and coatings depends on the nature of the lipid used, in particular on its structure, chemical arrangement, hydrophobicity, and physical state (e.g. solid or liquid), and on the lipid interactions with the other components of the film, such as proteins and polysaccharides (Rhim and Shellhammer, 2005).

Haggenmaier and Shaw (1990) tested the effect of stearic acid concentration on the WVP of HPMC composite films. The WVP of the composite films decreased about 300 times with the addition of 40-50% of stearic acid. With less stearic acid the permeability was higher. The permeability was sharply dependent on the stearic acid content and decreases slowly with stearic acid concentration increases. However, excessive levels of lipid materials result in the film becoming brittle. Therefore, stearic acid concentration of about 20% seems to be the optimum for modifying the water vapor barrier properties.

Yilmaz *et al.* (1999) studied the effect of glycerol on the morphology of starch- sunflower oil composites. Glycerol was used as a plasticizer and lecithin was used as an emulsifier, to improve the emulsion stability. Increasing glycerol concentration in the samples resulted in different dispersed phase morphologies in the starch-sunflower oil composites. It was observed that increasing glycerol concentration resulted in a decrease in the particle sizes and polydispersity of the oil droplets. The two determining parameters on the formation of the dispersed phase during emulsification; viscosity and interfacial tension between two phases, were investigated to evaluate the effect of glycerol on the system. Since glycerol was found not to affect the viscosity of the aqueous starch phase, it was concluded that glycerol affects the dispersed phase morphology due to its effect on interfacial tension between the oil and aqueous starch phase, during emulsification.

Gallo *et al.* (2000) studied the effect of the physicochemical characteristics of the lipid phase (hydrophobicity and physical state) and of its distribution within the methylcellulose-matrix on the mechanical and water vapor barrier film properties was investigated. The nature of the lipid phase had little influence on mechanical properties of emulsified films, but had a substantial effect on the water vapor barrier efficiency. Alkanes had moisture barrier efficiencies better than those of triglycerides. The solid–liquid ratio of the lipid phase had little influence on film mechanical properties because totally liquid lipids had a lubricant effect. Solid fat content (SFC) did not affect significantly ($p \geq 0.05$) the moisture barrier efficiency, because water vapor could pass through the hydrophilic methylcellulose. SFC increased the opacity of film. The distribution of the fat globules into the methylcellulose matrix affected only the elongation of films.

Yang and Paulson (2000) studied gellan/lipid composite films through emulsification and determine effect of lipid (beeswax and 1:1 blend of stearic-palmitic acids) on the moisture barrier, mechanical and optical properties of the films. Addition of the lipids to gellan films significantly improved the WVP ($p \leq 0.05$), but lowered the mechanical properties and caused the films to become opaque. Beeswax was more effective than stearic-palmitic acids in reducing the WVP and films with beeswax showed better mechanical properties overall than those with stearic-palmitic acids.

Anker *et al.* (2002) studied the water vapor barrier of whey protein isolate WPI films could be improved by adding a lipid and make laminate and emulsion films. The laminate whey protein-lipid film decreased the water vapor permeability WVP 70 times compared with the WPI film. The WVP of the emulsion films was half the value of the WPI film and was not affected by changes in lipid concentration, whereas an increased homogenization led to a slight reduction in WVP. The mechanical properties showed that the lipid functioned as an apparent plasticizer by enhancing the fracture properties of the emulsion films. This effect increased with homogenization. The maximum strain at break was 117% compared with 50% for the less-homogenized emulsion films and 20% for the pure WPI films.

Phase-separated emulsion films were produced with a concentration gradient of fat through the films, but pure bilayer films were not formed.

Morillon *et al.* (2002) reported that moisture transfers inside food products could be controlled or limited by the use of edible films. There are usually based on hydrophobic substances such as lipid to improve barrier efficiency. Water permeability of films is affected by many factors, depending on both the nature of barrier components, the film structure (homogeneous, emulsion, multilayer, ect.), crystal type, shape, size and distribution of lipids, and thermodynamics such as temperature, vapor pressure, or the physical state of water in contact to the films.

Srinivasa *et al.* (2007) studied the effect of plasticizers and fatty acids on mechanical and permeability characteristics of chitosan films. Chitosan films were prepared by blending with polyols (glycerol, sorbitol and polyethylene glycol (PEG)) and fatty acids (stearic and palmitic acids) and their mechanical and barrier properties studied. The tensile strength of the blended films decreased with the addition of polyols and fatty acids, whereas the percent elongation was increased in polyol blend films, but fatty acid blend films showed no significant differences. Glycerol blend films showed decrease, whereas sorbitol and PEG blend films showed increase in the water vapor permeability (WVP) values. No considerable differences in WVP were observed in fatty acid blend films. Equilibrium moisture content of all the films was low at lower water activity (aw), but increased at higher aw. Eight moisture sorption models were applied to experimental data. The various constants determined by linear fitting of the sorption equation with R^2 values were in the range of 0.92-0.99. The GAB model showed the best fit over the entire range of a_w with root mean square error (RMSE) of 2.75 and 6.36 for native and blended chitosan films, respectively. While the BET model was applicable in the low a_w (0.11-0.45) range with RMSE values ranging from 1.95 to 9.55.

Fabra *et al.* (2008) were study to improve the tensile properties and water vapor permeability of sodium caseinate films by adding adequate amount and kind of plasticizers (glycerol or sorbitol) and lipids (Beeswax and oleic acid). Oleic acid, pure or mixed with beeswax, has a plasticizing effect in the films, increasing their elasticity, flexibility and stretch ability, and reduce water vapor permeability with respect to sodium caseinate films.

Vargas *et al.* (2009) prepared edible films based on high molecular weight chitosan (CH) and different concentrations of oleic acid (OA). Film-forming dispersions (FFD) were characterized in terms of rheological properties, surface tension, particle size distribution and z-potential. In order to study the impact of the incorporation of OA into the CH matrix, the water sorption isotherms, water vapor permeability (WVP), mechanical properties, and optical properties of the dry films were evaluated. Results showed that the increase in OA promoted changes in the size and surface charge of the FFD particles, which had an impact on the rheological properties of the FFD. As regards the film properties, the higher the OA content, the lower the WVP and the moisture sorption capacity. In general, the addition of OA into the CH matrix leads to a significant increase in gloss and translucency and a decrease in the tensile strength, elongation at break and elastic modulus of the composite films.

Jimenez *et al.* (2010) studied the effect of lipid self-association on the microstructure and physical properties of hydroxylpropyl-methylcellulose edible films containing fatty acids. Edible films based on HPMC containing lauric (LA), miristic (MA), palmitic (PA), stearic (SA) and oleic (OA) acids were obtained with 1:0.15 polymer–lipid ratio. The particle size distribution and rheological behaviour of the film-forming emulsions were studied, as well as the cross-section and surface microstructure of dried films. The effect of the film microstructure on its mechanical, optical and water barrier properties was analyzed. Saturated LA, MA and PA formed bigger lipid micellar structures than SA and OA in the HPMC aqueous system, which grew notably during film drying, giving rise to crystallized lipid layers in the films which were not observed for SA and OA. Laminar structures improved the moisture barrier properties, but resulted in more brittle, less stretchable, more opaque and less glossy films, depending on the particle size.

Zahedi *et al.* (2010) prepared film from pistachio globulin protein (PGP), saturated fatty acids, and an emulsifier using the emulsification technique. The water vapor permeability (WVP) of the emulsified films was reduced by approximately 37-43% by fatty acid addition. The effect of fatty acid on the oxygen permeability (OP) of PGP films was indirectly determined as the oil peroxide value. The OPs of the emulsified films were lower than those of a PGP film without fatty acid, but the differences were not significant ($p \leq 0.05$). The mechanical properties of PGP films were also affected by fatty acid addition; the ultimate tensile strength (UTS) was diminished, and elongation at breaking (E) decreased considerably (35-70%). Furthermore, the incorporation of fatty acid increased the opacity of the films. Finally, differential scanning calorimetry showed that the glass transition temperature (Tg) of the PGP film was ~127 °C and was not considerably affected by fatty acid addition.

Objectives

1. To prepare and characterize of starch nanocrystals from rice starch.
2. To study the effect of content of starch nanocrystals from rice starch on the properties of rice starch films
3. To study the effect of types and concentration of lipid on water vapor permeability and properties of rice starch films reinforced with starch nanocrystals
4. To study the effect of relative humidity on the properties of rice starch film reinforced with starch nanocrystals
5. To study the potential application of rice starch film reinforced with starch nanocrystals for food application

CHAPTER 2

MATERIAL AND METHODS

1. Materials

1.1 Raw Materials

Rice grains of the “Chiang Phatthalung” were purchased from local grocery in Phatthalung province. Hommy cracker rice bran oil and margarine were purchased from local grocery in Songkla province. Commercial grade sorbitol, palmitic acid, and poly ethylene film (PE) was purchased from Vidyasom Co. Ltd. (Thailand).

1.2 Chemical Reagent

Analytical grade (AR) lithium chloride, megnesium chloride, megnesium nitrate, sodium chloride and potassium nitrate for saturated salt solutions (at 33% RH, 50% RH, 75% RH and 90% RH and 98% RH, respectively), sulfuric acid 2, 3, 4 and 5 M, sodium chloride 5%, sodium hydroxide 0.35% and commercial grade ethanol 70% purchased from High Science Co. Ltd. (Thailand).

2. Instruments

Instruments	**Model**	**Company/City/Country**
Magnetic stirrer	RO 10 power	IKA LABORTECHNIK Stanfen, Germany
Stirrer	RW 20n	IKA LABORTECHNIK Stanfen, Germany
Homogenizer	T 25	Ultra Terrax, Malaysia Memmert, Schwabach, Germany
Blender	MX-T700GN	National, Japan
Scanning electron microscope	JSM-5800 LV	JEOL, Tokyo, Japan

Instruments	Model	Company/City/Country
Thermogravimetric analyzer	Perkin-Elmer TGA-7	Norwalk, Conn., U.S.A.
Universal testing machine	LR 30K,	Lloyd Instruments Ltd,
Differential scanning calorimeters	Perkin-Elmer DSC-7	Hampshire, UK
Dynamic mechanical thermal analyzer	DMTA-V	USA Rheometric Scientific,
Laser particle size analyzer	LS-230	US
X-ray diffractometer	X'Pert MPD	Coulter, U.S.
Hunter associates laboratory	Inc., VA	Philips, Netherland,USA

3. Preparation of Raw Materials

Rice starch (Chiang Phatthalung) were prepared by soaked rice grains with water (1:2 w/v) for 6-12 hour then poured water and were wet-milled. The slurry was heated at 55°C and the sheet was milled and sieved through 106 mesh screen. Rice flour was then collected for making rich starch according to the method of Sawai and Morita (1968), rice flour granules were mixed with 5% sodium chloride solution (a ratio of rice starch/NaCl such as 1:4). The suspensions were then continuously stirred. After 2 hours the suspension was centrifuged and the slurry was mixed with 70% ethanol solution (a ratio of rice starch/ethanol such as 1:4). The suspensions was then continuously stirred. After 2 hours the suspension were centrifuged and the slurry was mixed with 0.35% sodium hydroxide solution (a ratio of rice starch/NaOH such as 1:5). The suspensions were then continuously stirred for 12 hours. After 10 hours the suspension were washed by successive centrifugations in distilled water for 3 times and adjust pH = 7. The slurry was heated at 55 °C and the sheet was milled and sieved through 106-mesh screen to get a rice starch. The results were test as followed;

- Morphology of starch granule by Scanning Electron Microscope
- Crystals content of starch granule by X-ray diffractometer
- Gelatinization temperature by Differential Scanning Calorimeter
- Chemical composition of starch;

- Amylose content by AOAC, 1999
- Protein content by AOAC, 1999
- Lipid content by AOAC, 1999
- Moisture content by AOAC, 1999
- Ash content by AOAC, 1999

4. Effect of Concentration of Sulfuric Acid on Properties of Starch Nanocrystals

4.1 Preparation of Starch Nanocrystals

Rice starch nanocrystals dispersion was prepared by a previously described method (Angellier *et al.*, 2004) with minor modifications. Native rice starch granules 36 g were mixed with 250 mL of sulfuric acid (H_2SO_4) solution (at 2, 3, 4 and 5 M) in a 500 mL Erlenmeyer flask. The suspensions were then continuously stirred at 100 rpm under 40 °C. After 5 days of hydrolysis, the suspensions were washed by successive centrifugations in distilled water until neutrality was achieved. This suspensions was freeze-dried using a freeze dryer. The resulted dried starch nanocrystals were tested as followed;

- The hydrolysis yield (wt %) was calculated as the ratio between the weight of freeze-dried hydrolyzed particles and the initial weight of native granules.
- Morphology of starch nanocrystals by Scanning Electron Microscope
- Crystals content of starch nanocrystals by X-ray diffractometer
- Size of starch nanocrystals by Laser particle size analyzer

Selected optimum condition from size (1-100 nm) and hydrolysis yield (wt %)

5. Effect of Content of Starch Nanocrystals on Properties of Rice Starch Films

5.1 Film Preparation

Starch solution with concentration of 3% (w/v) was prepared by dispersing rice starch in distilled water and heating the mixtures and stirring until it gelatinized (85 °C for 5 min. It was cooling to 45±2 °C. Sorbitol was added as 40% of starch. Subsequently, the starch nanocrystals dispersion was added at 5, 10, 15, 20, 25 and 30% of starch and stirred for 2 min. The mixtures were cast onto flat, leveled, non-stick trays to set. Once set, the trays were held overnight at 55 °C for 10 h undisturbed, and then cooled to ambient temperature before peeling the films off the plates. Film samples were stored in plastic bags and held in desiccators at 60 % RH for further testing. All treatments were made in triplicate. The resulted films were tested as followed;

- Film thickness by digital micrometer
- Tensile strength and elongation at break by ASTM D882-91 (ASTM, 1995)
- Water vapor permeability (McHung *et al.*, 1993)
- Morphological properties of films by Scanning Electron Microscope
- Differential Scanning Calorimeter
- Degradation temperature by Thermo Gravimetric Analyzer
- Dynamic Mechanical Thermal Analyzer

Selected optimum condition from mechanical properties (tensile strength and elongation at break) and thermal properties.

6. Effect of Types and Concentration of Lipid on Water Vapor Permeability and Properties of Rice Starch Films

6.1 Film Preparation

Starch solution with concentration of 3% (w/v) was prepared by dispersing rice starch in distilled water and heating the mixtures with stirred until it gelatinized (85 °C for 5 min), and then cooling to 45±2 °C. Sorbitol was added 40% of starch solution. Subsequently, the starch nanocrystals dispersion was added at 15% of starch and stirred 2 min. The mixtures was added lipid (palm oil, rice bran oil and margarine) at 3, 6, 9, 12 and 15 % of starch solution and homogenizer 6,000 rpm/min. After mixing, the mixers was degassed under vacuum then were cast onto flat, leveled, non-stick tray to set. Once set, the trays were held overnight at 55 °C for 10 h undisturbed, and then cooled to ambient temperature before peeling the films off the plates. Film samples were stored in plastic bags and held in desiccators at 60% RH for further testing. All treatments were made in triplicate. The resulted films were tested as followed;

- Film thickness by digital micrometer
-.Tensile strength and elongation at break by ASTM D882-91 (ASTM, 1995)
- Water vapor permeability (McHung *et al.*, 1993)
- Morphological properties of films by Scanning Electron Microscope
- Water uptake (Sreekala and Thomas, 2003)

7. Effect of Relative Humidity on the Properties of Rice Starch Film

Effects of relative humidity on the properties of rice starch film were determined by placing rice starch films into a controlled humidity environment at a constant temperature until equilibrium. After drying at 75 °C and < 1 mmHg pressure for 24 h (EYELA ^{TM}, Model VOS-300VD, Japan), the films were placed into environments of various relative humidities above salt solutions and constant temperature at 27±2 °C in desiccators. The relative humidities were 33% RH (megnesium chloride), 50% RH (megnesium nitrate), 75% RH (sodium chloride) and 90% RH (potassium nitrate). The sorption experiments were carried out by keeping approximately 1,000 mg of films (2 x 2 cm for weighing and 1.5 x 12 cm for Tensile strength) in desiccators, removing at frequent intervals and weighing until they reach constant weight (within ± 5%), after that, the films were tested by tensile strength.

8. Application of Rice Starch Film

Three types of rice starch films, comprising of rice starch film, rice starch film reinforced with starch nanocrystals and rice starch film reinforced with starch nanocrystals and containing palm oil and poly ethylene film (PE). All films were wrapped around crackers and conditioned at 75% RH and room temperature (27±3 °C) by placing them in desiccators over a saturated solution of sodium chloride, removing at frequent intervals (3 days). The resulted crackers were tested as followed;

- Texture of crackers by Instron texturometer
- Moisture content by AOAC, 1999

- Water activity (a_w)
- Color by Hunter Associates Laboratory

9. Determination of Rice Starch Films Properties

9.1 Conditioning

All films were conditioned prior to subjecting them to permeability and mechanical tests according to a standard method, D 618-61 (ASTM, 1993a). The films were tested for water vapor permeability (WVP), tensile strength (TS), and elongation (E) were conditioned at 60% RH and 27±2°C by placing them in desiccators over a saturated solution of $Mg(NO_3)_2.6H_2O$ for 72 h or more. For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators.

9.2 Film Thickness

Thickness of the films was measured with a precision digital micrometer (Digimatic Indicator, Mitutoyo Corporation, Japan) to the nearest 0.0001 (±5%) at five random locations on the film. The mean thickness values for each sample were calculated and used in water vapor permeability (WVP) and tensile strength (TS) calculations.

9.3 Water Vapor Permeability (WVP)

The gravimetric modified cup method based on ASTM E96-92 (McHung *et al.*, 1993) was used to determine the WVP of films. The test cups were filled with 20 g of silica gel (desiccant) to produce a 0% RH below the film. A sample was placed in between the cup and the ring cover of each cup coated with silicone sealant (high vacuum grease, Lithelin, Hanau, Germany) and held with four screws around the cup's circumference. The air gap was at approximately 1.5 cm between the film surface and desiccant. The rated water vapor transmission (WVTR) of each film was measured at 60±2% RH and 25±2°C. After taking the initial weight of the test cup, it was placed into a growth chamber with an air velocity rate of 135 m/min

(Model KBF115, Contherm Scient, Lower Hutt, New Zealand). Weight gain measurements were taken by weighing the test cup to the nearest 0.0001 g with an electronic scale (Satorious Corp.) every 3 h for 18 h. A plot of weight gained versus time was used to determine the WVTR. The slope of the linear portion of this plot represented the steady state amount of water vapor diffusing through the film per unit time (g/h). The WVTR was expressed in gram units, per square meter, per day. Steady state over time (slope) yielded a regression coefficient of 0.99 or greater. Six samples per treatment were tested. The WVP of film was calculated by multiplying the steady WVTR by the film thickness and dividing that by the water vapor pressure difference across the film.

9.4 Mechanical Properties

Tensile strength (TS) was measured with a LLOYD Instrument (Model LR30K, LLOYD Instruments Ltd., Hampshire, England) as per ASTM D882-91 Standard method (ASTM, 1993b). Ten samples, 1.5 cm x 12 cm, were cut from each film. The initial grip separation and crosshead speed were set at 70 mm and 30 mm/min, respectively. Tensile strength was calculated by dividing the maximum force by the initial specimen cross-sectional area, and the present elongation at break (E) was calculated as follows:

$$E = 100 \times (d_{\text{after}} - d_{\text{before}}) / d_{\text{before}}$$

Where, d was the distance between grips holding the specimen before and after the break of the specimen.

9.5 X-ray Diffraction

X-ray patterns of starch powders, starch nanocrystals, starch film and starch film reinforced with starch nanocrystals were analyzed. This was done by using an X-ray diffractometer (Philips X, Pert MPD, Japan) with Cu Kα radiation at a voltage of 40 kV and 30 mA. The samples were scanned between $2\theta = 3\text{-}40°$ with a scanning speed of 2°/min. Prior to testing the samples were dried and stored in desecrator. The relative crystallinity index was calculated using equation 1 (Koksel *et al.*, 1993; Muller *et al.,* 2009).

$$\%X_c = \frac{A_c}{A_t} = \frac{A_c}{A_c + A_a}$$

Where A_c is the crystalline area, A_a is the non-crystalline area and A_t is total area.

9.6 Scanning Electron Microscope

The morphology of the starch, starch nanocrystals, starch film and starch film reinforced with starch nanocrystals, were observed with Scanning electron microscope (SEM, JSM-5800 LV, JEOL, Tokyo, Japan)

9.7 Differential Scanning Calorimeter (DSC)

The thermal properties of rice starch films were analyzed with a Perkin-Elmer DSC-7 (Norwalk, Conn., U.S.A.) equipped with an intra-coolant Thermal Analysis Controller TAC7/DX (Perkin-Elmer). Samples (approx. 20 mg each, db) were weighed into stainless steel pans (Perkin-Elmer) designed to withstand high pressures. The stainless steel pan was sealed with an O-ring, and allowed to reach equilibrium of the moisture overnight. An empty DSC pan was used as a reference pan. The heating rate was programmed by holding the heat at -50°C for 1 min, followed by ramping the temperature rang 0f -50 °C to 180 °C at a rate of 20 °C/min, and holding it at 180 °C for 1 min. The measurements were made at least at least in duplicate for each treatment.

9.8 Thermo Gravimetric Analysis (TGA)

Thermo gravimetric analysis was performed to study the degradation characteristics of the films. The thermal stability of each sample was determined using a Perkin-Elmer, TGA7 with a heating rate of 10 °C/min in a nitrogen environment. It had a weighting capacity of 0.1 g. Samples were heated from room temperature to 500 °C.

9.9 Dynamic Mechanical Thermal Analysis (DMTA)

The small deformation analysis of the films was performed in tension in a dynamic mechanical thermal analyzer (Rheometric Scientific, DMTA V). The tested film strips were cut into small strips (20 x 5 mm) and champed in the instrument with an initial grip separation 5.5 mm. The films were subjected to a sinusoidal strain on top of static deformation. The testing was conducted at a constant frequency of 1 Hz and a strain of 0.02% over a temperature range of 50 to 200 °C, at a heating rate of 5 °C/min. The measurements at each experimental point were done at least in triplicates. Dynamic mechanical spectroscopy was employed within the linear viscoelastic regime to determine T_g. The storage and loss modulus (E’ and E’’) and loss tangent (tan δ = ΔE’/E’’) were measured as a function of temperature at a constant frequency and a selected heating or cooling rate.

9.10 Water Uptake

The kinetics of water absorption was determined for rice starch films addition lipid. The dimensions of specimens were 2 x 2 cm. The films were therefore supposed to be thin enough so that the molecular diffusion was considered to be one-dimensional. Samples were first dried overnight at 100° C. After weighing, they were condition at room temperature in desiccators containing sodium sulfate to ensure a RH ratio of 98%. The conditioning of samples in high moisture atmosphere were preferred to the classical technique of immersion in water because starch is very sensitive to liquid water and can partially dissolve after long time exposure to water. The sample were removed at specific intervals and weighed a using a four-digit balance. The water content or water uptake of the samples was calculated as follows:

$$\%\ \text{Water uptake} = \frac{M_t - M_0}{M_0} \times 100$$

9.11 Color

A CIE colorimeter (Hunter Associates Laboratory, Inc., VA, USA) was used to determine the film L*, a* and b* color value [(L* = 0 (black) to 100 (white); $a^* = -60$ (green) to +60 (red); and $b^* = -60$ (blue) to +60 (yellow)]. The standard plate (calibration plate CX0384, $L^* = 92.82$, $a^* = -1.24$, and $b^* = 0.5$) was used as a standard. Total color difference (ΔE^*_{ab}), hue angle (H), and chroma (C) were calculated using the following equation:

$$\Delta L^* = L^*_{\text{sample}} - L^*_{\text{standard}},\ \Delta a^* = a^*_{\text{sample}} - a^*_{\text{standard}},\ \Delta b^* = b^*_{\text{sample}} - b^*_{\text{standard}}$$

$$\Delta E_{ab}^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$$

$$C = [(a^*)^2 + (b^*)^2]^{0.5}$$

$$H = \tan^{-1}(b^*/a^*) \text{ when } a^* > 0 \text{ and } b^* > 0$$

$$H = 180^o + \tan^{-1}(b^*/a^*) \text{ when } a^* < 0$$

$$H = 360^o + \tan^{-1}(b^*/a^*) \text{ when } a^* > 0 \text{ and } b^* < 0$$

10. Experimental Design and Statistical Analysis

A completely randomized experimental design was used to determine the character of composite films. Analysis of variance (ANOVA) was used to compare mean differences of the samples. If the differences in mean existed, multiple comparisons were performed using Duncan’s Multiple Range Test (DMRT).

CHAPTER 3

RESULTS AND DISCUSSION

1. Characteristics of Rice Starch

1.1 The Chemical Compositions of Rice Starch

The chemical compositions of starch were found to be 30.40, 12.19, 0.56, 0.51 and 0.21 of amylose, moisture, protein, fat and ash, respectively (Table 1).

Table 1. Compositions of rice starch

Compositions	Amount (%)
Amylose	30.40 ± 0.01
Moisture	12.19 ± 0.03
Protein	0.56 ± 0.02
Fat	0.51 ± 0.01
Ash	0.21±0.07

1.2 Morphology of the Rice Starch Granules

SEM photograph of rice starch granules are shown in Figure 3. Rice starch granules have a smooth surface but angular and polygonal shapes with a size of about 5 µm (Figure 3). Rice starch granules are the smallest known to exist in cereal grains, with the size reported to range from 3-8 µm in the mature grain measured by SEM. There is some variation in starch granule size between different rice genotypes (Sodhi and Singh, 2003).

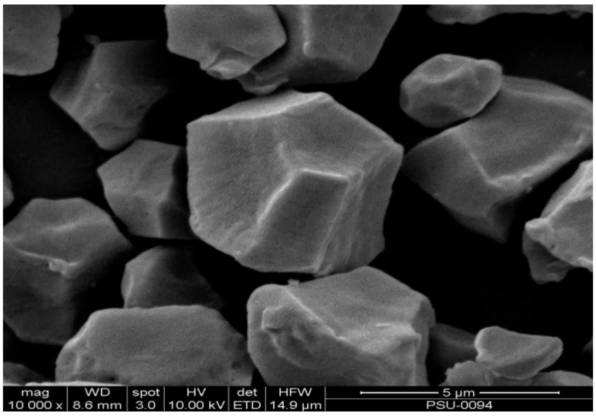

Figure 3. SEM photograph of rice starch granules (scale bar: 5µm).

1.3 Rice Starch Crystallinity

The X-ray diffractograms of the rice starch powder is shown in Figure 4. As observed, the rice starch powders have the characteristic typical A-type crystalline polymorph like other cereal starches (Wong *et al*, 2003; Kristo and Biliaderis, 2007). This result shows a reasonable agreement with experimental of Bourttoom and Chinnan (2008). Rice starch powder exhibited three strong intensity peaks at 16.8, 18.0 and 22.7°. The crystallinity values of rice starch were estimated as 23.19%.

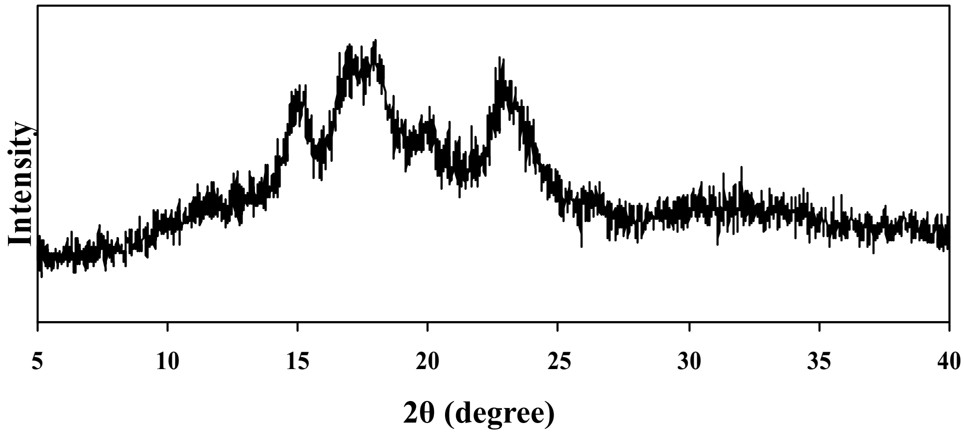

Figure 4. X-ray diffractograms of rice starch powder.

1.4 Gelatinization Temperature of Rice Starch

Gelatinization is the process that takes place when starch is heated in the presence of water, resulting in the irreversible disruption of molecular order within a starch granule. Evidence of this loss of order can be seen by irreversible granule swelling, loss of birefringence, and loss of crystallinity. For gelatinization to occure the regions of amorphous starch must first melt or undergo glass transition (Slade and Levine, 1989). Gelatinization temperature of rice starch of 79.67 °C was measure by Differential scanning calorimetry (DSC).

2. Effect of Concentration of Sulfuric Acid on Properties of Starch Nanocrystals

2.1 Hydrolysis Yield and Size of Starch Nanocrystals

The hydrolysis yield and size of the studied starch nanocrystals along with acid concentration are given in Table 2. The hydrolysis yield (wt %) was calculated as the ratio between the weight of freeze-dried hydrolyzed particles and the initial weight of native granules, and size of starch nanocrystals was measured by Laser particle size analyzer. No significant differences ($p>0.05$) were observed in hydrolysis yield but showed significant ($p>0.05$) in size of starch nanocrystals hydrolysis by sulfuric acid concentration at 2 and 3M. While, hydrolysis yield and size of starch nanocrystals hydrolysis by sulfuric acid concentration at 4M showed significant ($p>0.05$) decrease of hydrolysis yield, but higher size of starch nanocrystals than starch nanocrystals hydrolysis by sulfuric acid concentration at 2 and 3M. However, starch nanocrystals hydrolysis by sulfuric acid concentration at 3M showed higher percentage of size in nanometer range than other hydrolysis conditions about 50%. Angellier *et al.* (2005) reported that starch nanocrystals are observed in the form of aggregates having an average size around 4.4 μm, as measured by laser granulometry and the freeze-drying process most probably induced an aggregation phenomenon of the particles, leading to an underestimated value of the specific surface. In addition to, the results demonstrated that using 5M of H_2SO_4 leads to complete dissolution of rice starch. Therefore, 3M of H_2SO_4 was selected for starch nanocrystals preparation by sulfuric acid hydrolysis concentration at 3M for nanocomposite applications to be considered.

Table 2. Hydrolysis yield and size of starch nanocrystals

Acid concentration (M)	Yield (%)	Size (nm)	Percentage
Sulfuric acid 2 M	10.19 ± 0.06^{a}	86^{a}	20
Sulfuric acid 3 M	10.13 ± 0.05^{a}	78^{b}	50
Sulfuric acid 4 M	2.06 ± 0.04^{b}	119^{c}	10

2.2 Characteristics of Starch Nanocrystals

2.2.1 Morphology of Starch Nanocrystals

SEM photograph of rice starch granules and starch nanocrystals are shown in Figure 5. Rice starch granules have a smooth surface but angular and polygonal shapes with a size of about 5 µm (Figure 5A). After the acid treatment process, the granules have been destructured and degraded with average size range of 3.97-9.90 µm measured by Laser particle size analyzer (Figure 5B-C). The starch nanocrystals showed very close morphological characteristics to the native rice starch.

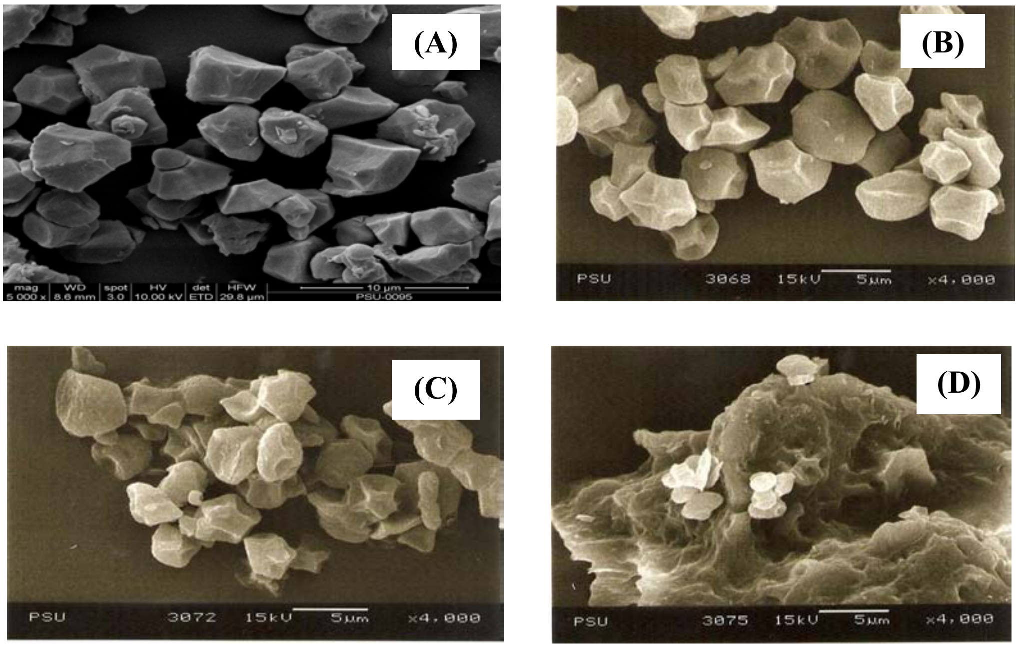

Figure 5. Scanning electron microscope (SEM) of rice starch (A), starch nanocrystals hydrolysis by sulfuric acid at concentration 2 M (B), 3 M (C) and 4 M (D).

2.2.2 Starch Nanocrystals Crystallinity

The XRD patterns of rice starch powder and rice starch nanocrystals are shown in Figure 6, and the corresponding data are listed in Table 3. Rice starch powder, have the characteristic typical A-type crystalline pattern with strong reflection at 16.8, 18.0 and 22.7°. Following the acid hydrolysis treatment, obvious changes happened in diffraction pattern of starch nanocrystals. The results showed that diffraction peak of starch nanocrystals were sharper than diffraction peak of rice starch powder. The sharper diffraction peak is an indication of higher crystallinity value in structure of the starch nanocrystals. The crystallinity values were estimated as 23.19, 30.19, 30.36 and 30.17 %, for rice starch powder and starch nanocrystals hydrolysis by sulfuric acid at concentration 2 M, 3 M and 4 M, respectively. During the acid hydrolysis process, acid attacked amorphous areas more rapidly than the crystalline ones (Putaux *et al.,* 2003).

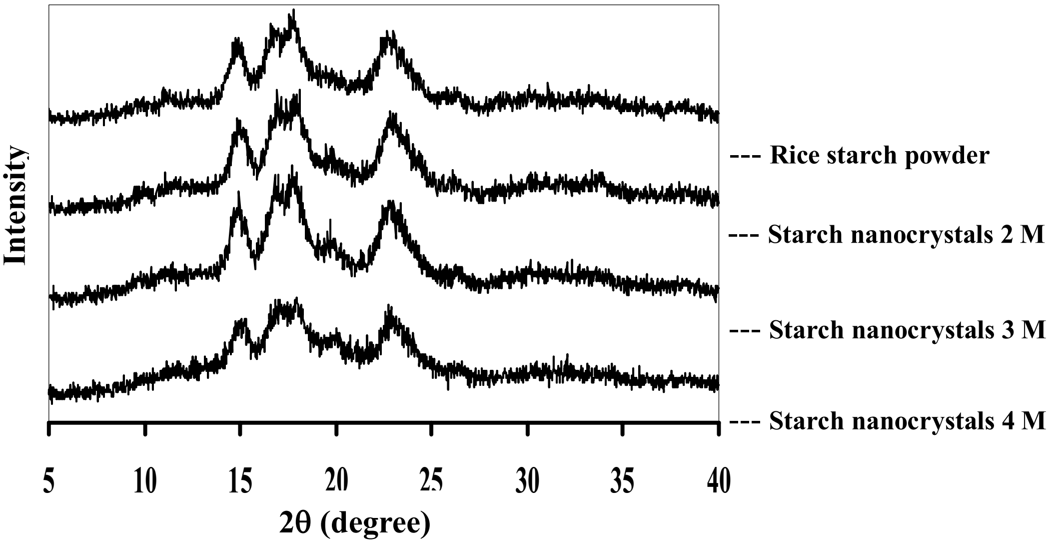

Figure 6. X-ray diffractograms of rice starch powder, starch nanocrystals hydrolysis by sulfuric acid at concentration 2, 3 and 4 M.

Table 3. The data of the XRD diffraction patterns of starch powder, starch nanocrystals (SNC) hydrolysis by sulfuric acid at concentration 2, 3 and 4 M.

Type of polymorphs	Rice starch	SNC 2 M	SNC 3 M	SNC 4 M
peaks	15	14.87	14.856	14.91
	16.8	16.81	16.824	16.679
	18	17.825	17.992	17.77
	22.7	22.7	22.6	22.7
% crystallinity	23.19	30.19	30.36	30.17

From the results, starch nanocrystals prepared by hydrolysis with sulfuric acid concentration at 3 M had the smallest size, higher % crystallinity and % hydrolysis yield compared with other conditions. Therefore, starch nanocrystals hydrolysis by sulfuric acid concentration at 3 M was chosen and used for further study.

3. Effect of Content of Starch Nanocrystals on Properties of Rice Starch Films

The thickness of resulted films were measured with a precision digital micrometer and the average thickness of rice starch films was approximate 0.121±0.01 mm. Mean thickness values of sample were calculated and used in tensile strength (TS), water vapor permeability (WVP) of rice starch films.

3.1 Tensile Strength (TS) and Elongation at Break (E)

Food packaging generally requires high stress with deformation in accord with the intended application. In general, food packaging must be an undeformable material to provide structural integrity or to reinforce food structure, or a deformable film for other applications (Gontard *et al.,* 1992). TS is the maximum tensile stress sustained by the sample during the tension test. If maximum tensile stress occurs at either the yield point or the breaking point it is designated the TS at yield or at break, respectively (ASTM, 1995). The dependence of the TS on the starch nanocrystals content of the rice starch films is shown in Figure 7A. The TS of the control films (unfilled starch nanocrystals) was 7.12 MPa. When the starch nanocrystals was added to rice starch films, the TS of the resulting films increased along with an increase in starch nanocrystals content. It reached a maximum point at about 20 wt% of the starch nanocrystals, thus achieving a TS of 16.43 MPa. However, when the starch nanocrystals content exceeded 20%, the TS of rice starch films tended to decrease (Figure 7A), due to, interface adhesion was not sufficient to withstand the imposed high stretching forces (Lui *et al*., 2010) and the heterogeneous size distribution and agglomerations of starch nanocrystals within in the starch matrix, starch nanocrystals did not act as reinforcing filler in starch film (Chen *et al*., 2009). But it was still much higher than that of the control film.

Owing to the very high specific surface area provided by nanoparticles, the filler-matrix interfacial interactions play a key role in the mechanical properties of nanocomposites (Wetzel *et al.,* 2003). Well distributed particles with an adequate interfacial bonding between filler and matrix allow the effective transfer of stress through a shear mechanism from the matrix to the particles. This can carry the load efficiently and enhance the strength of the composite (Sihna and Okamoto, 2003; Brown and Ellyin, 2005; Abdelmouleh *et al.,* 2005). Similarly Kristo and Biliaderis (2007) reported significant increases in the TS with increases in the starch nano filler content. This illustrated the great reinforcing effect of starch nanocrystals addition on plasticized pullulan films. Such a reinforcing effect was also observed in thermoplastic starch filled with waxy maize starch nanocrystals (Angllier *et al.,* 2005) and in cellulose fiber reinforced thermoplastic starch or silk fibroin (Averous *et al.,* 2001; Noshiki *et al.,* 2002).

Elongation at break (E) is an indication of the film's flexibility and stretchability (extensibility). This is determined at the point when the film breaks under tensile testing and is expressed as the percentage of stretching or extending from the original length of the specimen between the grips of a film. The dependence of E on the starch nanocrystals content of the nanocomposite films is shown in Figure 7B. The E of the rice starch films decreased from 53.46% to a minimum of 2.48% when the starch nanocrystals content increased from 5 to 30%. The experiments showed that the TS and E of nanocomposite films are almost inversely related. This is because the increased brittleness of the composite material induces a decrease in E.

The decrease in E with the rigid filler addition is a well known phenomenon that is related to the differences in the rigidity between the matrix and fillers. Because of the rigid nature of the fillers, most of the system deformation under high strain comes from the polymer. Consequently, the actual deformation experienced by the polymeric matrix only is much greater than the measured deformation of the sample. Thus, the polymer reaches the failure strain limit at a lower total of deformation (Angles *et al.,* 1999; Wetzel *et al.,* 2003). Similar results were observed by Angllier *et al.* (2006) who reported that the unfilled matrix is a viscoelastic material, characterized by a low TS and high E. As expected, the addition of nanocrystals allows the TS to be increased significantly but also leads to a drastic decrease of the E. They compare the reinforcing effect of starch nanocrystals to those of tunicin whiskers, because of the organic nature of both fillers and the similar nature of the matrix material used.

Chen *et al.* (2007) indicated that the addition of pea starch granules could improve the mechanical properties of the composite. This further proves that starch nanocrystals with smaller size disperses more homogeneously and forms stronger interactions with the matrix than native starch granules. Furthermore, (Liu *et al.,* 2010) reported that when the bamboo cellulosic crystals content was increased the TS of starch composite films increased sharply. This was because when the size of particle was greatly reduced, many interesting phenomena occurred as a result of the larger surface area and higher surface energy. However, the E increased when the bamboo cellulosic crystals decreased.

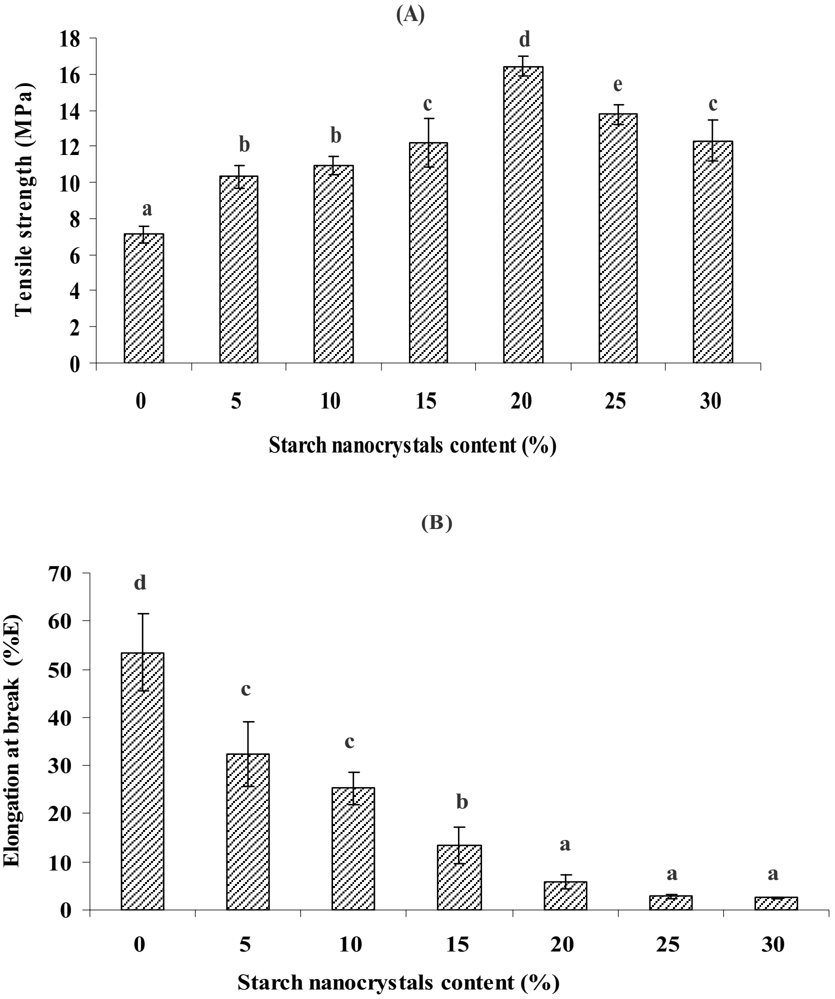

Figure 7.Effect of content of starch nanocrystals on tensile strength (A) and elongation at break (B) of rice starch films. Mean values with different letter are significantly different ($p<0.05$).

3.2 Water Vapor Permeability (WVP)

Water vapor permeability is proportionally constant and assumed to be independent of the water vapor pressure gradient applied across the films. However, hydrophilic materials such as protein films deviate from this ideal behavior due to the interactions of permeating water molecules with polar groups in the film's structure (Haggenmaier and Shaw, 1990). Deviation from the ideal behavior can also be induced by the effects of the structure on materials (Myers *et al.,* 1962). A main function of food packaging is often to avoid or least to decrease moisture transfer between the food and the surrounding atmosphere, or between two components of a heterogeneous food product. Thus the water vapor permeability should be as low as possible (Gontard *et al.,* 1992).

The results demonstrated that rice starch films containing starch nanocrystals at 25% and 30% were broken when measuring the WVP, because of the brittleness of the films. Hence, only 5% to 20% starch nanocrystals were dealt with in this study. The effect of starch nanocrystals content on the WVP of rice starch films is shown in Figure 8. It can be seen that WVP of rice starch films decreased significantly with the addition starch nanocrystals and decreased slowly with an increase in starch nanocrystals content. This was because the water resistance of starch nanocrystals was better than that of the rice starch matrix and the starch nanocrystals dispersed well in the matrix of starch. This provided fewer paths for the water molecules to pass through the composites (Chang *et al.,* 2010). The WVP of the control films (unfilled starch nanocrystals) was 6.25 g·mm/m^2·day·KPa, whereas rice starch film containing starch 20% nanocrystals had the lowest WVP at about 2.56 g·mm/m^2·day·KPa. The presence of such high filler concentrations probably introduced a tortuous path for water molecule to pass through. The longer diffusive path that the penetrant molecules must travel leads to the reduction of permeability (Sihna and Okamoto, 2003). The starch nanoparticles most likely to form a continuous pathway for the diffusion of water molecules through the composite are those where the filler content does not exceed 20% (w/w) at high loading levels. The starch nanocrystals probably forms a compact network structure, making the pathway of the permeate complex and decreasing its diffusion through the composite system (Beall, 2000).

A similar trend was observed in the composites prepare by Kristo and Biliaderis (2007). In addition, Tang *et al.* (2008) indicated that the WVP of starch/polyvinyl alcohol blend films decrease with an increase in nano-SiO_2 content. This might indicate the existence of intermolecular interactions and a decrease in the mobility of both the starch and PVA when the nano-SiO_2 was added to the solution. In addition, the WVP of potato starch film reinforced by chitin nanoparticle was decreased by increasing the chitin nanoparticles concentration (Chang *et al.,* 2010).

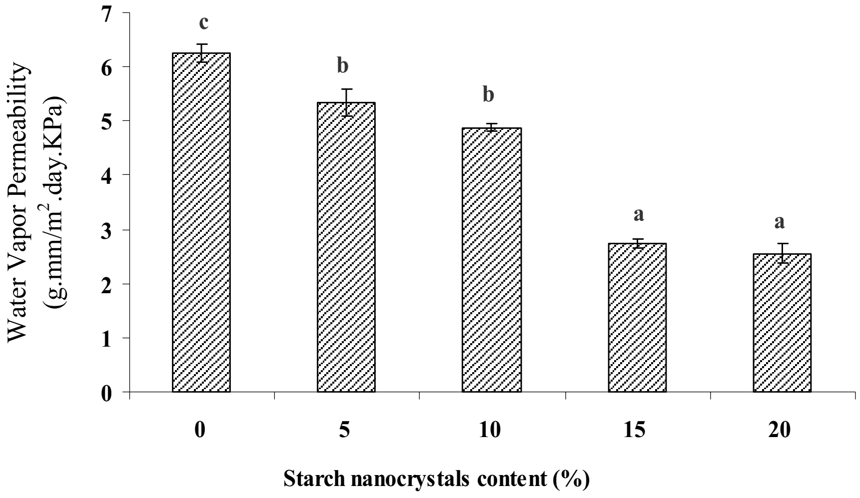

Figure 8. Effect of content of starch nanocrystals on water vapor permeability of rice starch films. Mean values with different letter are significantly different ($p<0.05$).

3.3 XRD Analysis

The XRD patterns of rice starch powder and rice starch nanocrystals are shown in Figure 9. Rice starch powder has a characteristic typical A-type crystalline pattern with strong reflection at 16.8, 18.0 and 22.7°. Following the acid hydrolysis treatment, obvious changes took place in the diffraction pattern of starch nanocrystals. The results showed that the diffraction peaks of the starch nanocrystals were sharper than the diffraction peaks of rice starch powder. The crystallinity values were estimated as 23.19 and 30.36 for rice starch powder and starch nanocrystals, respectively.

During the acid hydrolysis process, acid attacked amorphous areas more rapidly than the crystalline ones (Putaux *et al.,* 2003). This process became very sharp, which is associated with an increase in crystalline regions and with losses in amorphous fractions (Punchongkavarin *et al.,* 2003). Rice starch films showed an obvious diffraction peak at 16.9° and 19.5°, while the crystallinity value was estimated as 13.3% (Table 4). It decreases to nearly half after gelatinization indicating the destruction of crystalline starch granules during gelatinization

(Zhang *et al.,* 2007). When adding starch nanocrystals to the films, the results showed that crystallinity was increased. It reached a maximum point at about 20 wt% of the starch nanocrystals content, thus achieving 17.35%. When the starch nanocrystals content exceeded 20 wt%, the crystallinity of the composite film decreased along with an increase in starch nanocrystals content, due to self-aggregation of starch nanocrystals affect decreased the effective active surface of starch nanocrystals and increased the breakage of the original structure and interaction in starch matrixe and even induced microphase separation between starch nanocrystals and matrix (Chen *et al.*, 2007). However, it was still much higher than rice starch films without starch nanocrystals. This result was agreement with Yu *et al.* (2008). They reported that when agglomerates of starch nanocrystals formed in the nanocomposite with higher loading-levels of starch nanocrystals, a lower crystalline character of Poly(ε-caprolactone) can be observed. This was when the loading-level of starch nanocrystals was higher than 15 wt%. Its location was in good agreement with the diffraction of the Poly(ε-caprolactone) component, demonstrating the self-aggregation of starch nanocrystals. These results suggest that starch nanocrystals increased the crystallinity of rice starch films, as has been reported by other authors. They have attributed this phenomenon to the more crystalline nature of native starch (Chen *et al.,* 2007; Kristo and Biliaderis, 2007).

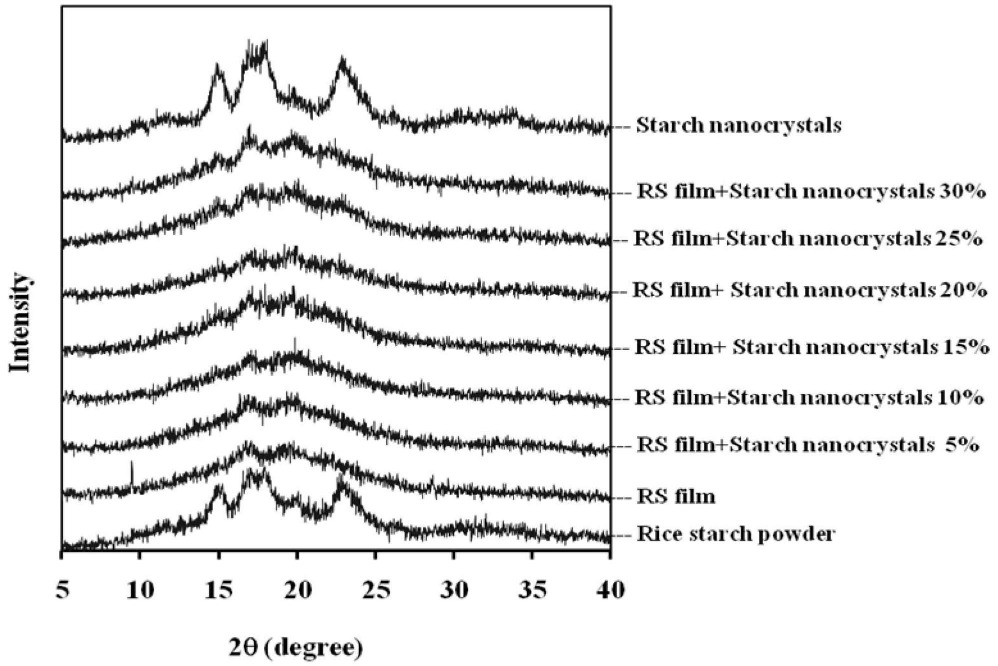

Figure 9. X-ray diffractograms of rice starch powder, starch nanocrystals and rice starch films (RS) reinforced with starch nanocrystals 5-30%.

Table 4. Crystals content of rice starch films reinforced with starch nanocrystals.

Starch nanocrystals content (%)	Crystallinity (%)
0	13.3
5	11.5
10	15.45
15	15.82
20	17.35
25	13.82
30	15.72

3.4 Thermal Properties

3.4.1 Thermogravimetric Analysis (TGA)

TGA thermograms and char yields of starch nanocrystals, rice starch film and rice starch films reinforced with starch nanocrystals (in the temperature range 50 to 500 °C under nitrogen) are shown in Figure 10. The initial weight loss of all samples at approximately 60 °C is due to the evaporation of water. The weight loss in the second range (250-300 °C) corresponds to a complex process including the dehydration of the saccharide rings and depolymerization (Mathew and Dufrene, 2002). According to the results, the char yield of starch nanocrystals at about 19.23% and the behavior of TGA curves were similar in the composites films (Figure 10). The TGA curves showed that all rice starch film reinforced with starch nanocrystals are stable up to 250 °C with a maximum rate of decomposition occurring at about 340 °C. The results showed that, the char yield of rice starch film was less (12.7%) than rice starch films reinforced with starch nanocrystals (13.4-14.5%). This was due to the greater thermal stability of the starch nanocrystals.

With regards to this result, the mainly composition of the starch nanocrystals are starch crystals that had strong structures. Therefore, the addition of starch nanocrystals into rice starch film can improve the thermal stability of rice starch films. It was found that the char yield of rice starch film was enhanced as starch nanocrystals filler content increased from 0 to 30% of the starch. These results indicated that addition of starch nanocrystals increased the thermal stability of rice starch films. This is in agreement with the results reported by Wang *et al.* (2010) for composites with starch nanocrystals and incorporating starch nanocrystals and cellulose whiskers in a waterborn polyeurethane (WPU) matrix. The films containing polysaccharide nanocrystals, especially the ternary system, exhibited higher thermal resistance than pure WPU.

It is worth noting that the addition of nanofillers displayed a more distinct effect on improving the thermal resistance of the hard segment. However the rate of the thermal decomposition of the soft segment was even faster than that of pure WPU. This could be explained by the strong hydrogen bonding interactions that existed between the nanofillers and the hard segments, especially in the ternary system. These interactions induced the microphase separation between the hard and soft segments. This led to the acceleration of the decomposition of the soft segments.

However, the incorporation of starch nanocrystals and cellulose whiskers also exhibited a much better reinforcing effect than starch nanocrystals reinforced in WPU. This was because jammed hydrogen bonding networks of polysaccharide nanocrystals and whiskers formed in the nanocomposite films. This caused the synergistic reinforcement of the WPU networks. Similarly, Kaushik *et al.* (2010) found that increasing the cellulose nanofibril content led to increased in the char yield of composites films. This is due to cellulose-based composites having a lower water content at equilibrium, compared to unfilled thermoplastic starch. As cellulose is crystalline, its crystallinity decreased the polar character of starch. Thus the addition of cellulose into a starchy matrix decreases the global water content with a resultant increase in the cellulose nanofibril content. Typically, the thermal properties are improved and the char yield of composites films increased with the addition fillers (Wilhelm *et al.,* 2003; Huang *et al.,* 2004; Cyras *et al.,* 2008).

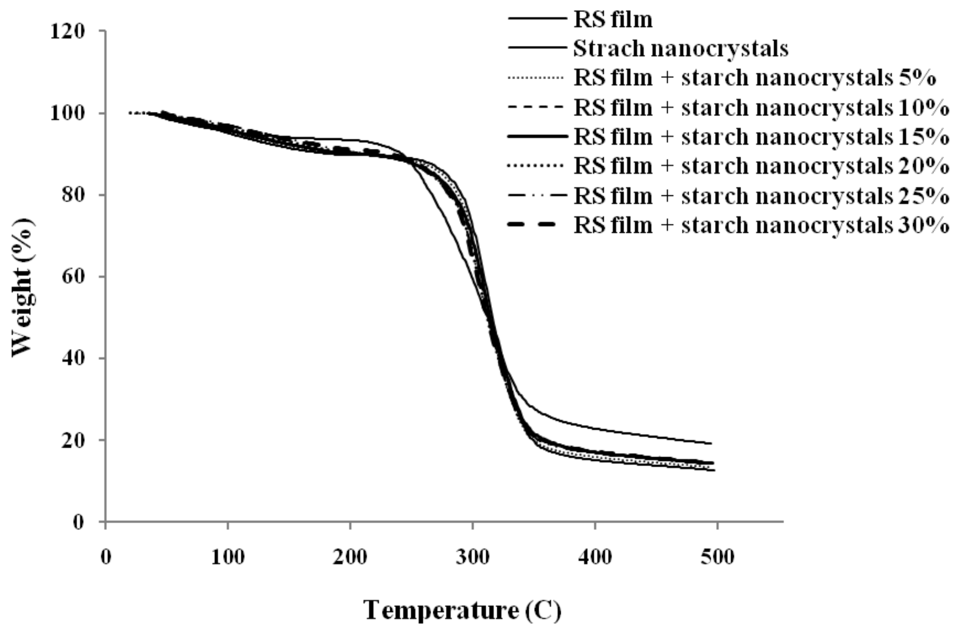

Figure 10. TGA thermograms of starch nanocrystals, rice starch (RS) films and rice starch film reinforced with starch nanocrystals 5-30%.

3.5 Dynamic Mechanical Thermal Analysis

The thermo-mechanical behavior of rice starch film and rice starch film reinforced with starch nanocrystals was studied using a dynamic mechanical thermal analyzer (DMTA). Figure 11A shows the storage modulus (E') as a function of the temperature of rice starch film and rice starch film reinforced with starch nanocrystals**.** The storage modulus, related to the composite stiffness when starch nanocrystals was introduced, of the rice starch film increased. For example, the storage modulus of rice starch film containing 30%(w/w) starch nanocrystals presented about 4.58×10^8 MPa which is about 2 times higher than that of the rice starch film without starch nanocrystals (2.52×10^8 MPa). These results agreed with the study conducted by Angllier *et al.* (2005). They found that, for starch nanocrystals reinforced in an acrylic polymer matrix, the storage modulus of composite was increased with increasing starch nanocrystals content compared to the pure acrylic polymer film. This reinforcing effect was similar to the experimental data reported by Kristo and Biliaderis, (2007) and Chang *et al.* (2010). These studies dealt with the increase of the storage modulus of starch matrix with increasing concentrations of starch nanocrystals and chitin nano particles, respectively.

Figure 11B shows the tan δ curve of rice starch film and rice starch film reinforced with starch nanocrystals as a function of temperature. Usually the position of the tan δ peak is used as the definition of glass transition temperature (T_g). The main tan δ peak shifts to a higher temperature as the starch nanocrystals content increases. Thus the T_g (tan δ peak temperature) of rice starch film at about 172°C (Table 5) increased along with an increase in starch nanocrystals content. It reached a maximum point at about 30 wt% of the starch nanocrystals content, thus achieving T_g at about 202°C (Table 5). The shift of the main relaxation to a higher temperature usually indicates restricted molecular movement. This is because of the strong interactions through hydrogen bonding between the amorphous polymeric matric and reinforcing particles. This interfacial H-bonding leads to a strong absorption of polymer chains on the surface of nanoparticles (Smith *et al.,* 2003).

Similarly, Chang *et al.* (2010) reported that the T_g of glycerol plasticized-potato starch film shifted to higher temperature with an increase in chitin nanoparticles. This indicated that the chitin nanoparticles improved the intermolecular interaction of glycerol plasticized-potato starch film in the starch-rich phase. This brought adjacent chains of starch close, restraining chain mobility, and reducing the free volume (Yu *et al.,* 2008). In addition, Angllier *et al.* (2006) indicated an increase in the relaxation temperature of thermoplastic waxy maize starch film when adding starch nanocrystals. The increased of T_g may be due to a direct contact between the starch nanocrystals and amylopectin-rich domains. This would confirm the presence of interactions between the filler and the matrixes due to the hydrogen bonding force and starch nanocrystals reducing the mobility of amylopectin chains. Most of the composites exhibited a higher relaxation peak than did the rice starch films. This is attributed to the incorporation of a significant quality in the starch nanocrystals and their elevated degrees of crystallinity, which is in agreement with the results of Liu *et al.* (2010). It was also noted that a double-peak of tan δ for rice starch films containing 20-30% starch nanocrystals was observed, which suggested an aggregation of starch nanocrystals. Aggregations of fillers would result in the heterogeneous collections of polymer matrix and filler (Chang *et al.,* 2010).

Table 5. Glasstransition temperature (Tg) of rice starch films and rice starch films reinforced with starch nanocrystals

Starch nanocrystals content (%)	Glass transition temperature (Tg)
0	172.8
5	170.31
10	175.3
15	179.9
20	186.4
25	184.2
30	202.1

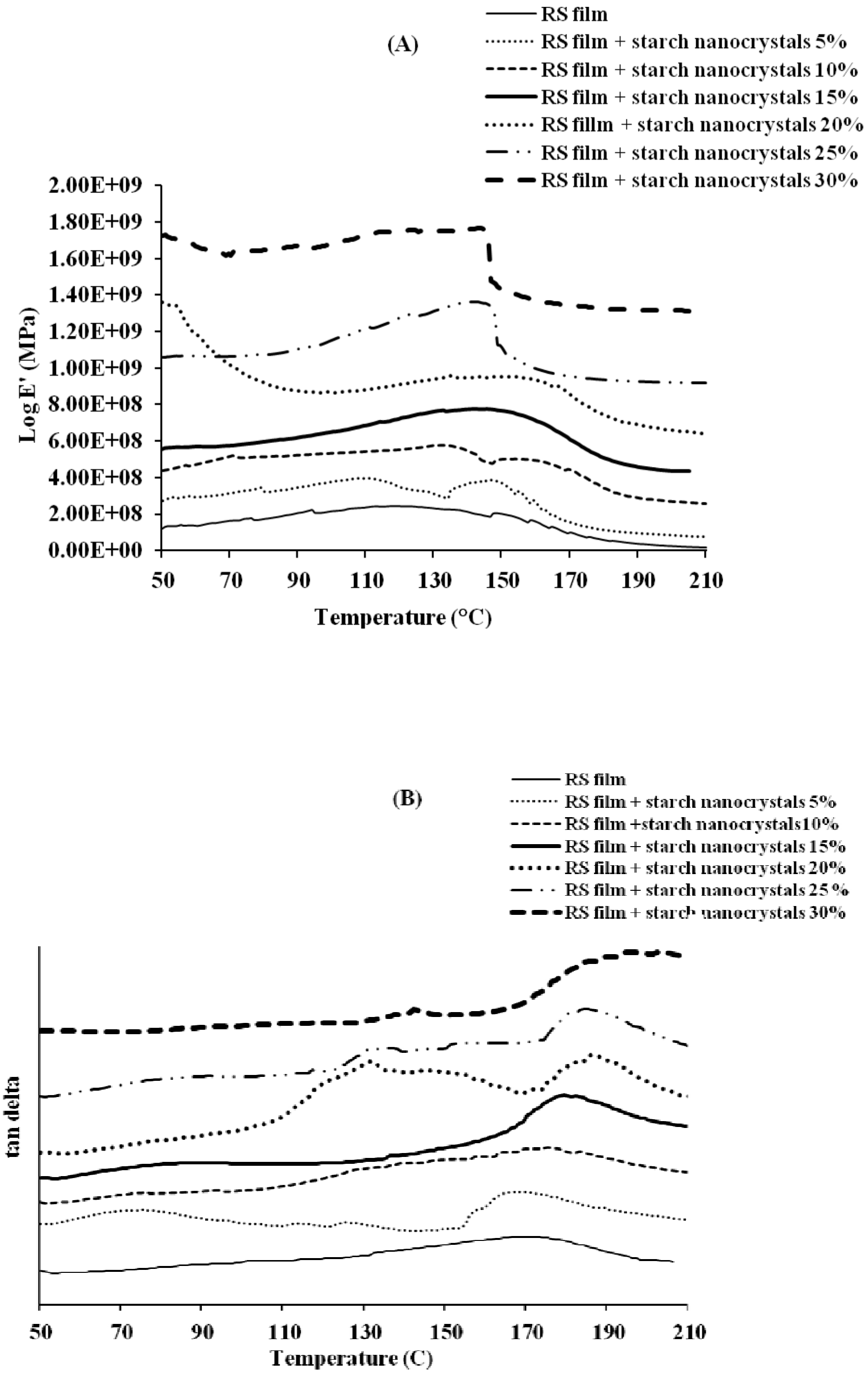

Figure 11. The dynamic mechanical behaviors of both (A) storage modulus (*E′*) and (B) loss factor (tan δ) as a function of temperature for rice starch films (RS) and rice starch films reinforced with starch nanocrystals 5-30%.

3.6 Differential Scanning Calorimeter (DSC)

Differential scanning calorimeter (DSC) measurements were performed for all the starch nanocrystals, rice starch films and rice starch films reinforced with starch nanocrystals at 5-30%. All the samples were heated from -50 to 180 °C with a heating rate of 20 °C/min. The DSC traces are shown in Figure 12. No endothermic peaks, assigned to the glass transition temperature (T_g) of the rice starch film. However, this can be observed from the increasing endothermic heat flow when starch nanocrystals is added to rice starch films. With the increase of the starch nanocrystals contents, the endothermic heat flow increased gradually. This was understood by taking into account the concomitant increase in the crystallinity of the starch matrix with filler content (Mathew and Dufresne, 2002).

This restricted mobility of amorphous amylopectin chains results from the physical cross-links induced by crystallization (Mathew and Dufresne, 2002). Similarly, Xiong *et al.* (2008) found that the addition of nano-silicon dioxide led to an increase in the thermal stability of starch/polyvinyl alcohol (PVOH) films. This was because the hydrogen bond and chemical bond C-O-Si were generated in nano-silicon dioxide and starch/PVA, which played a role by cross-linking points and restricted the movement of molecular chains. Hence, this resulted in an improved melting temperature of the composites films. This is in agreement with the results reported by Wang *et al.* (2010) for composites with starch nanocrystals and waterborne polyurethane matrix.

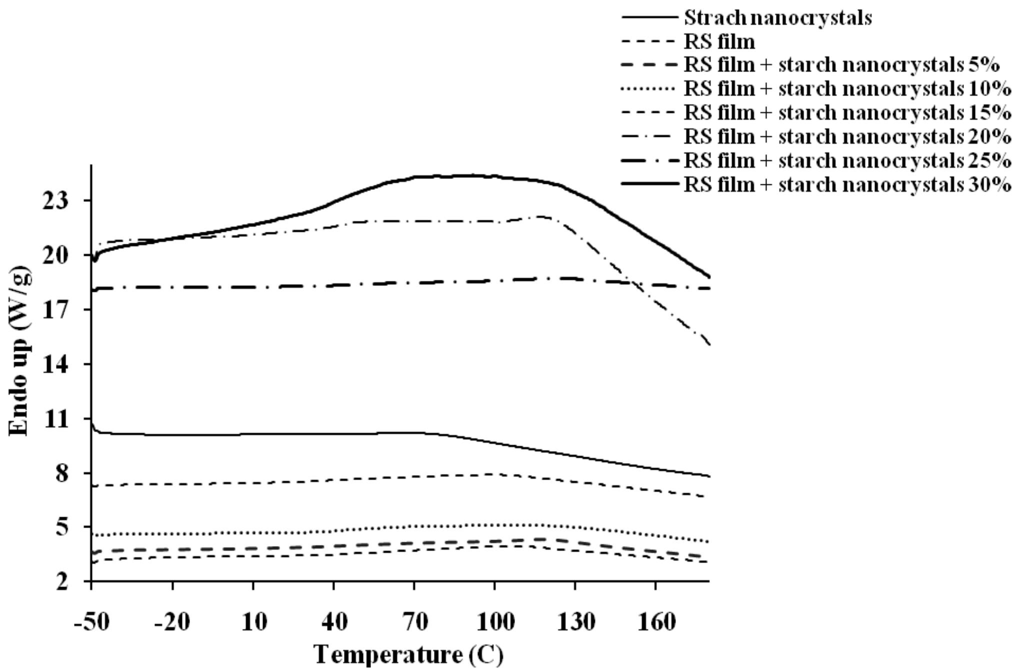

Figure 12. DSC curve of starch nanocrystals, rice starch films (RS) and rice starch films reinforced with starch nanocrystals 5-30%.

3.7 Morphology of the Films

The morphology of rice starch films and rice starch films reinforced with starch nanocrystals (at 5, 10, 15, 20, 25, and 30%) are shown in Figure 13. It was observed that the surface of rice starch film was smooth (Figure 13A): the rice starch films reinforced with starch nanocrystals became rougher with increased starch nanocrystals content. However, particles of starch nanocrystals in rice starch films were dispersed throughout the composited film matrix of rice starch films reinforced with starch nanocrystals 5 to 20% (Figure 13B and 13E). No obvious aggregations of nanocrystals and microphase separation were observed using scanning electron microscopy (SEM) photographs. In addition, nanopores were also observed throughout the rice starch film reinforced with starch nanocrystals at 25 and 30% (Figure 13F to 13G). This suggested that the high filler films contained nanoporous structures. The nanopores in nanocomposite films were formed due to the aggregation of starch nanocrystals and microphase separation between the nanoparticles and matrix (Chen *et al.,* 2007). That indicated that low filler dispersed more homogenously than high filler in rice starch matrix. This resulted in more chances to interact with rice starch film, thus forming stronger interaction and adhesion on interfaces of filler and matrix (Chen *et al.,* 2007). This is in good agreement with the mechanical properties and thermal properties.

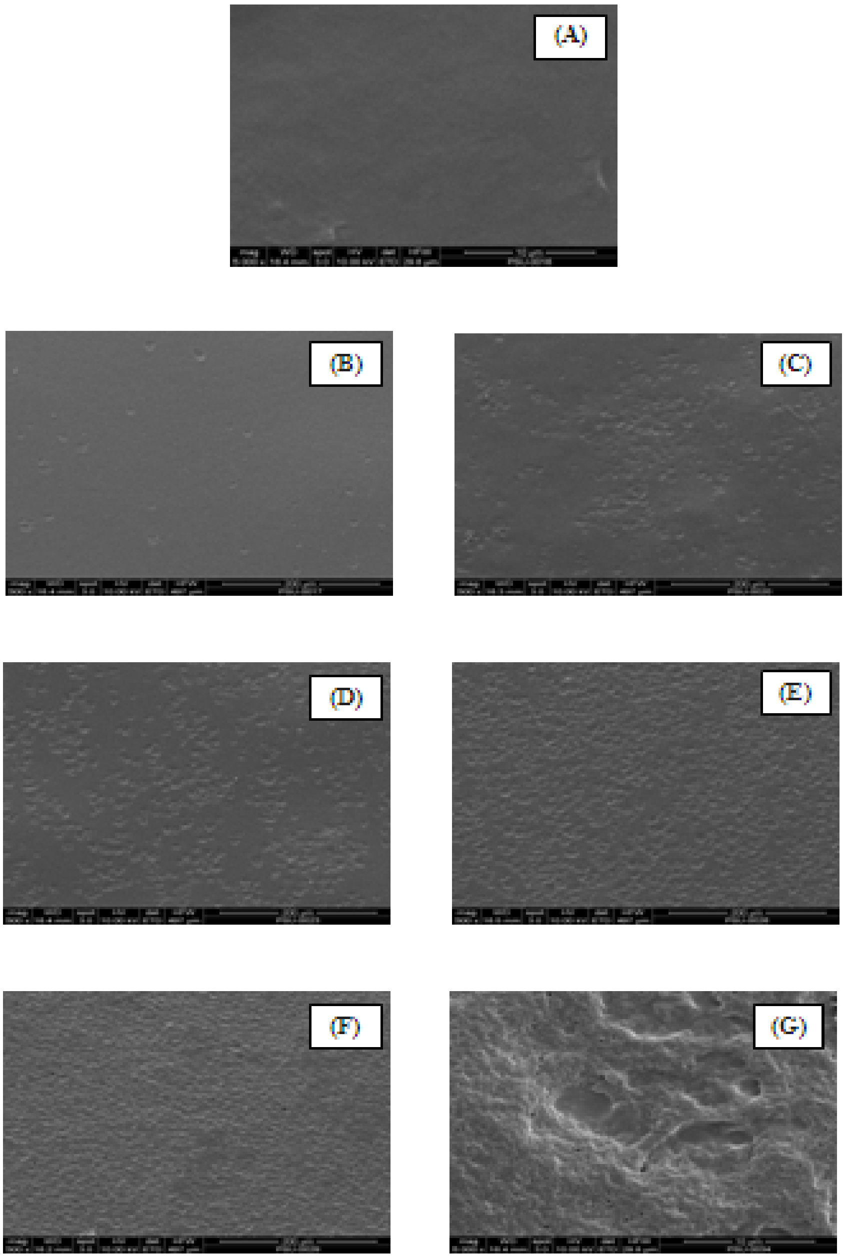

Figure 13. Surface of rice starch films (A), rice starch films reinforced with 5% (B), 10% (C), 15% (D), 20% (E), 25% (F), 30% (G) of starch nanocrystals.

From the results, rice starch film reinforced with starch nanocrystals 15% showed better properties including mechanical, water barrier and thermal properties. Therefore, rice starch film reinforced with starch nanocrystals 15% was chosen and used for further study.

4. Effect of Lipid Types and Concentration on Water Vapor Permeability and Properties of Rice Starch Films.

Rice starch film reinforced with starch nanocrystals were prepared and the influence of types and content of lipid (Palm oil, Margarine and Rice bran oil) on water vapor permeability, mechanical properties and water uptake of rice starch films were investigated. The starch nanocrystals content at 15% was selected according to mechanical, barrier properties and together with thermal properties. For this study, the effect of types and content of lipid on the properties of rice starch films was investigated. The selected type of lipid on rice starch films properties included palm oil, margarine and rice bran oil at various concentrations.

4.1 Tensile Strength (TS) and Elongation at Break (E)

Polysaccharides such as starch, cellulose, carrageenans and chitin generally form films with good mechanical properties, clear, flexible and have excellent oxygen barrier properties at relatively low humidities (Allen *et al.,* 1963; Mark *et al.,* 1966) but they are poor moisture barriers, because of their hydrophilicity. Conversely, lipid films are good moisture barriers, but they are usually opaque, relatively inflexible and unstable (rancidness) (Kamper and Fennema, 1984b). One way to achieve a better water vapor barrier is to produce a composite film by adding hydrophobic components such as lipid and wax materials (Greener and Fennema, 1989b). Hence, in this study the mechanical properties of rice starch films were improved by using three types of lipid (palm oil, margarine and rice bran oil) at different concentrations (3, 6, 9, 12 and 15 %). The mechanical properties of films were assessed by measuring their tensile strength and elongation at break. The results are shown in Figure 14. No data could be obtained for rice starch films contained lipid 15% because they were too brittle and cracked during drying. It was observed that the

TS of rice starch films reinforced with starch nanocrystals increased with the addition of lipid. Previously work demonstrated that the maximum occurred when no lipid was added (12.20 MPa). When the concentration of lipids increasing resulted in a decreased in TS of the films. However, these values are higher than TS of rice starch films without lipids (Figure 14). This is in agreement with findings of Vargas *et al.* (2009) reported that the addition of oleic acid caused a decrease in TS when the ratio oleic acid : chitosan was higher than 1:1 because at the 1:1 oleic acid : chitosan ratio molecular interactions could enhance the structural bonds in the polymer network leading to an increase in the TS of the films. Yang and Paulson (2000) showed that the TS of films composed of gellan and a blend of stearic/palmitic acids or beeswax decreased abruptly when the concentration of lipid substances increased above 14.3 and 34%, respectively, as compared to the simple film. In general, lipid addition induced development of heterogeneous film structure featuring discontinuities in the polymer network (Bravin *et al.,* 2004). The interactions between nonpolar lipid molecules and between the polar polymer and nonpolar lipid molecules are believed to be much lower than those between the polar polymer molecules (Yang and Paulson, 2000). Weller *et al.* (1998) stated that the decreased in TS accompanying the increase in lipid concentration was related to weakening effect of lipid on the protein network, due to the lack of structural integrity of the lipid. The result is a decrease in TS agreement with the findings of other researchers (Anker *et al.,* 2002; Bertana *et al.,* 2005). The addition of margarine and rice bran oil resulted in a higher TS of rice starch films than the addition of palm oil (Figure 14). The differences in TS between these films, depending on the characteristics of the lipid and its capacity to interact with the structural matrix of the polymer (Gontard *et al*., 1994). No significant difference ($p>0.05$) in TS occurred between the addition of palm oil and rice starch film without lipid.

The elongation at break (E) of rice starch films reinforced with starch nanocrystal 15% decreased significant ($p>0.05$) with the addition and increasing in concentration of lipids (Figure 14). This is probably because the lower water content of films containing lipids compared to films containing only hydrocolloid can explain the decreased E generally observed when lipids are dispersed in the hydrocolloid matrix (Martin-Polo, 1991). The same effect was observed by Vargas *et al.* (2009)

who showed an decrease in E of chitosan films with increase in lipid concentration caused by interaction between molecular of lipid and chitosan could enhance the structural bonds in the polymer network leading to decreasing its ability to stretch. Peroval *et al.* (2002) observed a decrease in E on incorporating fatty acids into arabinoxylan films, arguing that some lipids were unable to form a continuous, cohesive matrix, thus resulting in decreased E. The addition of rice bran oil resulting in greater reduction in E of rice starch film than the addition of palm oil and margarine (Figure 14). These resulted, depending on the lipid characteristics. While palm oil and rice bran oil are liquid and can flow at the temperatures considered, margarine is solid. During film stretching, lipid droplets in the films can be easily deformed when liquid, but this does not occur when they are solid. On the contrary, the solid droplets form a more rigid dispersed phase in the film and reduce its ability to stretch (Morillon *et al.,* 2002).

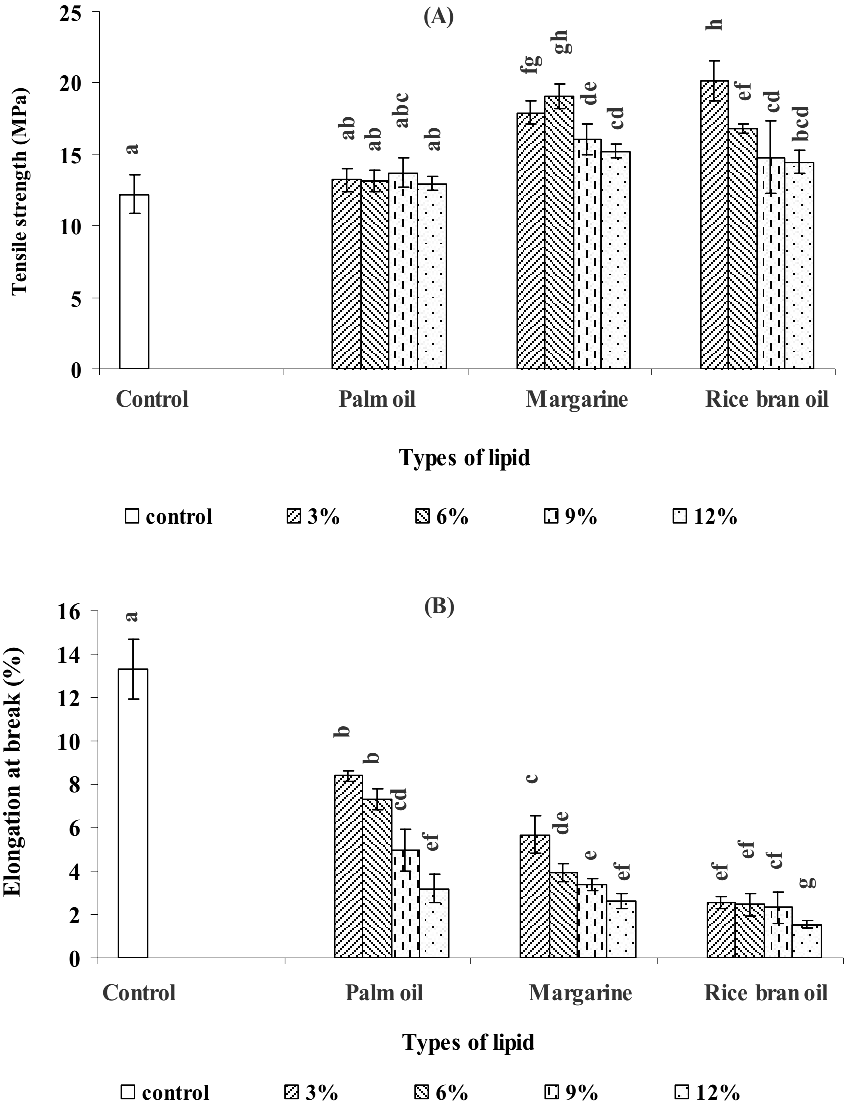

Figure 14. Effect of lipid type and concentration on tensile strength (A) and elongation at break (B) of rice starch films reinforced with starch nanocrystals. Mean values with different letter are significantly different ($p<0.05$).

4.2 Water Vapor Permeability (WVP)

The films produced with rice starch reinforced with starch nanocrystals and with the addition of lipids at the concentrations studied, were malleable and visually homogenous. Figure 15 shows the WVP of these films. Rice starch films without the addition of lipids were more permeable to water vapor

(10.62 g.mm/m^2.day.KPa) than incorporated films with lipids, reflecting the greater hydrophilic property of the films without the addition of lipids (Bertana *et al.,* 2005). The addition and increasing concentration of lipids resulted in a reduction in WVP of the films, due to the fact that, the WVP decreases with increasing hydrophobicity of the added lipid materials (Morillon *et al.,* 2002). These results agreed with the study conducted by Curtis *et al.* (1998); Anker *et al.*, 2002; Bertana *et al.* (2005). As expected, all lipids reduce the WVP values due to their low water affinity. Rice bran oil is less efficient at reducing moisture permeability which can be attributed to its highly unsaturated fatty acid (about 42.5% oleic acid), because they are more polar than saturated lipids. The strong interactions between the polar groups of oleic acid and water allow the oleic acid molecules to penetrate into the starch matrix film and establish attractive forces between plasticizer molecules and chain segments. These attractive forces reduce the cohesive forces between the polymer chains and increase the segmental mobility thus favoring water molecule diffusivity (Morillon *et al.,* 2002). Indeed, films containing palm oil and margarine have better performances to reduce WVP of rice starch films than rice bran oil. Similary, Limpisophon *et al.,* (2010) who found that incorporation of fatty acid into emulsion film can reduced WVP and addition of stearic acid (saturated fatty acid) could improve the water barrier property better than could oleic acid (unsaturated fatty acid) addition at the same fatty acid concentration. Although oleic acid and stearic acid have the same carbon number in the molecule. The addition of margarine could reduce the WVP more than the addition of palm oil, resulting from the high solid content of the films addition with margarine. The solid or liquid state of lipid compounds strongly influences the barrier efficiency of the film (Morillon *et al*., 2002). Gallo, *et al.* (2000) showed that the permeability through alkanes are two-three times lower than through triglycerides. This is the due the influence of the physical state of lipid in lipid or bilayer films has been already studied by some authors who observed that solid lipid showed better barrier properties than liquid lipid (Kamper and Fennema, 1985; Landmann, *et al*,. 1960). This was explained in terms of the relative water-vapor solubility in the liquid/solid phase and/or of the molecular organization of the lipids. Morever, the solid fat content in emulsion and bilayer edible films could influence the WVP (Martin-Polo *et al.,* 1992; Kester and Fennema, 1989). The WVP decreased by

5-20 times as a function of the lipid nature (alkanes or triglycerides) when the solid fat content varied from 0-80%. Indeed, CH_2 groups of liquid aliphatic chains have a greater volume, from 4 to 5.10^{-3} nm^3, than when they are crystallized (Hagenmaier and Baker, 1993). So, the solid structure of fats is more dense and limits the diffusion of water. Moreover, the solubility of water in solid lipid is also reduced (Kamper and Fennema, 1984). However, for solid fat contents higher than a critical value that depends of the lipid nature, permeability could increase due to structure defects within the film. In generally, the efficiency of the lipid materials in composite films and coatings depends on the nature of the lipids used, in particular on its structure, chemical arrangement, hydrophobicity and physical state (e.g. solid or liquid) and on the lipid interactions with the other components of the film, such as polysaccharides and proteins (Rhim and Shellhammer, 2005). For components having the same chemical nature, the chain length and degree of saturation modifies the barrier properties. McHung and Krochta (1994) state that moisture barrier efficiency of fatty alcohols and fatty acids increases with carbon number (from 14 to 18) because the apolar part of the molecule increases. However, when the cabon atom chain length is higher than 18 atoms, films have higher permeability. This is explained by very long chains inducing a heterogeneous structure of the polymer network (Koelsch and labuza, 1992). Furthermore, composite films containing unsaturated fatty acids have been shown to be permeable to water vapor than those containing saturated fatty acids (Kamper and Fennema, 1984a). The poor barrier properties of unsaturated fatty acids are a result of the expansion in the molecular volume that occurs with the introduction of double bond, and of the lower melting point with greater chain mobility (Greener and Fennema, 1992).

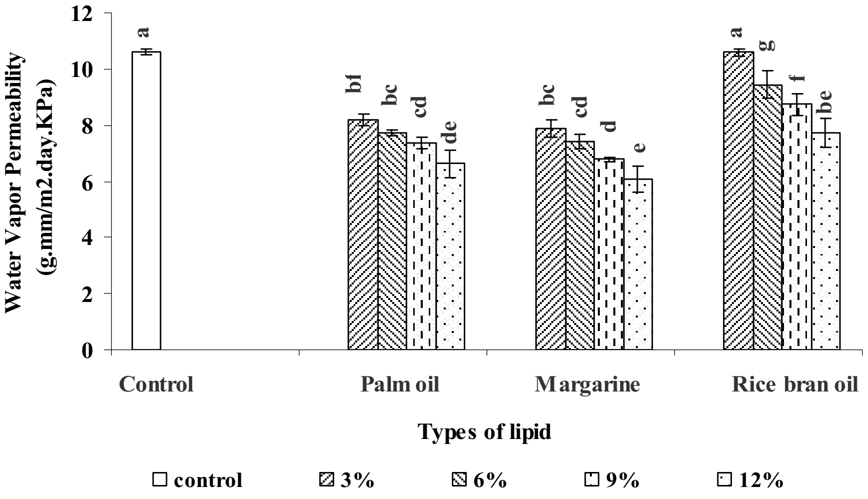

Figure 15. Effect of types and content of lipid on water vapor permeability of rice starch films reinforced with starch nanocrystals. Mean values with different letter are significantly different ($p<0.05$).

4.3 Water Uptake

The water uptake of rice starch films reinforced with starch nanocrystals with and without lipids conditioned at 98% RH, room temperature was plotted as function of time (Figure 16). The two stage water absorption pattern was obvious in all cases. At lower times (t < 2 days) the absorption kinetics is fast, whereas after that a slower absorption process follows, leading to plateau, corresponding to the water uptake at equilibrium. The rice starch film exhibited 54.09% water absorption when condition in sodium sulfate for 4 days. The decrease in water absorption is 48.02% for rice starch reinforced with starch nanocrystals, resulting from the phenomenon could be ascribed to the formation of a rigid three-dimensional starch nanocrystals network (due to the strong hydrogen bonding between the starch particles) that prevents the swelling of polymer matrix (Dufresne and Vignon, 1998; Dufresne *et al.*, 2000), or to strong interactions between the filler and polymer chains, decreasing the swelling and water absorbance of the polymeric chains located in the interfacial regions (Gopalan Nair and Dufresne, 2003). Results similar to the present study are presented by Lu *et al.* (2004) in chitin whiskers reinforced soy protein isolate thermoplastic, by Lu *et al.* (2006) in ramie crystallites containing starch films. The similar polar and hydrophilic character of matrix (biopolymers like starch or soy protein) and nanoparticles (tunicin or chitin whiskers, cellulose microfibrils, starch nanocrystals) leads probably to strong adhesion between the filler and matrix due to hydrogen bonding, which intensifies with increasing filler concentration, thus contributing to the evidenced lower water sensitivity of the highly hydrophilic composites (Kristo and Biliaderis, 2007). The water uptake at equilibrium of rice starch films reinforced with starch nanocrystals and addition of lipids decreased as the concentration of lipid increased (Figure 16). This is attributable to the fewer sites available for water sorption in the polymeric matrix due to the increase in the hydrophobic chains from the fatty acid (Vargas *et al.,* 2009). Fabra *et al.* (2010) reported the incorporation of lipids reduces water sorption due to the fact that lipids correspond to a function of solids with small water uptake capacity, especially beeswax which is very hydrophobic.

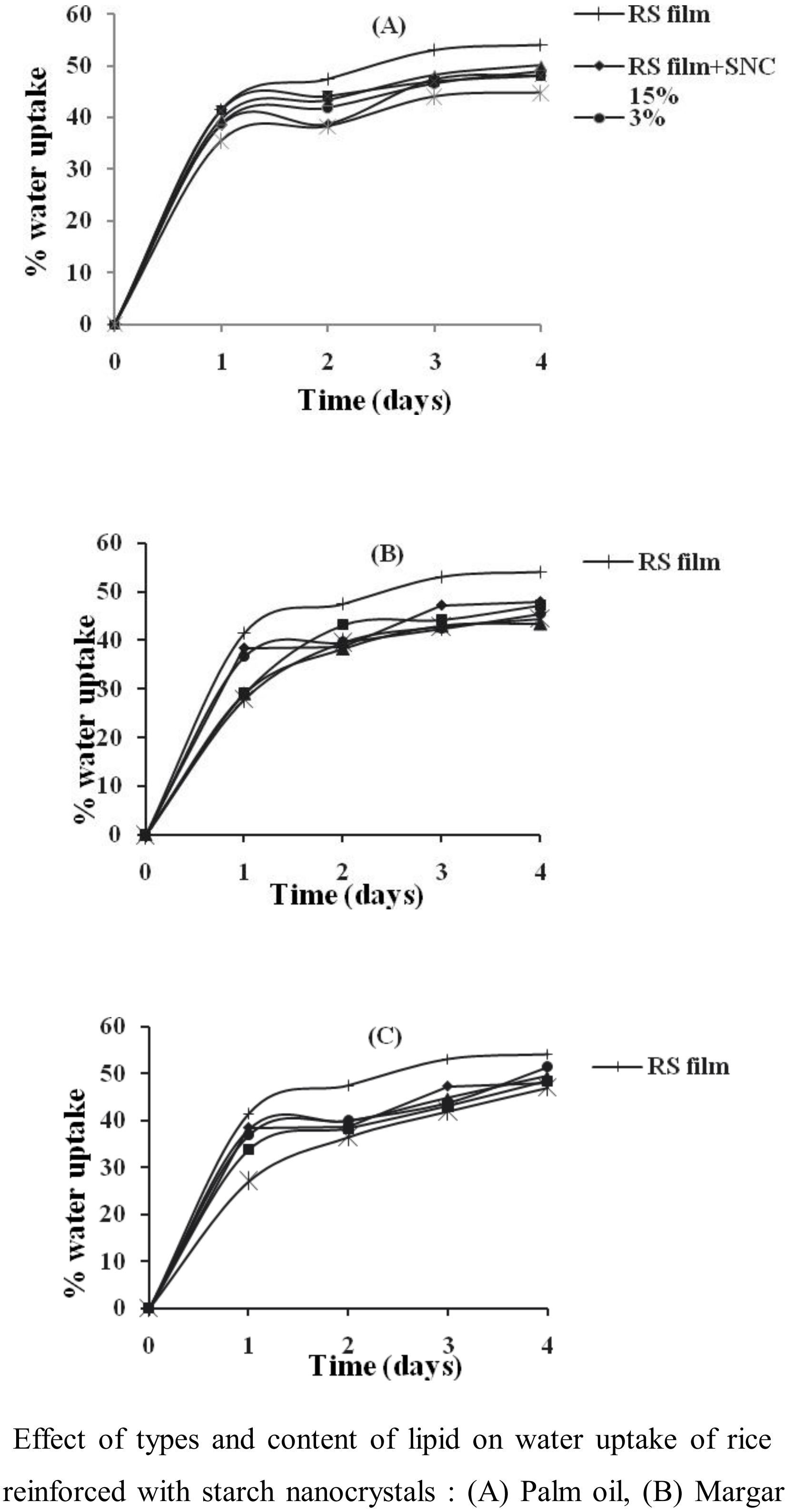

Figure 16. Effect of types and content of lipid on water uptake of rice starch films reinforced with starch nanocrystals : (A) Palm oil, (B) Margarine and (C) Rice brand oil.

6.4 Morphology of the Films

Figure 17 shows SEM micrograps of surface of the rice starch films reinforced with starch nanocrystals and addition of palm oil at various concentration. If compared with rice starch film at various concentration of palm oil (Figure 17 A-D) showed remarkable differences in term of surface microstructure. While a smooth, continuous presence was observed for the rice starch film at 3 and 6% of palm oil surface, the presence of higher palm oil caused discontinuities associated with the formation of two phases (lipid and polymer) in the matrix: lipid droplets embedded in a continuous polymer phase. The increase in the lipid content of the film leads to an appreciable change in the surface topography, revealing a different amount of lipid particles on the surface and particularly for the film with higher lipid, a bigger particle size, which coincides with the greater lipid concentration and bigger particle size (Vargas, *et al.,* 2009). Perada *et al.* (2010) reported that the incorporation of tung oil, led to a heterogeneous structure as the concentration of tung oil increased.

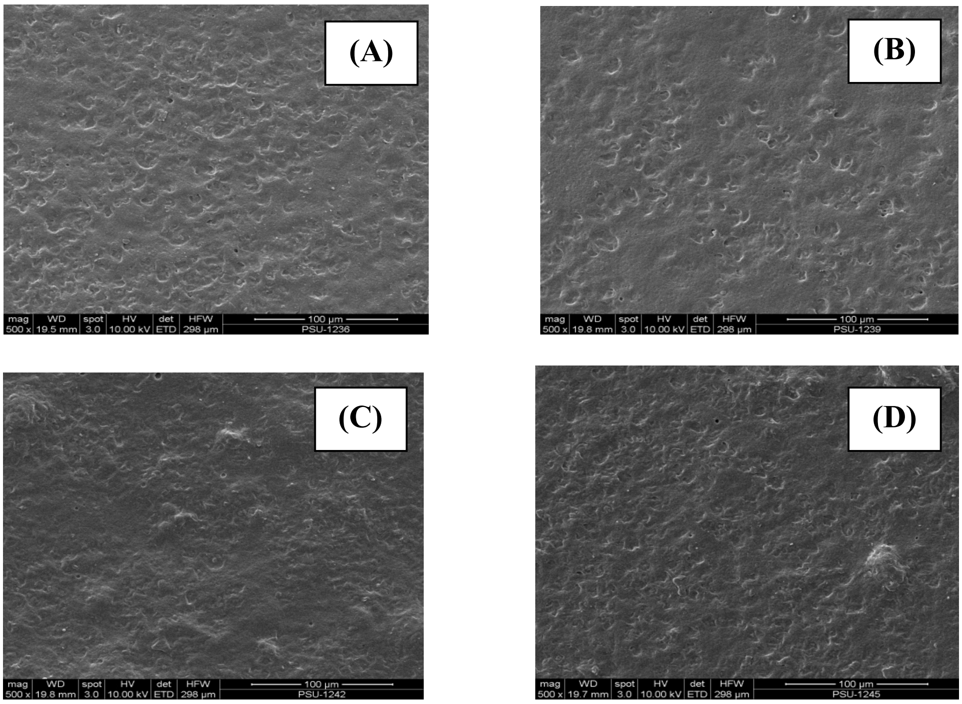

Figure 17. Surface of rice starch film reinforced with starch nanocrystals 15% and added various types of lipid ; (A) palm oil 3%, (B) palm oil 6%, (C) palm oil 9% and (D) palm oil 12%.

Comparing with the same concentration of lipids, the results showed that the incorporating lipid led to rougher film surfaces, as previously reported by Febra *et al.* (2009) in sodium caseinate based films. However, palm oil appeared to be well incorporated and embedded in the composite films resulting in a relatively smooth and continuous surface more than composite films with margarine and rice brain oil, respectively (Figure 18). The film with margarine had more irregular surface than rice bran oil, according to the different fatty acid and were coherent with the size of particles (Jimenez *et al.,* 2010).

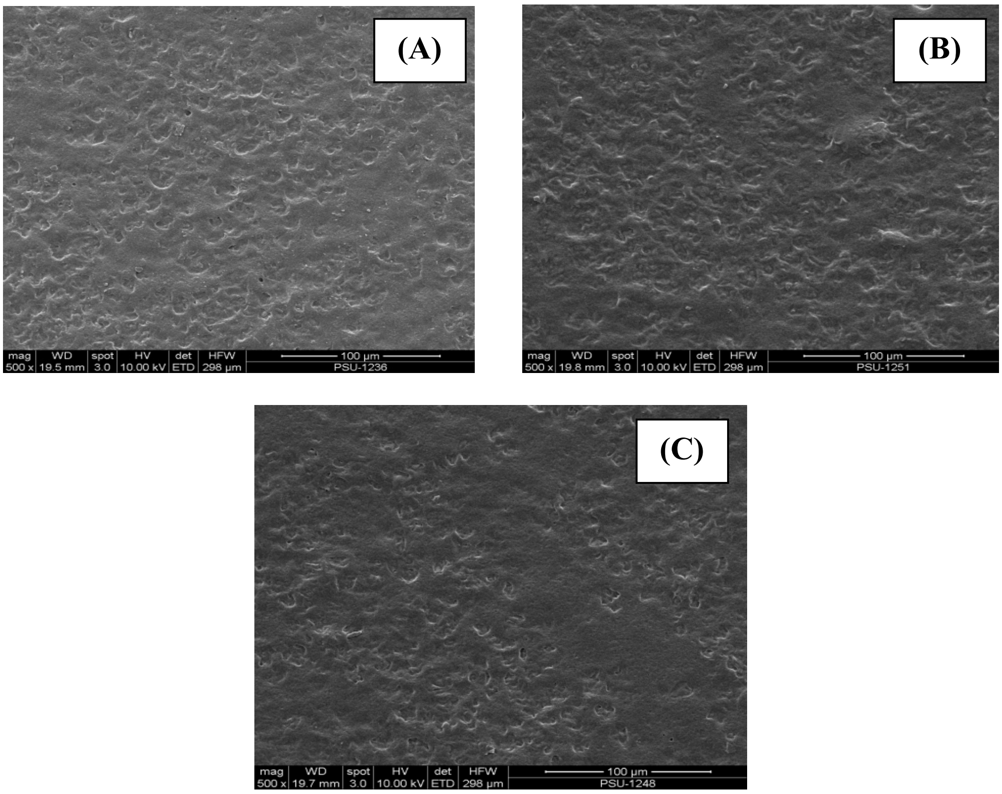

Figure 18. Surface of rice starch film reinforced with starch nanocrystals 15% and added of (A) palm oil 3%, (B) Margarine 3% and (C) Rice bran oil 3%.

From the results, rice starch film reinforced with 15% starch nanocrystals and contained 3% palm oil showed better properties including mechanical and water barrier than other lipids. Therefore, rice starch film reinforced with 15% starch nanocrystals and contained 3% palm oil was chosen and used for further study.

5. Effect of Relative Humidity on Properties of Rice Starch Film

Moisture sorption curves of rice starch films, rice starch film reinforced with 15% starch nanocrystals, rice starch film contained 3% of palm oil and rice starch film reinforced with 15% starch nanocrystals and contained 3% of palm oil were determined. Moisture adsorption was more rapid at the initial stages of the storage and lower amounts of water were adsorbed as time increased. Then, the moisture content of rice starch films reached a plateau indicating that they became equilibrated with storage RH. Moisture equilibrium time was influenced by storage RH; films stored at 90% needed more time to reach the equilibrium. The moisture adsorption of rice starch films were significantly increased with increasing the relative humidity ($p<0.05$) and higher moisture adsorption rate was observed when higher relative humidity was used. The addition of starch nanocrystals to rice starch films absorbed less water than rice starch films, the phenomenon could be ascribed to strong interactions between the filler and polymer chains, decreasing the swelling and water absorbance of the polymeric chains located in the interfacial regions (Gopalan *et al.,* 2003). The incorporation of lipids reduces the moisture sorption of rice starch film reinforced with starch nanocrystals significantly ($p<0.05$) lowest moisture content than other rice starch film at high relative humidity, due to the fact that lipids correspond to a fraction of solids with small water uptake capacity (Fabra *et al.*, 2010) and this is attributable to the fewer sites available for water sorption in the polymeric matrix due to the increase in the hydrophobic chains from the fatty acid (Vargas *et al.,* 2009). Pereda *et al.*, 2010 reported that, the moisture content of caseinate films depends on the tung oil content, being lower as the oil concentration increase due to the hydrophobic nature of the lipid. The result showed that moisture adsorption was related with the tensile strength of rice starch films. Figure 20 shows the tensile strength of rice starch films at different relative humidities. The results demonstrated that the TS of rice starch films, rice starch film rice starch film reinforced with starch nanocrystals 15%, rice starch film contained 3% of palm oil and rice starch film reinforced with 15% starch nanocrystals and contained 3% of palm oil were decreased as the relative humidity increased. The rice starch films were strongly sensitive to the moisture content. This phenomenon is ascribed to the starch being in the rubbery state at room temperature. Bravin *et al.* (2006) reported that the water adsorption isotherm of edible films increased for high RH values because high water solubility. Hydration causes matrix swelling, increased water mobility, and a rise in the diffusion coefficient. When incorporated with lipid, film was less hydroscopic resulted decreased in water adsorption.

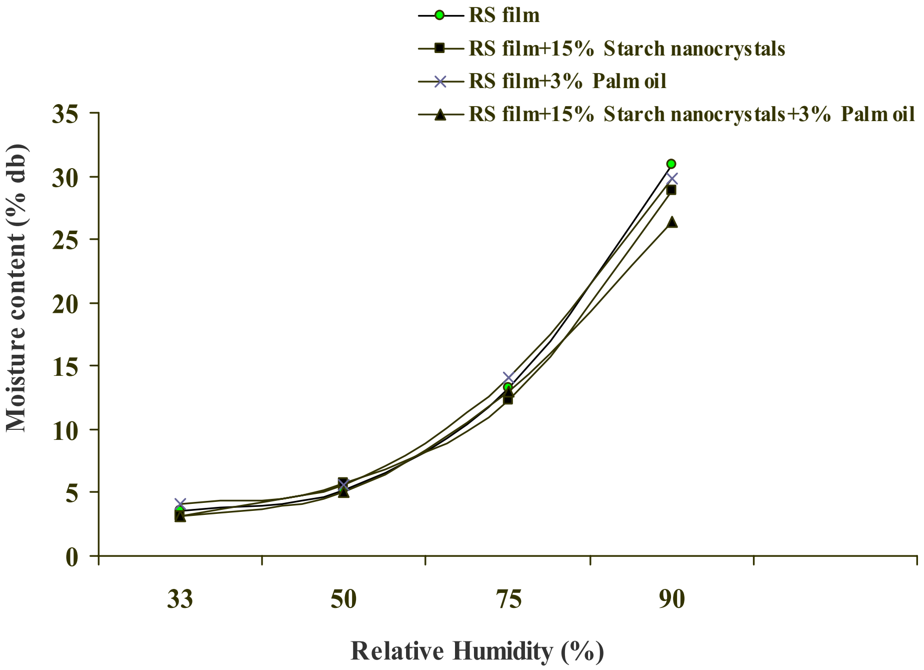

Figure 19. Moisture sorption isotherm curves of rice starch film, rice starch film (RS) reinforced with starch nanocrystals 15%, rice starch film added with 3% palm oil and rice starch film reinforced with starch nanocrystals 15% and added with 3% palm oil at various relative humidities (27±2 °C).

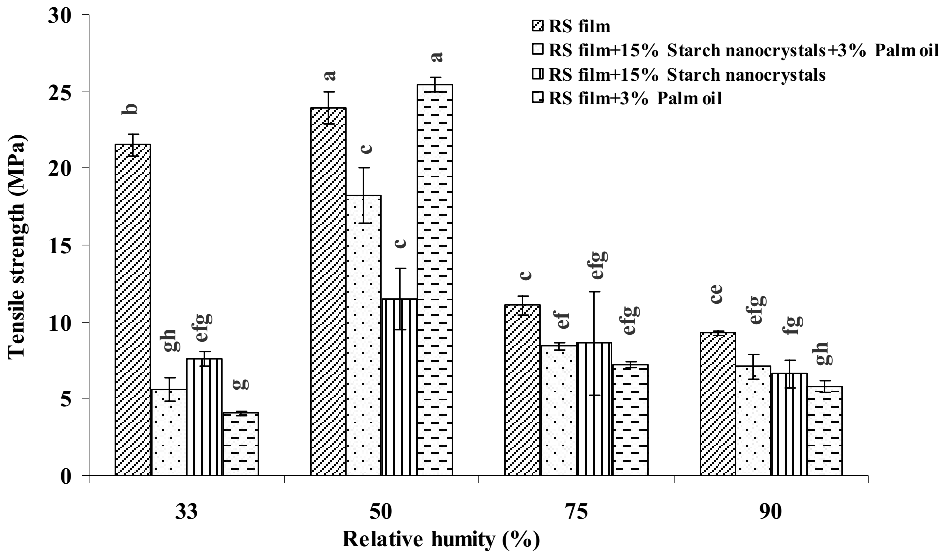

Figure 20. The tensile strength of rice starch film, rice starch film reinforced with starch nanocrystals 15%, rice starch film added with 3% palm oil and rice starch film (RS) reinforced with starch nanocrystals 15% and added with 3% palm oil at various relative humidities (27±2 °C). Mean values with different letter are significantly different ($p<0.05$).

6. Application of Rice Starch Films for Food Product

Three types of rice starch films, comprising of rice starch film, rice starch film reinforced with 15% of starch nanocrystals and rice starch film reinforced with 15% of starch nanocrystals and containing 3% of palm oil and poly ethylene film (PE). All films were wrapped around crackers and study the effect of types of film on the properties of cracker was investigated.

6.1 Effect of Rice Starch Film on Texture of Cracker During Storage

The efficiency of films in controlling moisture transfer in moisture-sensitivity products were evaluated by wrapping crackers, a low a_w type food. Texture is a primary sensory attribute for these products, and loss of desire texture (crispness) results in shorter shelf-life. Figure 21 shows the compression load for wrapping materials of crackers. This parameter can be used as indicator of cracker crispness. After 3 days storage, the crackers wrapped with all films were significantly higher compression value than the control at time 0 day. When the storage time increased resulted in an increased of compression load of crackers in all cases. The grow is associated with a loss of crispness due to the hydration (Bravin *et al.,* 2006). Polyethylene film can prevent crispness of cracker more than rice starch films and rice starch films reinforced with starch nanocrystals and contained lipid. However, rice starch films reinforced with starch nanocrystals and contained lipid reduced the crispness of the cracker less than rice starch film. Cracker wrapped with rice starch film reinforced with starch nanocrystals and contained lipid exhibited higher shelf-life (9 days) than cracker wrapped with rice starch films (6 days). The addition of lipids resulted in a reduction in WVP of the films, due to the fact that, the WVP decreases with increasing hydrophobicity of the added lipid materials (Morillon *et al.,* 2002). The loss in this texture attribute is the major cause of dry food rejection by consumers. Schou *et al.* (2005) reported that wrapping bread samples in the casienate films reduced hardness during 6 h storage at ambient temperature relative to unwrapped controls. However, the caseinate films were not as synthetic polyvinyl chloride film. This result from the bread wrapping study are in accordance with the trend observed in the WVP values. Bravin *et al.* (2006) who found that the coated cracker by polysaccharide-lipid-based coating exhibited higher shelf-life than uncoated. At 85% RH, coatings double the shelf-life of the bakery products. Results clearly suggest that polysaccharide-lipid-based edible coating was successful in delaying moisture uptake in a low a_w food.

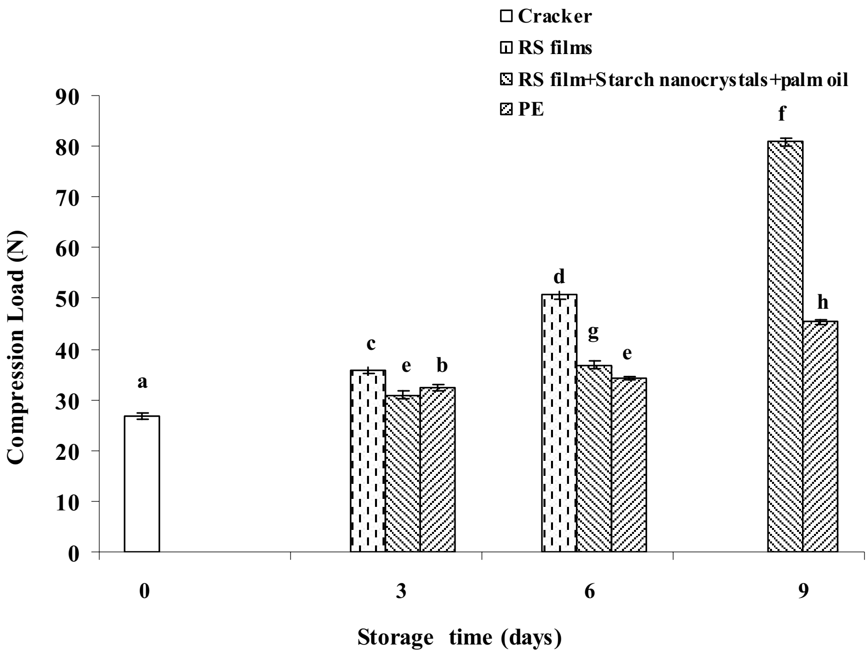

Figure 21 Effect of type of wrapping materials on compression load of wrapped cracker using rice starch film (RS), rice starch film reinforced with starch nanocrystals and added lipid and polyethylene condition at 75% RH and room temperature (27±3°C). Mean value with different letter are significant different ($p<0.05$).

6.2 Effect of Rice Starch Film on Color of Cracker

Figure 22 shows the results from the effect of type of films on color (L*, a* and b*) of crackers. The results demonstrated that cracker wrapped with rice starch film have shelf-life 6 days, therefore no measured color of cracker wrapped with rice starch film at 9 days of storage. The results showed that the types of films did not significant effect on the color of crackers. Crackers became more darker as evidenced by the decrease L* and a* value as the storage time increased from 0 – 9 days concomitant with decreased in b* value. This is indicated the decreasing lightness (L*) and yellowness (b*) of the crackers.

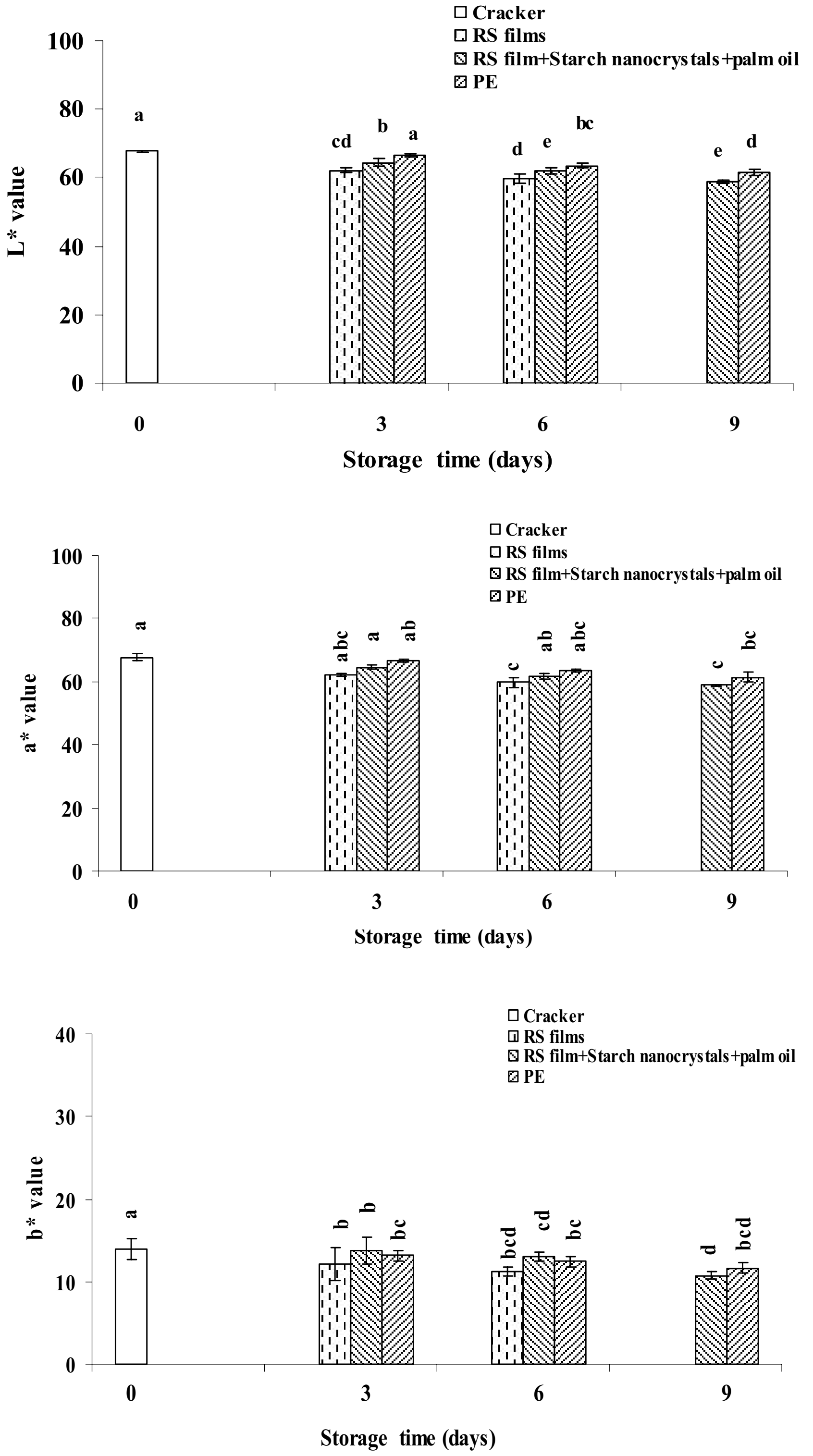

Figure 22 Effect of type of wrapping materials on L* (A); a* (B) and b* (C) of cracker condition at 75% RH and room temperature (27±3°C). Mean value with different letter are significant different ($p<0.05$).

6.3 Effect of Rice Starch Film on Moisture content and water activity of Cracker

Figure 23 show the evolution of moisture content with storage time of cracker wrapped with rice starch film, rice starch film reinforced with starch nanocrystals and contained lipid and polyethylene film condition at 75% RH and 27±2°C. The same trend were observed in all cases. The storage time increasing resulted in an increase in moisture content of cracker. After 3 days storage, the cracker wrapped with polyethylene film did not have a significantly ($p<0.05$) higher moisture content value than the control at time 0 day. However, moisture content of cracker wrapped with rice starch film reinforced with starch nanocrystals and contained were significantly ($p<0.05$) lower than cracker wrapped with rice starch film all storage time. The increased in moisture content of crackers during storage under high relative humidity conditions are affected to loss of crispness and softening. The loss in this texture attribute is the major cause of dry food rejection by consumers (Katz and Labuza, 1981; Roudaut *et al.*,2002). Results similar to the present study are presented by Bravin *et al.* (2006) in coated cracker with polysaccharide-lipid-based.

Figure 24 shows the results from the effect of type of films on water activity (a_w) value of the crackers. The same trend were observed in all cases. The storage time increasing resulted in an increase in water activity (a_w) value of cracker. After 3 days storage, water activity of crackers wrapped with all type of films were significantly ($p<0.05$) higher than cracker at 0 day. Consider, water activity of cracker wrapped with rice starch film reinforced with starch nanocrystals and contained were significantly ($p<0.05$) lower than cracker wrapped with rice starch film all storage time.

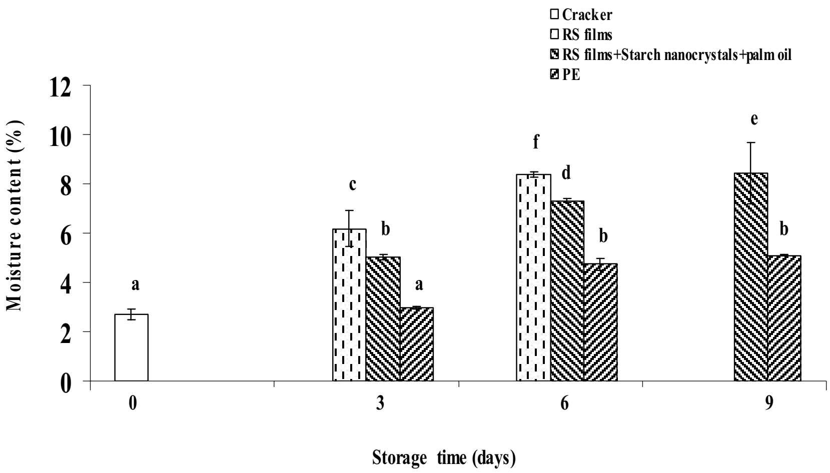

Figure 23 Effect of type of wrapping materials on moisture content evaluation with storage time of wrapped cracker condition at 75% RH and room temperature (27±3°C). Mean value with different letter are significant different (p<0.05).

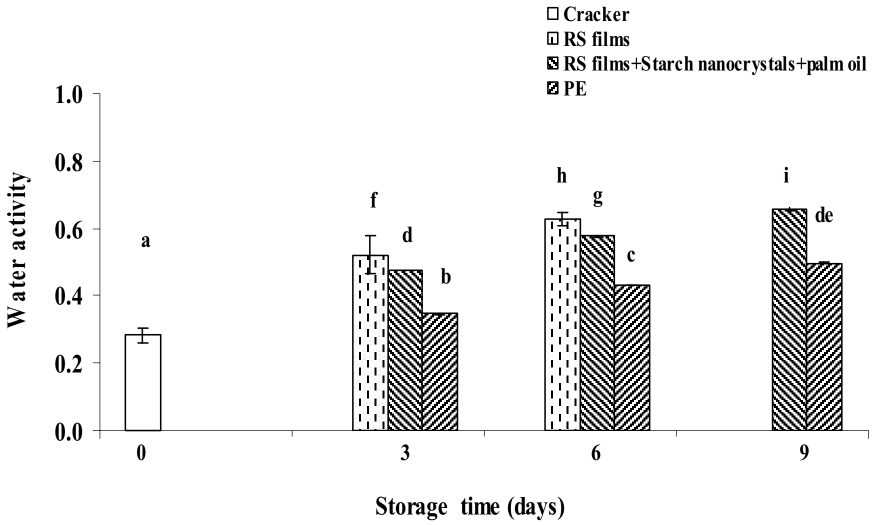

Figure 24 Effect of type of wrapping materials on water activity evaluation with storage time of wrapped cracker condition at 75% RH and room temperature (27±3°C). Mean value with different letter are significant different (p<0.05).

CHAPTER 4

CONCLUSIONS

1. Rice starch were prepared by alkaline process. The proximate composition of rice starch were found to be 30.40, 12.19, 0.56, 0.51 and 0.21 of amylose, moisture, protein, fat and ash, respectively. Rice starch granules have a smooth surface but angular and polygonal shapes with a size of about 8-10 µm. The rice starch powder have the characteristic typical A-type pattern with strong reflection at 16.8, 18.0 and 22.7°.

2. Rice starch nanocrystals were prepared by acid hydrolysis of rice starch with sulfuric acid. The optimal conditions of sulfuric acid hydrolysis to be obtained in the highest yield and smallest median size particle is starch nanocrystals hydrolysis by sulfuric acid concentration at 3M. Starch nanocrystals are generally observed in the form of aggregates having an average size around 3.97 µm. The acid hydrolysis treatment, obvious changes happened in diffraction pattern of starch nanocrystals. The results showed that diffraction peak of starch nanocrystals were sharper than diffraction peak of rice starch powder. The sharper diffraction peak is an indication of higher crystallinity value in structure of the starch nanocrystals.

3. The properties of the rice starch films were improved through the addition of starch nanocrystals. The mechanical properties of the film were enhanced by the addition of starch nanocrystals. Furthermore, increasing of starch nanocrystals content, the water barrier properties of the films was also improved. The glass transition temperature (*Tg*) shifted towards higher temperature with increasing amount of nanocrystals and the char yield of rice starch films was enhanced as starch nanocrystals increased. The results demonstrated that addition of starch nanocrystals 15% showed better properties including mechanical, water barrier and thermal properties.

4. Addition and increasing concentration of lipids resulted in a reduction in water vapor permeability and water uptake of the films. The addition of palm oil and margarine could reduce the water vapor permeability of rice starch film than the addition of rice bran oil, due to the rice bran oil is a highly unsaturated fatty

acid and more polar than saturated lipids. Moreover, the addition of lipid affected on mechanical properties of rice starch film. By increasing of lipid content, the mechanical properties are lower and reduce its ability to stretch. The addition of margarine and rice bran oil resulted in a higher tensile strength but lower elongation at break of rice starch films than the addition of palm oil. The improvement properties of the films can be attributed to the lipids used, in particular on its structure, chemical arrangement, hydrophobicity and physical state (e.g. solid or liquid) and on the lipid interactions with the other components of the film, such as polysaccharides and proteins. The incorporation of palm oil 3% showed better properties including water barrier and mechanical properties than other lipids.

5. Moisture adsorption of rice starch films was more rapidly in the initial stage of moisture adsorption and a little of moisture was adsorbed when adsorption time increased. The incorporation of lipids reduces the moisture sorption of rice starch film reinforced with starch nanocrystals was significantly the lowest at all relative humidity. The moisture adsorption data of rice starch biocomposite films were increase with increasing the relative humidity and higher moisture adsorption rate was observed when higher relative humidity was used. Moreover, the tensile strength of rice starch biocomposite film was decrease at high relative humidity.

6. Rice starch films reinforced with starch nanocrystal and contained lipid reduced the crispness of the cracker less than rice starch film. Besides, cracker wrapped with rice starch film reinforced with starch nanocrystal and containg lipid exhibited higher shelf-life (9 days) than cracker wrapped with rice starch films (6 days). Moisture content and water activity of cracker wrapped with all films increased when the storage time increased. In addition, moisture content and water activity of cracker wrapped with rice starch film reinforced with starch nanocrystal contained lipid showed significantly ($p<0.05$) lower than cracker wrapped with rice starch film all storage time.

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APPENDIX

ANALYTICAL METHODS

1. Moisture content (AOAC, 1999)

Method

1. Dry the empty dish and lid in the oven at 105°C for 30 min and transfer to desiccator to cool (30 min). Weigh the empty dish and lid.
2. Weigh about 5 g of sample to the dish. Spread the sample with spatula.
3. Place the dish with sample in the oven. Dry for 16 h or overnight at 105°C.
4. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried content.

Calculation

$$\% \text{ Moisture} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Where W_1 = weight (g) of sample before drying

W_2 = weight (g) of sample after drying

2. Fat (AOAC, 1999)

Reagent

- Petroleum ether

Method

1. Place the bottle and lid in the incubator at 105°C overnight to ensure that weight of bottle is stable.
2. Weigh about 3-5 g of sample to paper filter and wrap.
3. Take the sample into extraction thimble and transfer into soxhlet.
4. Fill petroleum ether about 250 ml into the bottle and take it on the heating mantle.
5. Connect the soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle.
6. Heat the sample about 14 h (heat rate of 150 drop/min).
7. Evaporate the solvent by using the vacuum condenser.
8. Incubate the bottle at 80-90°C until solvent is completely evaporate and bottle is completely dry.
9. After drying, transfer the bottle with partially covered lid to the desiccator to cool. Reweigh the bottle and its dried content.

Calculation

$$\%\ \text{Fat content} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

3. Ash (AOAC, 1999)

Method

1. The crucible and lid is firstly placed in the furnace at 550°C overnight to ensure that impurities on the surface of crucible is burned off. The crucible is then cool in the desiccator (30 min).
2. Weigh the crucible and lid to 3 decimal places.
3. Weigh about 5 g sample into the crucible. Heat over low Bunsen flame with lid haft covered. When fumes are no longer produced, place crucible and lid in furnace.
4. Heat at 550°C overnight. During heating, do not cover the lid. Place the lid after complete heating to prevent loss of fluffy ash. Cool down in the desiccator.
5. Weigh the ash with crucible and lid when the sample turns to gray. If not, return the crucible and lid to the furnace for the further ashing.

Calculation

$$\%\ \text{Ash content} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

4. Protein (AOAC, 1999)

Reagents

- Kjedahl catalyst: Mix 9 part of potassium sulphate (K_2SO_4) anhydrous, nitrogen free with 1 part of copper sulphate ($CuSO_4$)
- Sulfuric acid (H_2SO_4)
- 40% NaOH solution (w/v)
- 0.2 N HCl solution
- 4% H_3BO_3
- Indicator solution: Mix 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% bromocresol green (in 95% ethanol)

Method

1. Place sample (0.5-1.0 g) in digestion flask.
2. Add 5 g Kjeldahl catalyst, and 200 ml of conc. H_2SO_4.
3. Prepare a tube containing the above chemical except sample as blank. Place flasks in inclined position and heat gently unit frothing ceases. Boil briskly until solution clears.
4. Cool and add 60 ml distilled water cautiously.
5. Immediately connect flask to digestion bulb on condenser, and with tip of condenser immersed in standard acid and 5-7 indicator in receiver. Rotate flask to mix content thoroughly; then heat until all NH_3 is distilled.

6. Remove receiver, wash tip of condenser, and titrate excess standard acid distilled with standard NaOH solution.

Calculation

$$\% \text{ Protein} = \frac{(A-B) \times N \times 1.4007 \times 5.95}{W}$$

Where: A = volume (ml) of 0.2 N HCl used sample titration
B = volume (ml) of 0.2 N HCl used in blank titration
N = Normality of HCl
W = weight (g) of sample
14.007 = atomic weight of nitrogen
5.95 = the protein-nitrogen conversion factor for starch and its by-products

VITAE

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Educational Attainment

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Bachelor of Science (Food Science and Technology)	Rajamangala University of Technology Srivijaya	2006

List of Publication and Proceedings

Kaewpool, P. and Wittaya, T. 2010. Properties and Characteristics of Rice Starch Films Reinforced with Rice Starch Nanocrystals. In Proceedings of The International Conference on Applied Science Engineering and Technology 2010. Singapore. 25-27 August 2010.

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