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PREPARATION OF LAMINATED FILM FROM CASSAVA STARCH AND CHITOSAN

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การเตรียมฟิล์มลามิเนตจากแป้งมันสำปะหลังและไคโทซานเพื่อใช้เป็นบรรจุภัณฑ์อาหาร กระทำโดยการเคลือบสารละลายไคโทซานลงบนผิวของฟิล์มแป้งด้วยเครื่องปาดฟิล์มอัตโนมัติ โดยปริมาณความเข้มข้นของสารละลายไคโทซานเป็น 1-4 เปอร์เซ็นต์ (โดยน้ำหนัก) ในขณะที่ปริมาณความเข้มข้นของกลีเซอรอลซึ่งเป็นพลาสติไซเซอร์ในฟิล์มแป้งเป็น 2-6 เปอร์เซ็นต์ (โดยน้ำหนัก) ฟิล์มที่เตรียมได้จะถูกตรวจสอบลักษณะโครงสร้างทางกายภาพ ตลอดจนศึกษาสมบัติเชิงกลและสมบัติทางกายภาพ จากการตรวจสอบผลึกของฟิล์มลามิเนตด้วยเทคนิคเอกซเรย์พบว่า ตำแหน่งของผลึกอันประกอบด้วยผลึกจากฟิล์มแป้งและฟิล์มไคโทซานมีการเปลี่ยนแปลงไปสู่ระดับที่สูงขึ้น โดยผลึกของแป้งซึ่งเป็นประเภทบีพบที่ 17.2° (2θ) (ฟิล์มแป้งที่ไม่ผ่านการเคลือบไคโทซานพบผลึกที่ตำแหน่ง 17.0° (2θ)) ในขณะที่ผลึกของไคโทซานซึ่งมีปริมาณน้อยกว่าพบที่ 22.2° (2θ) (ฟิล์มไคโทซานที่เตรียมได้จากการหล่อแบบพบผลึกที่ตำแหน่ง 21.1° (2θ)) การเปลี่ยนแปลงดังกล่าว เกิดจากการจัดเรียงสายโซ่โมเลกุลใหม่ของฟิล์มทั้งสอง อันเป็นผลมาจากพันธะไฮโดรเจนระหว่างโมเลกุลของแป้งและไคโทซานที่เกิดขึ้นเพื่อให้ฟิล์มสามารถยึดติดกันได้ดี จากผลการทดลองพบว่า การเคลือบด้วยสารละลายไคโทซานช่วยปรับปรุงทั้งสมบัติเชิงกลและสมบัติทางกายภาพของฟิล์ม โดยเมื่อปริมาณความเข้มข้นของสารละลายไคโทซานสูงขึ้น ค่าความทนแรงดึงและมอดุลัสของฟิล์มลามิเนตซึ่งเตรียมจากฟิล์มแป้งที่มีปริมาณกลีเซอรอล 2 เปอร์เซ็นต์ (โดยน้ำหนัก) เพิ่มขึ้นอย่างเด่นชัด ในขณะที่เปอร์เซ็นต์การยืดตัว ณ จุดขาดลดลงทั้งในแนวแรงและแนวขวาง โดยความแข็งแรงของฟิล์มในแนวแรงสูงกว่าแนวขวาง ทั้งนี้เนื่องจากกระบวนการเคลือบฟิล์มส่งผลให้โมเลกุลในทิศทางดังกล่าวมีการจัดเรียงตัวสูงกว่า สำหรับสมบัติทางกายภาพพบว่า การปรากฏอยู่ของหมู่อะเซทิลในชั้นฟิล์มไคโทซานทำให้ปริมาณการรับน้ำของฟิล์มลามิเนตลดลงอย่างชัดเจนเมื่อเทียบกับฟิล์มแป้ง อันเป็นผลให้ความสามารถในการเปียก ความสามารถในการแพร่ผ่านของไอน้ำ ตลอดจนการดูดซึมน้ำของฟิล์มลามิเนตลดลงด้วย ซึ่งนับว่าเป็นข้อดีสำหรับการพัฒนาเพื่อนำไปใช้งานเป็นฟิล์มบรรจุภัณฑ์ต่อไป

ภาควิชา วัสดุศาสตร์ ลายมือชื่อนิสิต…………………………..
สาขาวิชา วิทยาศาสตร์พอลิเมอร์ประยุกต์และเทคโนโลยีสิ่งทอ ลายมือชื่ออาจารย์ที่ปรึกษา………………
ปีการศึกษา 2546 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม…………..

4472524923 : MAJOR APPLIED POLYMER SCIENCE AND TEXTILE TECHNOLOGY
KEY WORD : CASSAVA STARCH / CHITOSAN / LAMINATED FILM

CHOLWASA BANGYEKAN : PREPARATION OF LAMINATED FILM FROM CASSAVA STARCH AND CHITOSAN. THESIS ADVISOR : ASSOCIATE PROFESSOR DR. KAWEE SRIKULKIT, THESIS COADVISOR : ASSISTANT PROFESSOR DR. DUANGDAO AHT-ONG, 102 pp. ISBN 974-17-4838-8.

Chitosan/cassava starch laminated film designed for food packaging was prepared by coating viscous chitosan solution onto the free starch film using an automatic coating applicator. In this study, chitosan coating solutions varying from 1 to 4 wt% were coated onto the free starch films containing 2, 3, 4, 5, and 6 wt% glycerol. After allowing open air drying, the laminated films were investigated the physical structure, mechanical, and physical properties. The X-ray patterns of chitosan/cassava starch laminated films showed the reflection of B-type starch crystalline shifting to slightly higher degrees at about 17.2° (2θ) (free starch film found at 17.0° (2θ)) while that of chitosan appeared as a smaller peak at about 22.2° (2θ) (free chitosan film prepared by casting technique found at 21.1° (2θ)). The shifting in starch diffraction peak was probably due to the change in its chain orientation caused by hydrogen-bonding interaction between chitosan and starch molecules, indicating their good adhesion. Coating of chitosan solutions led to an improvement in several film properties including mechanical and physical properties. An increase in chitosan coating concentration resulted in a significant increase in tensile strength. The results on mechanical properties showed a significant increase in tensile stress at maximum load and tensile modulus, and a decrease in % elongation at break of laminated film containing low glycerol content. Film strength along machine direction is higher than that of transverse direction because of the higher molecular orientation during coating process. Concerning physical properties, a remarkable decrease in water uptake was observed due to the contribution of hydrophobicity of chitosan coating layer. The hydrophobic acetyl groups of chitosan causes a reduction of wettability as well as water vapor permeability which are preferable for packaging film application.

Department Matereials Science	Student’s signature………..
Field of study Applied Polymer Science and Textile Technology	Advisor’s signature………..
Academic year 2003	Co-advisor’s signature……..

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Chapter I
Introduction

Over the last few decades, there has been growing needs to find alternatives to petroleum-based plastics because of environmental concerns. Plastic waste becomes a serious problem because of its low weight-to-volume ratio and its inalterablity over very long time duration. Today, incineration is a common method to get rid of polyolefins, but this unfortunately leads to high emission of CO_2. One approach for solving the problem is to use biodegradable materials instead of non-renewable polymers. These materials have the potential to reduce environmental pollution by lowering solid disposal waste and reducing the need for inceneration [1].

Cassava (*Manihot esculenta* crantz) or tapioca is one of the economically important crops in Thailand and the cheapest raw material of starch production. Structurally, cassava starch consists of two types of molecules: amylose, a substantially linear polymer with a molecular weight of about 10^5; and amylopectin, a highly branched polymer with very high molecular weight of about 10^7. The approximate 17% of amylose content is responsible for strong film. This is due to association and aggregation of the straight chains through hydrogen bonding providing rapid gel formation, which develops into order regions leading to crystalline orders and chains converting into insoluble forms. Cassava starch can readily be cast into films. However, the cassava starch films are brittle and weak leading to inadequate mechanical properties. Overcoming the brittleness of the film can be accomplished by adding plasticizers. Common plasticizers used for starch films preparation are water, glycerol, sorbitol, and other low-molecular weight-polyhydroxy-compounds. Water is an excellent plasticizer, however, it has some disadvantages since water content varies with humidity. At low humidity there are problems with brittleness and at high humidity with softness. Glycerol and sorbitol are widely used as plasticizer because of their stability and edibility. Addition of plasticizers makes the brittle films more flexible, but also less strong. This problem has led to the development of mechanical properties of cassava starch film. Nowadays, film made from cassava starch with good mechanical properties and heated-sealability is used for food packaging.

Recently, biodegradable and edible cassava starch film which possesses some outstanding properties (1.77 kg mm^{-2} of tensile strength, 10.13 of percentage elongation, and 45 times of folding endurance) and is suitable for fatty food packaging either wrapping or sealed bag has been developed. The film has been used for wrapping some Thai fruit candy such as durian paste, banana paste and kalamae. Moreover, sealed film could be made as an oily seasoning sachet for instant noodle providing a convenience to consumers during cooking. It has also been reported that the product shelf-lives have been significantly prolonged and the packaging is more attractive and more practical than the conventional ones [2]. However, the applications of cassava starch film in this field are limited. This is because the hydrophilic nature of the raw material affects moisture barrier properties, and then tensile properties of the film. Blending or laminating with other materials could improve the disadvantages.

The scope of films made with starch combined with other polysaccharides was widened to include chitosan for several reasons. First, chitosan is a biopolymer, obtained by N-deacetylation of chitin, which is the second most abundant polysaccharide on the earth after cellulose [3]. It is commercially available from a stable renewable source, that is, shellfish waste (shrimp and crab shells) of the sea-food industry [4]. Second, chitosan forms good films and membranes. Chitosan films that were clean, tough, flexible, and good oxygen barriers were formed by solution casting [5]. In Japan, composited films from chitosan and cellulose have been made by casting dispersions on the steel or chrome plates at elevated temperatures from 70-100^{o}C [6]. Some of these films contained glycerol and had good tensile strength. They were readily biodegradable either in sea water or in soil [6, 7]. Third, the cationic properties of chitosan offer the film-maker an opportunity to take advantage of electrostatic interactions with other anionic polysaccharides. Laminated films from chitosan and pectin, patented by Hoagland and Parris [8], were prepared by casting with either glycerol or lactic acid as plasticizers. The films were high modulus, flexible and self-supporting, and were advantageous in that all materials derived from agricultural.

In addition, chitosan possesses useful properties such as biodegradability, biocompatibility, and non-toxicity leading to extensively use over a wide range of applications. For example, flocculant, clarifier, thickener, gas-selective membrane, plant disease resistance promoter, wound healing promoting agent and antimicrobial agent [9], including biodegradable, edible coating or film in food packaging [10-13]. Chitosan film has a potential to be employed as packaging, particularly as an edible packaging. This is due to its excellent oxygen and carbon dioxide barrier properties and interesting antimicrobial properties. For example, biodegradable and edible chitosan films were produced to protect foods from fungal decay [15] and modify the atmospheres of fresh fruits [14, 15].

In this research, chitosan/cassava starch laminated film was prepared by coating chitosan solution on cassava starch film where glycerol acted as a plasticizer. The combination of hydrogen bonding, opposite charge attraction between chitiosan cations and negatively charged starch film surface, hydrophilicity, and compatible water activities provided a good adherance between a starch film and a chitosan film. The more hydrophobicity of chitosan film could be expected to improve the moisture barrier properties and water resistance including dimensional stability of cassava starch film. To obtain the optimum condition for the laminated film preparation, the effects of glycerol content and chitosan coating concentration on the physical and mechanical properties of the laminated films were investigated.

Chapter II

Literature Survey

2.1 Native Starch and Properties

Starch is a natural polymer, which occurs widely in plants. The principal crops used for its production include potatoes, corn, cassava, and rice. In all of these plants, starch molecules are arranged in semicrystalline starch granules (1-100 μm). Size and shape of the granules and the properties of the starch depend on the plant source as shown in Figure 2.1.

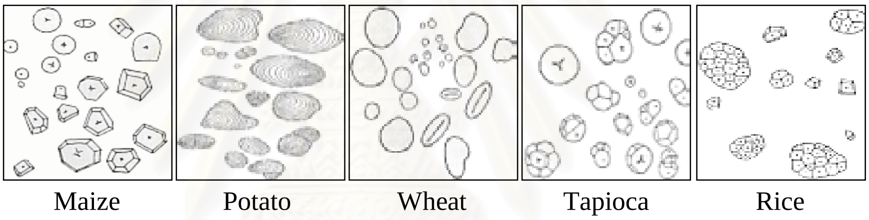

Maize Potato Wheat Tapioca Rice

Figure 2.1 Microscopic appearance of various starches [16]

2.1.1 Cassava Starch

Cassava (*Manihot esculenta* Crantz) is an important food crop in many tropical countries in Africa, South America and Asia. However, in Thailand, this crop has been well recognized as more than a subsistence crop. It is important commercially as the raw material for a large and complex industrial system that has a significant impact to the country's economics. The roots of this crop contain high starch content and approximately half of the total roots produced (20 million tons) are used for the starch industry. General properties of cassava starch are given in Table 2.1. The most important characteristics of cassava starch are:

- **Odorless** : The absence of unpleasant odor in cassava starch enables this product to be conveniently and readily blended with other flavoring ingredients.

- **Paste clarity** : Cassava starch, when cooked, provides a paste with clear appearance; the paste clarity of cassava starch (1%) as indicated by % light transmittance at 650 nm is in the range of 40-70%. The products of cassava are clear and suitable for combining with other coloring agents.

- Stickiness : With high ratio of amylopectin to amylose (83:17), cassava starch provides a high-peak viscosity but low potential for retrogradation, resulting in starch gel with good freeze-thaw stability.

These remarkable characteristics of cassava starch (high paste viscosity, high paste clarity and high freeze-thaw stability) are advantageous to many industries.

Table 2.1 General properties of cassava starch [17]

Property	Value
Chemical composition (% dry basis)	
- Protein	0.15 – 0.30
- Fat	0 – 0.01
- Ash	0.10 – 0.15
Granule size (μm by image analysis)	3 – 34
Amylose content (% by high performance size exclusion chromatography)	17 – 23
Amylose size (DP_n, by high performance size exclusion chromatography)	2040 – 4640
Swelling power at 85°C (0.1 g starch in 15 ml of distilled water)	40 – 62
Solubility (%) at 85°C (0.1 g starch in 15 ml of distilled water)	22 – 42
Paste viscosity (by Rapid Visco Analyzer using 3 g starch at 14% moisture content in 25 ml of distilled water)	
- Pasting temperature (°C)	67.0 – 74.0
- Peak viscosity (RVU)	350 – 490
- Trough viscosity (RVU)	110 – 210
- Final Viscosity (RVU)	180 – 290
- Breakdown (RVU)	160 – 340
- Setback (RVU)	50 – 110
Thermal analysis (by Differential Scanning Calorimeter using 30% starch)	
- Onset temperature (°C)	60.0 – 65.0
- Peak temperature (°C)	67.0 – 74.0
- Conclusion temperature (°C)	79.0 – 87.0
- Enthalpy (J/g)	14.0 – 17.0
Retrogradation (% by thermal analysis of starch gel kept at 4°C for 7 days using Differential Scanning Calorimeter)	28.0
Degree of hydrolysis (% using 1% each of α-amylase and glucoamylase at 37°C, 48 hrs)	25 – 60

2.1.2 The Constituents of Starch

Starch consists of two types of α-D-glucose polymers: amylose, an essentially linear polysaccharide with molecular weight up to 10^6; and amylopectin, a highly branched polymer with very high molecular weight of the order of 10^8. The abundance of hydroxyl groups results in hydrogen bonding being a key force influencing starch functions and properties. The ratio of the two types of starch molecules present varies with the origin of the starch. Corn starch, potato starch, and cassava starch contain 27, 20, and 17% of amylose, respectively. However, conventional hybrid breeding can create starch with 100% amylopectin (waxy maize starch) or progressively higher amylose content (e.g. 50-70% amylose and unique hybrid with greater than 90% amylose) [18].

Amylose molecules make up of single mostly unbranched chains with α-(1,4)-linkages (Figure 2.2). The length of amylose chains varies with plant source but in general the average length runs between 500-2000 glucose units. Aqueous solutions of amylose are very unstable due to the intermolecular attraction and association of neighboring amylose molecules. This leads to viscosity increase, retrogradation and, under specific condition, precipitation of amylose particles.

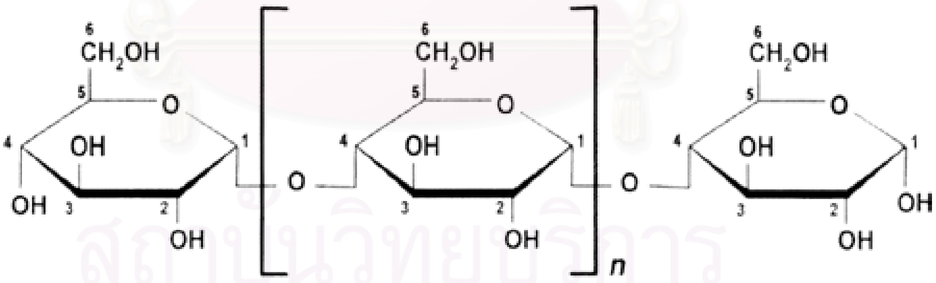


Figure 2.2 Amylose molecule structure

It is interesting to note that amylose and cellulose are very similar in structure with the single exception of the spatial arrangement of the bridging between the number 1 and 4 carbons (α-(1,4)-linkage) as presented in Figure 2.3. The beta glucose form found in cellulose results in a rigid molecule with strong intermolecular bonding, which is not digestible by humans, where as, the alpha linkage of amylose allows it to be flexible and humanly digestible.

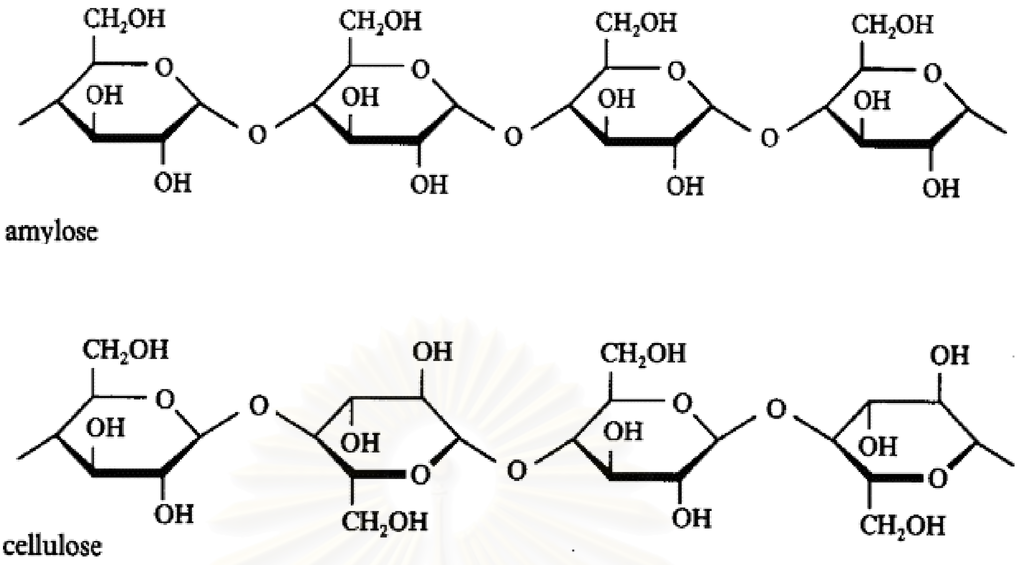


Figure 2.3 The difference of amylose and cellulose structure

The larger of the two components, amylopectin is highly branched with a much greater molecular weight. The structure is formed by non-random α-(1,6)-branching of amylose type α-(1,4)-D-glucose structure (Figure 2.4). This branching is determined by branching enzymes that leave each chain with up to 30 glucose residues. Each amylopectin molecule contains a million or so residues, about 5% of which form the branch points. Amylopectin has been found to form the basis of the structure of starch granules. This is because the short branched 1,4-chains (DP 15-18 of amylopectin molecule) are able to form helical structures which crystallize.

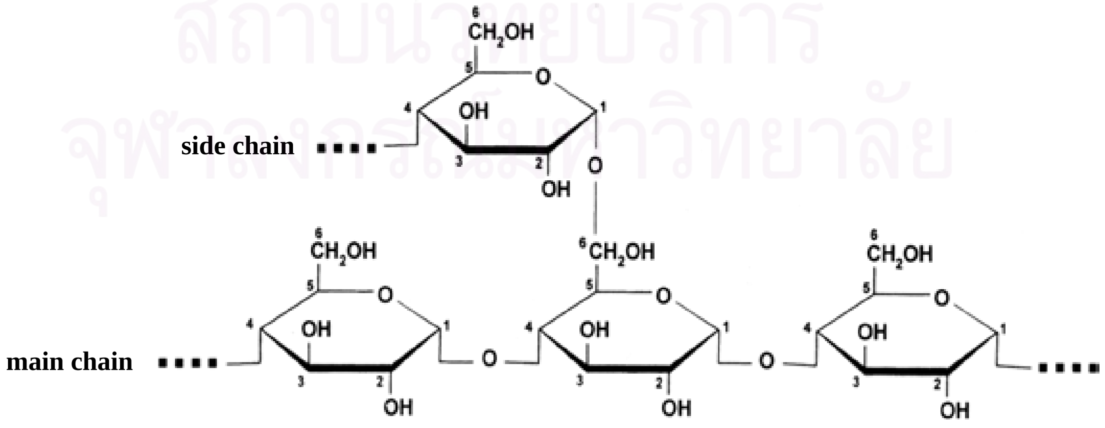


Figure 2.4 Amylopectin molecule structure

It is now accepted that amylopectin crystallizes according to a cluster structure as shown in Figure 2.5. Amylose fits into this structure in ways which are still not clearly understood. One amylopectin molecule spans several clusters and the branches can be labelled A, B or C. The A chains are the outer chains linked to an inner B chain. The B chains are linked to other B chains or the C chain. This is identified as the only chain having a free reducing end. There is only one C chain per amylopectin molecule. With this A, B, and C chain structure there are crystalline and amorphous regions. The extended B chains provide the rigidity to the granule structure by bridging the amorphous regions.

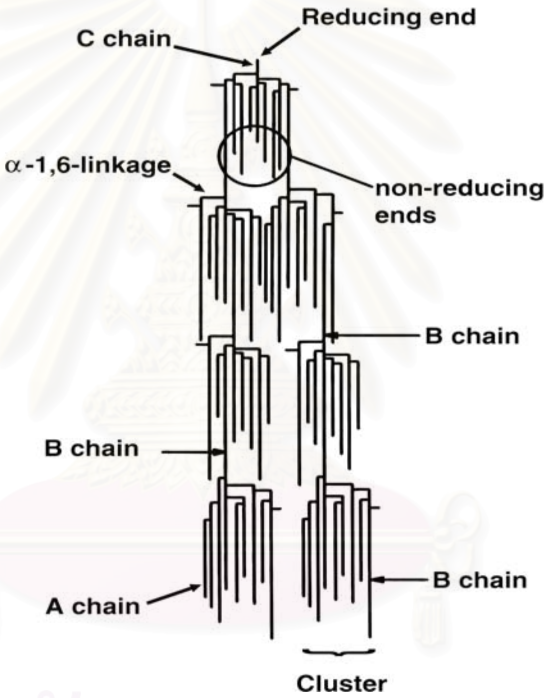


Figure 2.5 Cluster structure formed by amylopectin [19]

The amylopectin molecules are oriented radially in the starch granule and as the radius increases so does the number of branches required to fill up the space, with the consequent formation of concentric regions of alternating amorphous and crystalline structure. In general, the structure of native starch granule has crystallinity levels from 15-45%. Amylopectin is the key crystalline component in starch granule with its outer chains forming double helices arranged in thin lamella regions while amylose and the branching points of amylopectin form the amorphous regions. Amylose may also crystallize may arise into single helical structure [19].

A cross section of starch granule and the arrangement of amylopectin with in the semicrystalline granule are shown in Figure 2.6. In the diagram below: A - shows the essential features of amylopectin. B - shows the organization of the amorphous and crystalline regions (or domains) of the structure generating the concentric layers that contribute to the “growth rings” that are visible by light microscopy. C - shows the orientation of the amylopectin molecules in a cross section of an idealized entire granule. D - shows the likely double helix structure taken up by neighboring chains and giving rise to the extensive degree of crystallinity in granule. There is some debate over the form of the crystalline structure but it appears most likely that it consists of parallel left-handed helices with six residues per turn.

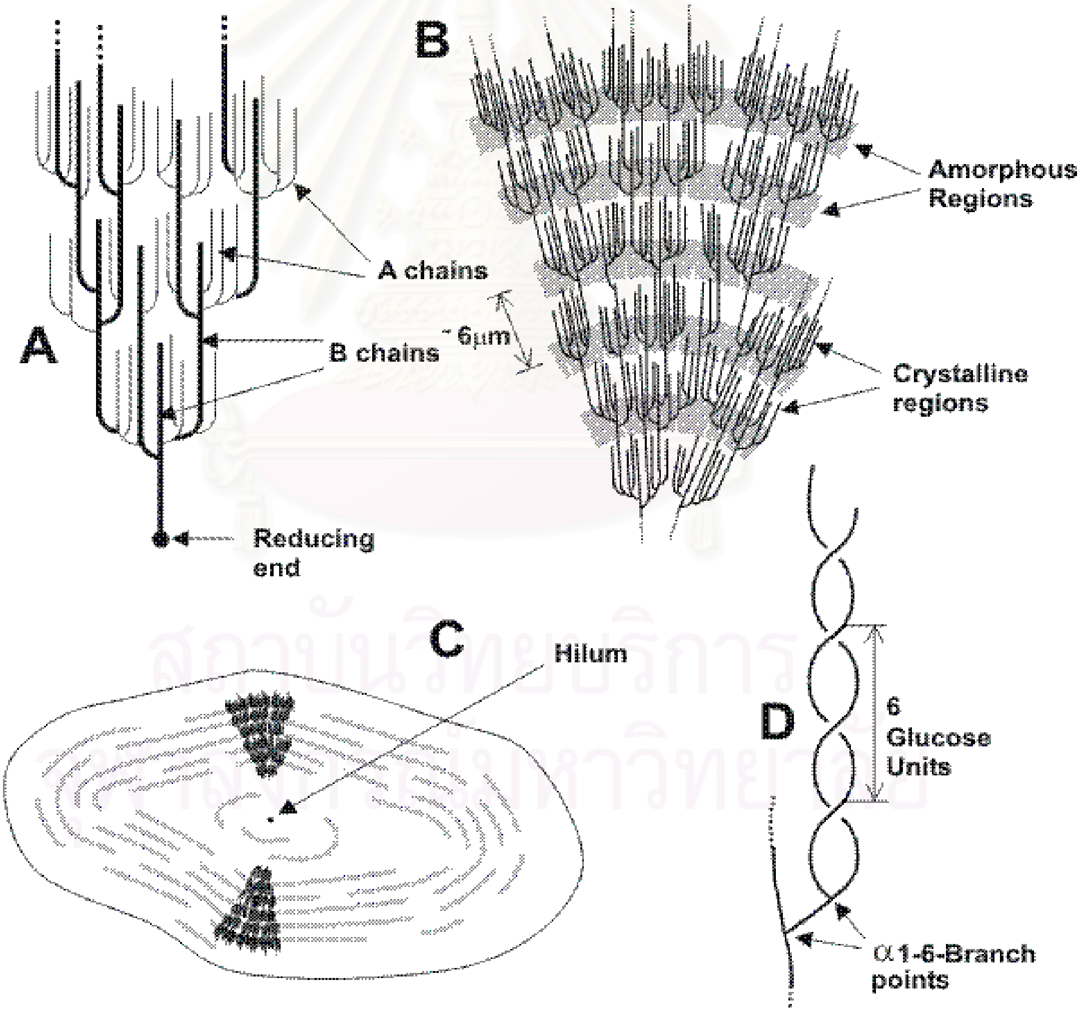


Figure 2.6 Amorphous and crystalline regions in starch granule [19]

There is an additional complexity relating to the nature of crystalline structures measured by X-ray techniques. By this method, different types of crystal structures can be analyzed and amount of crystalline and amorphous material can be measured.

In native starch granules, the crystal types called A, B, and their mixture C are formed in cereals, potatoes and legumes, respectively [20, 21]. Water content is also essential when investigating the crystallinity of starches. Dry starch has a completely amorphous X-ray pattern, and the crystallization of B-type starches has been shown to vary with varying water contents [22]. The B-type is converted to A-typed by means of heat-moisture treatment at 100-120°C [23]. Imberty et al. [24] proposed a crystal model of A-type starch. Left-handed double helices are densely packed in monoclinic units, with eight water molecules. The crystal unit cell is 2,124 nm wide, 1,172 nm thick and 1,069 nm high. In the B-type crystal model, the double helices are packed in a hexagonal unit, with 36 water molecules [22]. The width and thickness of the B-crystal unit cell is 1.85 nm and the height is 1.04 nm [24]. There is an amorphous area between the crystal clusters, which is mainly composed of branched points of amylopectin chains [25].

2.1.3 Starch Properties

2.1.3.1 Starch Gelatinization and Retrogradation

Starch is insoluble in cold water, alcohol, ether and most organic solvents. It is stable in storage for indefinite periods of time if kept dry. When starch is heated in aqueous conditions, the starch granules begin to swell and the molecular order within the starch granule collapses. Irreversible changes occur, such as crystallite melting, viscosity development and solubilization. This phenomenon, called gelatinization, takes place at about 55-80^{o}C depending on the type of starch. However, pasting is better defined as the phenomenon following gelatinization in the dissolution of starch, leading eventually to total disruption of the granules, which occurs at temperatures of about 100-160^{o}C [26, 27]. Yet a definition of pasting is that pasting is the sequence of events that starch undergoes when both heated and stirred to apparent solubilization. The solution formed is called a starch paste. Upon cooling, the starch begins to undergo retrogradation, which means that the starch molecules begin to reassociate in an ordered structure [27]. First, two or more molecules may form a simple juncture point, and the juncture points may then develop into ordered regions. Finally, a crystalline order may appear and the chains convert into an insoluble form. Starch retrogradation can be studied by rheological methods, X-ray diffraction, thermal analysis and spectroscopic methods [28]. When cooked starch pastes are cooled, retrogradation takes place and this is manifested by the formation of a precipitate or a gel [26]. As dilute (< 1.5%) amylose solutions are cooled, the amylose precipitates out, forming a gel of increasing modulus with increasing concentration [29]. The chain length of amylose affects the phase behavior as follow: chain lengths of < 110 residues form precipitates, those of 250-600 residues form both precipitate and gel, and gelation predominates for longer chain lengths (> 1100) [30]. However, the critical concentration for gelation (approximately 1%) is nearly independent of chain length [31]. The amylose concentration affects the properties of the formed gels. Amylose-rich gels exhibit higher mechanical and thermal resistance and they degrade less both chemically and enzymatically as compared with amylopectin-rich gels [32]. The shape of aggregates formed out of a steam jet cooked solution upon cooling has been investigated by Fanta and co-workers [33]. When normal maize starch, high amylose maize starch and rice starch were cooled, two different kinds of particles were formed. The small particles were disc or torus in shape, while the larger particles were almost spherical.

2.1.3.2 Glass Transition Temperature

The glass transition temperature (T_g) is the most important parameter in determining the mechanical properties of amorphous polymers and in controlling the kinetics of crystallization of amorphous materials [34-36]. For dry amylose and amylopectin, the T_g has been estimated to be at about 277°C, and the presence of 13% water has been observed to decrease the T_g to 56°C [35]. The T_g of gelatinized wheat starch containing 22% water was detected to be at room temperature [37]. Many studies have demonstrated the plasticization effect of water on starches, and the various techniques for analyzing the glass transition have been compared. When examining different techniques, the most frequently used DSC was observed to give 10-30°C higher T_g than pulsed NMR [38].

The effect of water on the T_g of amylose and amylopectin was recently analyzed using DSC [39]. The much-branched amylopectin had a somewhat lower T_g than the amylose polymer. Studies of the effect of glycerol and other plasticizers on the T_g of potato starch showed that the plasticization of starch follows Couchman's model [40]. When amylose and maltose were plasticized by glycerol, phase separation above 25% glycerol was found, based on dielectric thermal (DETA) analysis [41]. In a combination of water and glycerol as plasticizers of barley starch, two calorimetric glass transition temperatures were measured. The two transitions observed were an indication of phase separation [42]. The dynamic mechanical, the dielectric thermal, and the calorimetric behavior of binary amylose-glycerol system, assessed by DMTA, DETA, and DSC, respectively, indicated that the system was composed of amylose-rich and glycerol-rich phases [43].

2.1.4 Applications of Starch

Being a pure renewable natural polymer starch has a multitude of applications. Starch and starch products are used both in food and non-food applications. Starch enjoys wide usage in several non-food sectors, most notably in the sizing and coating of papers, as an adhesive, a thickener, and as a "green strength" additive to simple composite materials.

Fifty years ago, it was suggested that starch could be used as an edible films or coatings [46-47]. Recently, much research activity has focused on the use of starch as bioplastics [48], especially, in the packaging field because of biodegradability and low cost including a decrease of conventional film-forming resins. Agricultural mulch film is a good example of starch used as one of raw material in film production.

2.2 Thermoplastic Starch (TPS)

The terms of thermoplastic, destructed, or molten starches have been used in numerous patents during the last decade to describe the changes in starch granules during processing of plastic-type materials. Starch can be processed into a thermoplastic material only by the presence of plasticizers and under the influence of heat and shear.

One problem with the use of starch in plastics is its brittle nature caused by its relatively high glass transition temperature (T_g) and lack of chain relaxation. The glass transition temperature (T_g) and the melting temperature (T_m) of starch (230°C and 220-240°C, respectively) are higher than the temperature at which starch begins to decompose (about 220°C). Brittleness also increases with time due to free volume relaxation and retrogradation. Overcoming this problem, plasticizers (usually 1-2%) are added to reduce the intermolecular hydrogen bonds and depress T_g and T_m leading to more flexible starch chain, but less strong. Common plasticizers for starch are water, glycerol, and other low-molecular weight-polyhydroxy-compounds, polyethers and urea. Water is an excellent plasticizer for starch because of its favourable interaction with the hydroxyl groups of starch; thereby diminishing interactions between starch chains. However, water content varies with humidity. At low humidity there are problems with brittleness and at high humidity with softness. Moreover, water can induce physical aging. Due to these disadvantages, glycerol is most commonly used.

2.3 Starch as a Film Former

Early investigations on melted or dissolved starch were focused on film formation properties of starch polymers. A solvent casting procedure was applied, in which starch was dissolved at low concentration in water or in other solvents and a film was produced by evaporating the solvent [44, 45].

Starch films prepared by extrusion in the presence of glycerol was studied. Different mechanical properties could be obtained depending on the glycerol content and botanical origin of starch [47-49]. High amylose starch has been shown to form stronger and stiffer thermoplastic films than high amylopectin starch. On the other hand, the hydrophilic nature of the TPS made it sensitive to environmental humidity, and the addition of a high level of glycerol further accelerated these problems. During storage, TPS made of potato starch had a tendency to change its structures, becoming more brittle [50]. The changes observed in TPS were similar to retrogradation of normal starch gels, indicating that this process might be linked to the crystallization of amylopectin [51].

2.3.1 Factors Affecting Starch Films

Drying temperatures is the dominant factor affecting the orientation of amylose-rich starch film structures [52]. When a lower drying temperature is used, a B-type crystalline is formed, whereas at temperatures above 80°C a film with A-type crystalline is obtained. Drying temperature also affects the degree of crystallinity; films formed at room temperature (20°C) obtain a higher crystallinity than films formed at elevated temperature (68°C) [52]. An increase in air humidity increases the crystallinity of both starch films [52] and amylopectin films, but air humidity has no effect on the crystallinity of amylose films [53].

In addition to the environmental factors affecting starch film formation, the amylose/amylopectin ratio and co-materials, such as plasticizers, also affect it. In case of effect of amylose/amylopectin ratio, for unplasticized films, an increase in amylose content leads to a decrease in carbon dioxide and water vapor permeabilities [54] and an increase in both tensile strength and elongation [55]. For plasticized (glycerol) films, however, the tensile strength increases with amylose content while the elongation decreases. The tensile strength and the elongation reach a plateau, however, and the mechanical properties stay unchanged above an amylose content of about 30-40%. The amylose/amylopectin ratio also affects the morphology of the formed films: higher amylose content leads to a more homogeneous film, and higher amylopectin content increases phase separation [56].

Plasticizers are added to polymers to increase the ductility of the material. Glycerol, sorbitol, fructose, glucose, sucrose, xylose, lactic acid sodium, urea, diethylene glycol, polyethylene glycol (PEG), and glycerol diacetate are materials used as plasticizers in starch films [53, 57-60]. The first two excipients are the most widely studied. At low plasticizer contents, the plasticizer (glycerol or sorbitol) does not have the classical effect of elongating on starch films [40, 60]. This phenomenon, well known from synthetic polymer science, is called the antiplasticization effect [59]. The elongation starts to increase, with higher plasticizer contents than approximately 15% for glycerol and 27% for sorbitol. The tensile strength of starch films decreases continuously with the increasing plasticizer content.

2.3.2 Properties of Starch Films

Recent studies on starch films prepared by water casting and plasticized by glycerol have attempted to elucidate in more details the effects of plasticization on the structure and properties [52, 53, 55]. The structures of starch films were observed to be entirely amorphous [55]. The degree of crystallinity varied with the film forming conditions [53]. The mechanical and barrier properties of starch films depend on the environment and their applications.

The mechanical properties of pure amylose and pure amylopectin films are different, amylopectin films having a more brittle character than amylose [61]. It has been reported that the linear amylose fraction of starch yields stronger and more flexible films than the branched amylopectin. There is a non-linear positive correlation between tensile strength and amylose content. The water binding capacity of starch decreases with increasing in amylose content, possibly due to the increase of hydrogen bonding between and within amylose chains which reduces the number of hydroxyl groups available to water. It has been reported that pure amylose films were more stable in water than pure amylopectin films dispersing into the water during the same time [62].

Plasticizer content and the surrounding air humidity have some effects on barrier properties of starch film. The oxygen permeability of native starch film is low. A higher plasticizer content and/or higher air humidity lead to increasing oxygen permeability [58], whereas a higher crystallinity leads to a reduction in gas (O_2, N_2, and CO_2) and water vapor permeability [58, 63].

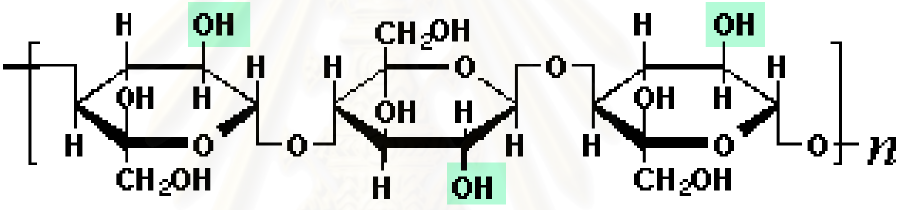
Amorphous starch films are in nonequilibrium state, characterized by molecular disorder. They may be either in glassy state or in rubbery state. The glassy state, when the material is hard and brittle, is formed by sufficiently rapid evaporation of the solvent or by rapid cooling. With an increase in temperature, glassy material may transform into the less viscous rubbery state. The state transition from glassy to rubbery is called the glass transition and it occurs over a specific temperature range. A significant decrease in stiffness and increase in molecular mobility take place at the glass transition temperature. The glass transition temperature of films depends on factors such as the particular polymer employed, plasticizer added [40], and the amount of plasticizer [57]. Water acts as a plasticizer for starch films [64]. Increasing the plasticizer content in films leads to a lower glass transition temperature. At high (50% of polymer weight) plasticizer (fructose) content the transition was broadened, which suggested a multiphase system containing amylopectin-rich and fructose-rich regions [57]. Lourdin and co-workers [40] showed that phase separation occurred for starch films containing more than about 13% of lactic acid sodium. A similar phase separation was not seen for glycerol and sorbitol, but the authors speculated that it would occur at higher plasticizer contents. Forssell and co-workers [42] observed phase separation for glycerol at intermediate (29%) glycerol and water contents.

2.3.3 Stability of Starch Films

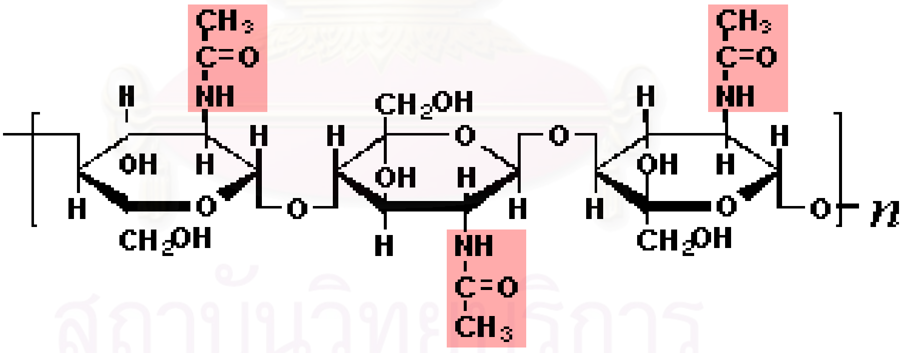
Several studies have reported changes in the mechanical properties of rubbery starch films during storage [50, 65]. The elongation of films decreases while the tensile strength increases. The changes occurring during storage have been explained by changes in water content, glass transition temperature [66], and crystallinity [65]. From calorimetric and X-ray diffraction studies, Forssell and co-workers [50] concluded that the major change in the mechanical properties of starch films is due to the reorientation and/or crystallization of amylose and amylopectin molecules. Kuutti and co-workers [67] concluded from atomic force microscope (AFM) friction measurements that the small molecular plasticizer, such as glycerol, was migrating to the surface during aging. Recently somewhat different results have been reported. Amylose films (plasticized with 0, 10 or 30% of glycerol) showed a B-type diffraction pattern, and no changes in crystallinity were observed during two months storage (relative humidity [RH] 0, 54, and 91% at room temperature) [62]. For most amorphous amylopectin films, no change occurred during the storage period. However, for amylopectin films with 30% of glycerol stored at RH 91%, the crystallinity increased from 10 to 19% within one month. The increasing crystallinity was attributed to the rubbery state of the highly plasticized amylopectin films [62]. Extruded starch rods in the glassy state have also shown a progressive embrittlement on aging [68].

2.4 Chitosan

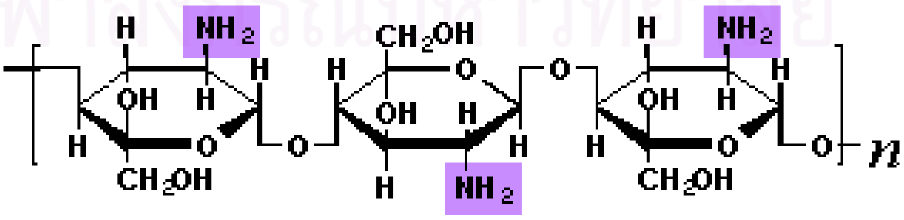
Chitosan, a partially N-acetylated 2-deoxy-2-amino-α-glucan polymer, is obtained by alkaline N-deacetylation of chitin, a naturally occurring macromolecule presented in the exoskeleton of invertebrates and the second most abundant polysaccharide resource after cellulose [1]. The structure of chitin is so similar to cellulose because both are β-(1-4) linked. The difference between them is that chitin has an amide group instead of a hydroxyl group, which cellulose has. Moreover, the structure of chitosan is also very similar to chitin. The difference is that chitosan has an amine group instead of an amide group. This means that chitosan does not have any carbons double bonded to oxygen and chitin does. Figure 2.7 shows the comparative structures of cellulose, chitin, and chitosan.



(a) cellulose



(b) chitin



(c) chitosan

Figure 2.7 Similarities of chemical structures of: (a) cellulose, (b) chitin, and (c) chitosan [69]

Being a biopolymer, chitosan is recommended as suitable resource material for various applications. It has uniquely excellent properties such as biodegradability, biocompatibility, non-toxicity, adsorption property and so on. However, the naturally abundant material also exhibits a limitation in reactivity and processability [70].

Chitosan has been extensively used over a wide range of applications. For example, a biomaterial in medicine either on its own or as a blend component, a water membrane filter for treatment, a biodegradable and edible coating or film in food packaging [71].

2.4.1 Production and Procedures

Commercial chitosan is available from plentiful and renewable sources primarily waste from the shellfish industry due to the main industrial sources of chitin are the shell wastes of shrimp, lobster, and crab. Besides, chitosan is also prepared from squid pens. Squid pen chitosan is synthesized from β-chitin (amine group aligned with the OH and CH_2OH groups) and crustacean exoskeleton chitosan is synthesized from α-chitin (anti-parallel chain alignment) [72, 73]. Briefly, the preparation of chitin and chitosan is presented in Figure 2.8.

Chitosan are commercially manufactured by a chemical method. The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a complete amino group ($-NH_2$) and chitosan versatility depends mainly on this high degree chemical reactive amino groups. Deacetylation of chitin with aqueous alkali is the most commonly used method to obtain the chitosan products. In general, alkaline deacetylation of chitin proceeds rapidly before the polymer is about 75-85% deacetylated and further treatment has only a very limited effect on the extent of deacetylation unless drastic conditions are used. So the degree of nearly 100% deacetylation is rarely achieved with the relatively mild and simple method.

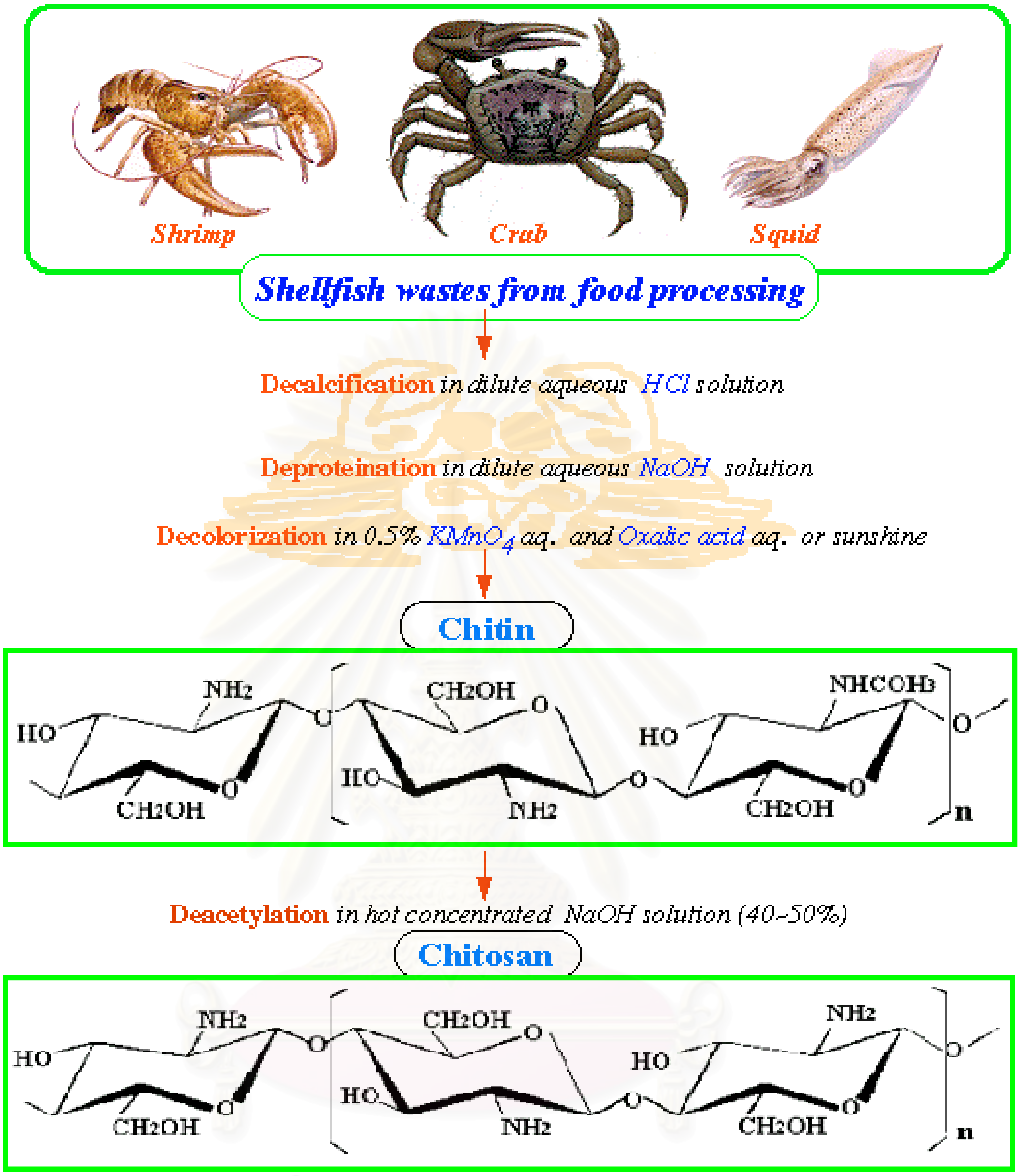


Figure 2.8 Preparation of chitin and chitosan [74]

An extraction method investigated by Hirano is one of examples [75]. Crab or shrimp shells are deproteinized by treatment with an aqueous 3-5% NaOH solution. The resulting product is neutralized and calcium is removed by treatment with an aqueous 3-5% HCl solution at room temperature to afford a white or slightly pink precipitate of chitin. The deacetylation of chitin is done by treatment with an aqueous 40-45% NaOH solution, and the precipitate is washed with water. The crude sample is dissolved in aqueous 2% acetic acid, and the insoluble material is removed.

The resulting clear supernatant solution is neutralized with aqueous NaOH solution to afford a purified sample of chitosan as a white precipitate. However, a novel method was recently established for preparing 100% deacetylated chitosan products. The facile and effective deacetylation obtained by only one-step alkali treatment after the low deacetylated chitosan sample was dissolved in acid solution and regenerated with the form of film from the solution by solvent evaporation [76].

The quality and properties of chitosan product may vary widely because many factors in the manufacturing process can influence the characteristics of the final product [77]. Various commercial grades of chitosan are considered from molecular weight, viscosity, degree of deacetylation, and purity.

2.4.2 Physical and Chemical Characterization

Chitosan can be characterized in terms of its quality, intrinsic properties (purity, molecular weight, viscosity, and degree of deacetylation), and physical forms.

Most of the naturally occurring polysaccharides, e.g. cellulose, dextran, pectin, alginic acid, agar, agarose, carragenans, are neutral or acidic in nature, whereas, chitin and chitosan are examples of highly basic polysaccharides. Like cellulose, chitosan naturally functions as a structural polysaccharide, but differs from cellulose in the properties. It especially has amino groups, which possess many properties different from cellulose such as solubility in dilute organic acid, effective capability of binding with metal ions and its biological effect including the unique properties such as polyoxysalt formation, ability to form films, and optical structural characteristics. For chemical characteristics, the degree of deacetylation, molecular weight, and solvent and solution properties are discussed in brief.

2.4.2.1 Degree of Deacetylation

Degree of deacetylation is one of the important chemical characteristics, which could influence the performance of chitosan in many of its applications [78, 79]. The degree of deacetylation determines the content of free amino groups in chitinous material that can be employed to differentiate between chitin and chitosan. Chitosan could be defined as chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be 80-85% or higher because the acetyl content of the chitosan product must be < 4-4.5%. Chitosan products are highly viscous, resembling natural gums.

Since degree of deacetylation depends mainly on the method of purification and reaction conditions, therefore, many routes have been developed to increase the degree of deacetylation values. For example, increase either in temperature or strength of sodium hydroxide solution could enhance the removal of acetyl groups from chitin, resulting in a range of chitosan molecules with different properties and hence its applications [79, 80].

2.4.2.2 Molecular Weight

Chitosan is polydisperse with chains different lengths. The random distribution of glucosamine to N-acetyl-glucosamine is often expressed as the degree of deacetylation. Chitosan molecular weight distributions have been obtained using HPLC. The weight-average molecular weight (M_w) of chitin and chitosan has been determined by light scattering.

Viscometry is a simple and rapid method for the determination of molecular weight; the constants α and K in the Mark-Houwink equation have been determined in 0.1 M acetic acid and 0.2 M sodium chloride solution. The intrinsic viscosity is expressed as

$$[\eta] = KM^{\alpha} \qquad ; [\eta] = \text{intrinsic viscosity}$$
$$\alpha = 1.81\times10^{-3}$$

The charged nature of chitosan in acid solvents and chitosan's propensity to form aggregation complexes requires care when applying these constants. Furthermore, converting chitin into chitosan lowers molecular weight, changes the degree of deacetylation, and thereby alters the charge contribution, which in turn influences the agglomeration. The weight-average molecular weight of chitin is 1.03×10^{6} to 2.5×10^{6}, but the deacetylation reaction reduces this to 1×10^{5} to 5×10^{5}. The increase in the number of deacetylation step promotes an increase in the degree of deacetylation followed by a decrease in the average of molecular weight and polydispersion.

2.4.2.3 Solution Properties

Chitosan is insoluble in water or in alkaline solutions at pH levels above about 6.5, or in organic solvents. It dissolves readily in dilute solutions of most organic acids, including formic, acetic, tartaric, and citric acids. Chitosan is soluble to a limited extent in dilute inorganic acids except phosphoric and sulfuric acids. Most properties of chitosan relate to its polyelectrolyte and polymeric carbohydrate character. In acid solutions, amine groups of chitosan are protonated to NH^{3+} leading to the cationic property as shown in Figure 2.9. Since most other soluble biopolymers become anionic in water, chitosan cations exhibit good affinity for other biopolymers.

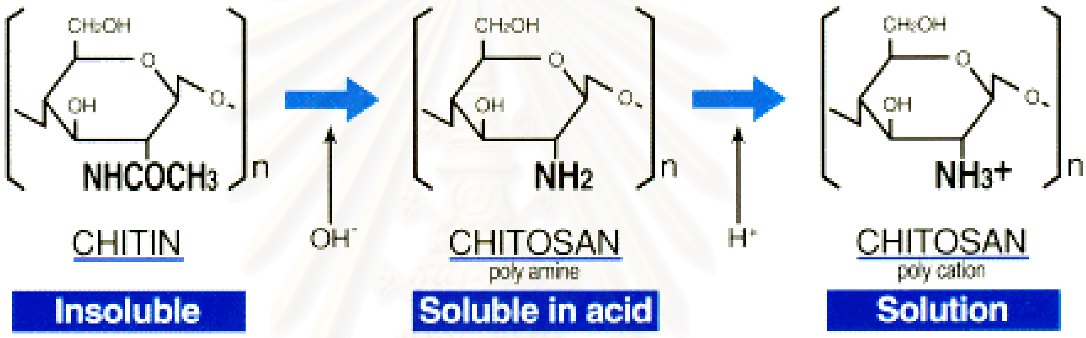


Figure 2.9 Cationic property of chitosan [74]

The cationic properties offer good opportunities to take advantage of electron interactions with numerous compounds during processing and incorporating specific properties into the materials. The cationic property may further be used for incorporation and/or slow release of active components, adding to possibilities for the manufacturer to tailor the properties [8]. Another interesting property of chitosan in relation to food packaging is their antimicrobial properties, and their ability to absorb heavy metal ions [15]. The former could be valuable in relation to microbial shelf-life and safety of food product and the latter could be used to diminish oxidation processes in the food catalyzed by free metals. So far, the major interest for chitosan as packaging material has been edible coatings. However, Makino and Hirata [82] have shown that a biodegradable laminate consisting of chitosan-cellulose and polycarprolactone can be used in modified atmosphere packaging of fresh produce.

Chitosan is not one chemical entity, but varies in composition depending on manufacture. The physicochemical properties of solutions of chitosan are expected to be governed by many factors, such as temperature, pH, ionic strength, surfactant concentration, and degree of deacetylation. In addition, it is known that the charge density along the chain increases with an increase in degree of deacetylation, and the chain flexibility of chitosan molecules can be manipulated by changing degree of deacetylation.

Nowadays, much attention has been paid to chitosan as a potential polysaccharide resource. Many efforts have been reported to prepare functional derivatives of chitosan by chemical modifications, which lead to improve its solubility in general organic solvents. Because the nitrogen content in chitin varies from 5-8% depending on the extent of deacetylation, whereas, the nitrogen in chitosan is mostly in the form of primary aliphatic amino groups. Chitosan, therefore, undergoes the reactions typical to amines, of which N-acylation and Schiff reaction are the most important. Chitosan derivatives are easily obtained under mild conditions and can be considered as substituted glucans N-acylation with acid anhydrides or acyl halides introduces amido groups at the chitosan nitrogen.

2.4.3 Chitosan Applications

A wide range of chitosan applications are based on the polyelectrolytic nature and chelating ability of amine groups of macromolecules. Many potential products using chitosan have been developed, including flocculating agents for water and waste treatment, chelating agents for removal of traces of heavy metals from aqueous solutions, coatings to improve dyeing characteristics of glass fibers, wet strength additives for paper, adhesives, photographic and printing applications, thickeners, and fibers and films. Especially, in films and coatings applications, it has been used as fruit coatings to prolong conserving period, as preservative, and as wound coating film to accelerate the wound healing process. Some novel applications involving chitosan include biodegradable fish hooks and surgical sutures, coated paper and transparencies for inkjet ink, biodegradable implants and vascular prostheses, and low-fat whipping cream and ice cream.

2.4.4 Chitosan Films and Properties

The examples of films or membranes produced from chitosan are as follows.

Films from chitosan and cellulose composites have been made by casting dispersions on steel or chrome plates at elevated temperatures from 70-100°C [6]. Some of these films contained glycerol and had good tensile strength. They were also readily biodegradable in either sea water or in soil [6, 7].

Chitosan membranes can also be formed by making rigid films with crosslinking agents, such as glutaraldehyde [83] or divalent metal ions, or with polyelectrolytes [84], including anionic polysaccharides, such as pectin. Chemically modified chitosan membranes can be used for separating ethanol from water by pervaporation [85], and chitosan membranes are being applied to water purification as well [86].

Chitosan coatings applied to fruits and vegetables can reduce water loss and extend shelf-life [12]. Chitosan films have been investigated for controlled release of pharmaceuticals [87].

Most mechanical properties of chitosan films are comparable to those of many medium-strength commercial polymers. The important factors are molecular weight and degree of deacetylation of chitosan. It has been suggested by Muzzarelli and co-workers [88] that tensile strength of chitosan film increased with increasing molecular weight of chitosan. Chitosan forms hydrogen bonds between hydroxyl groups and amino groups in chitosan film [83]. During the film formation, hydrogen bonding in the chitosan film increased with the increasing amount of amino and hydroxyl groups, due to the increase in concentration of chitosan. In addition, film-making conditions, including solvent pH, ionic strength, type of acid solutions used and annealing treatment, are parameters often manipulated to alter the mechanical properties and membrane porosity of chitosan films. Ionic strength or pH can be manipulated in order to reduce inter- and intramolecular electrostatic repulsion between chitosan chains, thus allowing the chains to approach each other and enhance the inter- and intramolecular hydrogen bonding during film formation [89]. Park and co-workers [90] explained the effects of molecular weight and organic acid solution properties on chitosan film properties. For example, molecular weight and molecular dimension of chitosan were measured by light scattering method. They showed that molecular weight of chitosan dissolved in acetic acid was larger than that dissolved in the other three acid solutions. In the acetic solution, chitosan forms dimmers indicating that the intermolecular interaction is relatively strong, which suggests that the chitosan film prepared with acetic acid had tighter structure than those prepared with other acid solutions.

Chitosan film has excellent O_2 and CO_2 barrier properties, but poor water vapor resistance. The O_2 permeability of the film was affected by type of acid and molecular weight of chitosan used. Generally, O_2 permeability value of low molecular weight chitosan film was lower than that of chitosan films with high molecular weight chitosan. As indicated by Butler and co-workers [91], O_2 permeability values of chitosan film are comparable with commercial polyvinilidene chloride or ethylene vinyl alcohol copolymer films. Low oxygen permeability of chitosan films can be exploited for food and medical packaging applications.

The use for unmodified chitosan films are limited because of their high moisture permeability and brittleness; however, the properties of chitosan films can be modified to improve barrier and mechanical properties. Wong et al. [11] reported the use of chitosan-lipid composite films to improve the moisture barrier properties of chitosan film. Hoagland and Parris [8] have developed a chitosan/pectin laminated film to alter water vapor permeability and water solubility of the film. Kittur et al. [1] have shown that chitosan films have moderate water vapor permeability and could increase the storage life of fresh produce and foodstuff with high water activity.

Films made from chitosan have two characteristics highly desirable to the food industry: they are biodegradable and they have low permeability to O_2. At present, those beneficial characteristics of chitosan films come at the expense of other desirable properties such as tensile strength. However, mechanical and barrier properties of chitosan films can be controlled through choosing appropriate molecular weight of chitosan and solvent system. The chitosan films with tailored properties can be useful in the food and medical packaging and applications. It has been recently reported that advanced genetic engineering techniques can potentially change the distribution of molecular weights in chitosan, particularly that derived from fungi. That would permit scientists to change such characteristic of films as tensile strength, flexibility, gas permeability, and rate of degradation in the environment.

2.5 Biodegradable and Edible Films for Packaging

Driven by the awareness the care should be taken not to exhaust the world's natural resources and deteriorate the environment by using non-degradable and non-recyclable materials, the development of edible and biodegradable polymers has been focused. In general, the fully recyclable materials are completely biodegradable within a considerably short period of time. There is an enormous potential for application of natural biopolymers as the packaging materials and coatings of the future generation.

Biopolymer films and packaging materials are generally designed using biological materials such as polysaccharides, proteins, lipids, and their derivatives. Films primarily composed of polysaccharides (cellulose and derivatives, starch and derivatives, gum, etc.) or proteins (gelatin, gluten, zien, etc.) have suitable overall mechanical and optical properties. In contrast, films composed of lipids (waxes, lipids or derivatives, etc.) or polyesters (poly-D-β-hydroxybutyrate, polylactic acid, etc.) have good water vapor properties, but are usually opaque and relatively flexible. Lipid films could also quite fragile and unstable. Certainly, edible bio-based materials, such as cellulose ethers, starch, hydroxypropylated starch, chitin and chitosan, corn zien, wheat gluten, soy proteins, milk proteins, etc., are acceptable for biodegradable materials. They, therefore, have received the greatest attention for use as edible films.

2.5.1 Starch-based Biodegradable and Edible films

In packaging fields, starch has been received great attention since it is inexpensive, edible, and very biodegradable. It has been investigated as a component in biodegradable films for application such as agricultural mulch. In early stage, starch has been used for many years as an additive to plastic for various purposes. It was added as filler to various resin systems to make films that were impermeable to water but permeable to water vapor. In order to enhance the overall degradability of the final plastic product, a starch-filled polyethylene was prepared. However, starch was only used as filling materials. From then on, several kinds of starch/synthetic polymer blends have been developed. The starch blending with polyvinyl alcohol (PVA) or polycaprolactone (PCL) produced transparent films. The starch component of the film is truly biodegradable; the other components of the film degrade during composting. This is significantly different to the older technology in which starch was blend with PE to produce a film that disintegrates, the starch component biodegrades, leaving a small particles of PE. Starch-PVA materials appear to have potential for replacing LDPE films in application where mechanical properties are critical for intended use and good moisture barrier properties are not necessary. This is because the materials, starch-PVA, are very sensitive to moisture [15].

Recently, researchers' interests have been focused on truly biodegradable starch based materials that should contain no non-biodegradable polymers or additives. To obtain these materials, referred to thermoplastic starch (TPS) or destructurized starch (DS), granular starch is processed with plasticizer to enable melting and disruption of the semi-crystalline starch granule. Despite their ease of preparation, the major drawback of TPS-based materials is their sensitivity to water. The plasticizers used are usually hydrophilic and can be washed by water. Moreover, it is necessary to add water to starch in the process. The evaporation of this nonequilibrium water during aging of material is accompanied by a change in material properties that is the material becomes brittle. Another drawback is the hydrophilic nature. Depending on the atmospheric relative humidity, starch can absorb more or less significant quantity of water. As T_g is very sensitive to water content, the mechanical properties of materials undergo changes with variation of relative humidity. Poor water resistance and low strength are limiting factors for the use of materials manufactured only from TPS, and hence it is often blended with other polymers or coated or encapsulated by a water impermeable substance for most current applications.

Despite all of the above factors, starch remains the most promising of the available polysaccharides for packaging. Recently, pure corn-based TPSs have some applications as a filler in packages and golf tees. In commercial products, the water resistivity of starch is improved by adding synthetic polycarprolactone (PCL/starch, MaterBi^{TM}, Novamont, Italy). These materials can be used in the production of applications such as the waste bag (Bioska^{TM}, Ylöjärvi, Finland) which can be composted.

For food packaging, starch is also widely used as a raw material for edible films or coatings, which have potential in a number of different areas. Poovarodom and Praditdoung [2] have prepared biodegradable films made from cassava starch used as wrapping or sealed bags for food. These products have excellent properties in that they are transparent, grease and oil resistance, heat-sealable, strong, and soluble in both hot and cold water. The film is also edible, which makes it suitable for fatty food packaging. In Thailand, the film is used for wrapping sweet fruit products such as banana paste, durian paste, and kalamae. The film has proven to extend shelf-life of product as well as being more practical than conventional packaging. However, applications of the products in this field are limited by moisture sensitivity. This is due to the hydrophilic nature of the raw material affects moisture barrier properties, and then tensile properties of the film. Blending and coatings with other biopolymers are methods applied for breakthrough the disadvantages. Fishman and Coffin [92] have patented the high modulus and flexible films fabricated from mixtures of pectin and starch. The films were biodegradable, water-soluble, and were advantageous in that all materials derived from agricultural products. They have reported that starch could be blended with the pectin to produce adhesives and edible films or food wrappings such as bags for soup, pharmaceutical compositions, and control release matrixes.

2.5.2 Chitosan as Edible Films

One of the polysaccharides used for edible films is chitosan. Due to its genetically film forming properties, chitosan is satisfactorily used as food wraps. Semipermeable chitosan films modify the internal atmosphere, decrease the transpiration and delay the ripening of fruits [12]. For the preparation of chitosan/pectin laminated films and chitosan/methylcellulose films several approaches have been used, including simple coacervation. Chitosan films are tough, flexible, and tear resistant, moreover they have favourable permeation characteristics for gases and water vapor.

For laminated films, chitosan is also an attractive raw material. Because it has some heterogeneity induced by the distribution of free amino groups that results from hydrolysis of some of acetyl groups of chitin. In acid solutions, the cationic properties of chitosan offer the film-maker an opportunity to take advantage of electrostatic interactions with other anionic biopolymers. Vary strong electrostatic interactions can be expected to produce a precipitate or thin membrane [84].

Since this work has been focused on the preparation of cassava starch/chitosan laminated films. Therefore, some related researches are briefly reviewed as follows.

Poovarodom, N. and Praditdoung, S. investigated the biodegradable packages made from cassava starch. Cassava starch film was prepared from starch solution consisting of native cassava starch (5%), sorbitol (30%), and water. The film was formed by casting method and possessed some outstanding properties as follows: transparent; excellent grease and oil resistance; good oxygen barrier; heat sealable; good mechanical strength; and soluble in both cold and hot water. According to these properties and its edibility, the film was very suitable for fatty food packaging either as wrapping or sealed bag. Oily seasoning for “Tom Yam” instant noodle could be stored at 35°C in this sealed bag for 5 months without significant change in quality and the estimated shelf-life was up to 9 months. This bag provided a great deal of convenience to consumers during cooking. Moreover, the film was used for wrapping some Thai fruit candy. It has been reported that the product shelf-lives were significantly extended and the packaging was more attractive and more practical than the conventional ones. For expanded package from cassava starch, it was produced by the thermal compression moulding. The starch mix consisted of native starch more than 90% (dry weight), some plasticizers, emulsifier, calcium carbonate and agar. The forming conditions were at temperature of 200 to 240°C for 1 to 3 minutes. The bulk density was in the range of 0.150 to 0.176 $g.cm^{-3}$. The package could hold water for about 75 minutes and liquid oil for several hours. This depended on the starch formulation and thickness of the package. These styrofoam liked packages have been found very practical in fast food services and one-way uses for ready-to-serve meal.

Hoagland, P. D. (1996) have patented biodegradable laminated films fabricated from pectin and chitosan prepared by casting with either glycerol or lactic acid as plasticizers. The films were high modulus, flexible and self-supporting, and were advantageous in that all materials derived from agricultural. The invention was useful for a number of applications including medicinal applications such as patches for the delivery of pharmaceuticals to skin; biodegradable and disposable pouches or bags for frozen or dried foods or soil additives; coatings for controlled release, adhesive bonding or protection; embedding and preserving agents for microscopic specimens; and encapsulation of living cells.

Hosokawa, J., Nishiyama, M., Yoshihara, K., and Kubo, T. (1990) studied biodegradable film derived from chitosan and homogenized cellulose. The composite film obtained by cast drying was hydrophilic but insoluble in water. It had a high oxygen-gas barrier capacity. The strength of the composite film changed with chitosan content, and the maximum tensile strength (more than 1000 kg cm^{-2}) has been attained at 10-20% w/w chitosan on cellulose. The composite film was assumed to have chemical bonds between chitosan and the cellulosic material. The flexibility of the composite film was improved by the addition of glycerol as a plasticizer. The composite film was degraded to fine fragments by cellulase and a ubiquitous chitosan-degrading bacterium isolated from soils in Japan. The period of its biodegradation was controlled by the conditions during film formation, such as temperature and the functional groups of cellulose.

Arvanitoyannis, I. S., Nakayama, A., and Aiba, S. (1998) investigated films of chitosan and gelatin prepared by casting their aqueous solutions ($pH < 4.0$) at 60°C and evaporating at 22 or 60°C (low- and high-temperature methods, respectively). The physical (thermal, mechanical, and gas/water permeation) properties of these composite films, plasticized with water or polyols, were studied. An increase in the total plasticizer content resulted in a considerable decrease of elasticity modulus and tensile strength (up to 50% of the original values when 30% plasticizer was added), whereas the percentage elongation increased (up to 150% compared to the original values). The low-temperature preparation method led to the development of a higher percentage renaturation (crystallinity) of gelatin which resulted in a decrease, by one or two orders of magnitude, of CO_2 and O_2 permeability in the chitosan/gelatin blends. An increase in the total plasticizer content (water, polyols) of these blends was found to be proportional to an increase in their gas permeability.

Sophanodora, P. (1998) studied the effects of film additives, e.g., cellulose derivatives, plasticizers, and fatty acids, on the properties of chitosan and composite films. Composite films between cellulose derivatives (methylcellulose; MC and hydroxypropyl methylcellulose; HPMC) and chitosan showed the maximum tensile strength at 10% cellulose. HPMC composite film showed more flexibility than MC composite film with a higher extension at break. At the same concentration of plasticizer, sorbitol exhibited higher tensile strength and lower extension at break and WVP than glycerol. There was very little effect of fatty acid on the water barrier property of the films. Lauric acid showed a better water barrier property than stearic acid.

Makino, Y. and Hirata, T. (1997) investigated use of a biodegradable laminate of a chitosan-cellulose and polycaprolactone as a film for modified atmosphere packaging (MAP) of fresh produce. The temperature dependence of O_2, CO_2, and N_2 gas permeability coefficients for the biodegradable laminate was examined. The coefficients increased linearly with increasing temperature in the range 10-25°C. The coefficients were validated by experiments on MAP with shredded lettuce and shredded cabbage. MAP systems with head lettuce, cut broccoli, whole broccoli, tomatoes, sweet corn, and blueberries were designed using the gas permeability coefficients. The gas composition in each biodegradable package including the fresh produce was simulated to be close to the optimal composition. The biodegradable laminate was found suitable as a packaging material for storage of fresh produce.

Jeon, Y. J., Kamil, J. Y. V. A., and Shahidi, F. (2002) studied chitosan as an edible film for quality preservation of herring and Atlantic cod. The effect of chitosan with different molecular weights as coatings for shelf-life extension of fresh fillets of Atlantic cod (*Gadus morhua*) and herring (*Clupea harengus*) was evaluated over a 12-day storage at refrigerated temperature (4 ± 1°C). Three chitosan preparations from snow crab (*Chinoecetes opilio*) processing wastes, differing in viscosities and molecular weights, were prepared. Their apparent viscosities (360, 57, and 14 cP) depended on the deacetylation time (4, 10, and 20 hrs, respectively) of the chitin precursor. Upon coating with chitosans, a significant reduction in relative moisture losses of 37, 29, 29, 40, and 32% was observed for cod samples coated with

360 cP chitosan after 4, 6, 8, 10, and 12 days of storage, respectively. Chitosan coating significantly reduced lipid oxidation as displayed in peroxide value, conjugated dienes, 2-thiobarbituric acid reactive substances and headspace volatiles, chemical spoilage as reflected in total volatile basic nitrogen, trimethylamine, and hypoxanthine, and growth of microorganisms as reflected in total plate count in both fish model systems compared to uncoated samples. The preservative efficacy and the viscosity of chitosan were inter-related; the efficacy of chitosans with viscosities of 57 and 360 cP was superior to that of chitosan with a 14 cP viscosity. Thus, chitosan as edible coating would enhance the quality of seafoods during storage.

Jayasekara, R., Harding, I., Bowater, I., Christie, G. B. Y., and Lonergan, G. T. (2003) investigated the preparation, surface modification, and characterization of solution cast starch-PVA blended films. The hydrophilic nature of the film surfaces was altered by surface modification with the chitosan chosen to render the surface more hydrophobic. Several blends have been prepared from polyvinyl alcohol, starch, and glycerol. The blend containing 20% polyvinyl alcohol has been modified by application of chitosan to the surface. The blend and its modified form have been characterized by atomic force microscopy, x-ray diffraction, fourier transform infrared spectroscopy, contact angle measurements, 13C-NMR, spectroscopy, and scanning electron microscopy. The blended films were flexible and homogeneous on a macroscopic scale but on a microscopic scale there seemed to be small patches of individual components. Surface modification altered some of the characteristics of the film. The blends had surface roughness intermediate between that of the pure components. The addition of chitosan made the film more hydrophobic than the unmodified film but slightly less than the starch film. There was no evidence of new bond formation among the individual components. Solution casting reduced the overall crystallinity in the blended films.

Chapter III

Experimental

3.1 Materials and Chemicals

- **Cassava Starch** : Cassava starch or tapioca was purchased from ETC International Trading Co., Ltd.(Thailand). The general specifications are as follows: 12.5-13.0% moisture content (max.), 0.20% ash (max.), 0.20% pulp (max.), pH value of 5.00-7.00, and viscosity of 600 BU. (Brabender unit). Cassava starch flour was dried in an oven at 50°C for 24 hours and kept in a desiccator prior to use.
- **Chitosan** : Commercial grade-chitosan flake (approx. 85% degree of deacetylation) with molecular weight of about 10^6 used to prepare a coating solution was purchased from Ebase Co., Ltd. (Thailand).
- **Glycerol** : Glycerol (approx. 96%) used as a plasticizer in starch films was purchased from Merck KGaA, Darmstadt, Germany.
- **Acetic acid** : Glacial acetic acid purchased from BDH Laboratory Suppiles (England) was diluted to prepare chitosan solutions.

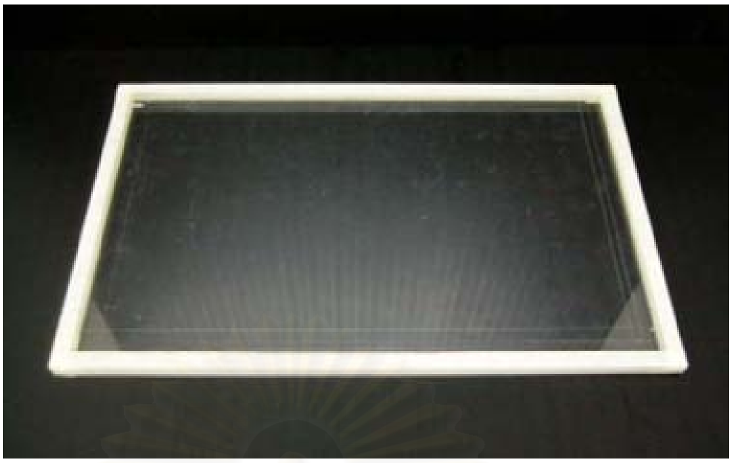
3.2 Instruments

Details of each instrument are classified according to the experimental procedure as follows.

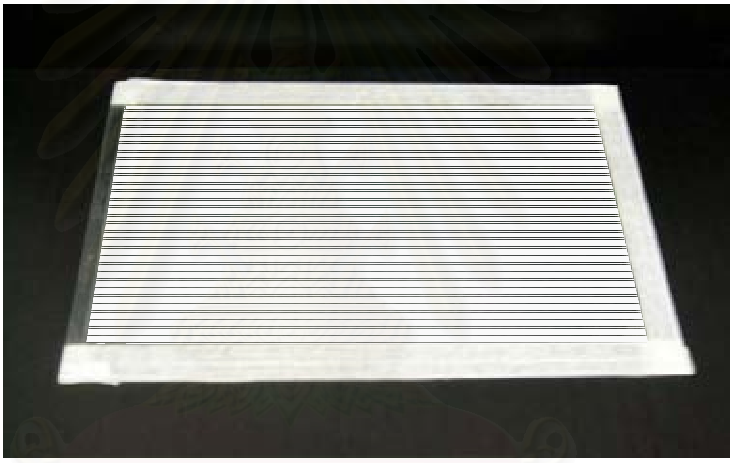
3.2.1 Sample Preparation

- **Acrylic Mold**

Cassava starch film was prepared by pouring the starch solution directly onto an acrylic mold. The acrylic sheet (0.5 cm in thick) cut into about 28 cm × 38 cm, 1 cm × 38 cm, and 1 cm × 26 cm was composed to be a removable edge mold in size of 0.5 cm (height) × 26 cm (width) × 36 cm (length).



(a)

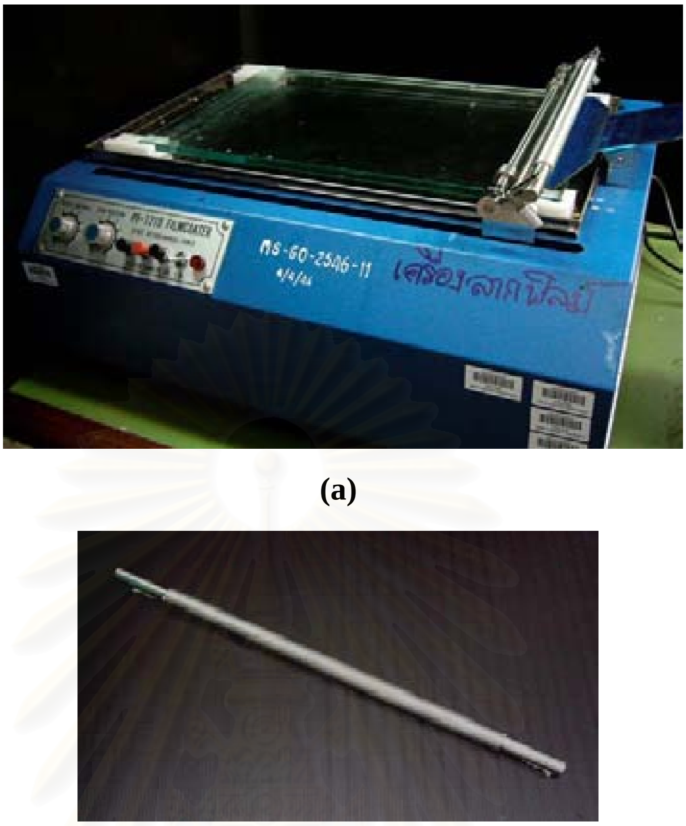


(b)

Figure 3.1 Acrylic mold: (a) acrylic mold, (b) starch film attached on acrylic sheet sealed with adhesive tape

- **Automatic Film Coater**

PI-1210 auto film applicator with wire bar coating rod number 75 giving wet thickness about 171.5 μm of Tester Sangyo Co., Ltd. (Japan) was used for coating chitosan solution onto starch film. The starch film attached on acrylic sheet substrate was placed over the glass plate of the coating machine and fixed with clamps, and then each end of a bar coater was pressed fitly to the spring-type clamp. Coating speed was set at about 70 mm sec^{-1}. The stop position was adjusted suitably to the length of acrylic sheet substrate with a scale of 5.



(a)

(b)

Figure 3.1 Automatic film coater of Tester Sangyo Co., Ltd.: (a) PI-1210 auto film applicator, (b) Wire bar no.75

3.2.2 Physical Property Equipments

- **Micrometer**

A micrometer (Peacock, Model G, Japan) was used to measure thickness of the film samples.

Figure 3.3 Micrometer

- **Gloss Meter**

Micro-gloss 60° with standard holder of BYK-Gardner GmbH, Germany was employed to measure specular gloss of the film samples according to the ASTM D 523 standard method.

Figure 3.4 BYK-Gardner Micro-gloss 60°

- **Spectrophotometer**

Macbeth Color-Eye 7000 spectrophotometer purchased from Kollmorgen Instruments Corporation, 1994 was employed to determine transparency of the film samples.

Figure 3.5 Macbeth Color-Eye 7000 Spectrophotometer

- X-ray Diffractometry (XRD)

A Philips x-ray diffractometer (model PW 3710) was employed to characterize structure of the film samples.

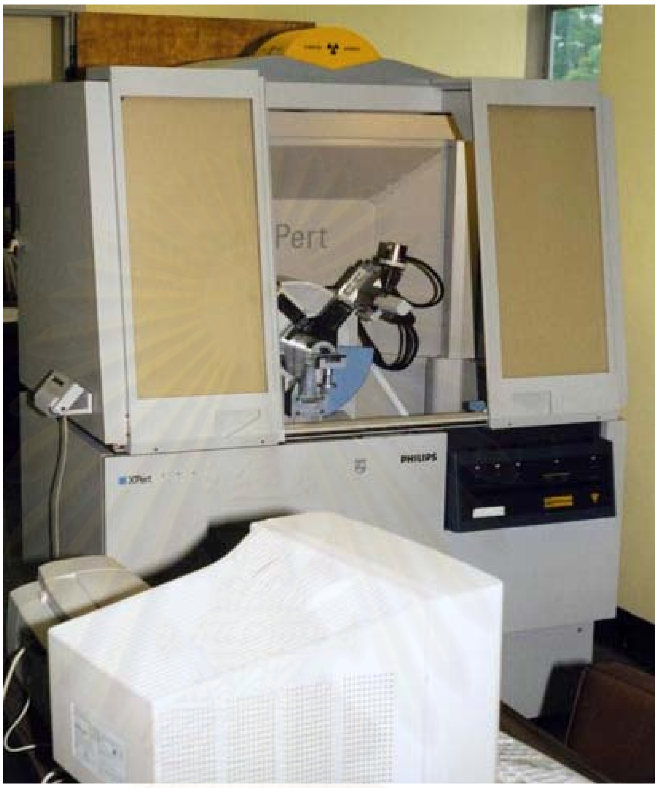


Figure 3.6 Philips PW 3710 X-ray Diffractometer

- Contact Angle Meter

A contact angle meter model CAM-PLUS MICRO (Tantec Inc., USA) was used to determine the wettability of the film samples.

Figure 3.7 Tantec CAM-PLUS MICRO contact angle meter

3.2.3 Mechanical Property Equipments

- Tensile Testing Machine

Tensile properties of the film samples were tested by universal testing machine LLOYD LR 100K according to the ASTM D 882.

Figure 3.8 Universal Testing Machine LLOYD LR 100K

3.3 Experimental Procedure

The experimental flow diagram for fabrication of chitosan/cassava starch laminated film is shown below in Figure 3.9.

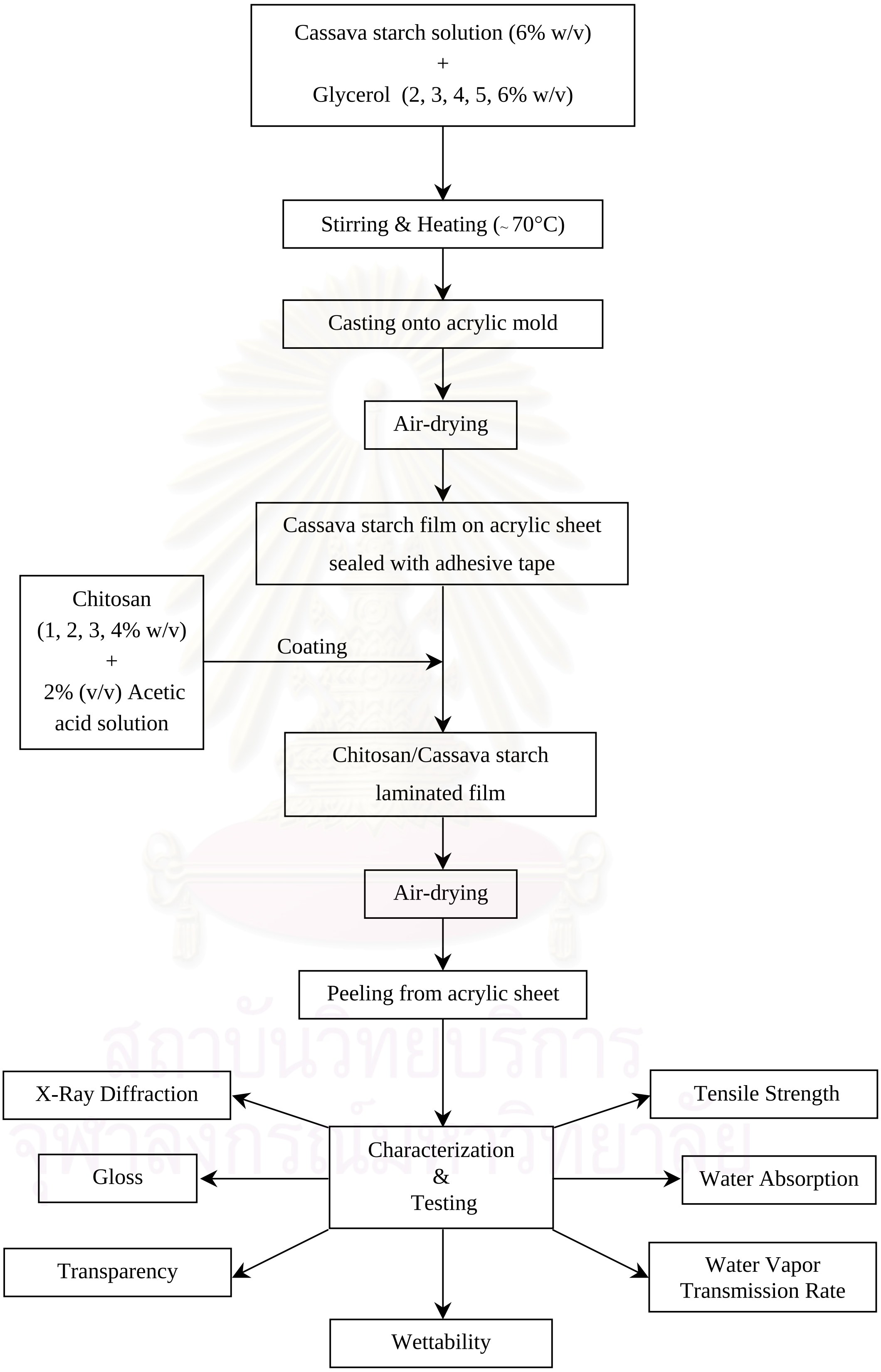


Figure 3.9 The flow diagram of experimental procedure

General preparation of laminated films was described in two steps as follows:

Step 1: Preparation of Starch Cast Films

A mixture of cassava starch solution (6% w/v in water) and glycerol used as a plasticizer (varied from 2, 3, 4, 5, and 6% (w/v)) was heated at the starch gelatinization temperature of 70°C under stirring until viscous and transparent solution was observed. After homogeneously mixing for 10 minutes this solution was poured into 5 mm thickness removable edge acrylic mold and allowed to dry freely at room temperature. After air drying, the edges of the mold were removed and four sides of the film were sealed with adhesive tape to prevent the underneath of starch film from getting contact with chitosan coating solution in the next step.

Step 2: Coating Process

Chitosan (varied from 1 to 4% (w/v)) was dissolved in 2% (v/v) acetic acid solution and stirred by a magnetic stirrer at about 700 rpm until the dissolution was completed, which typically taking about 2-3 hours. The solution was then filtered prior to coating application.

Coating chitosan solution (about 30 mL) onto the starch film was carried out by an automatic film coater with wire bar coating rod number 75. After coating, the bar coater was removed immediately from clamps and the remaining chitosan solution was wiped from the bar coater. The acrylic sheet substrate containing chitosan/starch laminated film was removed from glass plate of the film coater and stored at room temperature, allowing the laminate film to dry for at least 72 hours. The laminate film was peeled from the acrylic sheet and measured thickness by micrometer. The dry laminated film thickness was fallen within the range of 0.08-0.12 mm.

3.4 Characterization and Testing

3.4.1 Physical Properties

3.4.1.1 Film Thickness Measurement

Film thickness was measured by a micrometer. Five thickness values were taken along the length of the filmstrip and the mean value was used for tensile strength calculation. Similarly, five measurements were taken on each water vapor transmission rate (WVTR) samples, one at the center and four around the perimeter and mean values were used for calculation.

3.4.1.2 Structure Analysis

The crystalline structure of film samples was analyzed by a Philips diffractometer (Model PW 3710). The CuK$_\alpha$ radiation (λ = 1.542 Å) generated at 40 kV and 30 mA was monochromatized with a 15 μm Ni foil. The relative intensity was recorded in scattering range (2θ) of 3-40°.

3.4.1.3 Optical Properties Measurement

(a) Gloss

Gloss or specular reflectance is defined as the degree to which the finish of the surface approaches that of the theoretical specular gloss standard, or the perfect mirror, which is assigned a value of 1000. In practice, gloss measurements are made in comparison to a black tile with a refractive index of 1.567 and assigned an arbitrary value of 100 gloss units. The amount of specular reflectance of the black tile at a given angle is dependent on the index of refraction of the glass.

In this study, the specular gloss of film samples was determined by micro-gloss 60° at 23 ± 2°C according to ASTM D 523 standard method. Calibration of dark standard holder was 95.1 units. Five positions of each film sample were tested and gloss values were averaged to obtain a mean value.

(b) Transparency

The transparency of film samples was measured by Macbeth Color-Eye 7000 spectrophotometer. The percent transmittance of calibrated white standard and film samples was measured in the range of 400-750 nm.

3.4.1.4 Surface Property Determination: Wettability

The contact angles of film samples were measured using a CAM-PLUS MICRO (Tantec, Inc.) according to Tantec’s Half-Angle method. To calibrate the device, the height of the syringe was adjusted so that the needle tip image was at the lower border of the grid on the screen. The needle’s image was then focused on by moving the lens plate back and forth. The given specimen was placed onto the specimen holder under the syringe needle, and the knob was released clockwise to release the droplet of water. Bringing the specimen holder up slowly then down, one droplet of water was obtained, and was then focused. The line was then adjusted so that it was lined up with the left edge of the droplet. The line was then moved till it cross the apex of the droplet, and the angle was measured on the protractor. A contact angle of zero results in wetting, while an angle between 0 and 90° results in spreading of the drop (due to molecular attraction). Angles greater than 90° indicate the liquid tends to bead or shrink away from the solid surface.

3.4.1.5 Measurement of Water Vapor Transmission Rate (WVTR)

Water Vapor Transmission Rate (WVTR) of films was determined by the gravimetric (dish) method according to ISO 2528 standard method (1995(E)). At least three circular films with 90 mm of diameter for each sample were weighed and measured the thickness before testing. The aluminium dish filled with desiccant had a groove around the rim for sealing the film with wax. Films with an exposed area of 50 cm^2 were tested at 38°C, 90% RH. For each dish, represent the total increase in mass graphically as a function of time exposure, the test being completed when three or four points lie on a straight line, showing a constant rate of passage of water vapor. Using this straight line, the WVTR for each test piece is then calculated, in grams per square metre per 24 h ($g.m^{-2}.d^{-1}$), from the formula:

$$\frac{240 \times m_1}{S}$$

where m_1 is the rate of increase in mass, in milligrams per hour, determined from the graph;

S is the area, known to within 1%, in square centimeters, of the tested surface of the test piece.

3.4.1.6 Determination of Water Absorption

Water absorption of films was measured by twenty-four hour immersion method according to ASTM D 570 standard test (1995). Film samples cut into 25.4 mm × 76.2 mm were measured the thickness before testing. The films were conditioned by drying in an oven at 50 ± 3°C for 24 hours, cooling in desiccator, and weighing immediately. Then, the conditioned films were placed in a container of distilled water maintained at ambient temperature, and entirely immersed. At the end of 24 hours, the films were removed from the water one at a time, all surface water was wiped off with a dry cloth, and immediately weighed. The water absorption was calculated as the weight difference and reported as percentage of weight increase from the conditioned weight. The water absorption of films was calculated as follows:

$$\text{Increase in weight, \%} = \frac{\text{wet weight - conditioned weight}}{\text{conditioned weight}} \times 100$$

Three film samples were tested and the results were averaged to obtain a mean value.

3.4.2 Mechanical Properties

The tensile stress at maximum load, tensile modulus, and percent elongation at break of the film samples were measured by Universal Testing Machine (Model LLOYD LR 100K). The film samples in 150 mm × 15 mm were measured the thickness, and then conditioned for 24 hours at 25°C and 60% RH before testing. The test was carried out according to ASTM D 882 standard method, with initial grip separation of 100 mm, crosshead speed of 10 mm min^{-1}, and load cell of 100 N.

The tensile modulus is defined as the slope of the tangent to the stress strain curve at low strain. Tensile stress at maximum load is the value of the stress on the stress-strain curve where the curve occurred at maximum load. The percent elongation at break is the maximum strain exhibited by the test sample at the point of breakage. These values were measured both in longitudinal and transverse directions to observe whether any difference in the orientation of polymer chain occurs.

Chapter IV

Results and Discussion

4.1 Physical Properties Characterization

4.1.1 Physical Structure: X-ray Diffraction (XRD)

Free chitosan film, plasticized cassava starch film, and chitosan/starch laminated film were prepared according to the method described earlier in chapter III. The X-ray diffraction patterns of these films are displayed in Figure 4.1.

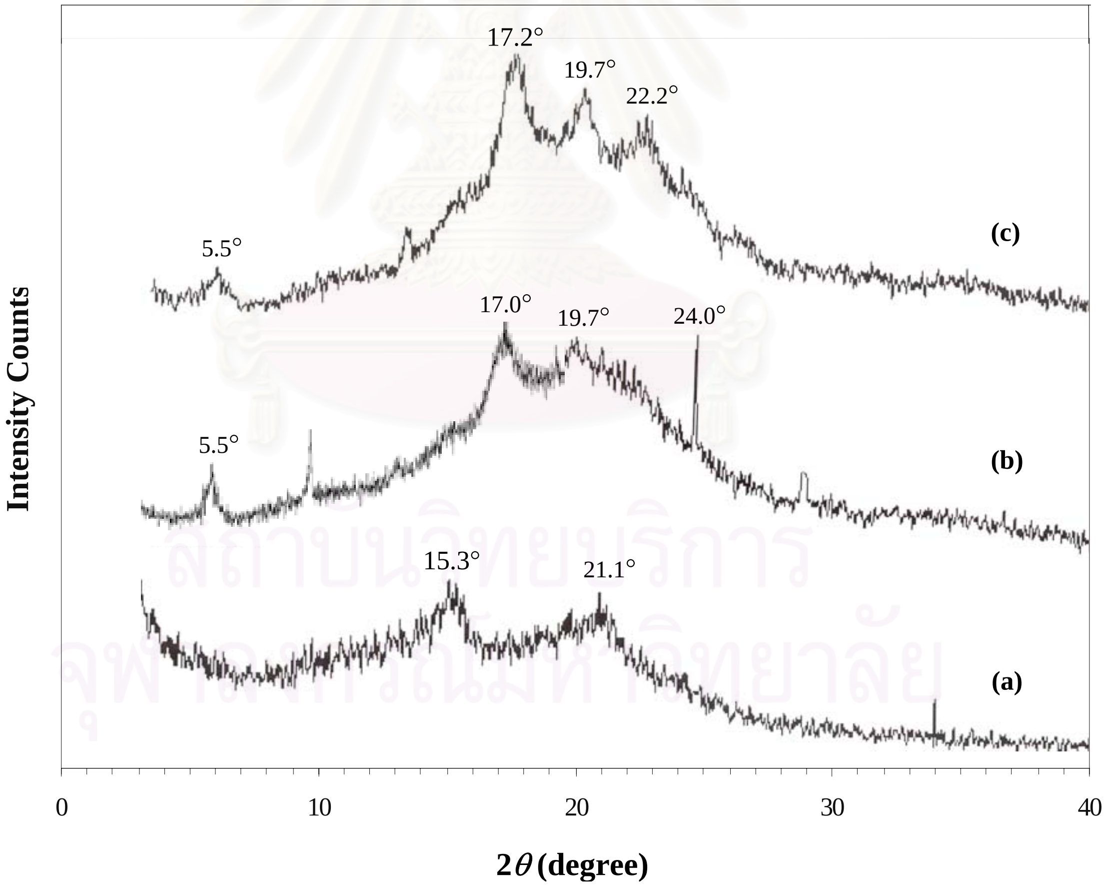


Figure 4.1 X-ray diffractograms of: (a) free chitosan film, (b) glycerol-plasticized starch film, and (c) chitosan/starch laminated film

The semicrystalline characters of free chitosan film and plasticized starch film were revealed from the diffractograms illustrated in Figure 4.1 (a) and (b), respectively.

The diffractogram of chitosan film presented in Figure 4.1 (a) shows the crystalline peaks (2θ) at 15.3° and 21.1°, which are typical fingerprint for chitosan film and very similar to the work of Wan et al. [93]. It was reported that the small peak at approximately 15° (2θ) was attributed to the anhydrous crystal of chitosan [94], whereas the diffraction peak at around 21-22° (2θ) was observed in chitosan film prepared from dissolving chitosan in acetic acid solution [95]. In this study, however, the intensity of the crystal peak at about 21.1° (2θ) is very low. It might be implied that chitosan film is in amorphous state to partially crystalline state. It is noted that the degree of deacetylation has obviously affected crystallinity of chitosan film. According to the previous research [93], it was observed that the crystallinity of the chitosan film increased gradually with increasing degree of deacetylation ranging from 70 to 90%. This may be attributed to the fact that chains of chitosan with higher degree of deacetylation are more compact leading to facilitate the hydrogen-bonding formation and in turn crytallinity formation in the film. Furthermore, higher degree of deacetylation chitosan contains more glucosamine groups that also facilitate the hydrogen-bonding formation. On the contrary, lower degree of deacetylation chitosan has more acetyl groups that hinder the chitosan chain packing due to their rigidity and steric effect [93]. Therefore, the chitosan films prepared from high degree of deacetylation chitosan contain a large crystalline region. It is quite rare that the production of chitosan with 100% degree of deacetylation is achieved. Therefore, commercial chitosan with various degree of deacetylation in the range of 75-85% is commonly found.

Figure 4.1 (b) shows the diffraction peak of free starch film at approximately 17° (2θ), which resembles to the characteristic of B-type crystalline structure. From previous study [62], amylose film showed the main peaks at about 5.5 and 17° (2θ) while amylopectin film showed broader reflection around 17-18° (2θ). The ordering of B-type structure with water uptake related to the peaks at about 5.5 and 24° (2θ) are the most sensitive to hydration. In addition, the diffraction peak at 19.7° (2θ) is similar to the B-type crystalline peak of amylopectin after storage seven days at 54% RH found in Myllärinen et al’s work [62]. The observation of the B-type crystalline in the free starch film should be attributed to the fact that crystallization of amylose is developed in the early stage of film formation, where as that of amylopectin is varied upon the humidity during film formation. The final degree of crystallinity of starch film is dependent on the ability of chains to form crystals as well as the mobility of chains during the crystallization process. The linear amylose and branched amylopectin polymers exhibit different behavior with regard to gelation and development of crystallinity. Amylose and amylopectin are recrytallizable in B-type crystalline form from a dilute solution [53]. In earlier study, X-ray diffraction of amylose solutions showed that the development of crystallinity though fast in the beginning was levelled off approximately 1 day after preparation. In amylopectin solutions, the development of crystallinity was much slower than in amylose solutions, and it had been shown to develop over a period of several days. The amylopectin film without glycerol was amorphous under all conditions because of insufficient time to crystallize before the water content reaching very low values during the drying process. When having glycerol as the plasticizer, however, B-type crystallinity was gradually produced with increasing humidity [62].

Compared to free chitosan film, the starch film exhibits higher crystallinity as indicated by sharper reflection with higher intensity. The result implies that the polymer chains of starch due to the absence of bulky side group exhibit more packing compact. The difference in molecular orientation is related to the physical appearance of starch and chitosan free films discussed in the next section.

The X-ray pattern of chitosan/starch laminated film displayed in Figure 4.1 (c) shows reflection of B-type starch crystalline shifting to the slightly higher degrees at about 17.2° (2θ) while that of the chitosan appears as a smaller peak at about 22.2° (2θ). The shifting in starch diffraction peaks may be due to the change in its chain orientation caused by chitosan coating. It is likely that hydrogen-bonding interaction between chitosan and starch molecules is responsible for this phenomenon. For chitosan diffraction peak, force and pressure of an automatic film coater probably cause the higher molecular orientation compared to casting technique. In addition, the smaller peak at 5.5° (2θ) and disappearance of peak at 24° (2θ) observed in the diffractogram indicate that the amount of water in the laminates is lower than that in starch film.

4.1.2 Physical Appearance

4.1.2.1 Gloss

It was observed that the physical appearance of the free chitosan film differed significantly from that of the free starch film in term of surface properties, particularly gloss. The gloss values of chitosan/starch laminated films including the free starch film and the free chitosan film are given and graphically shown in Table 4.1 and Figure 4.2, respectively.

Table 4.1 Gloss values of starch and chitosan free films and chitosan/starch laminated films

	Glycerol (wt%)	Chitosan Coating Solution (wt%)	Gloss* (units)
Chitosan Films	-	1	132.5 ± 2.6
	-	2	135.9 ± 4.2
	-	3	139.4 ± 4.3
	-	4	145.6 ± 2.5
Starch Films	2	-	54.3 ± 1.6
	3	-	52.4 ± 3.7
	4	-	53.2 ± 3.1
	5	-	60.1 ± 3.4
	6	-	58.3 ± 2.0
Chitosan/Starch Laminated Films	2	1	87.6 ± 3.9
		2	100.4 ± 2.4
		3	108.2 ± 5.5
		4	107.3 ± 2.7
	3	1	68.7 ± 4.1
		2	95.2 ± 4.7
		3	97.5 ± 3.1
		4	102.5 ± 3.1
	4	1	79.2 ± 7.6
		2	88.9 ± 4.3
		3	97.4 ± 3.5
		4	99.3 ± 4.1
	5	1	80.5 ± 1.5
		2	92.3 ± 3.9
		3	97.3 ± 3.8
		4	106.6 ± 2.1
	6	1	83.5 ± 4.7
		2	92.7 ± 4.5
		3	100.8 ± 2.4
		4	97.9 ± 4.2

* Gloss of standard black calibration = 95.1 units

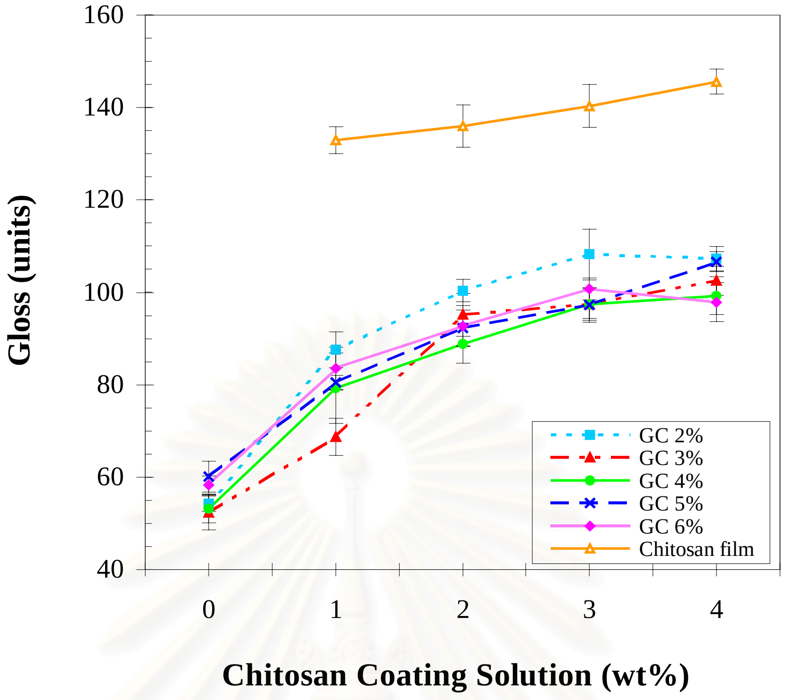


Figure 4.2 **The relationship between gloss and chitosan coating content of laminated films containing 2, 3, 4, 5, and 6 wt% glycerol**

The gloss values of free chitosan films are found in the range of 132.5-145.6 units, indicating a very glossy film probably due to a smooth surface. On the other hand, the gloss values of free starch films are between 52.4-60.1 units, reflecting the likelihood of uneven film surface. For the laminated film, it can be seen that the gloss values increase with an increase in the chitosan coating solution content. From the results, only 1 wt% chitosan coating solution brings about a significant increase in gloss values of film compared with those of free starch film. The greatest gloss value of the laminates received when applying the 4 wt% chitosan coating solution. As seen from this figure, the gloss values of 1 wt% chitosan-laminated films containing 2, 3, 4, 5, and 6 wt% glycerol are about 87.6, 68.7, 79.2, 80.5, and 83.5 units, respectively. Whereas those of 4 wt% chitosan-laminated films increase rapidly to 107.3, 102.5, 99.3, 106.6, and 97.9 units, respectively. This result may be due to the complete coverage of the chitosan with an increase in chitosan coating concentration.

It could be concluded that the smoother surface of chitosan film enhances the regularity of laminated film surface leading to increasing in the gloss value. In addition, the higher the chitosan coating content, the higher the gloss value.

4.1.2.2 Transparency

Transparency of packaging film is one of aesthetic factors in terms of general appearance and consumer acceptance. The transparency may be affected by various factors including film thickness. In this study, however, there is insignificant difference in average thickness among prepared films. The % transmittance values of laminated films including starch and chitosan free films are presented in Table 4.2.

Table 4.2 % Transmittance values of starch and chitosan free films and chitosan/starch laminated films

	Glycerol (wt%)	Chitosan Coating Solution (wt%)	% Transmittance* (at 650 nm)
Chitosan Films	-	1	95.55
	-	2	95.69
	-	3	95.36
	-	4	95.67
Starch Films	2	-	93.95
	3	-	93.87
	4	-	93.24
	5	-	91.32
	6	-	92.69
Chitosan/Starch Laminated Films	2	1	94.37
		2	94.70
		3	94.32
		4	94.66
	3	1	94.36
		2	94.30
		3	94.12
		4	94.23
	4	1	93.93
		2	94.17
		3	93.94
		4	94.09
	5	1	93.27
		2	93.02
		3	93.69
		4	93.92
	6	1	93.55
		2	93.82
		3	93.36
		4	93.87

* % Transmittance value of white standard (at 650 nm) = 96.52 %

The % transmittance of free starch films are found between 91.3-94.0% while those of free chitosan films are in the range of 95.3-95.7%. The transmittance of chitosan film is slightly higher than that of starch film. The smoother surface combined with more amorphous structure of chitosan film (from X-ray evidence) may be responsible for transparency. By coating chitosan solutions on these starch films, the little more transparency of the laminates compared with the starch films are observed as demonstrated in Figure 4.3.

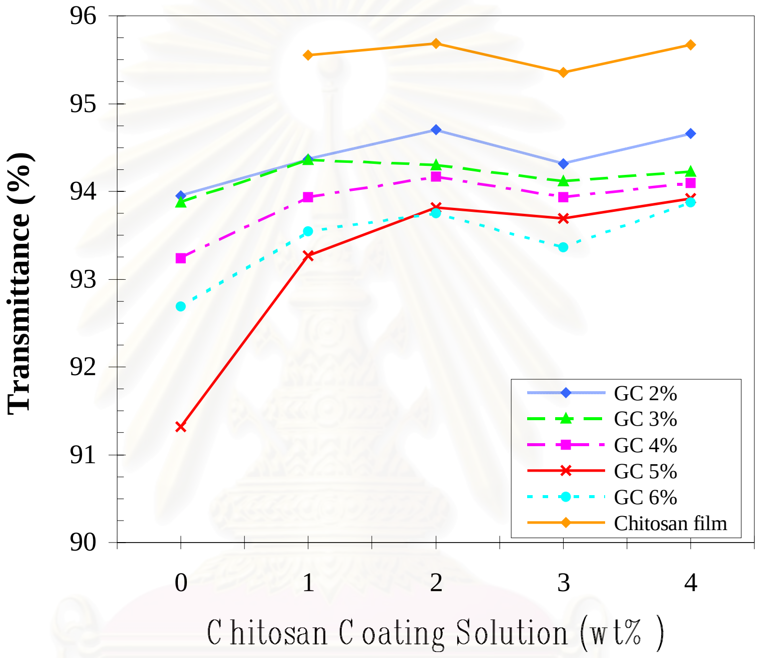


Figure 4.3 The relationship between % transmittance and chitosan coating content of laminated films containing 2, 3, 4, 5, and 6 wt% glycerol

However, there seems to be negligible effect from chitosan coating concentration. Comparing among all the laminated films, % transmittance of the films are found to lie in between the values of free starch films and free chitosan films, which is about 93.0-94.7%.

It could be concluded that chitosan/starch laminated film is more transparent than foundation starch film. However, there is a little effect of chitosan coating content on film transparency. This is probably due to the relatively high % transmittance values in both of free starch films and chitosan films are not much difference. Moreover, a fluctuation in % transmittance of laminated films as increasing chitosan coating content may be attributed to uneven of interface layer.

4.1.3 Surface Property: Wettability

Another interesting property of the laminated film is the surface hydrophilicity which was evaluated by means of contact angle determination. The contact angles of laminated films including starch and chitosan free films are summarized in Table 4.3.

Table 4.3 Contact angles of starch and chitosan free films and chitosan/starch laminated films

	Glycerol (wt%)	Chitosan Coating Solution (wt%)	Contact Angle (°)
Chitosan Films	-	1	79.4 ± 0.9
	-	2	90.0 ± 0.7
	-	3	94.2 ± 0.8
	-	4	100.6 ± 1.3
Starch Films	2	-	64.4 ± 1.1
	3	-	59.8 ± 0.8
	4	-	49.2 ± 0.8
	5	-	37.8 ± 1.6
	6	-	29.8 ± 1.3
Chitosan/Starch Laminated Films	2	1	73.4 ± 1.5
		2	89.0 ± 1.0
		3	92.4 ± 1.1
		4	99.0 ± 1.0
	3	1	67.8 ± 0.8
		2	73.6 ± 1.1
		3	85.8 ± 1.6
		4	97.2 ± 0.8
	4	1	59.4 ± 1.1
		2	71.4 ± 1.3
		3	77.7 ± 1.4
		4	86.0 ± 1.0
	5	1	54.0 ± 1.6
		2	58.8 ± 1.3
		3	67.0 ± 1.6
		4	76.0 ± 0.7
	6	1	40.6 ± 1.3
		2	48.2 ± 2.0
		3	55.8 ± 1.3
		4	68.6 ± 1.1

The contact angles of the free starch films containing 2, 3, 4, 5, and 6 wt% glycerol are 64.4, 59.8, 49.2, 37.8, and 29.8°, respectively. An increase in the amount of glycerol as plasticizer led to a gradually decreasing contact angle value of starch films due to hydrophilicity of glycerol. The important role of glycerol was to impart the thermoplasticity to the starch film. However, the addition of excess amount of glycerol caused the substantial increase in moisture absorption which reflected in the decreased contact angle, bringing about the deteriorating mechanical properties of the film discussed later. Coating of chitosan solution onto the starch film resulted in the change in surface properties including contact angle. The relationship between chitosan coating content and contact angle of laminated films with varying glycerol contents is demonstrated below in Figure 4.4.

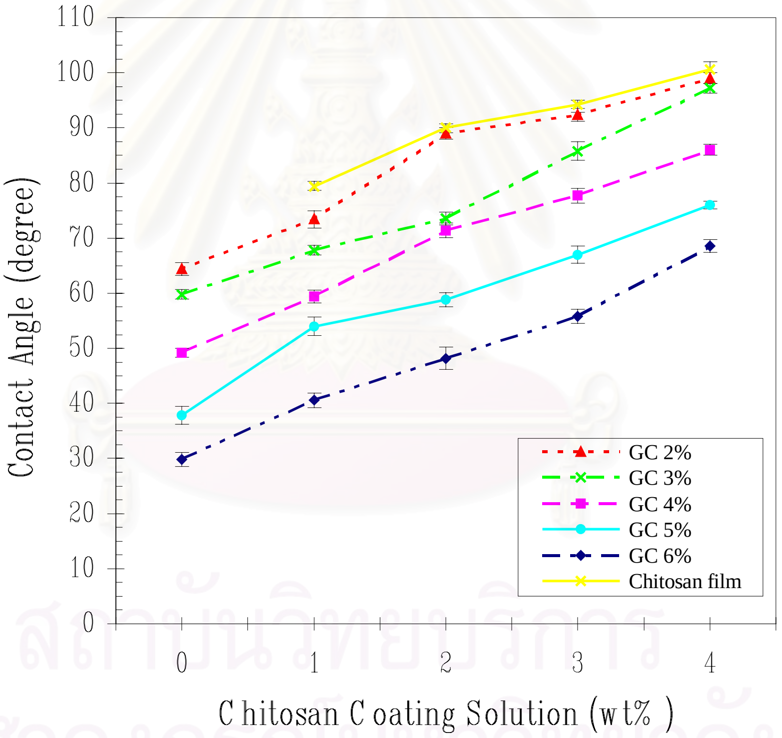


Figure 4.4 Effect of chitosan coating contents on contact angles of laminated films containing 2, 3, 4, 5, and 6 wt% glycerol

When considering at the particular glycerol content, the free starch film exhibits the smallest contact angle. It can be clearly seen that an increase in concentration of chitosan coating solution brings about a significant increase in contact angle values of the laminated films. In other words, wettability of the laminated films decreases with increasing the chitosan coating concentration. For example, at 6 wt % glycerol, the contact angle of starch film were 29.8°, whereas that of 1, 2, 3, and 4wt% chitosan-laminated film increased to 40.6, 48.2, 55.8, and 68.6°, respectively.

This finding evidence further supports the statement in the next section that chitosan film was more hydrophobic than starch film. It is probably due to the fact that the presence of hydrophobic acetyl groups in chitosan chain plays a role in contributing to higher hydrophobicity of chitosan.

4.1.4 Water Vapor Transmission Rate (WVTR)

The water vapor transmission rate (WVTR), often erroneously called water vapor permeability (WVP), is a mass of water vapor transmitted through a unit area in a unit time under specified condition of temperature and humidity as described earlier in Chapter III. Previous reports stated that the WVTR of polysaccharide films are related to their thickness [61]. In this study, it was expected to experience the difficulty of minimizing the film thickness variation which was commonly found from casting technique. The results of thickness measurement reveal that there is no significant change in thickness of the laminated film despite the variation of plasticizer concentration and chitosan coating content in each recipe.

- **Effect of Glycerol Contents**

Coating of 1wt% chitosan solution onto the series of plain starch films containing varying glycerol concentrations was carried out in order to investigate the effect of plasticizer. The WVTR values and the average thickness of these laminated films are given in Table 4.4. The relationship between WVTR and glycerol content is displayed in Figure 4.5.

Table 4.4 **Water vapor transmission rate and average thickness values of 1 wt% chitosan-laminated films containing various glycerol contents**

Laminated Films		Average Film Thickness (mm)	WVTR (g.m^{-2}.day^{-1})
Glycerol (wt%)	**Chitosan Coating Solution (wt%)**		
2	1	0.10	2289.8 ± 102.0
3	1	0.10	2793.6 ± 148.7
4	1	0.11	3143.0 ± 47.1
5	1	0.11	3098.8 ± 25.2
6	1	0.11	3133.8 ± 39.6

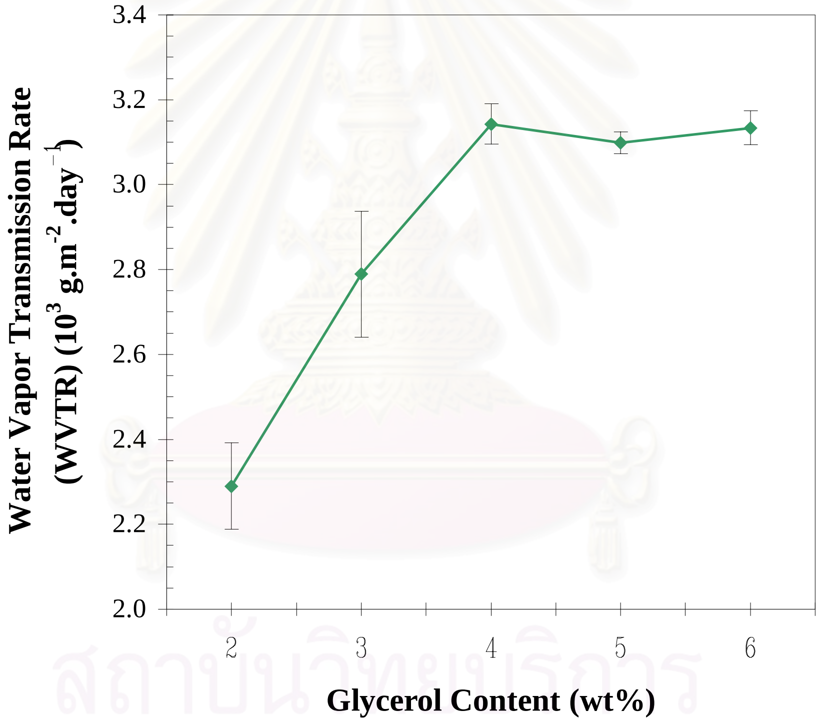


Figure 4.5 **Water vapor transmission rate values of laminated films with respect to glycerol content**

The results show that the WVTR values of the laminated films are between about 2290 and 3134 g.m^{-2}.day^{-1}. It is found that an increase in these values depends upon the increased amount of glycerol. The finding results are found in good agreement with the work of Arvanitoyannis and Biliaderis [96]. The presence of glycerol seemed to reduce the water vapor barrier properties of the laminated film. It is probably that its hydrophilic nature increases with the increased glycerol concentration. It is fact that the addition of plasticizer causes the polymer network less dense and consequently more water vapor permeable laminate film. An increase in free volume further raised the water vapor diffusion ability. Therefore, it is common to observe the high WVTR value of the typical edible film with the addition of hygroscopic plasticizer like glycerol. High water vapor permeability of the edible film is not desirable with respect to its usage performance. The amount of added glycerol, hence, is important in order to balance the thermoplastic property and good barrier properties of the film.

The results provide information that the WVTR value of the laminated film reaches its maximum value at 4 wt% glycerol. As seen, there is a sharp increase in the WVTR values of the laminated films containing 3 and 4 wt% glycerol, the WVTR values starts to level off afterwards. Hence, it should be recommended that the most suitable glycerol concentration added starch base film is in the range of 2-4 wt%. Moreover, the turning point of WVTR value occurring at 4 wt% glycerol leads to the use of laminated film prepared from 4 wt% glycerol plasticized-starch film in the next section.

- **Effect of Chitosan Coating Contents**

The laminated films containing 4 wt% glycerol were selected to study the effect of chitosan coating contents on WVTR. The results of the test are listed and presented in Table 4.5 and Figure 4.6, respectively.

Table 4.5 Water vapor transmission rate and average thickness values of 1, 2, 3, and 4% chitosan-laminated films containing 4 wt% glycerol

Laminated Films		Average Film Thickness (mm)	WVTR $(g.m^{-2}.day^{-1})$
Glycerol (wt%)	Chitosan Coating Solution (wt%)		
4	1	0.11	3143.0 ± 47.1
	2	0.11	3021.4 ± 41.8
	3	0.10	2793.8 ± 63.4
	4	0.10	2663.3 ± 83.3

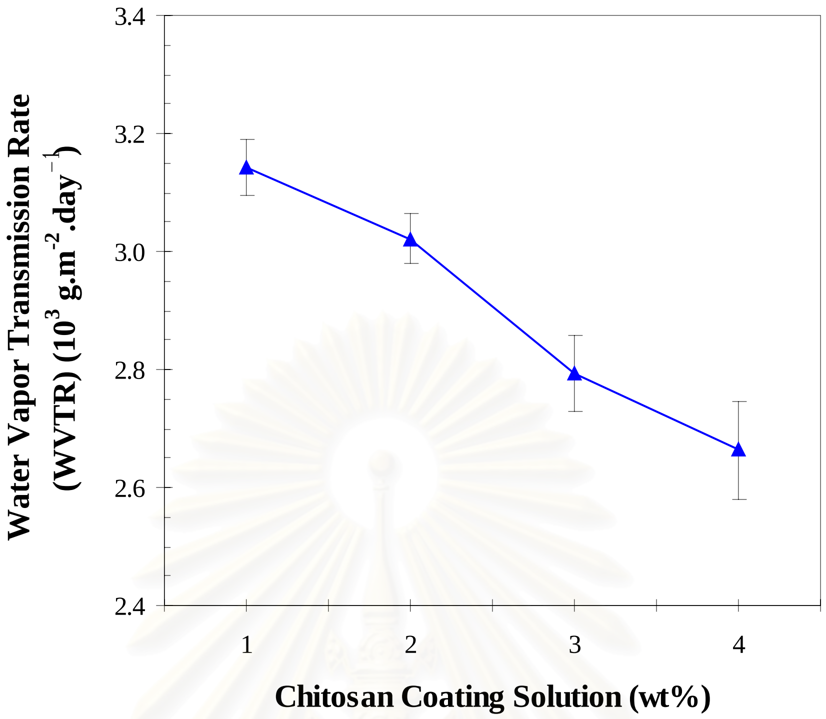


Figure 4.6 Effect of chitosan coating contents on water vapor transmission rate of laminated films containing 4 wt% glycerol

It can be clearly observed that effect of chitosan coating on WVTR is opposite to that of glycerol. The WVTR value of the laminated film decreases gradually as increasing the concentration of chitosan coating solution from 1 to 4 wt%. The decreasing WVTR value of the laminated film may be related to the hydrophobicity of chitosan due to the presence of hydrophobic acetyl groups of incompletely deacetylated chitosan. The residual acetyl groups played its part in hindering the transportation of water vapor. In other words, chitosan coating renders the hydrophobic characteristic to the laminated film surface. Moreover, the reduction of WVTR value of the 4 wt% chitosan-laminated film is probably that the single-coat surface of starch base film could be perfectly covered with high concentration of chitosan solution.

As a result, the chitosan-laminated film exhibited lower WVTR compared to the plain starch film. The chitosan/starch laminated film as packaging film therefore offers preferable performance.

4.1.5 Water Absorption

Water absorption values of starch and chitosan free films and laminated films are tabulated in Table 4.5. The effects of chitosan coating content on water absorption values of laminated films are graphically shown in Figure 4.6.

Table 4.6 Water absorption and average thickness values of starch and chitosan free films and chitosan/starch laminated films

	Glycerol (wt%)	Chitosan Coating Solution (wt%)	Average Film Thickness (mm)	Water Absorption (%)
Chitosan Films	-	1	0.10	66.2 ± 0.8
	-	2	0.11	59.7 ± 1.3
	-	3	0.10	46.3 ± 1.5
	-	4	0.10	30.4 ± 1.6
Starch Films	2	-	0.10	84.5 ± 0.7
	3	-	0.10	92.5 ± 2.6
	4	-	0.10	113.0 ± 1.2
	5	-	0.10	125.1 ± 2.6
	6	-	0.10	172.4 ± 2.1
Chitosan/Starch Laminated Films	2	1	0.10	71.8 ± 0.3
		2	0.10	53.5 ± 1.6
		3	0.10	45.2 ± 1.0
		4	0.10	35.7 ± 3.5
	3	1	0.10	78.4 ± 2.1
		2	0.11	56.5 ± 1.9
		3	0.11	54.5 ± 2.9
		4	0.10	49.0 ± 2.8
	4	1	0.11	92.2 ± 0.5
		2	0.11	77.5 ± 1.7
		3	0.10	64.0 ± 0.9
		4	0.10	49.6 ± 0.5
	5	1	0.11	113.8 ± 1.0
		2	0.10	82.5 ± 0.6
		3	0.10	76.7 ± 2.6
		4	0.10	73.7 ± 2.1
	6	1	0.11	125.4 ± 3.1
		2	0.10	100.5 ± 2.4
		3	0.11	78.5 ± 1.0
		4	0.10	74.8 ± 2.3

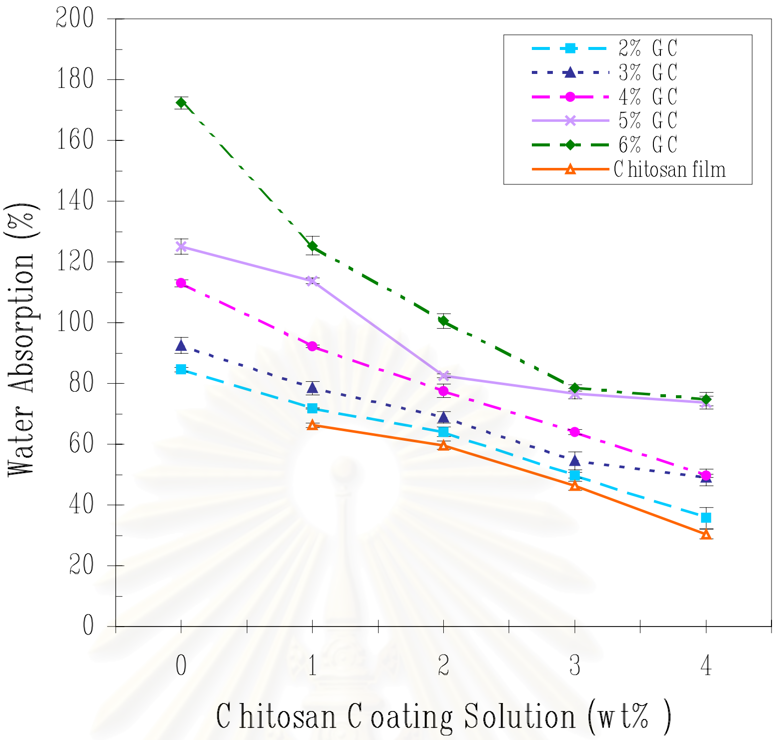


Figure 4.7 **Effect of chitosan coating contents on water absorption of laminated films containing 2, 3, 4, 5, and 6 wt% glycerol**

The result revealed that the plasticizer contents had great effect on the % water absorption values of free starch films and laminated films. The % water absorption values of 2, 3, 4, 5, and 6 wt% glycerol plasticized starch films are about 84.5, 92.5, 113.0, 125.1, and 172.4%, respectively. The similar trend can be observed in the laminated films, albeit lower % water uptake compared to the free starch film.

It is quite certain that the relatively lower water uptake of the laminated film resulted from the presence of chitosan coating since the ability of the water uptake of the laminated film decreases with increasing in chitosan coating content. The remarkable reduction in water uptake is seen with the laminated film containing 4 wt% chitosan coating. For example, at 2, 3, 4, 5, and 6 wt% glycerol, the water absorption values of 1 wt% chitosan-laminated films are about 71.8, 78.4, 92.2, 113.8, and 125.4%, respectively, whereas those of 4 wt% chitosan-laminated films rapidly decrease to 35.7, 49.0, 49.6, 73.7, and 74.8%, respectively. This result is in good agreement with the result from WVTR of the laminated films presented in Figure 4.5. The reason for these results is similar to that discussed in the previous section. Conclusively, the hydrophobic acetyl groups in the chitosan molecules would result in the lowered water absorption values and dissolution of the chitosan films. The higher the chitosan coating concentration, the higher the coverage film surfaces.

It should be noted that, however, coating chitosan solution onto starch film containing above 5 wt% glycerol brings about a little drop in percent water uptake. It is likely that the high amount of glycerol in starch film would be interfere the surface hydrophobic property of the laminated film due to the significant increase in water absorption of the starch base film.

4.2 Mechanical Properties Characterization: Tensile Properties Evaluation

4.2.1 Effect of Glycerol Contents

Figure 4.8 presents effect of glycerol content on tensile properties of the free starch films. It is obvious that the tensile strength, tensile modulus and % elongation at break are strongly influenced by concentration of glycerol.

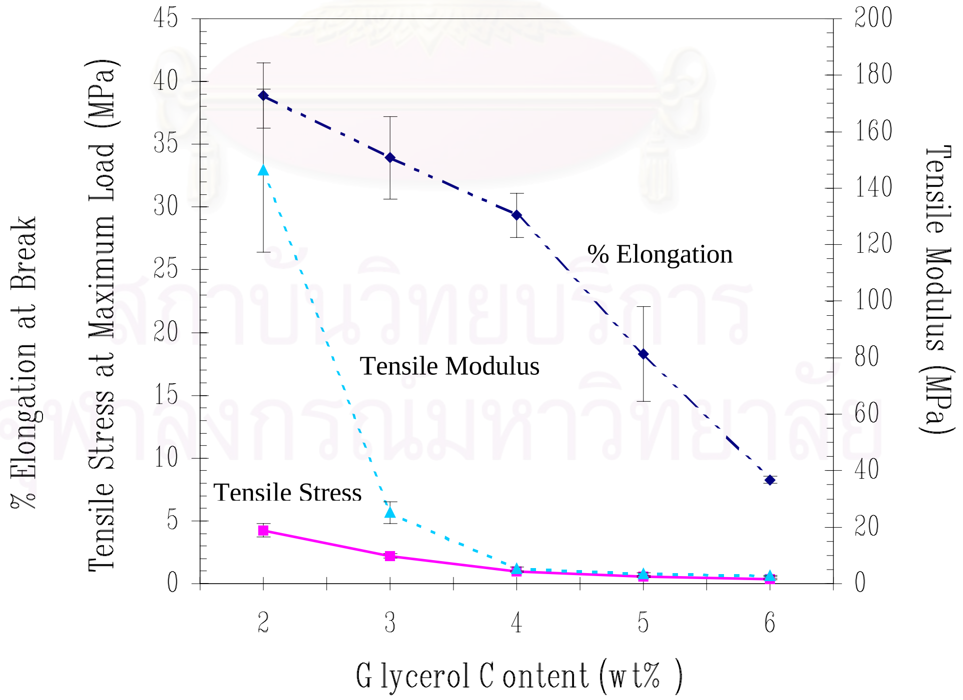


Figure 4.8 Effect of glycerol contents on tensile stress at maximum load, tensile modulus, and % elongation at break of free starch films

As increasing the amount of glycerol, the tensile strength and tensile modulus were decreased. The tensile strength values of starch films containing 2, 3, 4, 5, and 6 wt% glycerol were about 4.3, 2.2, 1.0, 0.54, and 0.36 MPa, respectively. The tensile modulus values were about 146.2, 25.1, 5.2, 3.4, and 2.8 MPa, respectively. Considering the % elongation at break, it is found that the % elongation values also decrease sharply with an increase of glycerol content. The % elongation at break of starch films containing 2, 3, 4, 5, and 6 wt% glycerol were 38.9, 33.9, 29.3, 18.3, and 8.3, respectively. It may be argued that the starch films are fairly ductile, especially 2, 3, and 4 wt% glycerol, since the % elongation at break exceeds 20%. However, as increasing glycerol content, the lower % elongation at break observed in this research did not mean that the starch films showed more brittle character. In contrast, the starch films were more stickiness resulting in easy breakage. As seen from the result, addition of 5 and 6 wt% glycerol the starch films had lost their strength clearly. The tensile strength was decreased to about 0.5 and 0.36 MPa, respectively. It is noted that the strength of plasticized starch film is strongly influenced by plasticizer. In the study of Rindlav-Westling et al. [53], it was found that amylopectin was more sensitive than amylose to plasticization caused by glycerol. At 10% GC, the mechanical properties measurement of amylopectin film was extremely difficult to perform, whereas stronger amylose film yielding higher mechanical properties. Nevertheless, effect of glycerol on overall behavior of both amylose and amylopectin films was similar. It was revealed that under low glycerol contents (< 20%) both tensile strength and percentage elongation decreased.

4.2.2 Effect of Chitosan Coating Contents

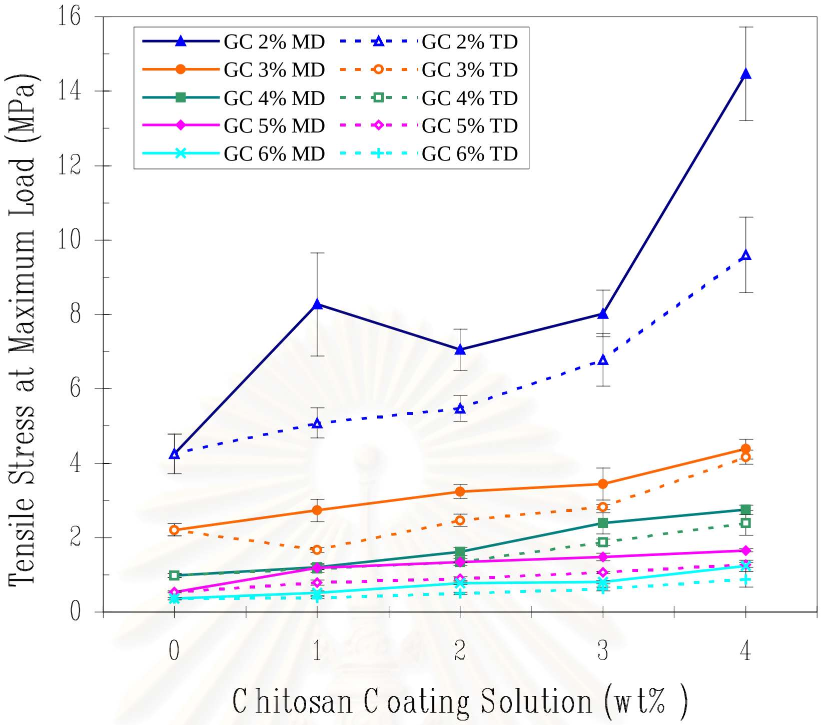
The tensile properties in machine direction (MD) and transverse direction (TD) of chitosan/starch laminated films are tabulated in Table 4.7 and 4.8, respectively. The effect of chitosan coating contents on tensile properties in both of MD and TD of laminated films containing 2, 3, 4, 5, and 6wt% glycerol are illustrated in Figure 4.9.

Table 4.7 Tensile properties and average thickness of starch and chitosan free films and chitosan/starch laminated films in machine direction

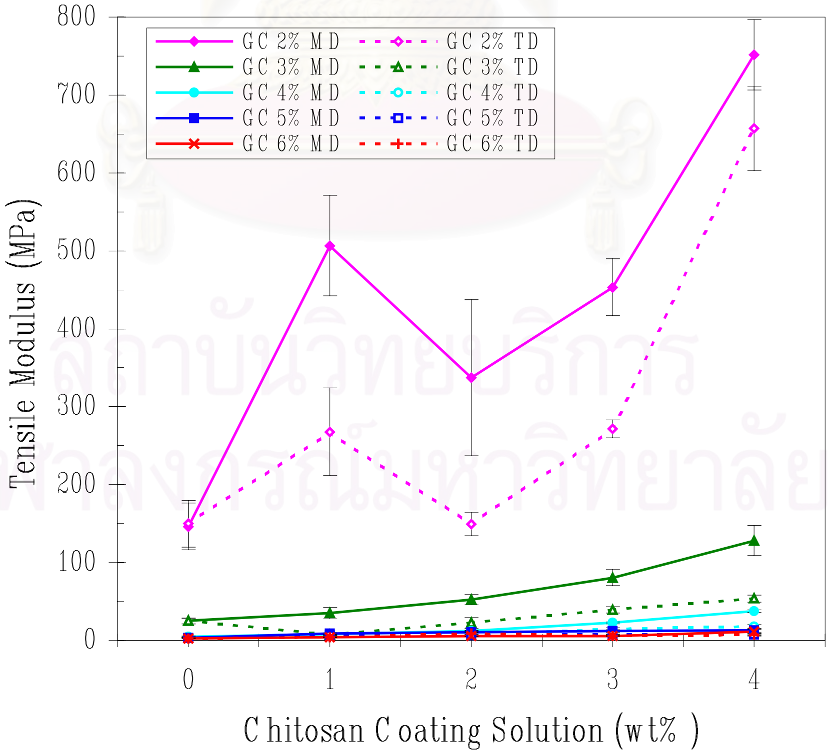
	Glycerol (wt%)	Chitosan Coating Solution (wt%)	Thickness (mm)	Tensile Stress at Maximum Load (MPa)	Modulus of Elasticity (MPa)	Elongation at Break (%)
Chitosan Films	-	1	0.10	10.71 ± 1.61	859.77 ± 71.29	3.16 ± 0.80
	-	2	0.10	14.47 ± 0.41	996.20 ± 97.5	3.45 ± 0.62
	-	3	0.10	17.17 ± 0.73	1133.06 ± 192.24	4.08 ± 1.11
	-	4	0.10	21.02 ± 2.01	1265.53 ± 59.75	4.42 ± 0.27
Starch Films	2	-	0.10	4.25 ± 0.53	146.23 ± 28.83	38.87 ± 2.61
	3	-	0.11	2.21 ± 0.17	25.08 ± 3.90	33.93 ± 3.29
	4	-	0.10	0.98 ± 0.05	5.19 ± 0.72	29.33 ± 1.78
	5	-	0.11	0.54 ± 0.03	3.44 ± 0.49	18.28 ± 3.77
	6	-	0.11	0.36 ± 0.04	2.79 ± 0.24	8.27 ± 0.28
Chitosan/ Starch Laminated Films	2	1	0.10	8.27 ± 1.38	506.29 ± 64.42	14.48 ± 3.57
		2	0.10	7.05 ± 0.56	336.96 ± 121.44	21.32 ± 3.22
		3	0.10	8.02 ± 0.63	453.30 ± 36.35	17.00 ± 1.83
		4	0.10	14.47 ± 1.25	751.34 ± 67.93	9.80 ± 1.06
	3	1	0.10	2.73 ± 0.30	35.36 ± 7.57	27.55 ± 3.73
		2	0.11	3.23 ± 0.19	52.50 ± 6.31	23.58 ± 1.43
		3	0.10	3.44 ± 0.43	80.44 ± 10.27	24.55 ± 4.81
		4	0.11	4.38 ± 0.26	128.32 ± 19.52	20.17 ± 3.22
	4	1	0.11	1.21 ± 0.06	8.29 ± 0.64	17.71 ± 1.26
		2	0.11	1.62 ± 0.11	12.69 ± 0.66	16.60 ± 1.69
		3	0.10	2.38 ± 0.28	22.79 ± 1.11	17.56 ± 2.47
		4	0.10	2.75 ± 0.13	37.70 ± 1.42	17.75 ± 0.73
	5	1	0.11	1.18 ± 0.12	8.82 ± 0.75	15.03 ± 0.86
		2	0.12	1.34 ± 0.01	10.85 ± 0.64	14.55 ± 1.98
		3	0.12	1.48 ± 0.10	12.54 ± 1.52	15.24 ± 1.95
		4	0.11	1.66 ± 0.04	12.94 ± 1.89	17.91 ± 1.04
	6	1	0.11	0.51 ± 0.06	3.71 ± 0.80	15.89 ± 1.33
		2	0.11	0.77 ± 0.03	5.54 ± 0.50	12.51 ± 1.08
		3	0.11	0.81 ± 0.04	5.85 ± 1.18	12.93 ± 1.67
		4	0.10	1.24 ± 0.15	11.71 ± 2.60	13.76 ± 1.01

Table 4.8 Tensile properties and average thickness of starch and chitosan free films and chitosan/starch laminated films in transverse direction

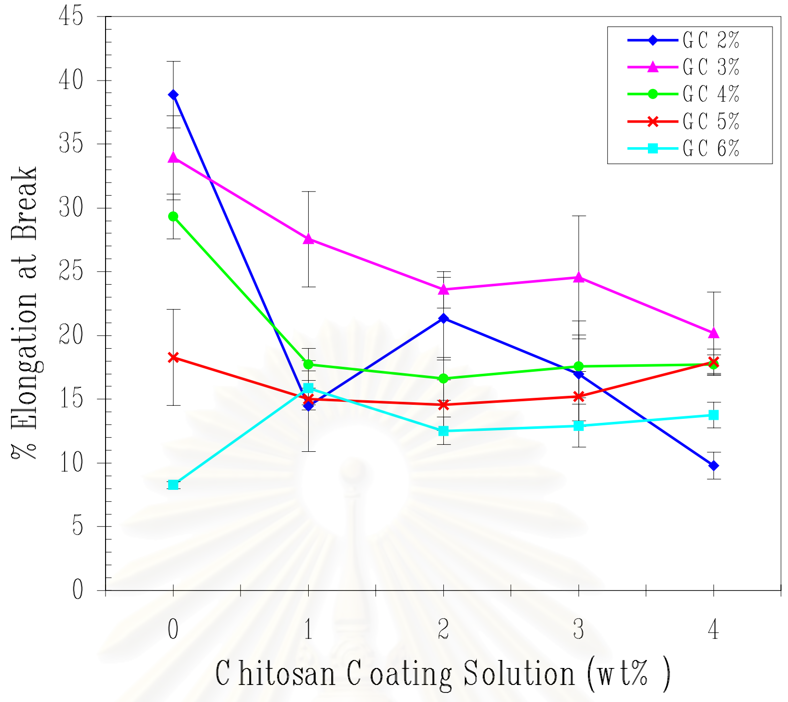
	Glycerol (wt%)	Chitosan Coating Solution (wt%)	Thickness (mm)	Tensile Stress at Maximum Load (MPa)	Modulus of Elasticity (MPa)	Elongation at Break (%)
Chitosan Films	-	1	0.10	10.71 ± 1.61	859.77 ± 71.29	3.16 ± 0.80
	-	2	0.10	14.47 ± 0.41	996.20 ± 97.5	3.45 ± 0.62
	-	3	0.10	17.17 ± 0.73	1133.06 ± 192.24	4.08 ± 1.11
	-	4	0.10	21.02 ± 2.01	1265.53 ± 59.75	4.42 ± 0.27
Starch Films	2	-	0.10	4.25 ± 0.53	146.23 ± 28.83	38.87 ± 2.61
	3	-	0.11	2.21 ± 0.17	25.08 ± 3.90	33.93 ± 3.29
	4	-	0.10	0.98 ± 0.05	5.19 ± 0.72	29.33 ± 1.78
	5	-	0.11	0.54 ± 0.03	3.44 ± 0.49	18.28 ± 3.77
	6	-	0.11	0.36 ± 0.04	2.79 ± 0.24	8.27 ± 0.28
Chitosan/ Starch Laminated Films	2	1	0.10	5.08 ± 0.40	267.84 ± 55.93	33.10 ± 4.88
		2	0.11	5.47 ± 0.34	149.17 ± 14.92	31.52 ± 2.15
		3	0.10	6.78 ± 0.70	271.67 ± 11.21	22.54 ± 1.87
		4	0.10	9.6 ± 1.01	657.01 ± 53.98	20.26 ± 2.45
	3	1	0.11	1.67 ± 0.07	7.68 ± 0.84	33.28 ± 3.00
		2	0.10	2.46 ± 0.16	22.63 ± 0.85	35.95 ± 2.81
		3	0.11	2.82 ± 0.07	39.06 ± 4.05	39.70 ± 2.48
		4	0.10	4.17 ± 0.19	53.84 ± 4.69	39.86 ± 1.87
	4	1	0.11	1.17 ± 0.04	6.47 ± 0.49	22.06 ± 1.19
		2	0.10	1.35 ± 0.01	7.23 ± 1.01	23.32 ± 3.37
		3	0.11	1.87 ± 0.08	14.41 ± 2.06	19.72 ± 1.11
		4	0.10	2.40 ± 0.34	18.28 ± 2.38	23.80 ± 2.13
	5	1	0.12	0.79 ± 0.07	6.80 ± 1.14	13.60 ± 1.00
		2	0.11	0.90 ± 0.05	6.68 ± 0.52	13.74 ± 1.34
		3	0.12	1.06 ± 0.02	7.67 ± 0.15	15.79 ± 1.08
		4	0.12	1.26 ± 0.06	7.60 ± 0.66	18.23 ± 2.82
	6	1	0.11	0.38 ± 0.03	4.11 ± 0.95	7.14 ± 1.54
		2	0.11	0.50 ± 0.04	9.05 ± 1.48	7.42 ± 1.08
		3	0.12	0.62 ± 0.05	5.96 ± 0.80	9.92 ± 0.53
		4	0.11	0.88 ± 0.21	7.35 ± 1.68	12.17 ± 1.97



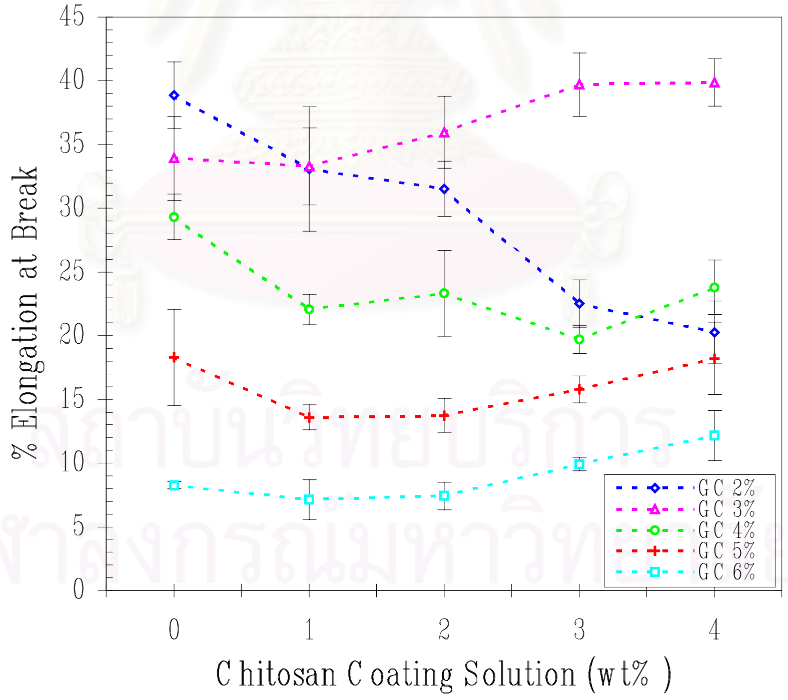
(a) Tensile stress at maximum load in MD and TD



(b) Tensile modulus in MD and TD



(c) % Elongation at break in MD

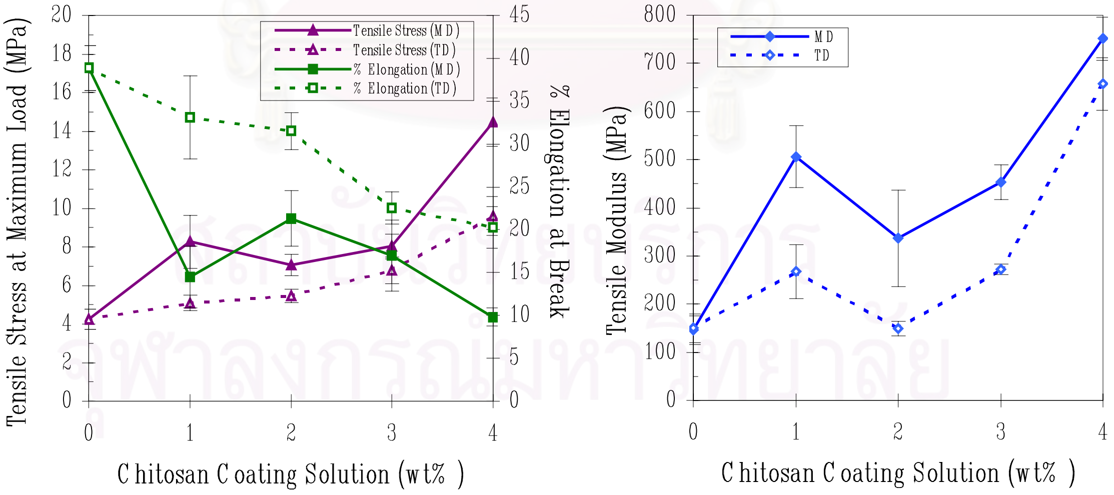


(d) % Elongation at break in TD

Figure 4.9 Effect of chitosan coating contents on tensile properties of laminated films containing 2, 3, 4, 5, and 6 wt% glycerol

It can be clearly seen that at individual glycerol content in the laminated film there is a significant change on the tensile stress at maximum load and tensile modulus in both of MD and TD upon increasing the amount of chitosan coatings. It was found that the tensile stress and tensile modulus values in MD were higher than in TD. This is probably due to polymer chain of chitosan aligned along the MD of automatic film applicator during applying force to wire bar coater. Regarding to percentage elongation at break, the % elongation values in both directions tended to be lower than that of uncoated or free starch films. In addition, % elongation at break in MD were found to be lower than in TD. These results indicate that by coating chitosan onto starch film the tensile properties, in particular tensile stress at maximum load and tensile modulus, of laminated film could be improved due to the strength reinforcement of chitosan to the laminates. However, it should be noted that concentration of glycerol in foundation starch film results in a change in anisotropic properties of the laminates. The detail will be discussed later.

Anisotropic properties of one-side coated film in the present study can be clearly explained from the laminated film containing 2 wt% glycerol. The relationship between chitosan coating contents and the tensile properties of laminated films is shown below in Figure 4.10.



(a) Tensile stress at maximum load and % elongation at break

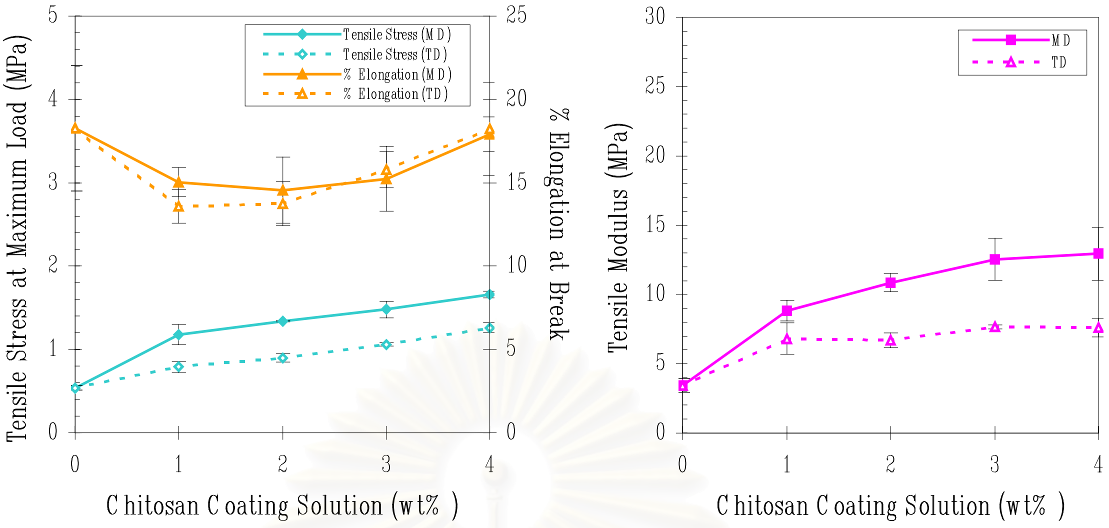
(b) Tensile modulus

Figure 4.10 Effect of chitosan coating contents on tensile properties of laminated film containing 2 wt% glycerol in MD and TD

Upon increasing chitosan coating contents, the tensile stress at maximum load and tensile modulus in both directions tend to increase, whereas % elongation at break tends to decrease. For example, in MD, tensile stress at maximum load, tensile modulus, and % elongation at break of 1 wt% chitosan-laminated film are about 8.3 MPa, 506.3 MPa, and 14.5%, respectively. When chitosan coating solution reaches to 4wt%, these values are found to be 14.5 MPa, 751.3 MPa, and 9.8%, respectively. In TD, tensile stress at maximum load, tensile modulus, and % elongation at break of 1 wt% chitosan-laminated film are about 5.1 MPa, 267.8 MPa, and 33.1%, respectively, whereas those of 4 wt% chitosan-laminated film are 9.6 MPa, 657.0 MPa, and 20.3%, respectively.

A significant difference in tensile properties between MD and TD results from the coating process performing on an automatic film applicator. The higher % elongation value found in TD is probably due to less molecular alignment of chitosan film in this direction due to lack of applying force compared to MD as mentioned earlier. In addition, with increasing chitosan coating concentration, a remarkable decrease in % elongation at break compared to laminated films containing 3, 4, 5, and 6 wt% glycerol may be attributed to the less plasticizing effect due to the minimized concentration of plasticizer in foundation starch film, including the effect of brittleness of chitosan film.

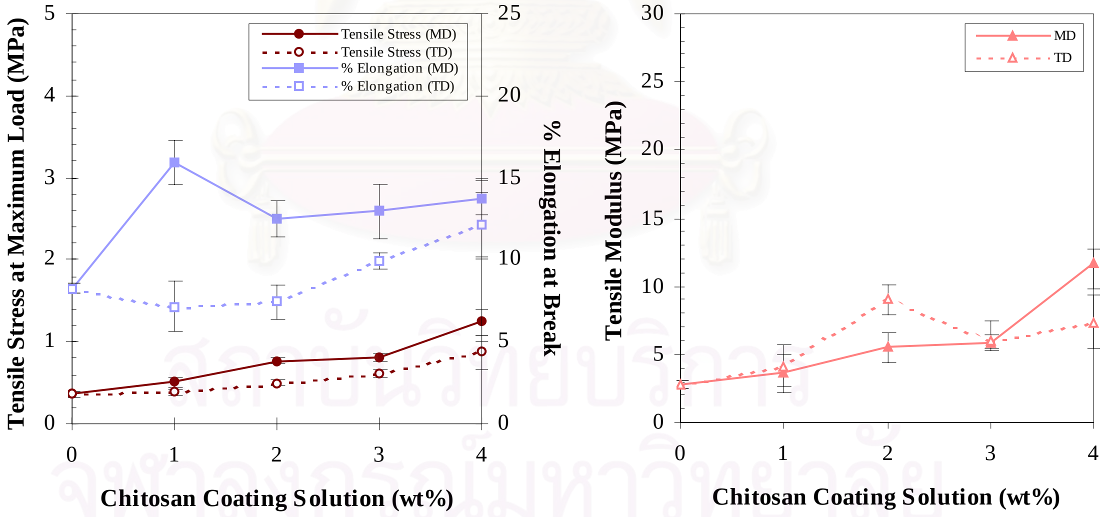
As stated earlier, high glycerol contents added to the foundation starch film result in lowered strength. This effect remains in the laminates. As increasing chitosan coating concentration, an increase in tensile stress at maximum load and tensile modulus in MD and TD of laminated films is observed. An increasing trend of laminated films containing higher glycerol content is inferior to that of laminated film containing 2 wt% glycerol. This is probably due to the plasticization in foundation starch film is more influence than reinforcement of chitosan film. Moreover, it seems that an increase in glycerol content brings about changing properties of the laminates from anisotropy to isotropy. It is observed that there is a little effect on tensile strength of the laminated film containing 5 and 6 wt% glycerol upon increasing chitosan coating concentration. Figure 4.11 presents the effect of chitosan coating contents on tensile properties of laminated film containing 5 wt% glycerol in both directions.



(a) Tensile stress at maximum load and % elongation at break

(b) Tensile modulus

Figure 4.11 Effect of chitosan coating contents on tensile properties of laminated film containing 5 wt% glycerol in MD and TD



(a) Tensile stress at maximum load and % elongation at break

(b) Tensile modulus

Figure 4.12 Effect of chitosan coating contents on tensile properties of laminated film containing 6 wt% glycerol in MD and TD

With high glycerol content, especially 5 and 6 wt% glycerol, tensile properties of the laminates in MD and TD are not much differences. Focused on MD of 1 and 4 wt% chitosan-laminated films containing 5 wt% glycerol as shown in Figure 4.11, tensile stress at maximum load and tensile modulus of the former are about 1.2 MPa and 8.8 MPa, respectively, whereas those of the latter slightly increase to 1.7 MPa and 12.9 MPa, respectively. At 6 wt% glycerol (Figure 4.12), although the improved tensile properties of laminates in MD and TD are attributed to an increase in chitosan coating concentration, the greatest tensile strength values obtained from 4 wt% chitosan coating are relatively low, i.e., in MD, the tensile stress at maximum load and tensile modulus are about 1.2 MPa and 11.7 MPa, respectively. Considering overall % elongation at break of laminated films containing high glycerol content, the % elongation at break in both directions, especially in TD, tended to increase with increasing the amount of chitosan coating. For example, in TD, the % elongation at break of 1, 2, 3, and 4 wt% chitosan-laminated films containing 5 wt% glycerol are about 13.6, 13.7, 15.8, and 18.2, respectively. For 6 wt% glycerol, these values are found to be 7.1, 7.4, 9.9, and 12.2, respectively.

High concentration of chitosan coating causes a simultaneously little increase in tensile properties of these laminates. However, the influence of chitosan coating concentration is less than that of glycerol content resulting in a relatively low strength of the films.

It could be concluded that the tensile strength and modulus values of the laminates are significantly larger than those of free starch films, but overall the elongation at break values are smaller. The higher the chitosan coating concentration, the higher the film strength. The brittle characteristic of chitosan film may be responsible for an increment of tensile strength and tensile modulus of the laminates in both directions. However, the amount of glycerol in foundation film affects the anisotropic properties and characteristic of the laminates. At higher glycerol content, tensile properties of the laminates in MD is similar to that in TD. The laminated film is more ductility. The % elongation at break of the film is not still low while the tensile stress at maximum load and tensile modulus of these laminates is close to be zero. The relationship between glycerol content added in foundation starch film and concentration of chitosan coating solution is a very important factor for preparing the laminated film with desired tensile properties.

Chapter V

Conclusions and Recommendations

5.1 Conclusions

Chitosan/cassava starch laminated films were prepared by film casting technique. The free starch films contained concentrations of glycerol as plasticizer varying from 2 to 6 wt%. Coating of the free starch film with chitosan solutions having concentrations in the range of 1-4 wt%. The laminates were subjected to structural characterization and evaluated for physical and mechanical properties. The results were summarized as follows.

1. The X-ray diffractogram of the laminated film showed the change in reflection characteristics of starch crystalline, indicating the chitosan-starch interaction occurring at the molecular level. This fact is attributed to the hydrogen-bonding interaction between chitosan and starch molecules, leading to structural reorientation and subsequent starch crystallinity.

2. The gloss values of laminated films increased significantly with increasing chitosan coating content. Comparing to the free starch films, the free chitosan films showed higher degree of gloss values due to its surface regularity. As a result, chitosan coating layer is attributed to the increase in the gloss value of the laminated film.

3. Comparing to free starch films, there was no significant change in transparency of the laminated films as increasing the chitosan coating concentration.

4. A significant sharp decrease in wettability of the laminated film was observed as indicated by a sharp increase in water contact angle. The higher the chitosan coating content, the higher the degree of contact angle. This is due to the likelihood of the complete coverage of the chitosan with an increase in chitosan coating concentration. The residual hydrophobic acetyl groups in chitosan chain are responsible for the improved surface hydrophobicity of the laminated film.

5. Glycerol content in foundation starch film was found to be adversely affect the water vapor permeability and water absorption of laminated film. This was because of hygroscopic character of glycerol leading to an increase in free volume of the system, consequently increasing the mass transfer through the film. This disadvantage was compensated with chitosan coating. The chitosan coating resulted in a decrease in water vapor transmission rate as well as the percent water uptake. This may be attributed to the presence of the hydrophobic acetyl group in chitosan, hence rendering the more hydrophobicity to the laminated film surface.

6. The results on mechanical properties showed that the tensile properties of the laminated films were superior to the free starch films. At low glycerol content, an increase in chitosan coating concentration contributed to a significant increment of tensile strength and tensile modulus in MD and TD while % elongation at break decreased. The coating process caused the more film strength in MD. The tensile response displayed by the laminates was consistent with that of coating materials, where in elongation values typically decreased as the brittleness of chitosan increase the tensile strength and modulus values. For laminated films containing high glycerol content, the tensile properties were slightly increased as increasing the concentration of chitosan coating solution. The increasing trend in % elongation at break indicated that these films were strengthen. However, the film strength influenced by concentration of chitosan coating solution was interfered with plasticizing effect. In addition, the similarity of film strength in both directions indicated that there was a change in film properties from anisotropy to isotropy. This result can be confirmed that the concentration of plasticizer is still an important factor for preparing the laminated film.

5.2 Recommendations

Due to time constraints placed on this investigation, not all of the desired experiments and experimental analyses could be conducted. Recommendations in this work are as follows.

1. The objective of this work was required to decrease the disadvantage of starch film properties, especially water vapor permeability and water absorption, using the benefit of chitosan film. Although these barrier properties were improved, the water vapor transmission rate and water absorption values could be lower because the laminates in this study were one-sided coatings. Therefore, two-sided coatings should be prepared to receive the better results.

2. Chitosan is known as an aminopolysaccharide. In dilute acid solutions, the positive charges of chitosan interfere with the negatively charged residues of macromolecule at the cell surface without the membrane leakage. Thus, it can be limit initial contamination on food surface. For development in food packaging areas, antimicrobial property of the laminated film should be investigated.

3. The replacement of acetic acid by citric acid, which is cheap, odorless, and nontoxic is recommended.

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Appendices

Appendix A

Physical Properties Characterization

Table A1 Gloss of starch and chitosan free films and chitosan/starch laminated films

Film Samples		Gloss (units)						
Glycerol (wt%)	Chitosan Coating (wt%)	Trial No.					Mean	SD
		1	2	3	4	5		
-	1	133.9	128.2	133.5	132.0	135.0	132.52	2.64
	2	131.5	136.0	134.4	142.8	134.6	135.86	4.21
	3	135.2	142.2	143.8	134.5	141.2	139.38	4.25
	4	149.5	144.0	146.4	144.9	143.3	145.62	2.46
2	0	54.4	55.4	52.3	56.3	53.1	54.30	1.63
	1	91.7	90.7	85.0	88.0	82.5	87.58	3.85
	2	101.9	98.8	103.9	98.1	99.2	100.38	2.44
	3	101.8	105.9	113.5	105.5	114.5	108.24	5.51
	4	105.7	110.5	109.1	103.8	107.4	107.30	2.66
3	0	50.4	59.0	51.5	50.7	50.3	52.38	3.73
	1	68.9	64.9	69.6	65.2	74.9	68.70	4.06
	2	99.5	89.9	91.3	95.2	100.2	95.22	4.66
	3	100.9	94.6	97.7	94.2	100.3	97.54	3.11
	4	103.2	102.8	100.4	98.9	107.0	102.46	3.09
4	0	52.8	49.7	52.0	58.1	53.2	53.16	3.08
	1	77.4	67.9	89.0	80.4	81.3	79.20	7.63
	2	89.0	90.7	91.1	81.5	92.1	88.88	4.27
	3	95.2	93.8	101.7	95.8	100.6	97.42	3.50
	4	94.3	103.5	98.2	97.0	103.7	99.34	4.14
5	0	58.8	60.0	64.3	62.1	55.4	60.12	3.37
	1	81.6	78.3	82.1	80.9	79.8	80.54	1.52
	2	98.1	92.3	87.8	90.1	93.4	92.34	3.87
	3	92.0	99.3	95.4	97.9	101.8	97.28	3.75
	4	104.5	108.3	106.1	109.4	104.9	106.64	2.14
6	0	61.0	56.7	57.1	59.8	56.8	58.28	1.99
	1	83.1	85.1	75.8	88.3	85.0	83.46	4.67
	2	98.2	94.3	88.3	94.7	87.9	92.68	4.45
	3	103.9	97.5	102.0	100.5	99.9	100.76	2.39
	4	95.2	102.1	93.7	102.8	95.8	97.92	4.21

Table A2 Contact angles of starch and chitosan free films and chitosan/starch laminated films

Film Samples		**Contact Angles (°)**						
Glycerol (wt%)	**Chitosan Coating (wt%)**	**Trial No.**					**Mean**	**SD**
		1	**2**	**3**	**4**	**5**		
-	1	79	80	78	80	80	79.4	0.89
	2	90	89	91	90	90	90.0	0.71
	3	93	95	94	95	94	94.2	0.84
	4	100	102	99	100	102	100.6	1.34
2	0	64	66	63	64	65	64.4	1.14
	1	71	74	74	75	73	73.4	1.52
	2	88	89	90	90	88	89.0	1.00
	3	92	93	94	92	91	92.4	1.14
	4	100	99	100	98	98	99.0	1.00
3	0	61	59	59	60	60	59.8	0.84
	1	68	69	67	68	67	67.8	0.84
	2	74	73	75	74	72	73.6	1.14
	3	87	85	88	85	84	85.8	1.64
	4	98	98	96	97	97	97.2	0.84
4	0	49	50	49	48	50	49.2	0.84
	1	60	59	58	61	59	59.4	1.14
	2	72	70	73	72	70	71.4	1.34
	3	77	78	76	78	80	77.7	1.37
	4	85	87	86	85	87	86.0	1.00
5	0	37	36	39	40	37	37.8	1.60
	1	54	51	51	56	55	54.0	1.64
	2	59	58	57	60	60	58.8	1.30
	3	68	69	66	67	65	67.0	1.58
	4	75	76	77	76	76	76.0	0.71
6	0	28	31	29	30	31	29.8	1.30
	1	40	42	39	40	42	40.6	1.34
	2	48	51	48	50	50	48.2	2.05
	3	54	57	56	55	57	55.8	1.30
	4	69	67	68	70	69	68.6	1.14

Table A3 Water vapor transmission rate of 1wt% chitosan free film, 1 wt% chitosan-laminated films containing 2, 3, 4, 5, and 6 wt% glycerol, and 1, 2, 3, and 4 wt% chitosan-laminated films containing 4 wt% glycerol

Film Samples		Water Vapor Transmission Rate ($g.m^{-2}.day^{-1}$)				
Glycerol (wt%)	Chitosan Coating (wt%)	Trial No.			Mean	SD
		1	2	3		
-	1	2085.48	1831.08	2103.23	2006.60	152.26
2	1	2190.60	2394.30	2284.48	2289.79	101.95
3	1	2947.68	2650.92	2782.35	2793.65	148.70
4	1	3149.88	3186.39	3092.86	3143.04	47.14
4	2	2854.08	3042.60	2973.34	2956.67	95.36
4	3	2727.72	3048.36	2799.48	2858.52	168.28
4	4	2736.00	2572.44	2681.54	2663.33	83.29
5	1	3085.92	3082.68	3127.78	3098.79	25.16
6	1	3150.12	3162.60	3088.68	3133.80	39.57

Table A4 Water absorption of starch and chitosan free films and chitosan/starch laminated films

Film Samples		Condition Weight (g)			Wet Weight (g)			Weight Increase (%)				
GC (wt%)	CS (wt%)	Trial No.			Trial No.			Trial No.			Mean	SD
		1	2	3	1	2	3	1	2	3		
-	1	0.1976	0.2014	0.1978	0.3302	0.3339	0.3280	67.10	65.77	65.82	66.23	0.75
	2	0.1945	0.1993	0.2177	0.3081	0.3205	0.3478	58.39	60.81	59.76	59.65	1.31
	3	0.1878	0.1956	0.2054	0.2718	0.2861	0.3035	44.73	46.29	47.76	46.26	1.52
	4	0.1932	0.2169	0.1887	0.2533	0.2851	0.2426	31.09	31.44	28.55	30.36	1.58
2	0	0.2910	0.3189	0.3614	0.5345	0.5912	0.6676	83.68	85.39	84.56	84.54	0.70
	1	0.2534	0.2466	0.2469	0.4362	0.4235	0.4233	72.14	71.74	71.45	71.78	0.28
	2	0.2938	0.2527	0.3125	0.4788	0.4167	0.5133	64.97	64.90	64.26	64.04	0.80
	3	0.2538	0.2471	0.2557	0.3778	0.3590	0.3794	48.86	51.94	48.38	49.72	1.58
	4	0.1859	0.2560	0.1662	0.2519	0.3367	0.2328	35.50	31.52	40.07	35.70	3.49
3	0	0.3790	0.4125	0.4255	0.7204	0.7869	0.8345	90.08	91.25	96.12	92.48	2.62
	1	0.1524	0.1527	0.1611	0.2682	0.2720	0.2919	75.98	78.13	81.19	78.43	2.14
	2	0.3110	0.3702	0.3398	0.5290	0.6149	0.5784	70.11	60.10	70.22	68.81	1.92
	3	0.2997	0.2958	0.3128	0.4598	0.4506	0.4937	53.42	52.33	57.83	54.53	2.91
	4	0.0039	0.1607	0.1874	0.3424	0.2364	0.2754	52.93	47.11	46.96	49.00	2.78
4	0	0.3997	0.3649	0.3073	0.8458	0.7826	0.6543	111.62	114.47	112.92	113.00	1.16
	1	0.2574	0.2475	0.3515	0.4958	0.4741	0.6768	92.62	91.56	92.55	92.24	0.48
	2	0.3420	0.3572	0.2538	0.5991	0.6384	0.4534	75.18	78.72	78.64	77.51	1.65
	3	0.4303	0.3612	0.3963	0.7106	0.5912	0.6465	65.14	63.67	63.13	63.98	0.85
	4	0.3202	0.3343	0.3209	0.4805	0.4976	0.4811	50.06	48.85	49.92	49.61	0.54
5	0	0.1999	0.1937	0.3140	0.4432	0.4368	0.7159	121.71	125.50	127.99	125.07	2.58
	1	0.2561	0.2421	0.2679	0.5443	0.5177	0.5758	112.53	113.84	114.93	113.77	0.98
	2	0.2329	0.3001	0.3250	0.4234	0.5522	0.5936	81.79	83.15	82.65	82.53	0.56
	3	0.3020	0.3049	0.3251	0.5413	0.5416	0.5631	79.23	77.63	73.21	76.69	2.55
	4	0.2750	0.2483	0.2410	0.4737	0.4273	0.4256	72.25	72.09	76.60	73.65	2.09
6	0	0.3016	0.2471	0.3018	0.8249	0.6773	0.8133	173.51	174.10	169.48	172.36	2.05
	1	0.1561	0.1656	0.1221	0.3468	0.3716	0.2802	122.17	124.40	129.48	125.35	3.06
	2	0.2084	0.2182	0.2156	0.4250	0.4347	0.4278	103.93	99.22	98.44	100.53	2.43
	3	0.3102	0.2634	0.3250	0.5536	0.4668	0.5842	78.47	77.22	79.75	78.48	1.03
	4	0.2607	0.2451	0.2413	0.4531	0.4234	0.4278	73.80	72.75	77.97	74.84	2.25

Appendix B

Mechanical Properties Characterization

Table B1 Tensile stress at maximum load (MPa) of starch and chitosan free films and chitosan/starch laminated films in machine direction (MD)

Laminated Films	Trial No.					Mean	SD
GC - CS*	**1**	**2**	**3**	**4**	**5**		
2-0	4.19	4.07	4.70	4.81	3.49	4.25	0.53
2-1	6.34	7.46	8.61	9.76	9.18	8.27	1.38
2-2	6.41	6.83	6.90	7.20	7.91	7.05	0.56
2-3	8.00	8.67	7.85	8.71	7.49	8.02	0.63
2-4	15.46	16.09	13.27	14.13	13.41	14.47	1.25
3-0	2.11	2.51	2.22	2.07	2.14	2.21	0.17
3-1	2.66	3.10	2.75	2.28	2.86	2.73	0.30
3-2	3.14	3.10	3.51	3.07	3.31	3.23	0.19
3-3	3.11	3.50	3.55	4.07	2.98	3.44	0.43
3-4	4.39	4.49	4.11	4.75	4.17	4.38	0.26
4-0	0.92	0.94	0.99	0.99	1.05	0.98	0.05
4-1	1.17	1.25	1.18	1.30	1.16	1.21	0.06
4-2	1.57	1.53	1.56	1.80	1.67	1.62	0.11
4-3	2.35	2.12	2.81	2.14	2.50	2.38	0.28
4-4	2.78	2.76	2.82	2.86	2.53	2.75	0.13
5-0	0.51	0.55	0.58	0.51	0.55	0.54	0.03
5-1	1.01	1.26	1.30	1.11	1.22	1.18	0.12
5-2	1.34	1.35	1.32	1.34	1.33	1.34	0.01
5-3	1.47	1.46	1.56	1.34	1.59	1.48	0.10
5-4	1.66	1.71	1.68	1.61	1.65	1.66	0.04
6-0	0.38	0.36	0.30	0.36	0.40	0.36	0.04
6-1	0.43	0.48	0.52	0.58	0.54	0.51	0.06
6-2	0.78	0.76	0.74	0.81	0.78	0.77	0.03
6-3	0.84	0.82	0.78	0.78	0.81	0.81	0.03
6-4	1.33	1.27	1.22	1.38	1.00	1.24	0.15
1 wt% chitosan film	11.39	10.33	13.15	9.22	9.47	10.71	1.61
2 wt% chitosan film	14.66	14.69	14.45	14.78	13.78	14.47	0.41
3 wt% chitosan film	16.14	17.60	17.35	16.77	17.99	17.17	0.73
4 wt% chitosan film	20.24	19.36	23.53	22.77	19.17	21.02	2.01

* **Glycerol - Chitosan**

Glycerol Content in Starch Film (2, 3, 4, 5, and 6 wt%) and
Chitosan Coating Content (1, 2, 3, and 4 wt%)

Table B2 Tensile stress at maximum load (MPa) of starch and chitosan free films and chitosan/starch laminated films in transverse direction (TD)

Laminated Films	Trial No.					Mean	SD
GC - CS*	1	2	3	4	5		
2-0	4.19	4.07	4.70	4.81	3.49	4.25	0.53
2-1	5.25	5.33	4.58	4.73	5.49	5.08	0.40
2-2	5.25	5.44	5.14	5.49	6.02	5.47	0.34
2-3	6.28	6.01	6.68	7.21	7.74	6.78	0.70
2-4	9.09	9.30	10.45	10.81	8.36	9.60	1.01
3-0	2.11	2.51	2.22	2.07	2.14	2.21	0.17
3-1	1.56	1.64	1.69	1.68	1.76	1.67	0.07
3-2	2.47	2.54	2.68	2.38	2.26	2.47	0.16
3-3	2.87	2.71	2.88	2.78	2.84	2.82	0.07
3-4	4.00	4.33	4.28	4.30	3.93	4.17	0.19
4-0	0.92	0.94	0.99	0.99	1.05	0.98	0.05
4-1	1.23	1.12	1.18	1.16	1.16	1.17	0.04
4-2	1.23	1.46	1.43	1.38	1.25	1.35	0.01
4-3	1.82	1.90	1.96	1.76	1.92	1.87	0.08
4-4	2.74	2.09	2.37	2.04	2.76	2.40	0.34
5-0	0.51	0.55	0.58	0.51	0.55	0.54	0.03
5-1	0.90	0.80	0.79	0.76	0.70	0.79	0.07
5-2	0.86	0.96	0.84	0.92	0.93	0.90	0.05
5-3	1.04	1.06	1.08	1.05	1.04	1.06	0.02
5-4	1.22	1.31	1.34	1.22	1.22	1.26	0.06
6-0	0.38	0.36	0.30	0.36	0.40	0.36	0.04
6-1	0.36	0.37	0.42	0.40	0.36	0.38	0.03
6-2	0.45	0.51	0.54	0.52	0.47	0.50	0.04
6-3	0.61	0.68	0.56	0.60	0.65	0.62	0.05
6-4	0.76	0.67	0.78	0.97	1.21	0.88	0.21
1 wt% chitosan film	11.39	10.33	13.15	9.22	9.47	10.71	1.61
2 wt% chitosan film	14.66	14.69	14.45	14.78	13.78	14.47	0.41
3 wt% chitosan film	16.14	17.60	17.35	16.77	17.99	17.17	0.73
4 wt% chitosan film	20.24	19.36	23.53	22.77	19.17	21.02	2.01

Table B3 Tensile modulus (MPa) of starch and chitosan free films and chitosan/starch laminated films in machine direction (MD)

Laminated Films	Trial No.					Mean	SD
GC - CS*	1	2	3	4	5		
2-0	106.11	156.94	135.45	187.67	144.98	146.23	29.83
2-1	435.19	438.80	532.81	563.86	560.81	506.29	64.42
2-2	273.85	246.98	286.82	386.05	527.09	336.96	121.44
2-3	542.48	465.87	411.92	480.10	467.87	453.30	36.35
2-4	765.93	796.87	830.93	687.77	675.20	751.34	67.93
3-0	18.94	24.85	29.69	26.43	25.48	25.08	3.90
3-1	33.14	38.71	35.97	44.76	24.22	35.36	7.57
3-2	50.74	46.50	60.46	57.72	47.05	52.50	6.31
3-3	75.16	78.14	72.26	78.42	98.25	80.44	10.27
3-4	102.45	116.50	119.79	155.28	147.57	128.32	19.52
4-0	5.02	4.22	6.20	5.04	5.45	5.19	0.72
4-1	8.06	8.69	8.05	9.16	7.50	8.29	0.64
4-2	12.74	12.54	12.47	13.76	11.98	12.69	0.66
4-3	23.75	22.86	23.20	23.25	20.89	22.79	1.11
4-4	38.88	36.05	37.00	39.46	37.09	37.70	1.42
5-0	2.93	3.09	3.35	3.75	4.11	3.44	0.49
5-1	8.91	9.11	9.88	8.01	8.21	8.82	0.75
5-2	11.22	11.75	10.40	10.15	10.74	10.85	0.64
5-3	12.51	14.33	13.23	10.16	12.52	12.54	1.52
5-4	10.35	11.76	13.84	13.56	15.17	12.94	1.89
6-0	2.96	2.56	2.61	2.71	3.12	2.79	0.24
6-1	3.97	3.48	2.50	3.94	4.65	3.71	0.80
6-2	5.46	5.35	6.41	5.11	5.38	5.54	0.50
6-3	6.83	7.37	4.91	4.77	5.34	5.84	1.18
6-4	15.58	11.81	9.08	12.54	9.58	11.71	2.60
1 wt% chitosan film	913.73	911.39	896.18	747.87	829.70	859.77	71.29
2 wt% chitosan film	1087.80	1102.70	956.51	962.67	871.32	996.20	96.5
3 wt% chitosan film	1086.22	1125.56	1460.60	1018.52	974.41	1133.06	192.24
4 wt% chitosan film	1243.70	1339.13	1287.32	1177.72	1279.80	1265.53	59.75

Table B4 Tensile modulus (MPa) of starch and chitosan free films and chitosan/starch laminated films in machine direction (TD)

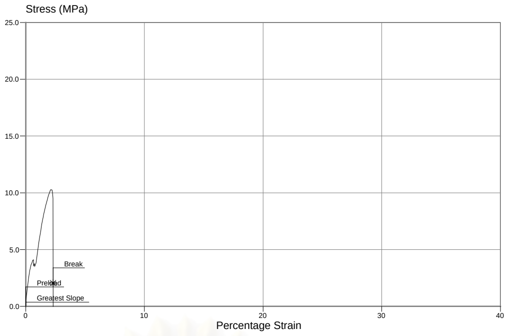
Laminated Films	Trial No.					Mean	SD
GC - CS*	1	2	3	4	5		
2-0	106.11	156.94	135.45	187.67	144.98	146.23	29.83
2-1	255.13	319.70	332.62	210.12	221.62	267.84	55.93
2-2	132.33	171.05	154.64	139.26	148.57	149.17	14.92
2-3	266.14	255.45	281.54	282.17	273.06	271.67	11.21
2-4	568.64	685.07	702.59	685.27	643.48	657.01	53.98
3-0	18.94	24.85	29.69	26.43	25.48	25.08	3.90
3-1	8.55	7.39	6.86	6.98	8.60	7.68	0.84
3-2	14.97	16.38	29.69	28.95	23.14	22.63	6.85
3-3	45.24	39.33	34.08	39.17	37.51	39.06	4.05
3-4	55.78	52.56	50.24	49.60	61.02	53.84	4.69
4-0	5.02	4.22	6.20	5.04	5.45	5.19	0.72
4-1	5.99	6.64	6.57	7.15	6.00	6.47	0.49
4-2	8.73	7.81	6.63	6.45	6.53	7.23	1.01
4-3	13.83	12.74	17.97	14.15	13.37	14.41	2.06
4-4	18.48	16.98	18.35	15.62	21.98	18.28	2.38
5-0	2.93	3.09	3.35	3.75	4.11	3.44	0.49
5-1	6.19	6.78	7.25	5.37	8.40	6.80	1.14
5-2	7.21	6.19	6.07	6.88	7.05	6.68	0.52
5-3	7.94	7.61	7.55	7.66	7.60	7.67	0.15
5-4	8.02	6.75	7.05	7.98	8.21	7.60	0.66
6-0	2.96	2.56	2.61	2.71	3.12	2.79	0.24
6-1	5.00	5.24	3.70	3.12	3.49	4.11	0.95
6-2	10.08	8.66	8.35	7.20	10.95	9.05	1.48
6-3	4.90	6.62	6.73	6.21	5.36	5.96	0.80
6-4	8.70	9.31	5.37	6.05	7.30	7.35	1.68
1 wt% chitosan film	913.73	911.39	896.18	747.87	829.70	859.77	71.29
2 wt% chitosan film	1087.80	1102.70	956.51	962.67	871.32	996.20	96.5
3 wt% chitosan film	1086.22	1125.56	1460.60	1018.52	974.41	1133.06	192.24
4 wt% chitosan film	1243.70	1339.13	1287.32	1177.72	1279.80	1265.53	59.75

Table B5 Percent elongation at break of starch and chitosan free films and chitosan/starch laminated films in machine direction (MD)

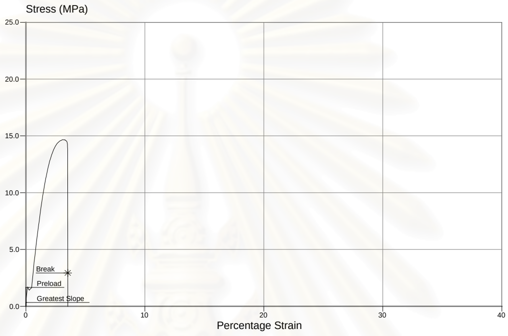
Laminated Films	Trial No.					Mean	SD
GC - CS*	1	2	3	4	5		
2-0	41.72	37.70	41.11	38.47	35.32	38.87	2.61
2-1	14.31	19.36	15.75	13.44	9.53	14.48	3.57
2-2	23.44	19.23	23.71	23.57	16.64	21.32	3.22
2-3	16.17	21.88	15.88	21.61	13.52	17.00	4.16
2-4	11.41	10.19	8.63	9.46	9.32	9.80	1.06
3-0	34.78	38.34	31.90	34.94	29.69	33.93	3.29
3-1	26.22	31.12	25.61	31.73	23.08	27.55	3.73
3-2	22.68	23.15	25.54	22.01	24.51	23.58	1.43
3-3	23.47	29.04	29.96	21.31	18.95	24.55	4.81
3-4	20.92	21.62	18.95	23.98	15.40	20.17	3.22
4-0	27.15	27.97	30.77	29.42	31.34	29.33	1.78
4-1	16.09	18.88	17.53	17.01	19.05	17.71	1.26
4-2	15.82	14.68	15.98	19.04	17.49	16.60	1.69
4-3	16.75	15.86	21.23	15.14	18.85	17.56	2.47
4-4	17.83	17.83	18.18	18.38	16.52	17.75	0.73
5-0	14.41	15.84	23.91	17.20	20.03	18.28	3.77
5-1	14.09	15.95	15.75	15.19	14.19	15.03	0.86
5-2	12.66	13.47	16.09	17.20	13.34	14.55	1.98
5-3	14.08	12.37	16.99	16.36	16.37	15.24	1.95
5-4	18.71	19.29	17.29	16.84	17.43	17.91	1.04
6-0	8.40	8.50	7.78	8.29	8.37	8.27	0.28
6-1	15.70	14.09	15.94	17.83	15.89	15.89	1.33
6-2	12.03	12.82	12.03	14.24	11.45	12.51	1.08
6-3	10.74	11.53	13.90	14.06	14.43	12.93	1.67
6-4	13.43	13.06	12.87	14.04	15.37	13.76	1.01
1 wt% chitosan film	3.93	2.30	3.27	3.93	2.36	3.16	0.80
2 wt% chitosan film	3.52	2.82	2.09	4.34	3.68	3.45	0.62
3 wt% chitosan film	3.5	4.44	2.66	4.21	5.62	4.08	1.11
4 wt% chitosan film	4.18	4.25	4.29	4.85	4.52	4.42	0.27

Table B6 Percent elongation at break of starch and chitosan free films and chitosan/starch laminated films in transverse direction (TD)

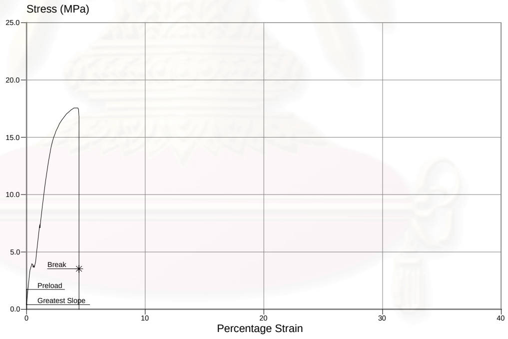
Laminated Films	Trial No.					Mean	SD
GC - CS*	1	2	3	4	5		
2-0	41.72	37.70	41.11	38.47	35.32	38.87	2.61
2-1	26.38	37.22	31.67	31.76	38.50	33.10	4.88
2-2	31.38	30.54	28.55	33.92	33.23	31.52	2.15
2-3	21.72	21.72	21.33	22.08	25.85	22.54	1.87
2-4	22.88	22.75	19.73	18.31	17.63	20.26	2.45
3-0	34.78	38.34	31.90	34.94	29.69	33.93	3.29
3-1	33.53	34.25	37.66	30.37	30.94	33.28	3.00
3-2	37.47	38.96	35.67	36.20	31.47	35.95	2.81
3-3	39.87	40.98	35.72	39.60	42.35	39.70	2.48
3-4	39.94	42.93	39.77	39.31	38.36	39.86	1.87
4-0	27.15	27.97	30.77	29.42	31.34	29.33	1.78
4-1	23.15	20.20	22.96	21.66	22.35	22.06	1.19
4-2	21.50	23.89	27.52	24.97	18.70	23.32	3.37
4-3	18.95	21.09	18.29	19.94	20.32	19.72	1.11
4-4	25.07	20.74	24.14	22.78	26.25	23.80	2.13
5-0	14.41	15.84	23.91	17.20	20.03	18.28	3.77
5-1	14.28	12.89	15.00	13.21	12.64	13.60	1.00
5-2	12.99	15.73	12.33	13.24	14.42	13.74	1.34
5-3	17.24	14.36	15.88	16.23	15.23	15.79	1.08
5-4	17.13	20.85	20.94	18.01	14.19	18.23	2.82
6-0	8.40	8.50	7.78	8.29	8.37	8.27	0.28
6-1	5.46	5.74	7.17	8.37	8.94	7.14	1.54
6-2	6.06	7.27	8.79	8.17	6.81	7.42	1.08
6-3	9.04	9.96	10.50	10.09	10.00	9.92	0.53
6-4	10.72	10.14	11.45	14.35	14.20	12.17	1.97
1 wt% chitosan film	3.93	2.30	3.27	3.93	2.36	3.16	0.80
2 wt% chitosan film	3.52	2.82	2.09	4.34	3.68	3.45	0.62
3 wt% chitosan film	3.5	4.44	2.66	4.21	5.62	4.08	1.11
4 wt% chitosan film	4.18	4.25	4.29	4.85	4.52	4.42	0.27



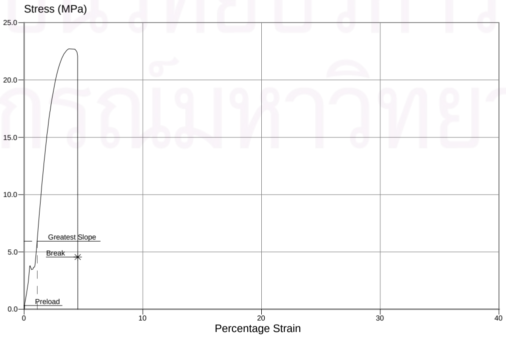
(a) 1 wt% chitosan film



(b) 2 wt% chitosan film

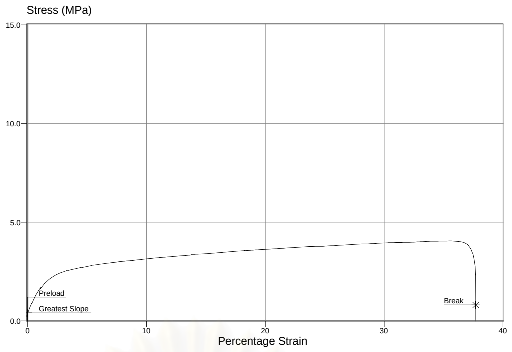


(c) 3 wt% chitosan film

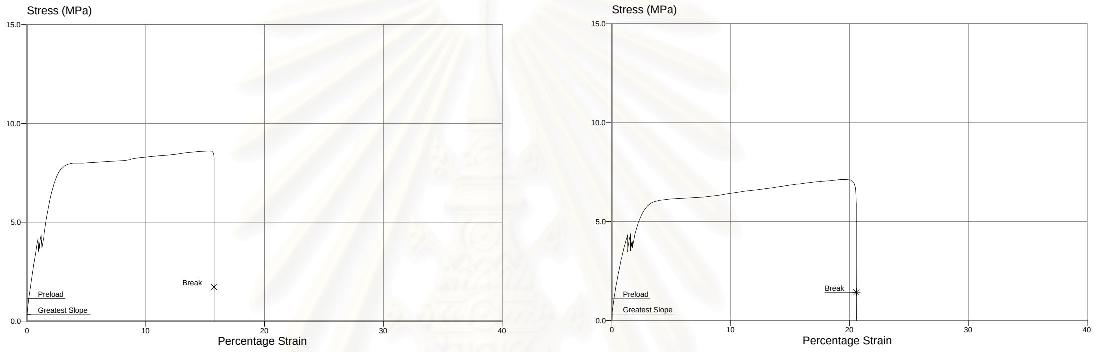


(d) 4 wt% chitosan film

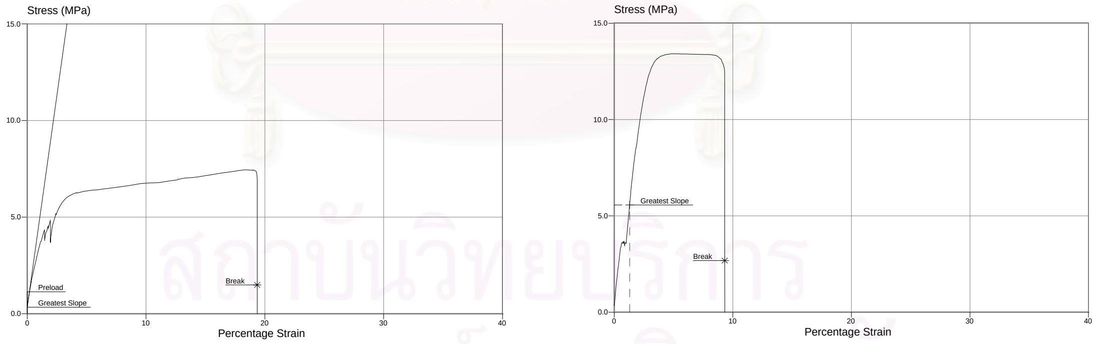
Figure B1 Stress-strain curves of free chitosan films



(a) 2 wt% glycerol-plasticized starch film

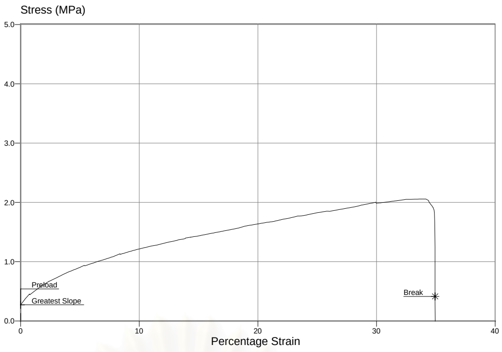


(b) 1 wt% chitosan coating (c) 2 wt% chitosan coating

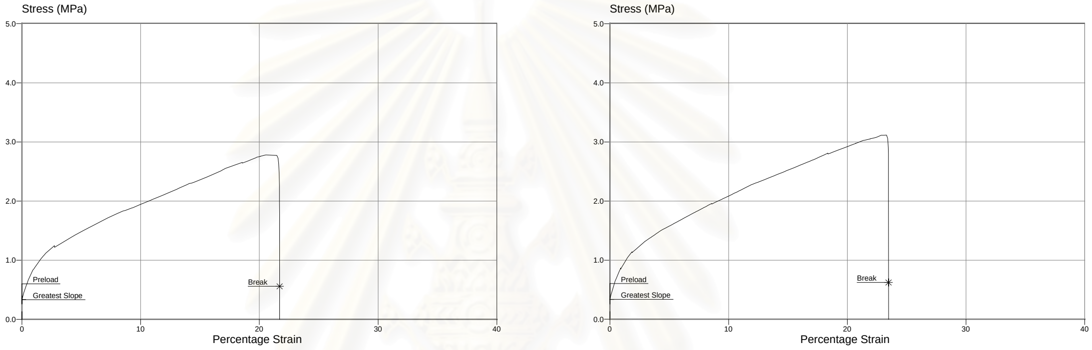


(d) 3 wt% chitosan coating (e) 4 wt% chitosan coating

Figure B2 Effect of chitosan coating contents on tensile properties of laminated films containing 2 wt% glycerol in MD

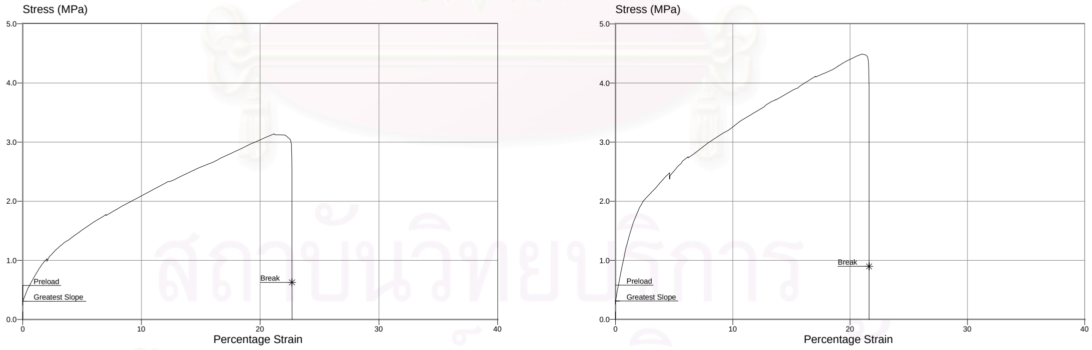


(a) 3 wt% glycerol-plasticized starch film



(b) 1 wt% chitosan coating

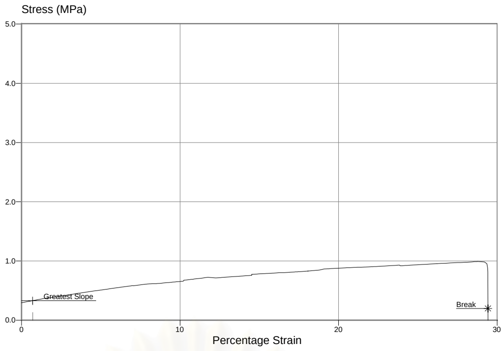
(c) 2 wt% chitosan coating



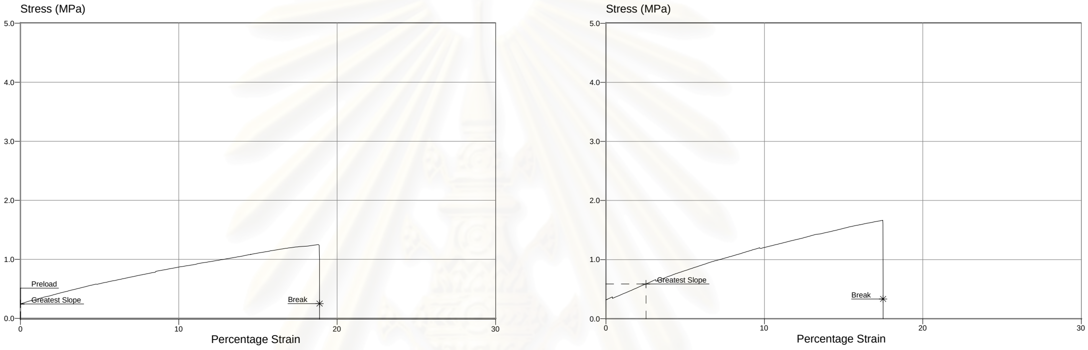
(d) 3 wt% chitosan coating

(e) 4 wt% chitosan coating

Figure B3 **Effect of chitosan coating contents on tensile properties of laminated films containing 3 wt% glycerol in MD**

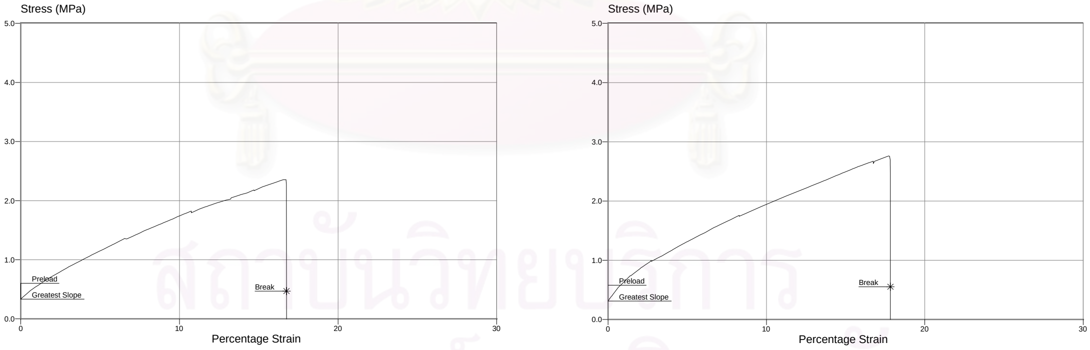


(a) 4 wt% glycerol-plasticized starch film



(b) 1 wt% chitosan coating

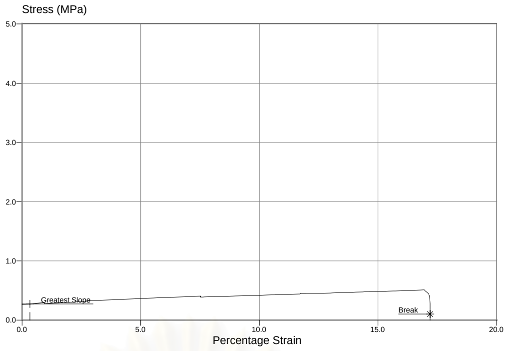
(c) 2 wt% chitosan coating



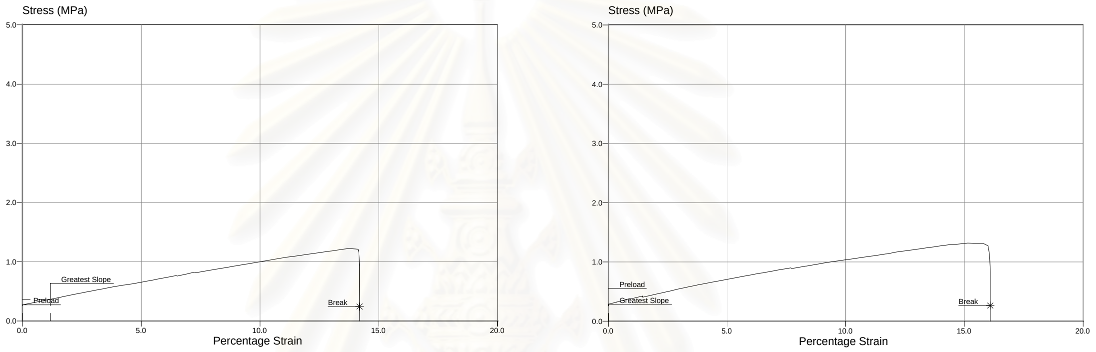
(d) 3 wt% chitosan coating

(e) 4 wt% chitosan coating

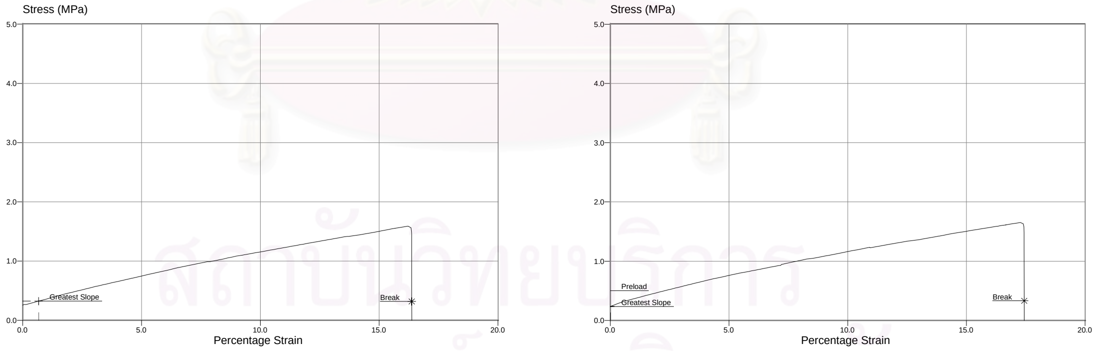
Figure B4 Effect of chitosan coating contents on tensile properties of laminated films containing 4 wt% glycerol in MD



(a) 5 wt% glycerol-plasticized starch film

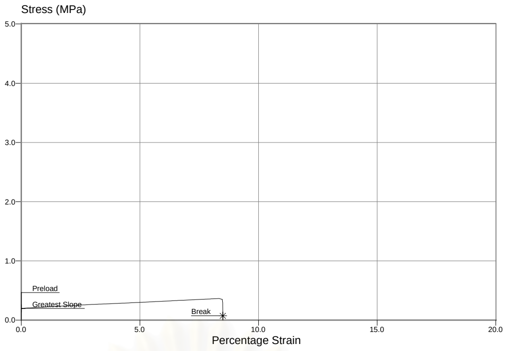


(b) 1 wt% chitosan coating (c) 2 wt% chitosan coating

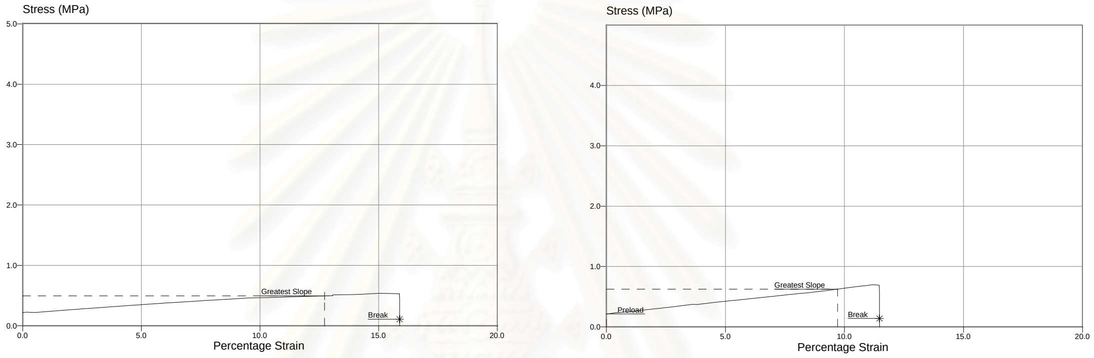


(d) 3 wt% chitosan coating (e) 4 wt% chitosan coating

Figure B5 **Effect of chitosan coating contents on tensile properties of laminated films containing 5 wt% glycerol in MD**

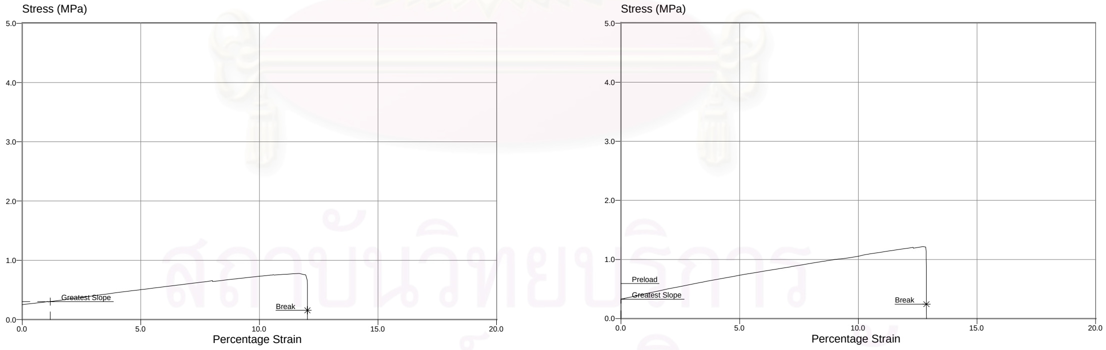


(a) 6 wt% glycerol-plasticized starch film



(b) 1 wt% chitosan coating

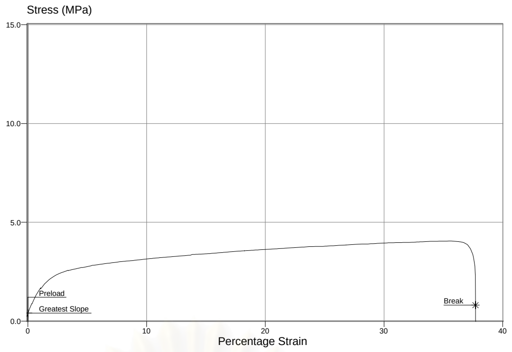
(c) 2 wt% chitosan coating



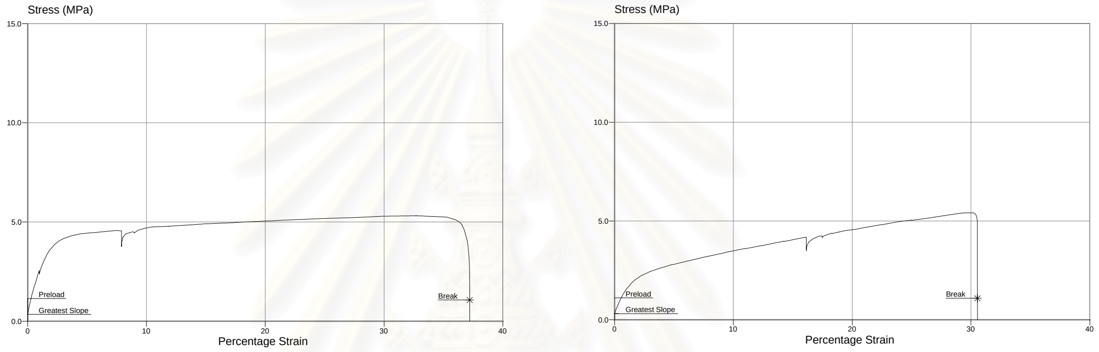
(d) 3 wt% chitosan coating

(e) 4 wt% chitosan coating

Figure B6 Effect of chitosan coating contents on tensile properties of laminated films containing 6 wt% glycerol in MD

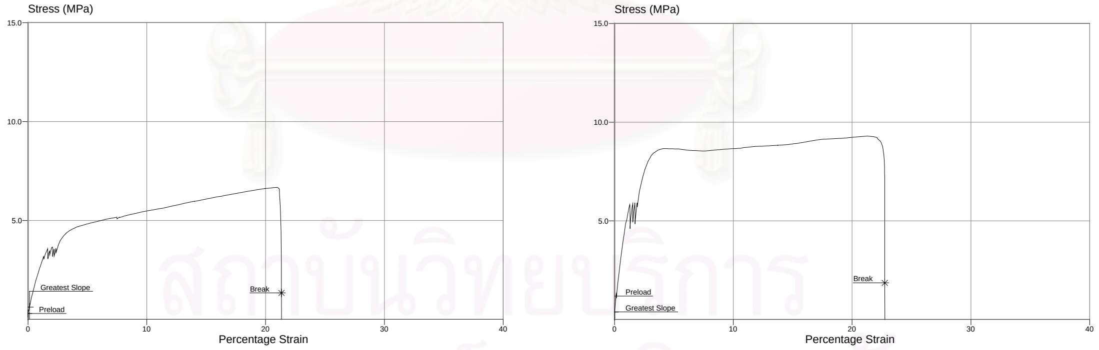


(a) 2 wt% glycerol-plasticized starch film



(b) 1 wt% chitosan coating

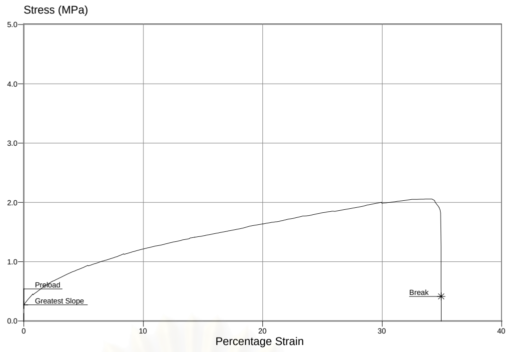
(c) 2 wt% chitosan coating



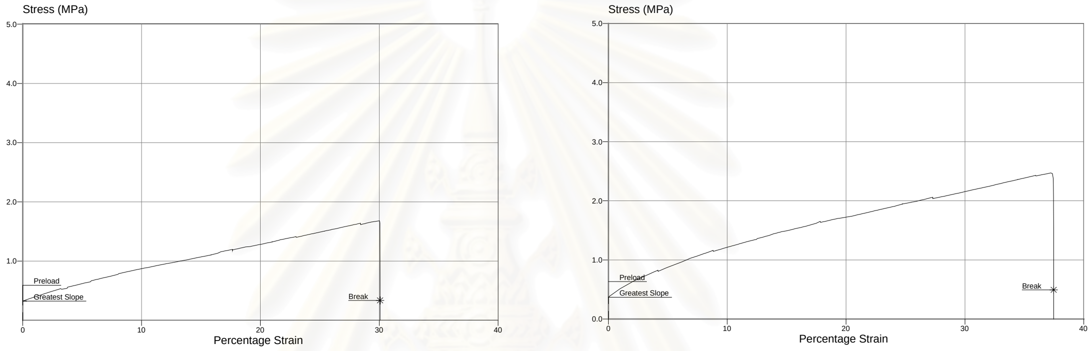
(d) 3 wt% chitosan coating

(e) 4 wt% chitosan coating

Figure B7 Effect of chitosan coating contents on tensile properties of laminated films containing 2 wt% glycerol in TD

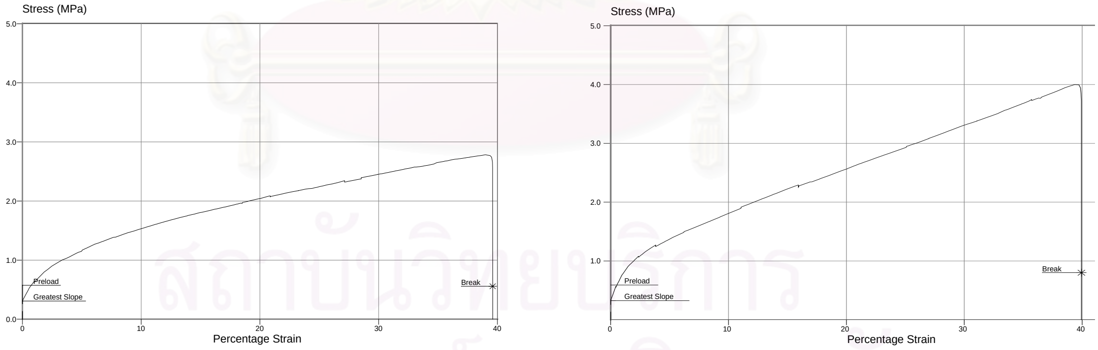


(a) 3 wt% glycerol-plasticized starch film



(b) 1 wt% chitosan coating

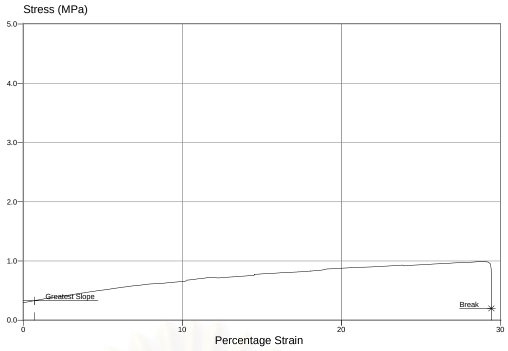
(c) 2 wt% chitosan coating



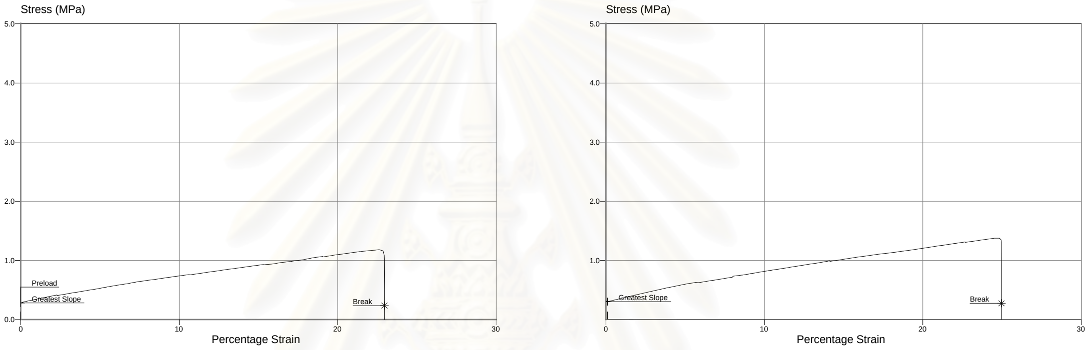
(d) 3 wt% chitosan coating

(e) 4 wt% chitosan coating

Figure B8 Effect of chitosan coating contents on tensile properties of laminated films containing 3 wt% glycerol in TD

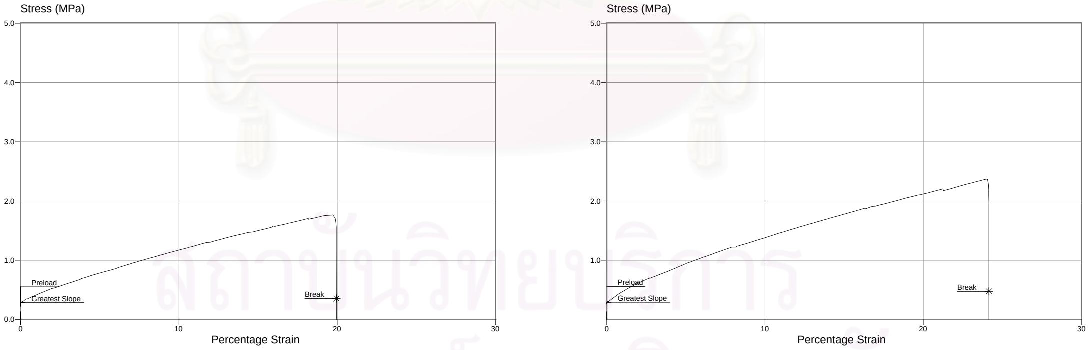


(a) 4 wt% glycerol-plasticized starch film



(b) 1 wt% chitosan coating

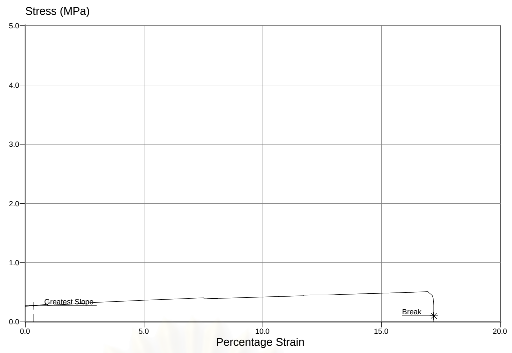
(c) 2 wt% chitosan coating



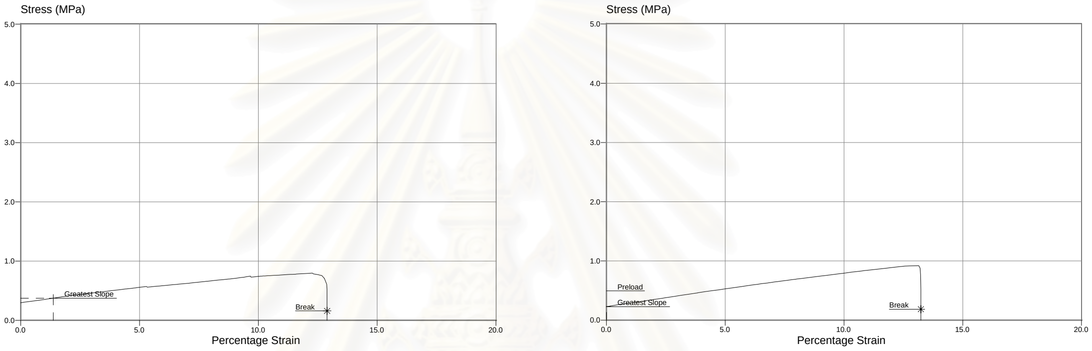
(d) 3 wt% chitosan coating

(e) 4 wt% chitosan coating

Figure B9 **Effect of chitosan coating contents on tensile properties of laminated films containing 4 wt% glycerol in TD**

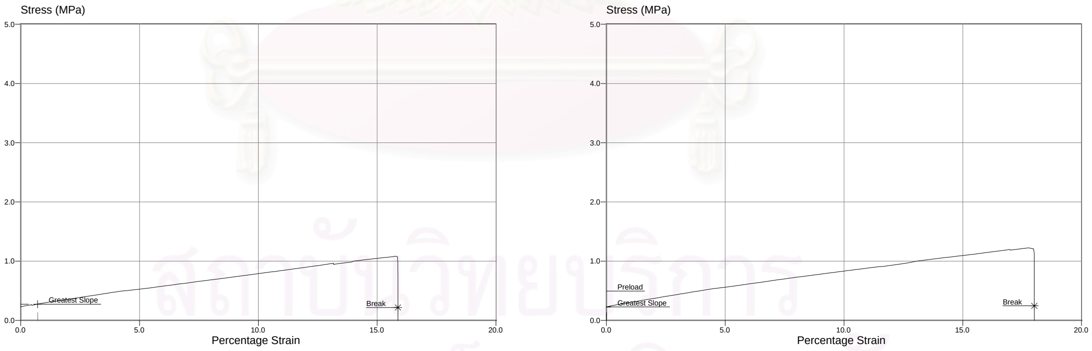


(a) 5 wt% glycerol-plasticized starch film



(b) 1 wt% chitosan coating

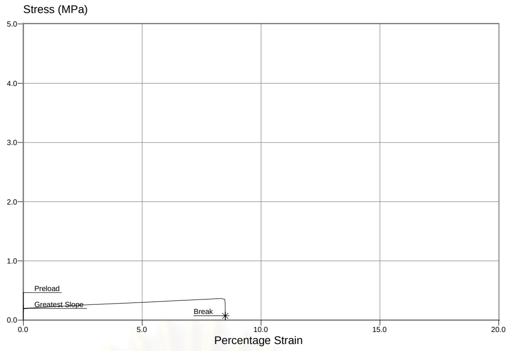
(c) 2 wt% chitosan coating



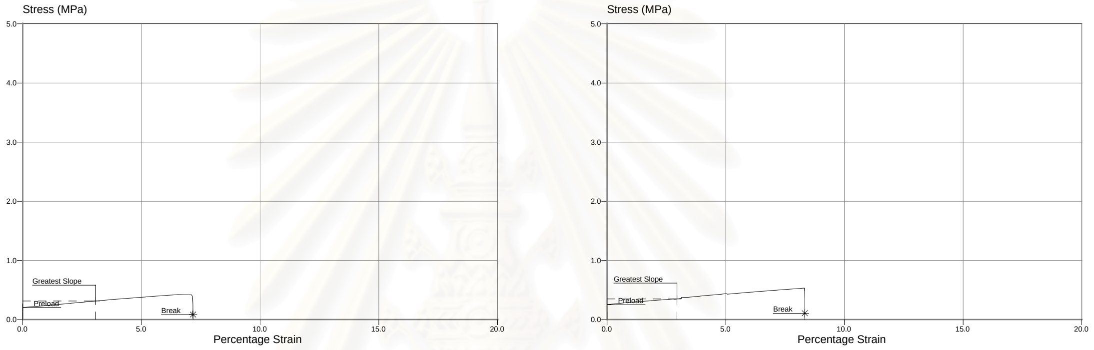
(d) 3 wt% chitosan coating

(e) 4 wt% chitosan coating

Figure B10 Effect of chitosan coating contents on tensile properties of laminated films containing 5 wt% glycerol in TD

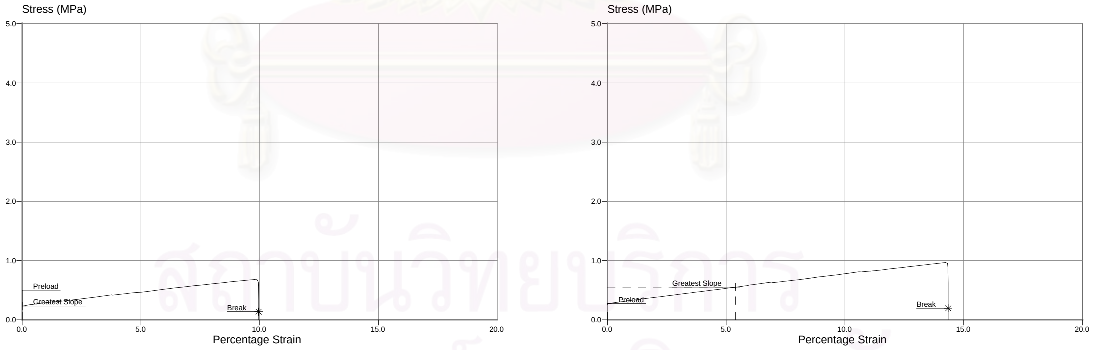


(a) 6 wt% glycerol-plasticized starch film



(b) 1 wt% chitosan coating

(c) 2 wt% chitosan coating



(d) 3 wt% chitosan coating

(e) 4 wt% chitosan coating

Figure B11 Effect of chitosan coating contents on tensile properties of laminated films containing 6 wt% glycerol in TD

Biography

Miss Cholwasa Bangyekan was born in Bangkok, Thailand, on 8th November, 1980. She received the Bachelor of Science (Second Class Honors) degree majoring in Materials Science from the Department of Materials Science, Faculty of Science, Chulalongkorn University in March 2001. Afterward, She persuaded her post graduate degree at the Department of Materials Science, Faculty of Science, Chulalongkorn University in November 2001. She completed the program and obtained her Master degree in Applied Polymer Science and Textile Technology in April 2004.