PHYSICAL AND MECHANICAL PROPERTIES OF CHITOSAN-GELATIN BASED FILM

SUPPAJIT SUKKUNTA

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ABSTRACT

The present study was aimed to utilize waste from the frozen black tiger shrimp industry as raw material for biodegradable cling film production. The physical and chemical properties of the produced chitosan were determined. In addition, the solubility of chitosan was tested in lactic acid, propionic acid and citric acid. Chitosan film from different acids and varying concentrations was prepared. The physical and mechanical properties of the film were measured and improved to the market standard using 2 different plasticizers: glycerol and sorbitol.

The results showed that the produced chitosan has 6.85 % moisture content, 6.34 % nitrogen content 0.1 % fat, 0.25 % ash, 86.7 % deacetylation and 230 cps viscosity. These properties are similar to the properties of the commercial chitosan.

For production of biodegradable film, several parameters that could influence properties of the film were optimized, including chitosan content, types and concentrations of organic acids, volume of chitosan solution, chitosan: gelatin ratio, as well as types and required amount of plasticizer. The optimum conditions for preparing 20×25 cm^2 biodegradable chitosan film were found to be 150 ml. of 1% chitosan-gelatin solution (1:1 chitosan: gelatin ratio) in 5% propionic acid and 0.5% glycerol. The properties of the film were: tensile strength 70.385 N/ mm^2; elongation at break 13.463 mm/ mm; tearing resistance 616.7 mN; weight 2.243 mg/ piece; film thickness 0.032 mm; and water vapor permeability 0.1564 x 10^{-3} g.mm/ mm^2 hr mm Hg.

Comparable to mechanical properties of commercially available polyethylene film, the produced chitosan-gelatin film was superior in tensile strength and tearing resistance, while elongation at break and water vapor permeability were inferior.

KEY WORDS: CHITOSAN/ FILM/ CHITOSAN-GELATIN FILM PROPERTIES.
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คุณสมบัติทางกายภาพและทางกลของแผ่นฟิล์มไคโตซาน-เจลาติน (PHYSICAL AND MECHANICAL PROPERTIES OF CHITOSAN-GELATIN BASED FILM)

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บทคัดย่อ

งานวิจัยนี้ เป็นการศึกษาการใช้เศษเหลือจากอุตสาหกรรมกุ้งกุลาดำแช่แข็ง ไปเป็นวัตถุดิบในการผลิตแผ่นฟิล์มยืดชีวภาพที่สามารถย่อยสลายได้ โดยทำการศึกษาคุณสมบัติทางเคมีและกายภาพของไคโตซานที่ผลิตได้ ศึกษาการละลายของไคโตซานในกรดอินทรีย์ 3 ชนิด คือ กรดแลคติก โพรพิโอนิค และซิตริก รวมถึงการนำสารละลายไคโตซานที่ได้ไปผลิตเป็นแผ่นฟิล์มไคโตซาน-เจลาติน ทำการปรับปรุงคุณสมบัติทางกายภาพ และทางกลของแผ่นฟิล์มให้ได้มาตรฐานโดยการเติมพลาสติกไซเซอร์ 2 ชนิด คือ กลีเซอรอล และซอร์บิทอล เพื่อให้ได้แผ่นฟิล์มไคโตซานที่มีคุณสมบัติดี

ผลจากการทดลองพบว่า ไคโตซานที่ผลิตได้ในห้องปฏิบัติ มีค่าความชื้นร้อยละ 6.85 ปริมาณโปรตีนร้อยละ 6.34 ไขมันร้อยละ 0.1 เถ้าร้อยละ 0.25 ระดับการกำจัดหมู่อะซิติลร้อยละ 86.7 และค่าความหนืด 230 เซนติพอยส์ ซึ่งมีคุณภาพใกล้เคียงกับไคโตซานที่มีจำหน่ายในท้องตลาด

ในการนำไคโตซานที่ผลิตได้นี้ไปทดลองผลิตเป็นแผ่นฟิล์มชีวภาพ ได้ทำการคัดเลือกปัจจัยต่าง ๆ ที่มีผลต่อคุณสมบัติของฟิล์ม เช่น ปริมาณไคโตซาน ชนิดและความเข้มข้นของกรดอินทรีย์ ปริมาตรของสารละลาย ไคโตซาน อัตราส่วนของเจลาติน รวมถึงชนิดและปริมาณของพลาสติกไซเซอร์ที่เหมาะสม พบว่าฟิล์มขนาด 20 x 25 ซม^2 ที่เตรียมจากสารละลายไคโตซาน-เจลาติน ร้อยละ 1 (อัตราส่วนหนึ่งต่อหนึ่ง) ในกรดโพรพิโอนิกเข้มข้นร้อยละ 5 ปริมาตร 150 มล และมีส่วนผสมของกลีเซอรอล ร้อยละ 0.5 ให้คุณสมบัติของแผ่นฟิล์มดีที่สุด โดยมีคุณสมบัติ ดังนี้ ค่าการต้านแรงดึง 70.385 นิวตัน/ มม^2 ค่าการยืดตัวเมื่อขาด 13.463 มม/ มม การต้านแรงฉีกขาดเท่ากับ 616.7 มิลลินิวตัน น้ำหนัก 2.243 มก/ แผ่น ความหนา 0.032 มม และมีค่าการซึมผ่านไอน้ำ 0.1667 x 10^{-3} กรัม มม/ มม^2 ชั่วโมง มมปรอท

แผ่นฟิล์มชีวภาพจากไคโตซานที่ผลิตได้นี้ เมื่อนำมาเปรียบเทียบในแง่คุณสมบัติเชิงกลกับแผ่นฟิล์มที่ผลิตจากโพลีเอธิลีนที่ขายทั่วไปตามท้องตลาด พบว่าแผ่นฟิล์มไคโตซานที่ผลิตขึ้นได้ใหม่มีค่าการต้านแรงดึง และการต้านแรงฉีกขาดที่สูงกว่าแผ่นฟิล์มโพลีเอธิลีน แต่มีค่าการยืดตัวเมื่อขาดและค่าการซึมผ่านไอน้ำต่ำกว่าแผ่นฟิล์มโพลีเอธิลีน

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CHAPTER I

INTRODUCTION

1.1 State of problems

Plastic packaging has become a central focus of waste reduction efforts because they compose large portion, almost two-thirds of the volume or 30 wt%, of the total municipal solid waste (MSW) (Hunt et al., 1990). A record of as high as 13 wt% of MSW, was alone contributed to plastic food packaging (Rowatt, 1993).

These materials are routinely discarded into the MSW system resulting in serious environmental problems. These include insufficient landfill sites, as well as harmful smoke generated during incineration (Hunt et al., 1990). Although several efforts have been focused on recycling the discarded plastics, the process has been neither complete nor economical for certain end-use applications such as agricultural mulch films and planting pots (Srinivasa et al., 2002).

Edible and biodegradable polymer films offer an alternative practice for lessening pressure on the environment (Krochta and Johnston, 1997). The major components of edible and biodegradable polymers have been normally prepared from natural sources such as carbohydrate (Cuq et al., 1995 and Debeaufut et al., 1995) and protein (Fairley et al., 1996; McHugh et al., 1994; Narkraksa and Tunyaporn, 1998). Wax and some polymers have been added (John, 1994) to improve water impermeability properties of these films (Kester and Fennema, 1989). For enhancing the flexibility of the edible and biodegradable film, plasticizers have also been blended to the components of such films (Sothornvit and Krochta, 1984). Several studies have reported the possibility of using chitosan to produce chitosan edible and biodegradable films (Kitturt et al., 1998; Wiratthikowit, 2001; Srinivasa et al., 2002).

Chitin is the second most abundant biopolymer on the earth after cellulose (Muzzaralli, 1977). Structurally similar to cellulose, chitin is translucent and contains nitrogen in its molecule (Kungsuwan, 1998). The major source of chitin is found in the exoskeleton of invertebrates and the cell walls of fungi (Shahidi et al., 1999).

Chitosan, derived from chitin, is a copolymer of β-(1- 4)-acetamido-D-glucose andβ-(1-4)-2-amino-2-deoxy-D-glucose units in various proportions (Muzzaralli, 1977). There are three functional groups in chitosan, including an amino group, primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively (Furusaki et al., 1996). Chemical modifications of these groups provided numerous useful materials in different fields of applications (Kurita, 1996). As a biomaterial, chitosan can be degraded quite readily under natural conditions (Hirano, 1997). It is, however, not water soluble and can only dissolve in diluted aqueous organic acid, such as acetic acid. The dissolved chitosan become cationic polyelectrolyte with high viscosity. As a result, chitosan solution can be processed into many forms such as gel, powder, fiber, colloid and coating materials (Goosen, 1997). Polyethylene film, a more common food packaging material than that from biomaterial sources, has, however, many disadvantages. These include fermentation of products causing by oxygen depletion, as well as water condensation resulting from temperature fluctuation during storage that promotes fungal growth (Shahidi et al., 1999). In addition, polyethylene films are usually nonrenewable materials, which are not readily degradable (Peterson et. al. 1999). Thus, the present studies was concentrated on production and quality improvement of chitosan films using varying concentrations of 3 different organic acids, i.e. lactic, citric and propionic acids, as well as one additive, i.e. gelatin and 2 different plasticizers, i.e. glycerol and sorbitol.

1.2 Objectives

1. To extract chitosan from black tiger shrimp waste obtained from frozen shrimp industry

2. To observed solubility of chitosan in three organic acids, including lactic, propionic and citric acid.

3. To determine physical and mechanical properties of chitosan-gelatin based films with varying concentrations of 2 plasticizers, i.e. glycerol and sorbitol.

1.3 Hypothesis

Physical and mechanical properties of chitosan-gelatin based film were similar to or higher than that of polyethylene film.

1.4 Scopes of this study

For the present study, black tiger shrimp waste obtained from a frozen shrimp factory was used for chitosan film production. Three main parts of this study were conducted as follows:

1. Chitosan was produced from Black Tiger shrimp waste. Its protein, lipid, ash contents, as well as percentage of deacetylation of the produced chitosan were analyzed.

2. The effects of type and concentration of organic acids, i.e. lactic, propionic and citric acids used for producing chitosan film were observed. Physical and mechanical properties of chitosan film prepared from different acids were determined.

3. Gelatin was used as an additive to improve strength of the prepared film. For improve flexibility of the prepared films, two plasticizers, i.e. glycerol and sorbitol were tested.

1.5 Anticipated benefits

1. To produce value added products from waste resulting from frozen food industry
2. To produce readily degradable, non toxic and environmental friendly films
3. To lessen high demand for new land field area required for ever increasing amount of municipal solid wastes

1.6 Definitions

Edible film:	Edible film is defined as a thin layer of edible material applied on food products as coating or placing on or laminating between food components.
Plasticizer:	Plasticizer is a substance, which added to a plastic film or biodegradable film to make them more flexible, resilient and easy to

handle. It should not volatile (or only very slightly volatile) and its degree of retention should be high.

Biodegradable polymer: Biodegradable polymer is a polymer that is degraded by microbes, macroorganisms, photodegradation and chemicals. It is particularly attractive because their mechanical and physical properties can readily be adjusted by varying the preparation techniques and molecular structure.

1.7 Conceptual Framework

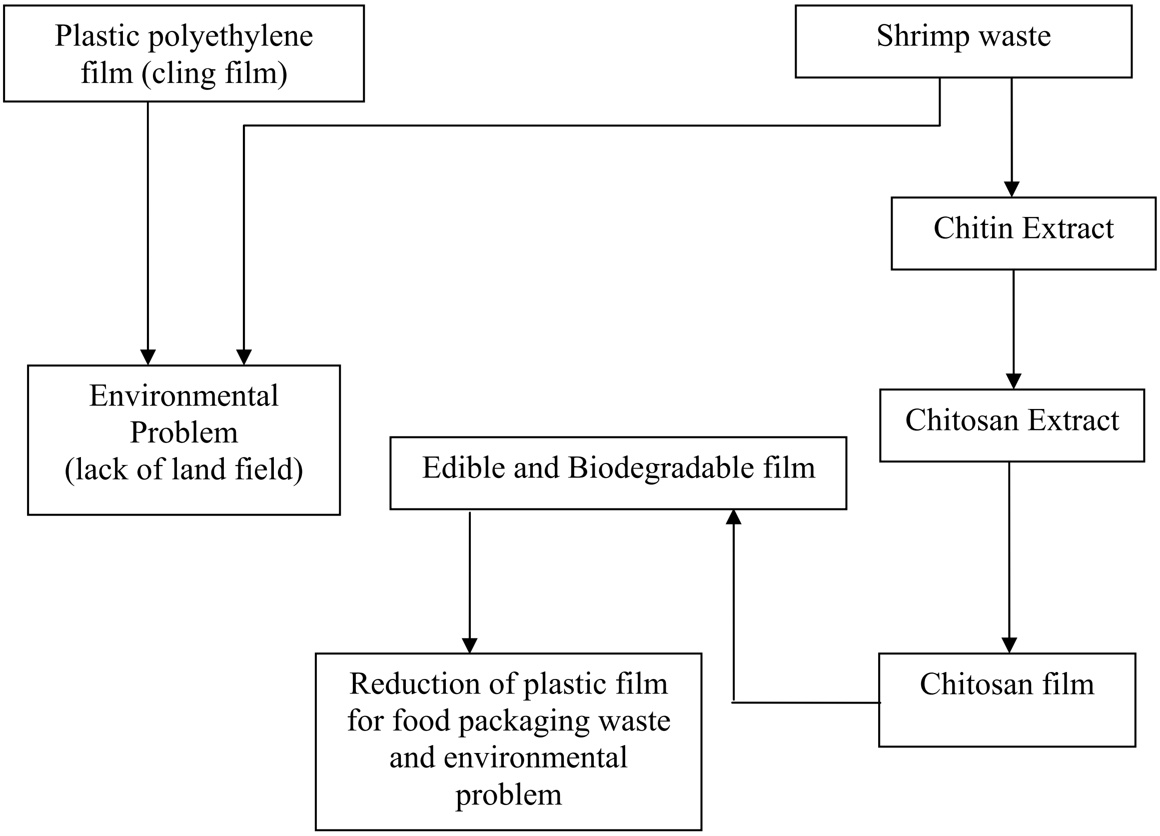

Figure 1.1 Conceptual framework

CHAPTER II

LITERATURE REVIEW

2.1 Plastic film for food packaging

Food packaging produced from plastic film has been applied in several aspects, for example in-store bags (such as apples and potatoes); all non-frozen baked goods (such as rolls and breads); bakery bread and bun bags; tray covers for delivering of bakery products; bags-in-a-box (film used to contain fluid in a supportive box, such as boxed wine); boil-in-bags (film used to contain food prepared by keeping it in the package and placing it in boiling water); candy and confection bags and wrappers; carton liners (for such products as cake mixes); and meat, poultry and seafood wraps (such as hot dog and bacon film) (American Plastic Council, 1996). Plastic packaging is safe, strong, light-weight, and economical. Presently, the majority of plastic films are made from polyethylene (PE) (Figure 2.1).

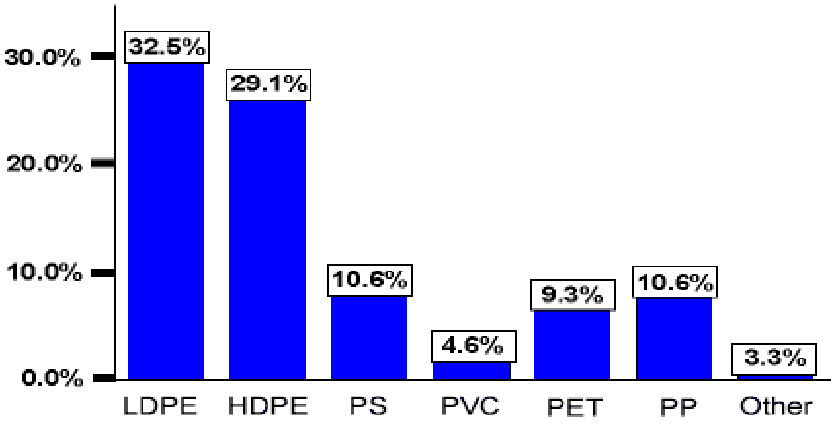

Figure 2.1 Plastic packaging classified by type of resin (LDPE = Low density polyethylene ; HDPE = High density polyethylene; PS = Polystyrene ; PVC = Poly vinyl chloride ; PET = Polyethylene teraphthalate ; PP = Polypropylene ; Other = Crosslinked polyethylene, Ultra low density polyethylene.

2.1.1 Polyethylene

PE packaging materials have revolutionized the food industries, which are, increasing food shelf life, reducing spoilage and enhancing the availability of different food products to public. Since they are lightweight, delivery costs are decreased. Krohn et al. (1999) reported that 3.5 billion pounds per year of PE was consumed in the food packaging industry and 45 percent were used specifically in single and multi-layer film structures.

PE is a polymer produced from ethylene that is the product of petroleum gas or dehydration of alcohol (Winter, 1994). A molecule of PE is a long chain of carbon atoms with two hydrogen atoms attached to each carbon atom. Figure 2.2 shows a chain of PE.

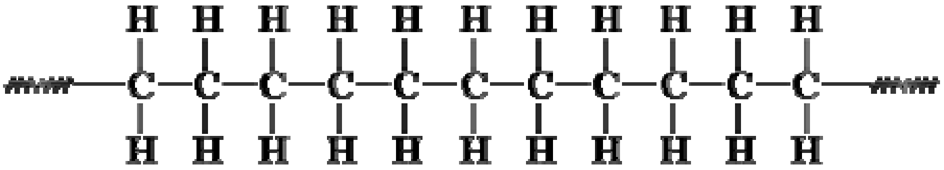

Figure 2.2 PE structure

Polyethylene can be categorized in a group of lightweight thermoplastic that have a good resistance to chemicals, low moisture absorption, and good insulating properties (Winter, 1994). It also has very low glass transition temperature, good mechanical properties, flexibility and impact resistance at low temperature. Therefore, it is suitable to use as chewing-gum based ingredient, ingredients in hand lotions, as well as to produce films and sheets for packaging and roughage replacement in feedlot rations for cattle. No known skin toxicity was reported but implanting of large amounts in rats caused cancer (Kuntz, 1992). Ingestion of large oral doses had produced kidney and liver damage. Billmeyer (1966) and Wangsoub (1998) classified Polyethylene into six types as follows:

2.1.1.1 Low density polyethylene (LDPE)

The production of LDPE involves very high pressure and leads to highly branched polymer with corresponding to low crystallinity and low density. Its density is approximately 0.91-0.925 g/cm^3. Its applications include films, coating on foil or paper to impart flexibility and heat sealability. LDPE provided water vapor and liquid resistance. However, it is poor oxygen barrier.

2.1.1.2 High density polyethylene (HDPE)

The polymerization process requires much lower pressure with special catalysts and leads to much less branch, higher crystallinity and density. The terms low pressure, linear and high density polyethylene are associated with the more rigid PE. It is characterized by density above 0.94 - 0.965 g/ cm^3 and produces a rigid plastic used for preparing containers such as milk bottles. HDPE exhibits lower water vapor permeability, greater inertness, high gas transmission, low odor barrier and a narrower heat-sealing range than the other polyethylene.

2.1.1.3 Linear low density polyethylene (LLDPE)

A new line of PE is obtained by copolymerizing ethylene at low pressures and in the presence of catalysts with small amounts of α-olefin comonomers (butene, hexene, octene), which play a role of uniform short branches along a nearly linear backbone. LLDPE, when compared to conventional LDPE of the same density and melt flow index in applications such as films or flexible molded products, was claimed to have better impact, tear, or puncture properties and improved environmental stress-cracking resistance, allowing in particular a downgrading of films.

2.1.1.4 Ultra low density polyethylene (ULDPE)

It is a relatively new class of linear PE, with density between 0.89 and 0.915 g/ cm^3. It provides flexibility, toughness and a broad operating temperature range film and sheet applications.

2.1.1.5 Ultra high molecular weight polyethylene (UHMPE)

It is possible to produce extremely high molecular weight PE at least ten times higher than that of regular polyethylene ($3x10^6$ to $6x10^6$). The polymerization process leads to linear molecular associated with HDPE.

2.1.1.6 Crosslinked polyethylene (XLPE)

An introduction of chemical crosslink into PE has some desirable effects on its chemical and temperature resistance. This could be achieved by the addition of small amounts of organic peroxides, which obviously must not cause significantly crosslinking before the polymer had acquired its final shape in processing.

The PE has good toughness and pliability over a wide temperature range. Since its density falls off rapidly above room temperature, some fabrication methods are difficult to achieve owing to the large dimensional changes. The relative low T_m (about 115°C) limits the temperature range of good mechanical properties. PE is very chemically inert. It does not dissolve in any solvent at room temperature but at high temperatures it can be slightly swelled by solvents such as benzene and carbon tetrachloride. It is very resistant to acids and alkalis. At 100°C, it could withstand 24 hours in concentrate sulfuric or hydrochloric acid but become charred in concentrated nitric acid. It can be used as container for acids, including hydrofluoric acid (Sarakarnkosol, 2000).

PE-based films have established a major rank in the food packaging (Table 2.1). HDPE-based multilayer films were the material of choice for many of these packaging applications (Krohn et. al., 1999) Fresh products and some meat packaging applications required a to extend their shelf life. In order to extend shelf life of fresh products, as well as meat packaging by controlling oxygen and carbon dioxide exposure of the products, packaging made from LDPE and ULDPE–based films could be applied (Ross and MacAdams,1996).

Because polyethylene is a product of petrochemical processes, it was not readily biodegradable (Peterson et. al., 1999). Environmental problems, thus, could result from using non-renewable raw materials and accumulation of such non-biodegradable packaging. As a solution to this dilemma, biopolymers could be used to formulate edible and biodegradable packaging to reduce environmental problems

Table 2.1 Consumption of plastic films packaging in the USA

Film application	Consumption (million pounds)		
	HDPE	LDPE	LLDPE
Food	144	1132	197
Non-Food	70	989	618
Merchandise Bags	306	247	282
Trash and Can Liners	139	105	198
Other Packaging	107	372	894
Other Non-Packaging	64	598	213
Total	830	3443	2402

Source: Osborn and Jenkins (1992).

2.2 Edible and biodegradable films

Edible and biodegradable films are defined as a thin layer of edible and biodegradable materials formed on food as a coating or placed (pre-formed) on or between food components (Baker et al., 1994). Depending on the product, coating might be used for preventing the loss of aromas, moisture, moisture absorption, and transfer of moisture between components that contain different water activity in a heterogeneous food system, formation of pack ice in frozen foods, as well as exposure to oxygen or diffusion of carbon dioxide. Moreover, it carries food ingredients (antioxidants, antimicrobials and flavor) and improved mechanical integrity or handling characteristics of the food.

In some cases, edible and biodegradable films with good mechanical properties could be used instead of synthetic packaging films (Krochta and Mulder-Johnston 1997; Youncharad 1992). The appropriate type of films that could be used in some applications are presented in Table 2.2 (Donhowe and Fennema, 1994)

Table 2.2 Possible usage of edible and biodegradable film coatings

Use	Appropriate Type of Film
Retard moisture migration	Lipid, composite*
Retard gas migration	Hydrocolloid, lipid or composite
Retard oil and fat migration	Hydrocolloid
Retard solute migration	Hydrocolloid, lipid or composite
Improve structural integrity or handling properties	Hydrocolloid, lipid or composite
Retard volatile flavor compounds	Hydrocolloid, lipid or composite
Convey food additive	Hydrocolloid, lipid or composite

* A composite film consists of lipid and hydrocolloid components combined to form a bilayer or conglomerate.

Source: Donhowe and Fennema (1994)

2.2.1 Advantages of edible and biodegradable films

Gennadios and Weller (1990) summarized advantages of edible and biodegradable films over other traditional non-edible and non-degradable polymeric packaging materials as follows:

1) Edible and biodegradable films could be consumed without peeling the film off the products. This is obvious critical importance since it represents an environmental ideal package and there is no package to dispose off.

2) Even if the films were not consumed, they could still contribute to the reduction of environmental pollution. Edible and biodegradable films are produced exclusively from renewable and edible ingredients. They are, thus, anticipated to degrade more readily than polymeric materials.

3) The films could enhance the organoleptic properties of packaged food. Flavorings, colorings and sweeteners could be incorporated to them.

4) The films could supplement the nutritional value of the food, especially the films that made from proteins.

5) The films could be used for individual packaging of small portions of food, especially particular products that are not packaged individually, such as peas, beans, and strawberries.

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

7) The films could carry antimicrobial and antioxidant agents. In these applications they could also be used at the surface of foods to control the diffusion rate of preservative substances from the surface to the interior of the food.

8) The films could be conveniently used for microencapsulation of food flavoring and leavening agents to efficiently control the release rate into the interior of foods.

9) Edible and biodegradable films could be used in multilayer food packaging materials together with non-edible films. In this case, the edible films would be the internal layer that contacted directly with food.

2.2.2 Film formation

When polymer films are applied to a food matrix, there were two types of forces, which are, cohesion force that is applied among the polymer molecules in film and adhesive force that is applied between the film and the substrate (Banker, 1966). The degree of cohesion affected film properties consists of density, compactness, porosity, permeability, flexibility and brittleness while processing parameters affects with cohesion and adhesion. The cohesive film strength has been found to increase with film thickness, up to a certain fixed value, and after that the film strength was constant (Guilbert, 1986). Cohesion and adhesion are related to the polymer structure such as the molecular weight, regularity of chain structure, branching, polarity and distribution of polar groups along the polymer chain. Maximum coating, solution salvation and polymer chain extension would produce the most efficient films. A high viscosity was also a good indication of the adequacy of salvation and chain extension according to the formulation and method of preparation prior to film application (Guilbert 1986; Guilbert and Biquet 1996).

2.2.3 Type of edible films

Edible and biodegradable films can be divided into 3 types based on different source of materials, which are, polysaccharide film, lipid film, and protein film (Younchalad 1998; Krochta et al., 1994; Guilbert and Biquet 1996).

2.2.3.1 Polysaccharide films

In general starch-based packaging materials need improvement before they could be used as a base for food packaging. To date, the polysaccharides of interest for material production have been cellulose, starch and gum (Weber, 2000).

a) Cellulose and its derivatives

As one of polysaccharide, cellulose is the structural material found in plant cell walls. It composes of linear chines of (1->4)-β-D-glucopyranosyl unit (Figure 2.3).

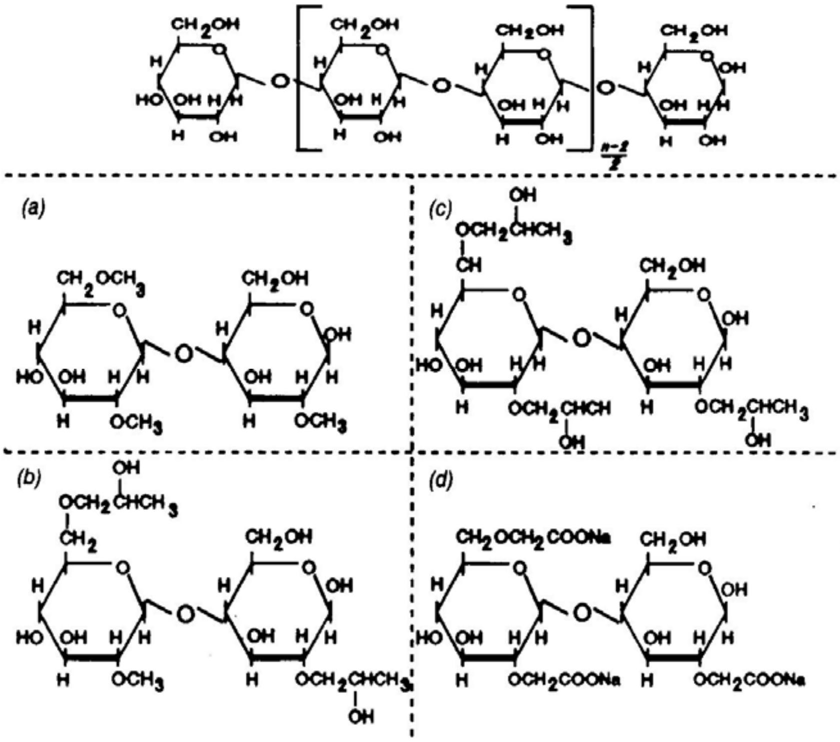

Figure 2.3 Cellulose structure (a) methylcellulose, (b) hydroxypropyl methylcellulose, (c) hydroxypropylcellulose, and (d) Na^+ salt of carboxy methylcellulose (from Nisperos-Carriedo, 1994)

Although cellulose is a low cost material, it is difficult to use because of its hydrophilic nature, water insolubility and crystalline structure. One of cellulose compound, cellulose acetate is a thermoplastic material. Cellulose acetate films could be obtained by either extrusion or solvent casting. They are clear and tough. Although they have poor barrier properties against moisture and gases, they are good oil and fat barrier, and excellent for certain high-moisture products because it breathes and does not fog up (Hanlon, 1992). Cellulose could be chemically modified with ether to produce ether cellulose (EC) that is biodegradable but not edible (Weber, 2000).

b) Starch and its derivatives

Starch, the polysaccharide stored in cereals, legumes and tubers, is a widely available raw material suiting for a variety of industrial uses. Although films could be produced from starch, their mechanical properties are inadequate to become a packaging material (Weber, 2000). Its properties could, however, be improved by blending with plasticizer or other materials. Starch can be derived from tubers (potato, tapioca, arrowroot, and sweet potato), stem (sago), cereals (corn, waxy maize, wheat, and rice), and starch-based (Nisperos-carriedo, 1994; Sanderson, 1981; Shamekh et al., 2000; Bunyaputtipong, 2000). In Europe, potato starch was an important ingredient in food, while in North America, corn or maize starch is the most widely used (Sanderson, 1981). The two distinct polymers containing in starch granules are amylose (Figure 2.4 a) and amylopectin (Figure 2.4 b). Amylose is a linear polymer composed of (1- 4)-α-D-glucopyranosyl monomers ranging from 200-3,000 depending on the sources. The amylose backbone with side unit of D-glucopyranosy linked by α-1, 6-glycosidic bonds are contained in amylopectin (Nisperos-carriedo, 1994).

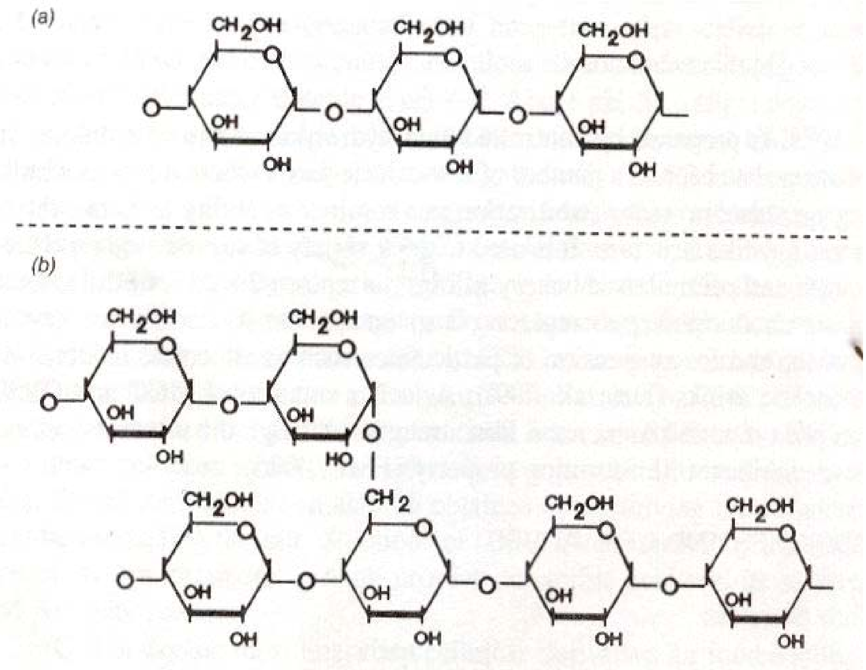

Figure 2.4 Structure of (a) amylose and (b) amylopectin (from Nisperos-Carriedo, 1994)

Amylopectin is applied where viscosity, stability, and thickening power need to be concerned. For film-forming and preparation of strong gels, amylose is required (Weber, 2000).

c) Pectin

Pectin is a complex group of structural polysaccharides, which widely occured in land plants (Aspinall, 1970). The properties of pectin depend on the content of methyl esters or degree of esterification (DE). Miers et al. (1953) and Swenson et al. (1953) employed a two-step technique, i.e., an aqueous solution of low-methoxyl pectin and treatment with a calcium solution to promote gel. These films have high water permeability and can prevent dehydration by acting with sacrificial agents.

d) Carrageenans

Carrageenans can be extracted from several species of red seaweeds, mainly *Chondrus crispus*. They are complex mixture of several polysaccharides (Nisperos-carriedo, 1994). Commercial carrageenans are mixtures of three fractions and skill is required in selecting the best combination for a given application. In food systems, carrageenans are mainly used in gel formation, in stabilizing suspension and emulsion, and for gelation and structural viscosity of milk-based products. Carrageenans gels could also be used as food coating (Meyer et al., 1959).

e) Alginates

Alginates are salts of alginic acid and occur naturally as the major structural polysaccharides of brown seaweeds known as Phaeophyceae. They possess good film-forming properties making them particularly useful in food applications. Alginate films tend to be quite brittle after drying but might be plasticized by the inclusion of glycerol (Glicksman, 1983). Films could be made from a sodium alginate solution by rapid reaction that involves the G-block regions.

2.2.3.2 Lipid film.

The traditional packaging is sufficient to retard moisture loss or gain in food but can not be applied to isolate or separate different components varying in water activity within a composite food. Edible and biodegradable barriers are developed mainly from hydrophobic substances such as lipids (Morillon et al., 2002). A large number of lipid compounds, such as, oil, animal and vegetable fats, acetoglycerides, natural waxes, or surfactants have been used to produce edible films or coatings (Guilbert and Biquet, 1996). Edible films and coatings can be used to encapsulate food, candy, medication, vitamins or flavoring agents for convenient ingestion, and to protect flavor. In the case of fruits and vegetables, they are used for preventing weight loss (retain moisture), slowing down aerobic respiration, and improving appearance by providing gloss (Hernandez, 1994).

a) Acetoglycerides

The reaction of glycerol monostearate with acetic anhydride that was called acetylation, yielded 1-stearodiacetin. This acetylated monoglyceride displays the unique characteristic of solidifying from the molten state to become a flexible, wax-like solid. Most lipids in the solid state could be stretched to about 102 % of their original length before fracturing. Acetylated glycerol monosterate, however, could be stretched up to 800 % of its original length. It has been determined that high stretchable, solid phase acetylated monoglycerides existing in the alpha (α) polymorphic form exhibited high stretchability (Kester and Fennema, 1986).

The barrier properties of acetoglyceride films were found by Lovegren and Feuge (1954, 1956). They stated that water vapor barrier properties were highly depended on the water vapor pressure difference on either side of the film and permeability increased rapidly after contacting with a moist atmosphere. As the degree of acetylation increased, the barrier properties are also improved due to the substitution of the polar hydroxyl groups (Guilbert and biquet, 1996). Because of their particular rheological properties (flexibility, plasticity), acetoglycerides are sometimes added to films or coatings in solid fats or wax in order to avoid the formation of cracks, particularly during low temperature storage (Lowe et al., 1963).

b) Waxes

Waxes are used as barrier films coated on skin of fresh fruits or dried fruits to control gas and moisture diffusion and to improve also the surface appearance of various foods. If a thick layer was applied on the surface, it must be removed before consumption. On the other hand, it was edible if a thin layer was applied. Guilbert (1986) suggested that waxes are the most efficient edible compounds providing a humidity barrier.

Paraffin is one of the most efficient waxes due to the absence of polar groups (Guilbert and Biquet, 1996). Paraffin wax is derived from distillated fraction of crude petroleum. They are permitted for use as protective coatings for row fruit and vegetables and for cheese (Hernandez, 1994).

Carnauba wax produced in Brazil from leaves of the palm tree *Copernica cerifera*, is a GRAS (generally recognized as safe) substance commonly added to edible coating formulations for fruits and vegetables (Weller et al., 1998). It has the highest melting point and specific gravity of commonly found natural waxes and is added to other waxes to increase melting point, hardness, toughness, and shine (Hernandez, 1994).

Beeswax is excreted by honey bees for comb building. The wax is harvested by centrifuging the honey from the wax combs, and then melted with hot water, steam, or solar heating. This wax is very plasticate at room temperature, but becomes brittle at colder temperatures. It is soluble in most other waxes and oils (Hernandez, 1994).

2.2.3.3 Protein films

The film-forming ability of several proteinaceous substances could be utilized in industrial applications. In particular, food industry that recognizes film-forming properties of several proteins as significant source of materials for production of edible, protective food films and coatings (Gennadios et al., 1994).

a) Corn zein

Corn zein is produced by corn grains. It has protein content in the range of 7 to11% (Wall and Paulis, 1978). Zein is the only corn protein that has been produced commercially. It was characterized by its ability to form tough, glossy, hard, grease-proof coatings after evaporation of aqueous alcoholic solution (Gennadios and Weller, 1990). Zine is insoluble in water (except at extremes), as well as in anhydrous alcohols. Typical solvent systems used for zein are aqueous aliphatic alcohol solutions. In general, suitable solvents for zein are either mixtures of water with an organic compound, such as alcohols, acetone, and acetonylacetone, or mixture of two anhydrous organic compounds, such as alcohols and chlorinated hydrocarbons or glycols (Pomes, 1997).

In the past, zein was used in numerous non-edible applications based on its coating-forming ability. Small quantities of zein, however, are used for some of the above products at the moment. The major amount of zein is currently used as edible coating for pharmaceutical tablets, confectionery products and shellac replacement (Gennadios and Weller, 1990). Advantages of corn zein-based coatings over shellac consist of faster drying rate, increasing stability under long-term storage, high temperature and humidity conditions

b) Wheat gluten

Wheat protein is a general term for water insoluble proteins of wheat flour and accounted for 8-15 % of the dry weight of wheat kernels. It is composed of mixture of polypeptide molecules considered as globular proteins. The amino acid composition of gluten related to its properties. For glutamine, there is high content of glutamic acid (about 37 %) (Gennadios and Weller, 1990).

Although emulsifying and foaming properties of gluten are poor because of low solubility in the neutral pH range, these properties are important for various food processing. (Takeda et. al, 2001)

c) Soy Protein

The protein content of soybean has been reported about 38-44 %. It was much higher than the protein content of cereal grains that contains about 8-15 % (Snyder and Kwon, 1987). A widely nomenclature system for soy protein is based on relative sedimentation rates of protein ultracentrifuge fractions (Gennadios et.al., 1994). Soy protein films are flexible, smooth, transparent, and clear compared with other films obtaining from plant proteins (Were et. al., 1999). Soy protein has also been manufactured as sausage casings and water soluble pouches (Guilbert, 1986; 1988).

d) Collagen

Collagen is a fibrous, flexible polymer or structural protein in animal tissue, particularly skin, bones and tendons, with a common repeating unit such as glycine, proline and hydroproline. Since it composes of complex helical and fibrous structures, collagen is very insoluble and difficult to process. Collagen is a basic raw material for the production of gelatin, a common food additive with potential for film and foam production (Korchta and Mulder-Johnston, 1997). Gelatin is produced via either partial acid or alkaline hydrolysis of collagen. Such treatments disrupt the tight, helical structure of collagen and produce water-soluble fragments that might form sticky gels, films, or light foams. Gelatin is a material that could be processed easily but it is extremely moisture sensitive. For prolonged use in packaging, therefore, research has been focused on the chemical modification of gelatin to improve moisture sensitivity (Weber, 2000). Collagen is the most commercial successful edible protein film. Collagen castings have largely replaced natural gut castings for sausages (Korchta and Mulder-Johnston, 1997).

e) Milk Proteins

Milk obtained from mammary glands of all mammal species contains about 33 g/ l protein. Naturally, milk composes mainly of whey protein and casein. Milk protein had high nutritional quality, water solubility and emulsifing capability and can be used for producing edible film (Gennadios et.al., 1994).

Casein is the major protein in milk, representing 80 % of the total milk proteins (Weber, 2000). The total concentration of casein in milk ranged between 2.5-3.2 %. Casein is produced as water soluble pouches. Caseinate coating retained sorbic acid on the surface of the model food system and on the surfaces of intermediate moisture fruits and, when combined with lipid, protected fresh vegetables, dried fruits and vegetables, and frozen fish form moisture migration and/ or oxidation (Korchta and Mulder-Johnston, 1997).

Whey protein, accounting for 20 % of total milk proteins, is characterized by their solubility at 4.6 (Gennadios et.al., 1994) and readily processable. An exterior film is produced by adding gelatin into whey protein and can be developed to reduce moisture sensitivity (Weber, 2000). Whey protein coating carries antioxidant for frozen fish effectively, significantly reduced oxygen uptake and rancidity in roasted peanuts, as well as reduced disintegration of freeze-dried food. Whey protein and whey protein-acetylated monoglyceride coatings also reduce moisture migration into breakfast cereal and reduced stickiness of raisins (Korchta and Mulder-Johnston, 1997).

2.3 Chitin and chitosan

Chitin was the second most plentiful natural biopolymer after cellulose and widely distributed throughout nature (Sanford, 1989). The most available sources are the protective shells of crustaceans such as crab and shrimp. In addition, it found in fibrous exoskeleton of insects, in fungal cell walls and other biological materials (Sanford, 1989; Jeuniaux et.al., 1989).

Chitin is *N*-aceetyl-2-aminocellulose or 2-acitamido-2-deoxycellulose and composed of *N*-acetyl-D-glucosamine units link β- D- (1→ 4) (Whistler, 1993). Chitin existed in three polymorphic forms, that is, α-, β- and γ-chitin (Minke and Blackwell, 1978; Gardner and Blackwell, 1975) with different molecular chains. The crystallographic structure shows the main chains arrange in an anti-parallel fashion with strong intermolecular hydrogen bonding (Gardner and Blackwell, 1975). Chitin was a highly insoluble material and low chemical reactivity. Its structure is almost the same as that of cellulose. Nevertheless, there is an acetamido group substituting hydroxyl group at C-2 position. Like cellulose, it functions naturally as a structural polysaccharide. Chitin is a white, hard, inelastic, nitrogenous polysaccharide and is the major source of surface pollution in coastal areas (Kumar, 2000). It has limited applications because of strong inter- or intra-molecular hydrogen bonding caused by acetamido groups in glucopyranose residues. Nonetheless, solubility, reactivity and processability of chitin can be improved by chemical modification (Kim et.al., 1994).

Low acetyl forms of chitin composed primarily of glucosamine, 2-amino-2-deoxy-D-glucose, are mainly used (Sanford, 1989; Peniston and Johnson, 1980). Glucosamine and its *N*-acetylated form are the most abundant amino sugars occurring in polysaccharides, glycoproteins etc (Sanford, 1989). The structures of cellulose, chitin and chitosan are shown in Figure 2.5. Chitin and chitosan are of commercial interest due to their high percentage of nitrogen (6.89 %) compared to synthetically substituted cellulose (1.25 %).

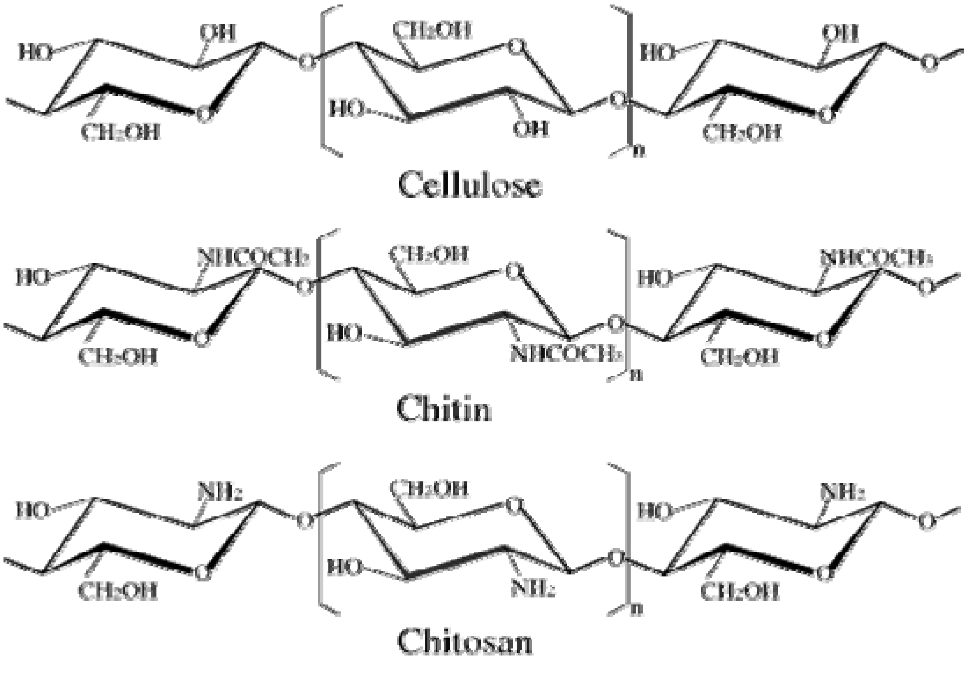

Figure 2.5 Chemical structures of cellulose, chitin and chitosan (from Winterowd and Sandford, 1997)

Chitosan could be defined as sufficiently deacetylated chitin to form soluble amine salts. To obtain a soluble product, the degree of deacetylation must be 80 to 85 % or higher. The content of acetyl groups in chitosan products must be less than 4 - 4.5 %. Chitosan products were highly viscous, resembling natural gums (Peniston and Johnson, 1980).

2.3.1 Chemical and Physical Properties of chitosan

Although the molecular structures of chitin and chitosan are rather similar, their chemical reaction and physical properties are different. Both compounds possess reactive hydroxyl and primary amine groups but chitosan is less crystalline than chitin (Winterowd and Sandford, 1997).

2.3.1.1. Common Organic Reaction

Chitin and chitosan are considered as weak bases and undergo the typical neutralization reactions of alkaline compounds. Chitosan is insoluble in alkaline aqueous solution. Presumably, the multitude of cationic site along the chain allows for the water solubility by increasing both the polarity of the compound and degree of polymer-to-polymer electrostatic repulsion. Evidently, chitin possesses an insufficient percentage of glucosamine units to permit water solubility, even under acidic conditions (Winterowd and Sandford, 1997).

Chitin and chitosan were susceptible to acid- or base-catalyzed hydrolysis. Under acidic or alkaline conditions, acetic acid could be liberated as the *N*-acetyl groups at C2 are converted to primary amine (Muzzarelli, 1977). Acidic conditions also result in some degree of depolymerization as hydrolysis of the β-glycosidic linkages takes place (Madhaven and Ramachandran, 1974). Depolymerization occurs in the low alkaline environments. Normally, chitosan could be degraded by treatment with nitrous acid (Allan and Peyron, 1989).

2.3.1.2. Heavy Metal Complexes

The free doublets of nitrogen atoms and possibly those of oxygen atom beard by the two kinds of monomers can be use to elaborate interactions of chelate type with numerous metals. Uptake of metals such as Hg, Cd, Pb, Zn, Ni, Cr, Cu, Fe, Mn, Ag, and Pt in chitosan has been reported in the range between 0.5-5.0 meq/g chitosan (Ratananontachi, 1998). There have been several reports on the interactions of chitin and chitosan with metals (Yaku 1978; Skaugrud 1989 and Chui et.al. 1996). Complexes do not form between these polymers and metals in groups 1 or 2 (Winterowd and Sandford, 1997). The tendency of chitin and chitosan to form complexes with heavy metals has significant impact on their toxicity. Severe toxicity could occur if chitosan contaminated with mercury or lead was consumed and the heavy metal released into the intestinal tract (Winterowd and Sandford, 1997).

2.3.1.3. Enzymatic Reactions

Chitin and chitosan are degraded by lysozyme under the proper conditions of temperature, ionic strength and pH (Sashiwa et.al.1989; Kumar 2002). Hydrolysis by chitinase, chitosanase, lysozyme and other enzymes could also occur (Winterowd and Sandford, 1997).

To be taken orally, chitin and chitosan are considered to be essentially nondigestible by human because there is no bacteria in gut flora of human that possesses chitinases or chitosanases, whereas animals, such as fish, hens and rabbits are capable to digest chitin or chitosan (Winterowd and Sandford 1997, Hirano et.al. 1990). Low concentration of lysozyme is found in the saliva and the stomach fluids of human (Hirano et.al. 1990). The enzyme, nevertheless, degrades a small extent of chitin or chitosan. Hydrolytic degradation could also occur in the stomach at pH between 1 and 2 (Winterowd and Sandford, 1997).

2.3.1.4. Solubility

The solubility of chitosan in water depends on the balance between the electrostatic repulsions occuring at protonated amine functions and the hydrogen bounding due to the free amino groups. The upper limit of pH for solubility depends on the value of pK_a (Domard and Rinaudo, 1989). In the free amine form, chitosan is not soluble in water at neutral condition but in acidic condition amino groups in chitosan are protonated to form cationic amine groups and become soluble (Sanford, 1989). The solubility is observed when pH is lower than 6 or 5.5 (Domard and Rinaudo, 1989). Chitosan is insoluble in sulfuric acid and phosphoric acid while other mineral acids such as hydrochloric, nitric, and perchloric acid dissolve chitosan. Solubility of chitosan is, however, enhanced in organic acids such as acetic, lactic, propionic, formic and citric acid (Muzzareli, 1977). Commonly, acetic acid is used as a reference for solubility of chitosan (Domard, 1996; Sitthai 1994 and Park et.al. 2002).

2.3.1.5. Viscosity

Just as with other food matrices, viscosity is an important factor in the conventional determination of molecular weight of chitosan and in determining its commercial applications incomplex biological environments such as in the food system (Muzzareli, 1977). Higher molecular weight chitosans often render highly viscous solutions, which may not be desirable for industrial handling. Some factors during processing such as the degree of deacetylation, molecular weight, concentration of solution, ionic strength, pH, and temperature affect the production of chitosan and its properties (Moorjani et al., 1975). For instance, chitosan viscosity decreases with an increased time of demineralization (Moorjani et al., 1975). Viscosity of chitosan in acetic acid tends to increase with decreasing pH but decrease with decreasing pH in HCl, giving rise to the definition of 'Intrinsic Viscosity' of chitosan which is a function of the degree of ionization as well as ion strength (Muzzareli, 1977).

2.3.2 Chitosan applications

Chitosan is natural, non-toxic, biodegradable and high molecular weight polymer. It has been produced commercially in North America on a large scale as solution, flake, fine powder, bead and fiber (Sandford, 1989). The industrial production of chitosan has been steadily increased since the 1970s. In Japan, the production of chitosan increased 37 % annually from 1978 to 1983 and the total production was 311 and 1,270 tons in 1983 and 1986, respectively (Li et al., 1997).

A wide variety of medical applications for chitin and chitin derivatives have been reported over the last three decades. It was suggested that chitosan may be used to inhibit fibroplasia in wound healing, to promote tissue growth and to differentiate in tissue culture (Hirano, 1997). The poor solubility of chitin has been a major factor for utilization, investigating properties and structure. Various applications of chitin and modified chitins as a raw material for man-made fibres have been reported. They have been used as absorbable sutures and wound-dressing materials (Hirano, 1997). The chitin sutures resisted bile, urine and pancreatic juice, which are problem areas of other absorbable sutures. It has been claimed that wound dressings made of chitin and chitosan fibers have been potentially applied in wastewater treatment where the removal of heavy metal ions by chitosan through chelation has received much attention (Kumar 2000, Knorr, 1991, Hirano 1997). Several chitosan applications are shown in Table 2.3.

The difference value between the chitosan products and the low-cost polymers was one of the main driving forces for studying about new applications of chitosan (ref.). Biotechnology has attempted large-scale production using by products, such as monoclonal antibodies. This project invested about 1.2 billion US dollars on the world market in 1991 Chitosan membranes and gels have also great potential for utilizing immobilized cell culture systems (Li et. al., 1997).

Chitin and chitosan have a wide range of applications they could solve numerous problems in environmental and biomedical engineering. Chitin derivatives and partially deacetylated chitosan could be easily molded to various forms. Recently, the transdermal absorption promoting characteristics of chitosan, especially for nasal and oral delivery of polar drug, including peptide and proteins, as well as for vaccine delivery, have been exploited (Miyazaki et.al., 1996). These properties, together with its very safe toxicity profile, make chitosan an exciting and promising excipient for the pharmaceutical industry for present and future applications (Kumar, 2000).

Table 2.3 Chitosan applications

Applications	Examples	References
Water treatment	Removal of Metal Ions	Milot et.al., 1996
	Flocculant/Coagulant of	
	Protein	Juang et.al., 2001
	Dyes	Gildberg and Stenberg, 2001
	Amino acids	Goodsen, 1997
	Filtration	Juang and Shiau, 2000
Pulp and Paper	Surface Treatment	Jok and Hayasi, 1996
	Photographic Paper	Goodsen, 1997
	Carbonless Copy Paper	Goodsen, 1997
Medical	Bandages, Sponges	Goodsen, 1997
	Artificial Blood Vessels	Goodsen, 1997
	Blood Cholesterol Control	Goodsen, 1997
	Tumor Inhibition	Kumar, 2000
	Membranes	Goodsen, 1997
	Dental/Plaque Inhibition	Goodsen, 1997
	Skin Burns/Artificial Skin	Kumar, 2000
	Eye Humor Fluid	Goodsen, 1997
	Contact Lens	Goodsen, 1997
	Controlled Release of Drugs	Nuntahnid et.al., 1996
	Bone Disease Treatment	Goodsen, 1997

Table 2.3 Chitosan applications (continued)

Applications	Examples	References
Cosmetics	Make-up Powder	Lang and Clausen, 1989
	Nail Polish	Hirano, 1989
	Moisturizer	Lang and Clausen, 1989
	Fixtures	Lang and Clausen, 1989
	Bath Lotion	Hirano, 1989
	Face, Hand and Body Creams	Hirano, 1989
	Toothpaste	Lang and Clausen, 1989
	Foam Enhancing	Hirano, 1989
Biotechnology	Enzyme Immobilization	Goodsen, 1997
	Protein Separation	Goodsen, 1997
	Chromatography	Goodsen, 1997
	Cell Recovery	Goodsen, 1997
	Cell Immobilization	Kumar, 2000
	Glucose Electrode	Bough, 1978
Agriculture	Seed coating	Goodsen, 1997
	Leaf coating	Kumar, 2000
	Hydroponic/ fertilizer	Goodsen, 1997
	Controlled agrochemical Release	Goodsen, 1997
Food	Removal of dyes, solids, acids	Kumar, 2000
	Preservatives	Kungsuwan et.al., 1996
	Color stabilization	Kumar, 2000
	Animal feed additive	Hirano et.al., 1990
Membranes	Reverse osmosis	Goodsen, 1997
	Permeability control	Kumar, 2000
	Solvent separation	Goodsen, 1997

Another application of chitosan is edible film that is made by dissolving chitosan in acid solution, then blending with plasticizer and other additives. The chitosan edible film possessed excellent gas barrier properties and some physical properties depending on many factors, including chitosan concentration, type and amount of acid, as well as concentration of gelatin and plasticizer (Mahae and Sophanodora, 1998; Suchinpongpan, 1998).

2.4 Factors affecting properties of chitosan films

Factors that could potentially influence properties of chitosan film were as follows:

2.4.1 Chitosan content

The effects of varying chitosan concentrations on the properties of chitosan films have been observed (Mahae and Sophanodora, 1998; Suchinpongpan, 1998). Because of the hydrophilic nature of these films, water vapor permeability of chitosan film tended to be enhanced as the chitosan concentrations and thickness increased resulting to higher surface density and tensile strength.

2.4.2 Types of Acid

Chitosan is insoluble in pure water or organic solvents but is soluble in aqueous solutions of organic acid, such as, acetic, citric, formic, glycolic, lactic, malic and propionic acids at specific conditions (Muzzareli, 1977). These acids were selected because of their low toxicity to human (Muzzareli, 1977). In the acid solution, amine groups of chitosan are protonated to become NH_3^+, and thus polyelectrolyte and chelating properties of chitosan were mainly governed by the acidity of the NH_3^+ (Park et.al., 2002). After it was dissolved in the acids mentioned above to become viscous solutions, functional films were formed and could be produced as edible coating (Krochta and Mulder-Johnston, 1997). However, types and concentrations of acid affected physical and mechanical properties of chitosan film (Ritthidej et. al., 2002). Caner et. al. (1996) and Nguyen (2002) used citric, lactic and propionic acid as solvents for chitosan film production.

2.4.2.1 Citric acid

Citric acid is one of the most widely used acids in the cosmetic industries and was extracted from citrus fruits (Winter, 1994). It is found naturally in coffee and peach. It is a clear, crystalline and water-absorbing chemical. Citric acid has been widely used as a flavoring, pH adjuster, curing meats, removing trace metals and brightens color in various commercial products, preservative, sequestering agent etc. It is also used for dissolving urinary bladder stones.

2.4.2.2 Lactic acid

Lactic acid is odorless, colorless and normally found in blood and muscle tissue as a product of the metabolism of glucose and glycogen. It also presents in sour milk, beer, sauerkraut, pickle and other food products made by bacterial fermentation. Lactic acid has been produced commercially by fermentation of whey, corn starch, potatoes and molasses and normally used as acidulate, flavorings, skin fresheners. Nevertheless, it has been reported that lactic acid in cosmetic products might cause stinging in sensitive people and particularly in fair-skinned women (Winter, 1994).

2.4.2.3 Propionic acid

Propionic acid occurs naturally in apples, strawberries, tea and violet leave. It is oily liquid with slightly pungent and rancid odor. It has been used as butter and fruit flavorings, inhibitor and preservative to prevent mold in baked goods and processed cheeses, perfume bases, mold inhibitor, antioxidant and preservative in cosmetics. Its salts have been used as antifungal agents to treat skin mold. Although there were many applications, propionic acid might cause migraine in those susceptible to migraine and skin irritations in bakery workers. It was reported that large oral dose in rat was lethal (Winter, 1994).

2.4.3 Gelatin

Gelatin is a protein resulting from partial hydrolysis of collagen (Krochta et. al., 1994) and was composed of 18 kinds of amino acid linked by peptide bonds to yield along, branched molecule with exposed guanidine (basic) and carboxylic groups. Gelatin formed thermally reversible gels when warm aqueous suspensions of the polypeptide are cooled. The gelation mechanism involves formation of ionic crosslinkes between amino and carboxyl groups of amino acid side chains, as well as secondary interactions such as hydrogen bonding (Glicksman, 1982; Achet and He, 1995).

Principal raw materials for commercially used gelatin manufacture are pork skins and bovine hides, skins and bones. These raw materials, however, need preparation, such as, degreasing, size reducing and demineralization of bones prior to further processing. It was noted that pork skins were the largest supply of raw material for edible gelatin production in the U.S. (Alleavitch et. al., 1989).

Gelatin is tasteless, odorless, colorless or slightly yellowish, transparent and brittle. It has been produced in 3 forms, that is, sheets, flakes and powder and solubled in hot water, glycerol, acetic acid and insoluble in organic solvents (Budavari, 1996).

Gelatin absorbed water about 5-10 times of its weight to become gel at 30-35°C. Gel point of fish extracted gelatin was reported in the range of 5-10°C. Under stress, the viscosity of gels increased and then was thermally reversible. Gelatin had a unique protein structure that provides a wide range of functional properties (Hudson, 1994). These proteins formed compound (triple) helix in aqueous solution.

The use of gelatin in the elaboration of edible and biodegradable films was very well studied until 1960s. There were many patents, mainly in the pharmaceutical area (Gennadios et al., 1994). This due to good film coating properties of gelatin that has no restrictions in fabricated foods. Klose et al. (1952) applied a gelatin coating to the surface of cut poultry meat prior frozen storage. The coating provided a limited degree of protection against the development of oxidative rancidity. The effectiveness, however, was greatly increased by incorporating antioxidant into the gelatin layer. A typical formula for producing gelatin films was 20-30% gelatin, 10-30% plasticizers and 40-70 % water (Mathlouthi, 1986).

From the early 80's, gelatin has returned to the attention of several researchers on edible films for food application. Guilbert (1986) conducted quality improvement of the films by mixing varying concentrations of sorbitol to chitosan-gelatin solution. Avanitoyannis et al. (1998) studied effects of polyol and water to edible films that were produced from hydroxypropyl starch and gelatin. Water vapor permeability, mechanical and thermal properties of edible films based on bovine hide and pigskin gelatins were also studied. Effects of polyol and water to edible films that were produced from hydroxypropyl starch and gelatin were conduct (Sobral et.al., 2001).

2.4.4 Plasticizer

A plasticizer is a substantial nonvolatile, high boiling, nonseparating substance. It functions is to improve physical and mechanical properties of materials. Banker (1966) stated that plasticizers are added into polymeric films in order to reduce brittleness, improve flow, impart flexibility and increase toughness, strength, tear resistance and the impact resistance. These are due to reduction of the cumulative intermolecular forces along the polymer chains and the glass transition temperature, which is below room temperature. The energy of activation for diffusion (E_d) of gases and vapors through plasticized plastic films decreases resulting from reducing amount of energy required to separate adjacent polymer chains (Banker, 1966; Kester and Fennema, 1986). This probably leads to increase in the diffusion constant and the permeability of gases and vapors through the film (Kester and Fennema, 1986). Normally, films required plasticizers approximately 10-60 % (on dry basis) depending on the rigidity of the polymer.

Plasticizers, which are generally added into edible and biodegradable films, are as follow (Caner et. al., 1996)

1) Mono-, di-, and oligo- saccharides (mainly glucose or fructose-glucose syrups, honey)

2) Polyols (mainly sorbitol, glycerol, glycerol derivatives, and polyethylene glycols)

They are polysaccharides that aldehyde and ketone groups are reduced. Although some polyols presented in nature, particularly in vegetable, it was scarce. Consequently, they have been manufactured industrially by catalytic hydrogenation of the corresponding saccharides (Billaux et al., 1991).

3) Lipids and its derivatives (mainly fatty acids, monoglycerides, ester derivative, other emulsifiers).

For hydrophilic polymer film, the first and second groups mentioned above are normally added, while the third group generally blended into wax or fat-based films (Guilbert and Biquet, 1996).

CHAPTER III

MATERIALS AND METHODS

The study was concentrated on production of chitosan-gelatin edible films from shrimps shell wastes. The procedure of process shows in Figure 3.1.

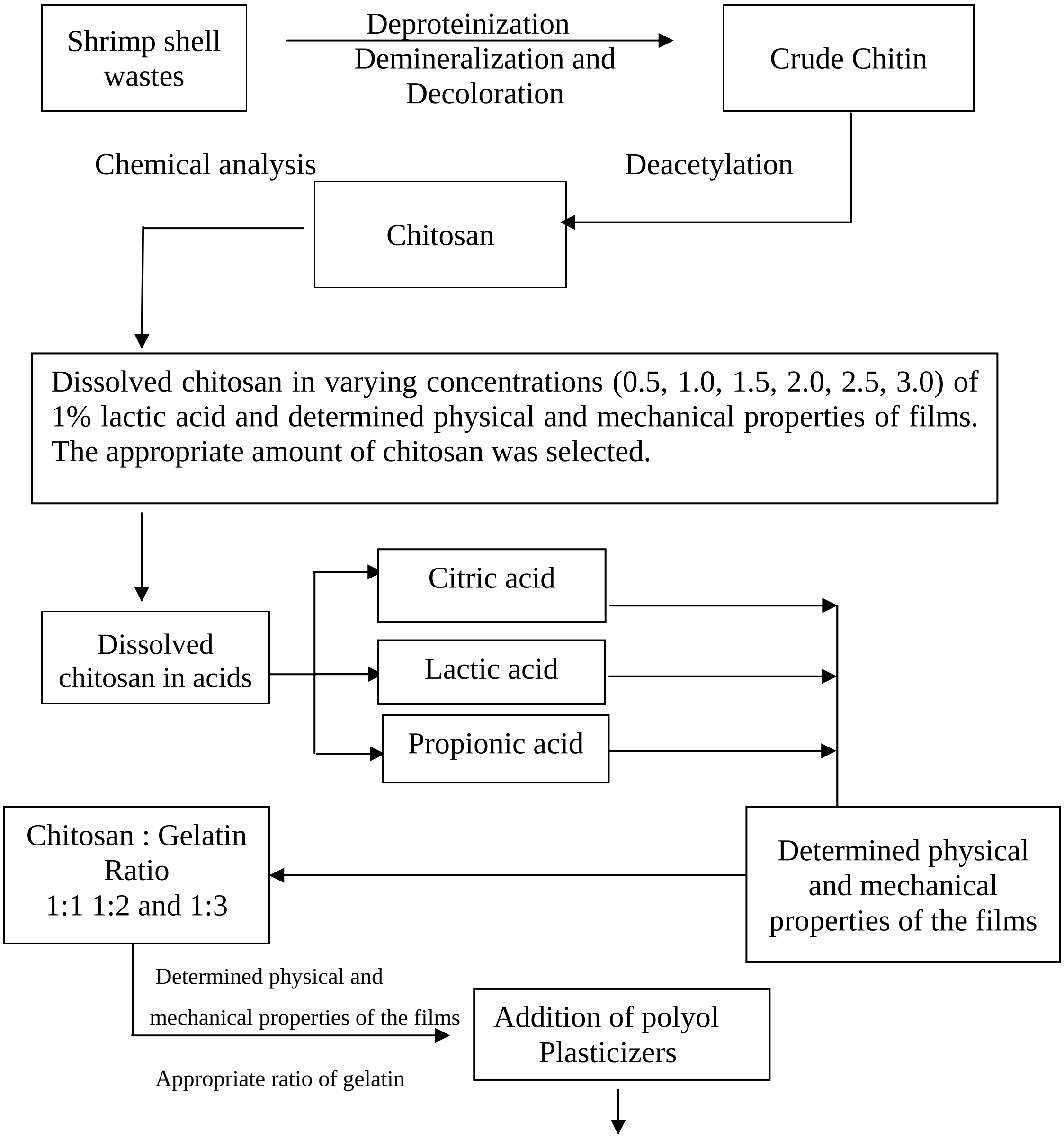

Figure 3.1 Experimental procedure

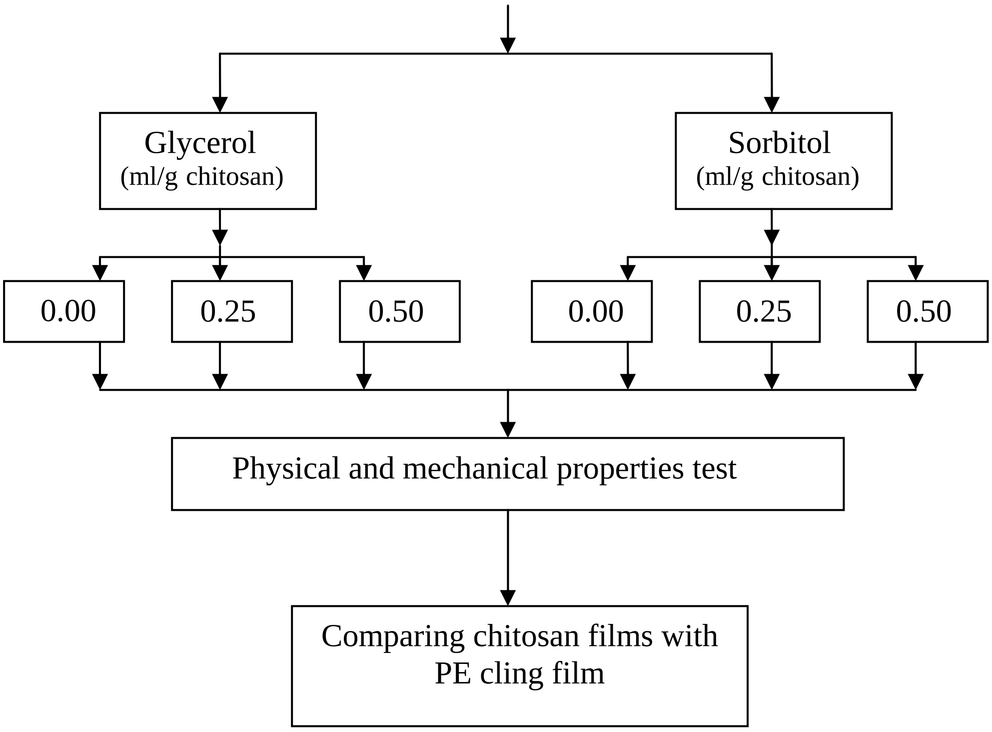

Figure 3.1 Experimental procedures (continued)

3.1 Chemical reagents and equipments

Chemical reagents

1) Boric acid (Merck)

2) Bromocrysol green indicator (Merck)

3) Citric acid (Carlo erbr reagenti)

4) Copper sulfate pentahydrate (Merck)

5) Diethyl ether (Merck)

6) Gelatin (Ajax Finechem)

7) Glycerol (Carlo erbr reagenti)

8) Grease (Thai wax)

9) Hydrochloric acid (Merck)

10) Lactic acid (Ajax Finechem)

11) Methyl red indicator (Merck)

12) Petroleum ether (Merck)

13) Phenolphthalein indicator (Merck)
14) Potassium hydroxide (Analar)
15) Propionic acid (Carlo erbr reagenti)
16) Sodium azide (Merck)
17) Sodium hydroxide (Merck)
18) Sodium sulfate (Merck)
19) Sorbitol (APS Chemical)
20) Sulfuric acid (Merck)

Equipments

1) Hot air oven (Binder)
2) Blender (Mulinex)
3) Hot plate and magnetic stirrer (Binder)
4) Electric balance (Binder)
5) Water bath (Binder)
6) Kjeldahl apparatus (Kjeldahltec)
7) Muffle furnace (Binder)
8) Brookfield viscometer (Brookfield)
9) Tensile Testing Machine (LLOYD)
10) Tearing Testing Machine (ETS)
11) Thickness Tester (Micrometer Mar)

3.2 Experimental methods

The present study was divided into two main parts. The first part was concentrated on the extraction and the analysis of physical and chemical properties, as well as determination of heavy metal contents of chitosan that was extracted from shells and heads of black tiger shrimp. The analytical methods were conducted after Muzzarelli (1977, 1985). For the second part, chitosan films were prepared from the extracted chitosan. Effects of some parameters on the properties of the films were investigated. These parameters consisted of chitosan content, type and concentration of acid solution, chitosan: gelatin ratio, as well as types and amounts of plasticizer. Finally, the properties of the films were compared with that of commercially available PE cling films.

3.2.1 Extraction and analysis

The methods for chitosan extraction was adapted from Benjakul and Sophanodora (1991, 1993), Waiprip (1993), Wanichpongpan (1999) and Wiratthikowit (2001). The purpose of this step was to produce chitosan for later experiments. Properties of the produced chitosan, including protein, fat, ash, % deacetylation, moisture content, viscosity and heavy metal content were also analyzed.

3.2.1.1. Deproteinization

Shells and heads of black tiger shrimp were washed with deionized water and dried in hot air oven at 65°C until constant weight was observed. The dried shells and heads were then ground to 1.4 - 4.0 mm in particle size and deproteinized with 4% NaOH at room temperature for 24 hr. The ratio of solid particles to the base was 1:10 (w/v). The deproteinized particle was neutralized with tap water and filtered through muslin cloth (Benjakul and Sophanodora, 1990; Srikumlaithong et. al. 1996, Wiratthikowit, 2001).

3.2.1.2 Demineralization

To produce chitin, the solid particle from 3.2.1.1 was demineralized with 1M HCl. The solid to acid ratio was 1:10 (w/v). It was conducted at ambient temperature. The reaction lasted 24 hr. The prepared chitin was neutralized with tap water and finally filtered through muslin cloth (Benjakul and Sophanodora, 1990; Suchinpongpan, 1998, Wiratthikowit, 2001).

3.2.1.3 Decoloration

Crude chitin from 3.2.1.2 was dried in a hot air oven at 65°C for 6 hr. The dried chitin was then allowed to bleach under sunlight. This process lasted 2 days.

3.2.1.4 Deacetylation

To prepare chitosan, acetyl groups in the prepared chitin have to be hydrolyzed with strong base (Muzzareli, 1977). This could be accomplished by reacting with 50 % (w/w) NaOH at 60°C for 72 hr. The solid to base ratio was 1:10

(w/v). The chitosan was neutralized with tap water. It was later dried in a hot air oven at 60°C until constant weight was observed. The dried chitosan was later stored in a plastic bag to protect it from dust and moisture.

3.2.1.5 Chemical composition analysis and heavy metal content

The chemical analysis of shrimp shells and heads, as well as chitosan was performed after official methods of analysis from Association of Official Analytical Chemistry (AOAC, 1990), Whaiprip (1991) and Varian Australia Pty. Ltd.(1991). These were conducted as listed in Table 3.1 and 3.2.

Table 3.1 Chemical analysis of shrimp shells and heads

Chemical compositions	Analysis methods	References
Protein	Kjeldahl method	AOAC(1990)
Fat	Soxhlet extraction method	AOAC(1990)
Ash	Ignition	AOAC(1990)

Table 3.2 Chemical composition analysis of chitosan

Chemical compositions	Analysis methods	References
Protein	Kjeldahl method	AOAC(1990)
Fat	Soxhlet extraction method	AOAC(1990)
Ash	Ignition	AOAC(1990)
% Deaectylation	Titrimetric method	Whaiprip (1991)

3.2.1.6 Physical properties analysis

The analysis of physical properties of extracted chitosan was performed after official methods of analysis from Association of Official Analytical Chemistry (AOAC, 1990) and ASTM (1990)

Table 3.3 Physical analysis of chitosan

Physical properties	Analysis methods	References
Moisture	Direct heating method (Hot air oven)	AOAC (1990)
Viscosity	Brookfield viscometer	ASTM (2001)

3.2.2 Production of chitosan films

Solubility of chitosan in three different acids, including citric, lactic and propionic acids was observed. Quality improvements of chitosan film by addition of an additive, i.e. gelatin, as well as two plasticizers, i.e. glycerol and sorbitol were conducted. The experiments were conducted as follows:

3.2.2.1 Film formation

A film forming solution was prepared by dissolving the amount of chitosan in a solvent. The mixture was stirred for 24 hr. and then filtered with muslin cloth. The filtered solutions were poured on 20 x 25 cm. plastic plate and spreaded out at room temperature. Then the plates were placed in an environmental chamber at 45°C and 15-30% RH for 12 hr. For neutralization, the films were soaked in 1 % NaOH solution and then washed with distilled water. After that the films were conditioned in the environmental chamber at 45°C and 15-30% RH until the films became dry. Finally, the films were stored at ambient condition.

3.2.2.2 Effect of chitosan content on the properties of films

Chitosan films were dissolved in 1% lactic acid to become 0.50, 1.00, 1.50, 2.00, 2.50 and 3.00 % w/v. 125, 150 a 175 ml of each chitosan solutions were taken. The properties of these films were determined by using the method in Table 3.4. The appropriate chitosan content was selected.

3.2.2.3 Effect of types and concentrations of acids

The appropriated chitosan content obtained from previous section was dissolved in 100 ml acid solutions, that is, citric acid, lactic acid and propionic acid. For lactic and propionic acids, 1% and 5% solution were prepared while 2% and 5% solution were prepared for citric acid. The mixtures were mixed for 24 hr and then filtrated with muslin cloth. The filtered solutions were poured on 20x25 cm. plastic plate and prepared the films using the method in section 3.2.2.1. The properties of film were determined according to Table 3.4.

3.2.2.4 The appropriate gelatin concentration

Gelatin was added in the filtered solutions obtained from previous section with 3 ratios (1:1, 1:2 and 1:3) and then mixed at 45°C for 24 hr. After that the solutions were poured on 20x25 cm. plastic plate and prepared the films using the method in section 3.2.2.1. The properties of film were determined according to Table 3.4 The appropriate gelatin ratio was selected.

3.2.2.5 Effects of plasticizer

The plasticizers consisted of glycerol and sorbitol was added into the filtered solutions that contain the appropriate gelatin that obtained in section 3.2.2.4. The concentrations of plasticizers were 0.25 and 0.5 ml/g chitosan. Then the solutions were mixed and poured on the 20x25 cm. plastic plate. The chitosan films were prepared using method in secgtion 3.2.2.1 and the properties of these films were determined according to Table 3.4.

Table 3.4 Determination methods of film’s mechanical and physical properties (ASTM, 1990)

Properties	Analysis instruments or methods	References
Tensile strength	Tensile testing machine	ASTM D-822 (2001)
Elongation at break	Tensile testing machine	ASTM D-822 (2001)
Thickness	Thickness tester	ASTM D-645M-97 (2001)
Tearing resistance	Tearing resistance tester	ASTM D: 5734 (2001)
Water vapor permeable	Water vapor permeable	ASTM D: 96 (2001)

3.2.2.6 Comparison between chitosan films and PE cling film

The mechanical properties of chitosan-gelatin films obtained from section 3.2.2.5 were compared with PE cling film.

3.3. Data analysis

All physical properties measurement of chitosan film was repeated six times. The mean and standard deviation were calculated. The differences among them were compared using analysis of variance (ANOVA). When F-ratios of data were statistically different at level 0.05, the average differences in each film type were calculated using Duncan new multiple range test (DMRT). All of the statistical analysis was calculated with computer program SPSS for window.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Chitosan Production

For the present study, chitosan extracted from Black tiger shrimp waste from the frozen food industry was used for the production of biodegradable film. The results were as follows:

4.1.1 Properties of shrimp waste

The properties of shell and head of black tiger shrimp in terms of examined chemical components, i.e. protein, ash, fat and moisture were shown in Table 4.1. Protein content was found to be the main component of shrimp shell and head similar to what was also found in several studies, including Whaiprip (1991), Benjakul and Sophanodora (1993), Srikumlaithong et.al. (1998), Wiratthikowit (2001). Ash content, the other major component, meanwhile, accounted for 25.7 % of the total amount of shell and head, similar to the results of the study by Benjakul (1993) and Whaiprip (1991).

Table 4.1 Physical and chemical properties of black tiger shrimp shell and head

Physical and chemical properties	Whaiprip (1991)	Benjakul and Sophanodora (1993)	Srikumlaithong et.al. (1998)	Wiratthikowit (2001)	Present study
Protein (%)	48.25	42.8	48.1	35.8	42.1
Fat (%)	0.58	1.2	1.7	0.6	0.9
Ash (%)	29.30	20.8	25.4	38.1	25.7
Moisture (%)	7.66	9.7	10.1	-	8.7

4.1.2 Chitosan Properties

The physical and chemical properties of chitosan prepared from black tiger shrimp crust were presented in Table 4.2. The produced chitosan contained 6.83 % of moisture and 0.25% of ash similar to those of Srikumlaithong et.al. (1998).

Comparable to previous reports (Filar and Wirick, 1978; Srikumlaithong et.al, 1998), viscosity of chitosan solution in the present study was quite low. This phenomenon was probably the results of different demineralization and deacetylation processes employed (Moorjani et.al, 1978; Madhavan and Ramachandrannair, 1974)

Table 4.2 Physical and chemical properties of the prepared chitosan

Physical and chemical properties	Srikumlaithong et.al. (1998)	Nopparat (1998)	Wiratthikowit (2001)	Present study
Nitrogen (%)	7.2	7.28	6.52	6.34
Fat (%)	-	-	0.11	0.1
Ash (%)	0.26	0.02	0.21	0.25
Moisture (%)	9.23	5.77	8.97	6.85
Viscosity (cPs)	567	521	172	230
Deacetylation (%)	82.3	97.20	87	86.7

Chitosan with a high degree of deacetylation could dissolve easily in a diluted organic acid. However, high degree of deacetylation might result in shorter polymer lines, which would lead to reduction in viscosity of chitosan solution. This could affect considerably to film properties (Averbach, 1978). For the present study, chitosan samples were demineralized in 1.0 M hydrochloric acid for 24 hrs. The maximum deacetylation observed in this study was 86 %, which could be rated as medium degree of deacetylation, which fell in a medium range as classified by Filar and Wirick's (1978). It dissolved completely in 1% acetic acid.

4.2 Chitosan film preparations

Effects of several parameters on chitosan film preparation were determined. The results were as follows:

4.2.1 Effects of chitosan content on viscosity of prepared films

Wiratthikowit (2001) implied that the degree of deacetylation of chitosan affected its viscosity, as well as its film formation. However, degree of deacetylation was not the only factor influence viscosity of chitosan solution, another important factor that might be considered was the chitosan content.

In order to determine suitable amount of chitosan that produced chitosan solution with acceptable viscosity for film formation, effects of varying chitosan concentrations on its viscosity were determined. The results showed that increasing amount of chitosan significantly enhanced the solution’s viscosity (Figure 1). The viscosity obtained from 0.5 to 3.0 % chitosan solution was between 230- 2,900 centipoises, which represented medium to high viscosity range (Filar and Wirick, 1978).

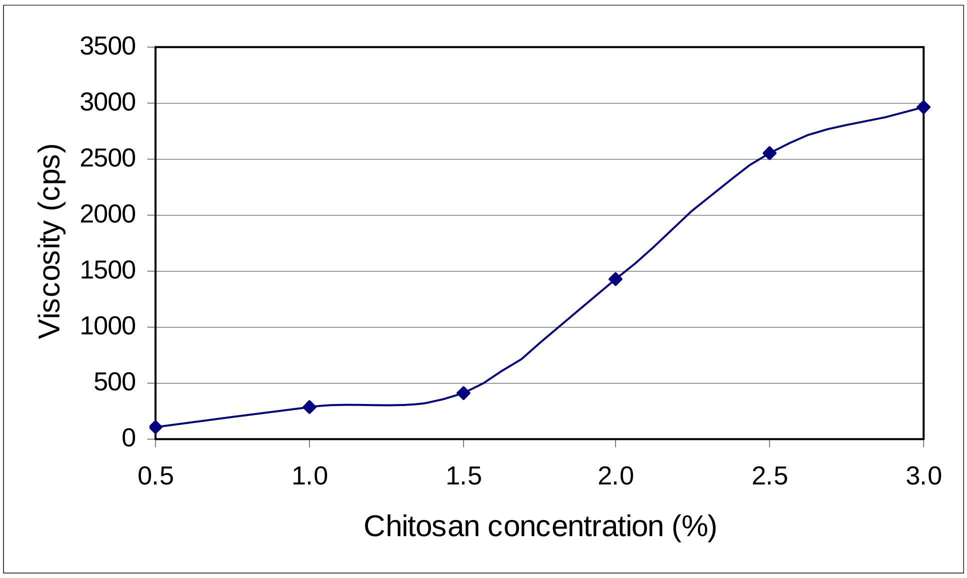

Figure 4.1 Relationship between varying concentrations of chitosan solution and their viscosity

4.2.2 Effects of chitosan content on film properties

The important factors that influence the chitosan film properties were chitosan concentration and volume of chitosan solution. Effects of varying concentrations and volume of chitosan solution on film properties were, thus, observed. For this study, the chitosan solutions were prepared from 1% concentrated lactic acid. Three different concentrations of chitosan solution, including 125, 150 and 175 ml were utilized.

The appearance of the prepared chitosan films such as coarseness, shine, toughness and acid smell were observed (Table 4.3). The film's surface exposed to the air was coarse, while the reverse surface touching plastic was smooth. The films were tough, hard to tear, and was flexible to a certain extent. With greater chitosan concentration, the film's hardness tended to increase. Their flexibility decreased. When dissolved into water the film would gradually dissolve and extend like glue because free $-NH^{3+}$ group capture the ion of water (Muzzareli, 1977). Due to their water soluble property, the chitosan film was needed to be neutralized to prevent it from being water-soluble and, at the same time, to help reduce its acidic smell. As by-product from neutralization process water molecules were then eliminated. This neutralization decreased the hydrophilic groups, thus decreased the percentage of water solubility (Kubota et al., 1993). Table 4.4 showed that after neutralization, characteristics of the films, which were not now water-soluble. Their air-exposed surface became smoother, while reverse surfaces contacting the plastic remained smooth and shining; and their acidic smell is either minimal or none. The other characteristics, meanwhile, remain unchanged. These results were similar to Ritthidej et.al., (2002), which reported that neutralization and drying of the film could reduced the acidic smell of the film but not change other properties of films.

Table 4.3 Characteristics of chitosan film before neutralization

Chitosan film		**Characteristics**					
Chitosan concentration (%)	**Volume of chitosan solution** (ml)	**Transparency**	**Acid smell**	**Flexibility**	**Coarseness of film surface**		**Water solubility**
					Exposed to air	**Contacting plastic**	
0.5	125	/	S1	F4	/	-	/
	150	/	S1	F4	/	-	/
	175	/	S1	F4	/	-	/
1.0	125	/	S1	F4	/	-	/
	150	/	S1	F4	/	-	/
	175	/	S1	F3	/	-	/
1.5	125	/	S1	F3	/	-	/
	150	/	S1	F3	/	-	/
	175	/	S1	F2	/	-	/

Notes:

/ = Showing characteristics

- = Not showing characteristics
S = Acid smell (S1<S2<S3<S4<S5)
F = Flexibility (F1<F2<F3<F4<F5)

Table 4.3 Characteristics of chitosan film before neutralization (continued)

Chitosan film		**Characteristics**					
					Coarseness of film surface		
Chitosan concentration (%)	**Volume of chitosan solution** (ml)	**Transparency**	**Acid Smell**	**Flexibility**	**Exposed to air**	**Contacting plastic**	**Water solubility**
2.0	125	/	S1	F2	/	-	/
	150	/	S1	F2	/	-	/
	175	/	S1	F1	/	-	/
2.5	125	/	S1	F1	/	-	/
	150	/	S1	F1	/	-	/
	175	/	S1	F1	/	-	/
3.0	125	/	S1	F1	/	-	/
	150	/	S1	F1	/	-	/
	175	/	S1	F1	/	-	/

Notes:

/ = Showing characteristics

- = Not showing characteristics
S = Acid smell (S1<S2<S3<S4<S5)
F = Flexibility (F1<F2<F3<F4<F5)

Table 4.4 Characteristics of chitosan film after neutralization(continued)

Chitosan film		**Characteristics**					
Chitosan concentration (%)	**Volume of chitosan solution** (ml)	**Transparency**	**Acid smell**	**Flexibility**	**Coarseness of film surface**		**Water solubility**
					Exposed to air	**Contacting plastic**	
0.5	125	/	-	F2	-	-	-
	150	/	-	F2	-	-	-
	175	/	-	F2	-	-	-
1.0	125	/	-	F1	-	-	-
	150	/	-	F1	-	-	-
	175	/	-	F1	-	-	-
1.5	125	/	-	F1	-	-	-
	150	/	-	F1	-	-	-
	175	/	-	F1	-	-	-

Notes:

/ = Showing characteristics

- = Not showing characteristics
S = Acid smell (S1<S2<S3<S4<S5)
F = Flexibility (F1<F2<F3<F4<F5)

Table 4.4 Characteristics of chitosan film after neutralization (continued)

Chitosan film		**Characteristics**					
					Coarseness of film surface		
Chitosan concentration (%)	**Volume of chitosan solution** (ml)	**Transparency**	**Acid smell**	**Flexibility**	**Exposed to air**	**Contacting plastic**	**Water solubility**
2.0	125	/	-	F1	-	-	-
	150	/	-	F1	-	-	-
	175	/	-	F1	-	-	-
2.5	125	/	-	F1	-	-	-
	150	/	-	F1	-	-	-
	175	/	-	F1	-	-	-
3.0	125	/	-	F1	-	-	-
	150	/	-	F1	-	-	-
	175	/	-	F1	-	-	-

Notes:

/ = Showing characteristics

- = Not showing characteristics
S = Acid smell (S1<S2<S3<S4<S5)
F = Flexibility (F1<F2<F3<F4<F5)

Effects of varying chitosan contents on chitosan film's properties at were shown in Figure 4.2-4.8. It was found that increasing chitosan content in the solution led to significant increase in the films' thickness, weight, tensile strength, tearing resistance and water vapor permeable (WVP)($p<0.05$), while a slight, insignificant change in their elongation at break ($p>0.05$). The results were similar to what was reported in other previous studies (Nopparat, 1998; Wiratthikowit, 2001; and Muzzareli,1977).

Banker et.al. (1966), as well as Park and Chinnan (1996) also reported that the water vapor permeable constants of hydrophilic films increased linearly with film thickness over rang of thickness studies. However, the relationship between the film's thickness and its elongation at break, and between its tensile strength and elongation at break tended to decrease along with the increase in its thickness and tensile strength.

In the present study, it showed that chitosan film containing more or less than 1% of chitosan yielded either too soft or too hard product. Chitosan solution containing 1.0% chitosan with the volume of 150 ml could be used for preparing a suitable film for further experiments. It had good film properties, including high tensile strength, clear, thin and medium viscosity.

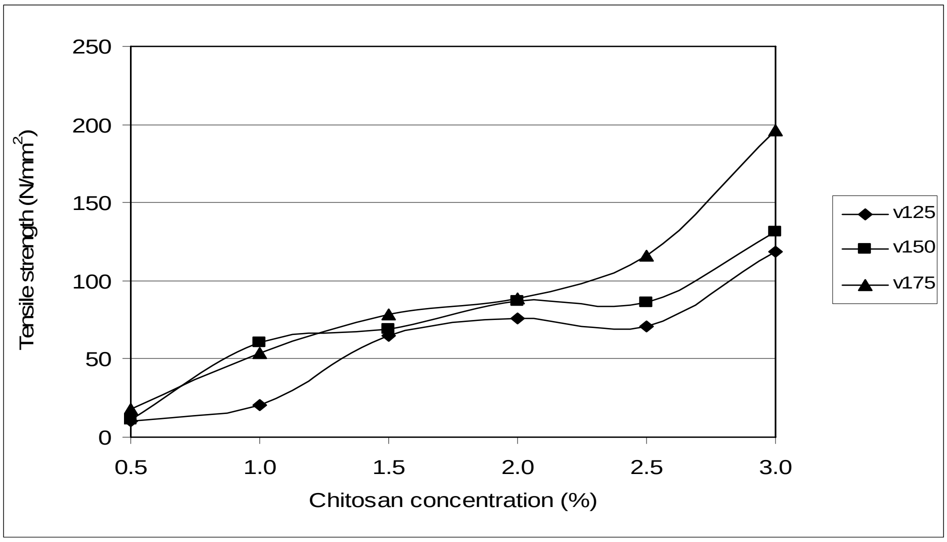

Figure 4.2 Relationship between chitosan concentration (%) and tensile strength (N/mm^2) (v125 = 125 ml of chitosan solution, v150 = 150 ml of chitosan solution, v175 = 175 ml of chitosan solution)

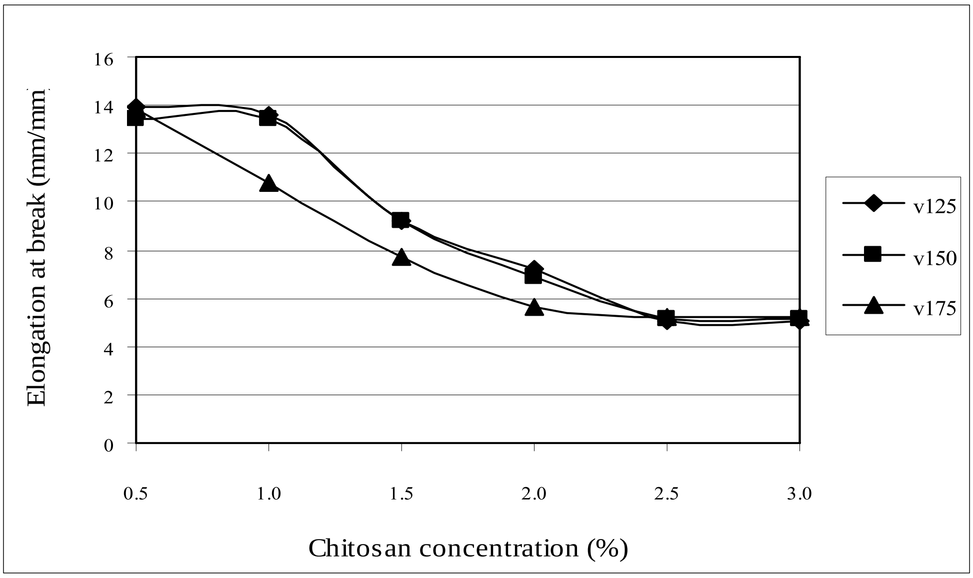

Figure 4.3 Relationship between chitosan concentration (%) and elongation at break. (mm/mm) (v125 = 125 ml of chitosan solution, v150 = 150 ml of chitosan solution, v175 = 175 ml of chitosan solution)

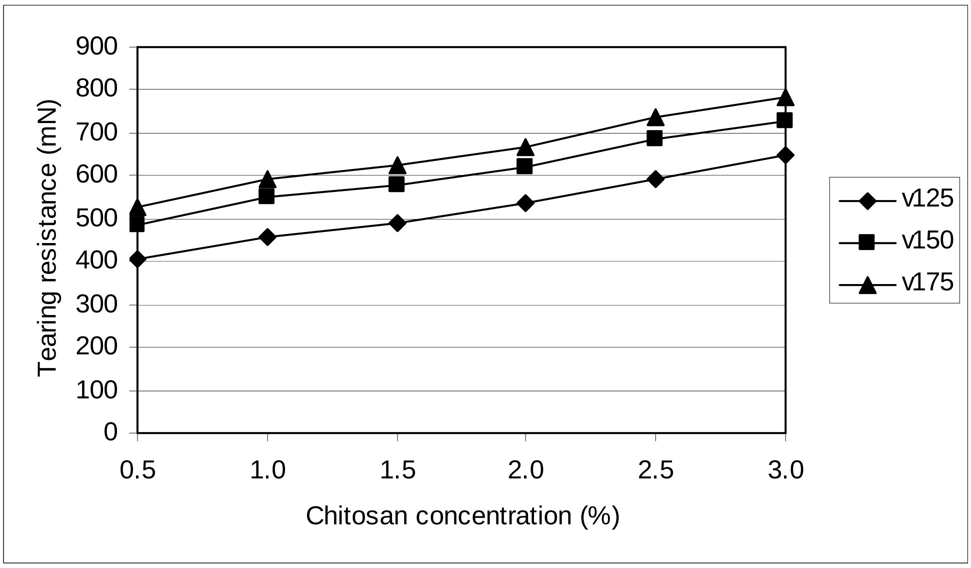

Figure 4.4 Relationship between chitosan concentration (%) and tearing resistance (mN) (v125 = 125 ml of chitosan solution, v150 = 150 ml of chitosan solution, v175 = 175 ml of chitosan solution)

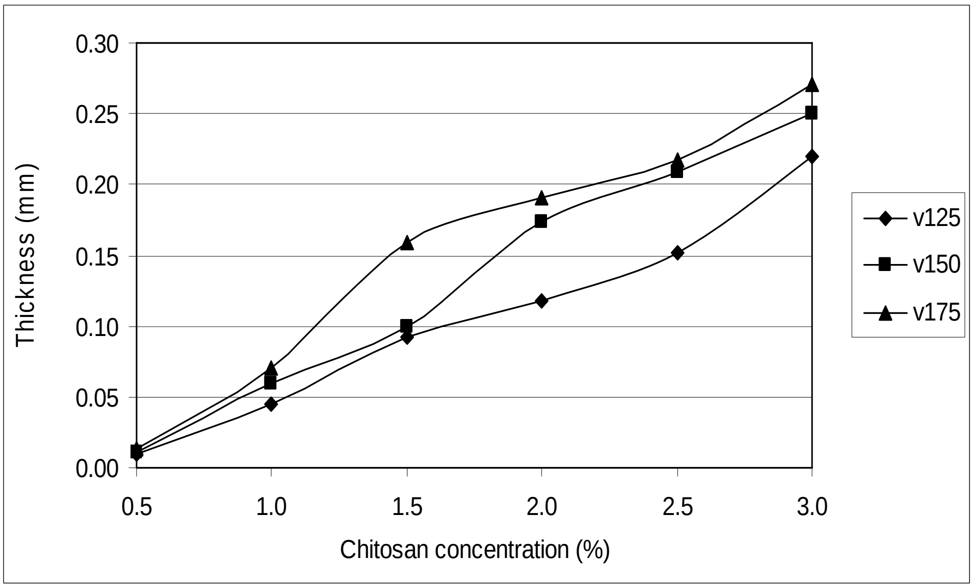

Figure 4.5 Relationship between chitosan concentration (%) and thickness (mm) (v125 = 125 ml of chitosan solution, v150 = 150 ml of chitosan solution, v175 = 175 ml of chitosan solution)

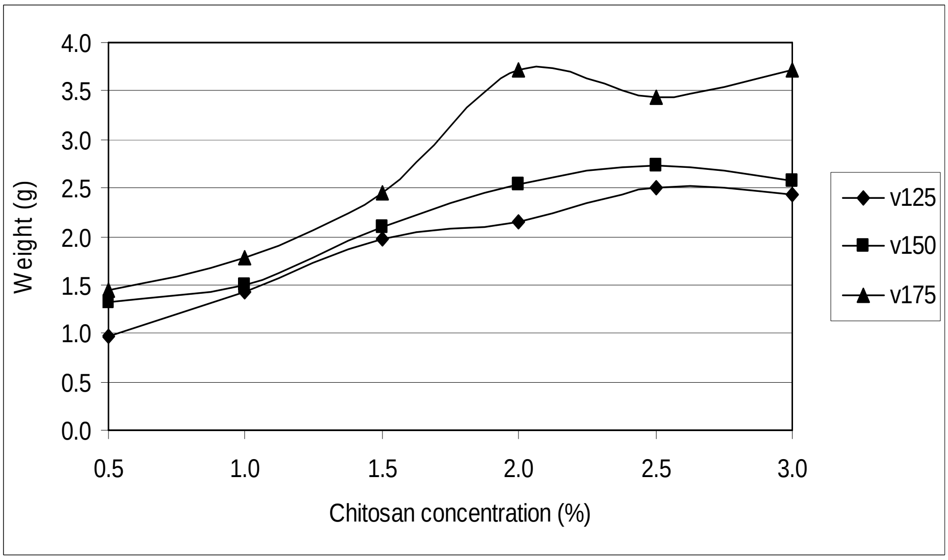

Figure 4.6 Relationship between chitosan concentration (%) and weight (g) (v125 = 125 ml of chitosan solution, v150 = 150 ml of chitosan solution, v175 = 175 ml of chitosan solution)

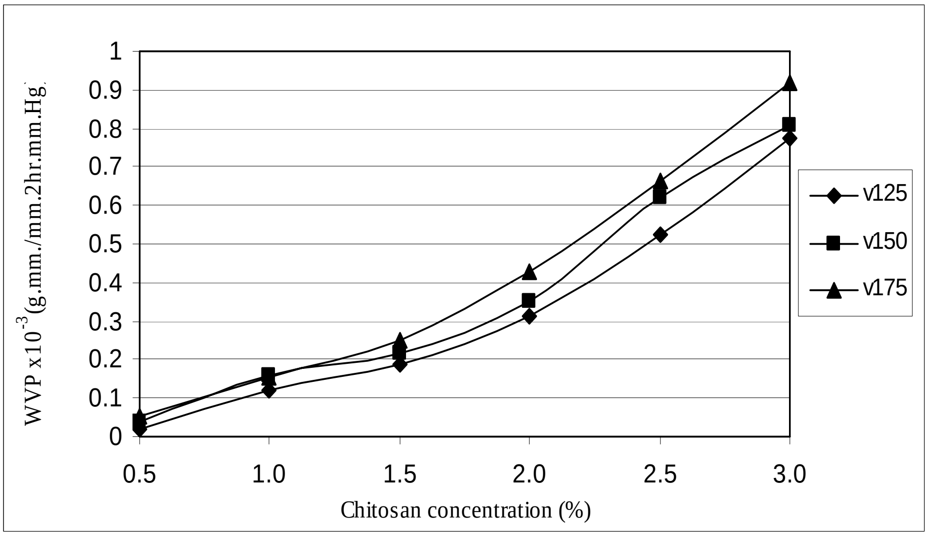

Figure 4.7 Relationship between chitosan concentration (%) and water vapor permeable. (g.mm/mm^2.hr.mmHg) (v125 = 125 ml of chitosan solution, v150 = 150 ml of chitosan solution, v175 = 175 ml of chitosan solution)

4.2.3 Effects of organic acids on properties of chitosan film.

Muzzareli (1977) reported that chitosan could dissolve in various organic acids and yielded films with different properties. Therefore, effects of different concentrations of lactic acid, citric acid and propionic acid, i.e. 1 and 5% lactic and propionic acids; 2 and 5% citric acid of 3 organic acids, on properties of films were determined. Physical characteristics of the film, i.e. coarseness of film surface, shining, toughness and residue acid smell were observed and presented in Table 4.5.

The prepared films were transparent, tough and flexible to a certain degree. Since the films were water soluble, neutralization of the film were required. After neutralization, the films were no longer water soluble (Table 4.6). The surface of the films also were smoother, especially the side that expose to air, while other side contacting plastic cast remained smooth and shining. The acidic smell was very slight or none discernable. The flexibility of the films changed. They became harder due to water lost resulting from reaction with NaOH solution (Ritthidej et.al., 2002).

Table 4.5 Characteristics of chitosan film prepared from lactic, propionic and citric acids before neutralization

Chitosan film		**Characteristics**					
Acid	**Concentration** (%)	**Transparency**	**Acid smell**	**Flexibility**	**Coarseness of film surface**		**Water solubility**
					Exposed to air	**Contacting plastic**	
Lactic	1 5	/ /	S2 S3	F2 F2	/ /	- - -	/ /
Propionic	1 5	/ /	S4 S5	F1 F1	/ /	- - -	/ /
Citric	2 5	/ /	- -	- -	/ /	- - -	/ /

Notes:

/ = Showing characteristics

- = Not showing characteristics
S = Acid smell (S1<S2<S3<S4<S5)
F = Flexibility (F1<F2<F3<F4<F5)

Table 4.6 Characteristics of chitosan film prepared from lactic, propionic and citric acids after neutralization

Chitosan film		**Characteristics**					
Acid	**Concentration** (%)	**Transparency**	**Acid smell**	**Flexibility**	**Coarseness of film surface**		**Water solubilty**
					Exposed to air	**Contacting plastic**	
Lactic	1 5	/ /	- -	F1 F1	- -	- - -	- -
Propionic	1 5	/ /	- -	F1 F1	- -	- - -	- -
Citric	2 5	/ /	- -	- -	- -	- - -	- -

Notes:

/ = Showing characteristics

\- = Not showing characteristics
S = Acid smell (S1<S2<S3<S4<S5)
F = Flexibility (F1<F2<F3<F4<F5)

The results showed that chitosan films prepared from citric acid were not transparent and quite brittle. Ritthidej et.al. (2002) indicated that the chitosan-citrate film was amorphous form. Its brittleness characteristic was likely due to the solid state of the acid being used. Regarding the acidic smell, the film obtained from propionic acid showed the strongest acidic smell, followed by that obtained from lactic and citric acids, respectively. After neutralization, acidic smell of all the films was disappearing. The flexibility of lactic and citric films was reduced. The coarseness of all the films was reduced or disappeared. The films also lost their water solubility.

The film's mechanical properties obtained from different acids at different concentrations were shown in Table 4.7. The tearing resistance of film prepared from 1%propionic acid yielded a maximum value of 662.5 mN, followed by that of films prepared from 5% propionic acid yielding 635 mN and that of films prepared from 5%citric acid yielding the lowest tearing resistance of 451.25 mN. The film prepared from 5% lactic acid had the highest tensile strength of 63.252 N/mm^2, followed by that prepared from 5% propionic acid (tensile strength = 61.141 N/mm^2) and that prepared from 2% citric acid (tensile strength = 20.961 N/mm^2). In terms of elongation at break, the film prepared from 5% lactic acid had the greatest elongation at break of 13.55 mm/mm, while films prepared from 2% citric acid showed the lowest value, i.e. 8.945 mm/mm. This finding was similar to that was reported in the study of Park, et.al. (2002), as well as Caner et.al. (1998).

The films' thickness ranged from 0.027-0.059 mm, the thinnest film, 0.027 mm, was prepared from 5% lactic acid. The films' weight ranged from 1.2175-1.975 g. The film prepared from 5% lactic acid was the lightest. Each film weighted 1.2175 g. Water vapor permeability of the prepared films tended to decrease in relation to increasing acid concentrations. Films prepared from citric acid yielded the lowest mechanical properties, including tearing resistance, tensile strength and elongation at break. This was probably due to hydrolysis reaction between the acid and chitosan chains, which resulted to inferior mechanical properties than those of films prepared from lactic and propionic acids (Ritthidej et.al., 2002).

Table 4.7 Properties of chitosan film prepared from varying concentrations of lactic, propionic and citric acids

Chitosan film		Properties					
Acid	**Concentration**	**Tearing Resistance (mN)**	**Tensile Strength (N/mm^2)**	**Elongation at break (mm/mm)**	**Thickness (mm)**	**Weight (g)**	**WVP 10^{-3} ($g.mm/mm^2.hr.mm.Hg$)**
Lactic	1	556.25±4.97a	60.583± 0.30a	13.40± 0.56a	0.059±0.005a	1.502±0.00a	0.205±0.0002a
	5	548.75±7.42a	63.252± 0.01a	13.55± 0.02a	0.027±0.0005b	1.217±0.02b	0.067±00.0001b
Propionic	1	662.50±7.25a	44.660± 0.13b	12.51± 0.04a	0.053±0.0008a	1.287±0.00b	0.165±0.0012a
	5	635.00± 4.22b	61.141± 0.03a	12.80± 0.02a	0.031±0.0008b	1.452±0.04a	0.089±0.0002b
Citric	2	442.50± 5.90a	20.962± 0.09b	8.94± 0.01b	0.044±0.001a	1.911±0.01a	0.167±0.0006a
	5	451.25± 7.42a	30.083±0.02 a	9.171±0.10 a	0.033±0.005b	1.975±0.07a	0.114±0.0001b

*The same letter means no significant difference at 95% confidence limit

4.2.4 Effects of gelatin on properties of chitosan film

The effect of gelatin on the strength of chitosan film was conducted by adding varying amount of gelatin to chitosan solution. Chitosan solutions were prepared from lactic, citric and propionic acids at different concentrations (1 and 5% in lactic and propionic acids, and 2 and 5% in citric acid). Gelatin was added to each chitosan solution to produce film with three different chitosan: gelatin ratios, i.e. 1:1, 1:2 and 1:3, respectively. The main characteristics, including film transparency, odor, flexibility, coarseness, and water solubility of chitosan-gelatin film prepared from lactic, propionic and citric acid were shown in Table 4.8, 4.9 and 4.10, respectively.

Lactic and propionic acids yielded transparent, light to dark yellow films, depending on type and concentration of acid, as well as chitosan:gelatin ratio but citric acid resulted to turbid white film at all chitosan:gelatin ratio. Because the chitosan citrate film was amorphous form and its brittle which likely due to the solid stare of the acid used (Ritthidej et.al., 2002).

The chitosan-gelatin film texture was tough and hard to tear, flexible to a certain degree. The films were water soluble and showed slight acidic smell. This indicated hydrophilic properties of the film. Therefore, film neutralization was required. Characteristics of the prepared films after neutralization were shown in Table 4.9, 4.10 and 4.11. It was found that the neutralized film was no longer water soluble. The air-exposed surface of the films became smoother, while its reverse side contacting plastic cast remained smooth and shining, its acidic smell was minimal or none at all. The film flexibility was also changed.

The film produced from propionic acid showed the strongest acidic smell, followed by the one prepared from lactic and citric acids, respectively. Lactic film was found to be the most flexible, followed by propionic film and citric film, respectively.

Table 4.8 Characteristics of chitosan-gelatin film prepared from lactic acid before and after neutralization

Chitosan film			Characteristics					
Neutralization	**Concentration (%)**	**Chitosan: Gelatin ratio**	**Transparency**	**Acid smell**	**Flexibility**	**Coarseness of film surface**		**Water solubility**
						Exposed to air	**Contacting plastic**	
Before	1	1:1	/	S1	F1	/	-	/
		1:2	/	S1	F2	/	-	/
		1:3	/	S1	F3	/	-	/
	5	1:1	/	S1	F3	/	-	/
		1:2	/	S1	F4	/	-	/
		1:3	/	S1	F5	/	-	/
After	1	1:1	/	-	F1	-	-	-
		1:2	/	-	F2	-	-	-
		1:3	/	-	F2	-	-	-
	5	1:1	/	-	F2	-	-	-
		1:2	/	-	F2	-	-	-
		1:3	/	-	F2	-	-	-

Notes:

/ = Showing characteristics

\- = Not showing characteristics
S = smell (S1<S2<S3<S4<S5)
F = Flexibility (F1<F2<F3<F4<F5)

Table 4.9 Characteristics of chitosan-gelatin film prepared from propionic acid before and after neutralization

Chitosan film			Characteristics					
Neutralization	**Concentration (%)**	**Chitosan: Gelatin ratio**	**Transparency**	**Acid smell**	**Flexibility**	**Coarseness of film surface**		**Water solubility**
						Exposed To air	**Contacting Plastic**	
Before	1	1:1	/	S3	F1	/	-	/
		1:2	/	S3	F2	/	-	/
		1:3	/	S3	F3	/	-	/
	5	1:1	/	S5	F3	/	-	/
		1:2	/	S4	F4	/	-	/
		1:3	/	S3	F5	/	-	/
After	1	1:1	/	-	F1	-	-	-
		1:2	/	-	F1	-	-	-
		1:3	/	-	F1	-	-	-
	5	1:1	/	-	F2	-	-	-
		1:2	/	-	F2	-	-	-
		1:3	/	-	F2	-	-	-

Notes:

/ = Showing characteristics

- = Not showing characteristics
S = Acid smell (S1<S2<S3<S4<S5)
F = Flexibility (F1<F2<F3<F4<F5)

Table 4.10 Characteristics of chitosan-gelatin film prepared from citric acid before and after neutralization

Chitosan film			Characteristics					
Neutralization	Concentration (%)	Chitosan: Gelatin ratio	Transparency	Acid smell	Flexibility	Coarseness of film surface		Water solubility
						Exposed To air	Contacting Plastic	
Before	1	1:1	/	-	F1	/	-	/
		1:2	/	-	F1	/	-	/
		1:3	/	-	F1	/	-	/
	5	1:1	/	-	F1	/	-	/
		1:2	/	-	F1	/	-	/
		1:3	/	-	F1	/	-	/
After	1	1:1	/	-	F1	-	-	-
		1:2	/	-	F1	-	-	-
		1:3	/	-	F1	-	-	-
	5	1:1	/	-	F2	-	-	-
		1:2	/	-	F2	-	-	-
		1:3	/	-	F2	-	-	-

Notes:

/ = Showing characteristics

- = Not showing characteristics
S = Acid smell (S1<S2<S3<S4<S5)
F = Flexibility (F1<F2<F3<F4<F5)

After neutralization, the acidic smell disappeared and lactic film’s flexibility decreased because the protonate amino group and carboxylate ion were equilibrated to the free amine and free carboxylic acid (Ritthidej et.al., 2002). Water molecule was then eliminated as by-product. Citric film was fragile and easily breakable with the increase in the ratio of chitosan to gelatin. It was also found that film coarseness was undetectable and the film was not soluble in water.

In terms of mechanical properties, tensile strength, which proved the strength of the film, was found to be higher in the films prepared from chitosan-gelatin solution than the films prepared from chitosan solution only. The highest tensile strength was obtained from films prepared from 5% lactic acid with the chitosan : gelatin ratio of 1:1, followed by films prepared from 5% propionic acid with 1:1 chitosan : gelatin ratio. Such high tensile strength might result from the reaction between the molecules of chitosan and gelatin, leading to 3-D bondage, which made the film even stronger (Arvanitoyanis et al., 1998). However, the increase of gelatin in the ratio led to significant reduction ($p< 0.05$) in the films’ tensile strength. This could be explained by the fact that at the ratio over 1:1, gelatin could interfere with chitosan structure resulting to low tensile strength of the films. Arvanitoyanis et al. (1998) also indicated that the lower chitin : gelatin ratio and high temperature did not help increase the film’s strength In addition, it was also found that the film became stronger yellow in relation to the increase of gelatin in the ratio, making it unacceptable for utilization. Details of the chitosan-gelatin films’ tensile strength were shown in Figure 4.8.

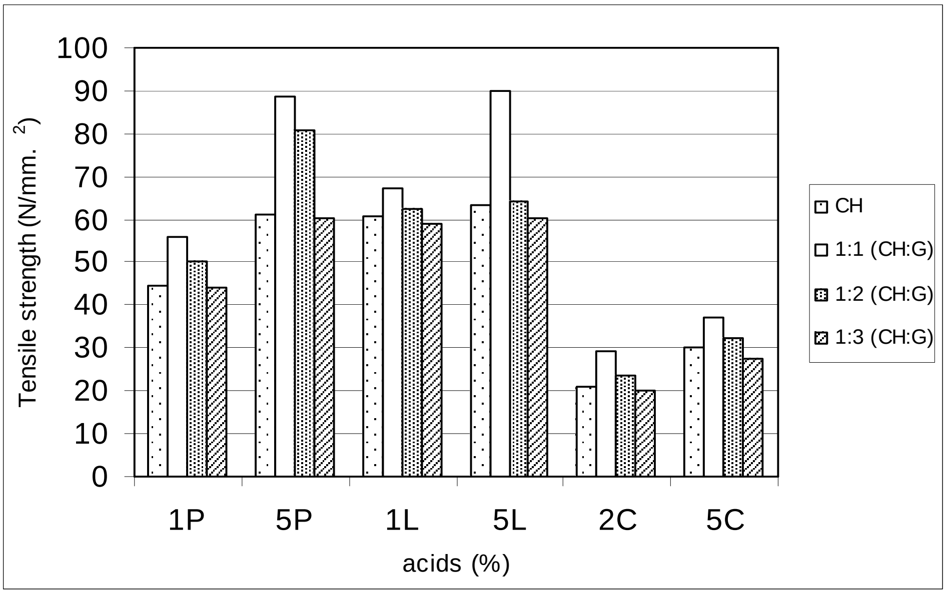

Figure 4.8 Relationship between different chitosan:gelatin ratios and tensile strength of the prepared film (CH = films contained 1 part chitosan; 1:1 CH:G = the ratio of chitosan:gelatin was 1:1; 1:2 CH:G = the ratio of chitosan:gelatin was 1:2; 1:3 CH:G = the ratio of chitosan:gelatin was 1:3; 1P = films prepared from 1% propionic acid; 5P = films prepared from 5% propionic acid; 1L = films prepared from 1% lactic acid; 5L = films prepared from 5% lactic acid; 2C = films prepared from 2% citric acid; 5C = films prepared from 5% citric acid)

Regarding elongation at break, the results showed that chitosan film's elongation at break decreased significantly ($p< 0.05$) in accordance with the increase of gelatin in the ratio of chitosan-gelatin film. Because of too high gelatin content, the film was fragile, crispy and easily breakable. The film prepared from 5% lactic acid with 1:1 chitosan to gelatin ratio yielded the highest elongation at break, followed by that prepared from 1% lactic acid with 1:1 chitosan to gelatin ratio. This might due to the fact that at this ratio chitosan could completely react with gelatin resulting to films with high tensile strength and elongation at break than any other ratio This finding was similar to what reported by Arvanitoyanis et al. (1998). The films prepared from lactic acid yielded higher elasticity than that prepared from the other two acids. It was also found that the films prepared from both concentrations of citric acid were fragile and readily breakable and yielded lower elongation at break than that produced from the other two acids with the same ratio of chitosan to gelatin. Details were shown in Figure 4.9.

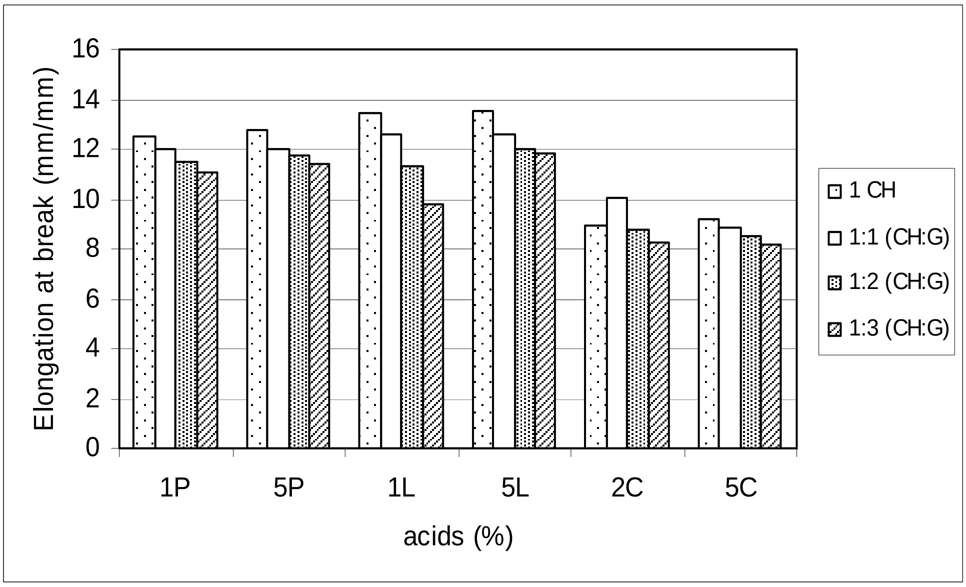

Figure 4.9 Relationship between different chitosan:gelatin ratios and elongation at break of the prepared film (CH = films contained 1 part chitosan; 1:1 CH:G = the ratio of chitosan:gelatin was 1:1; 1:2 CH:G = the ratio of chitosan:gelatin was 1:2; 1:3 CH:G = the ratio of chitosan:gelatin was 1:3; 1P = films prepared from 1% propionic acid; 5P = films prepared from 5% propionic acid; 1L = films prepared from 1% lactic acid; 5L = films prepared from 5% lactic acid; 2C = films prepared from 2% citric acid; 5C = films prepared from 5% citric acid)

Tearing resistance is another important properties used in indicating the film's strength through its resistance against tearing (James and Wilmer, 1991). For the present study, tearing resistance of each film with the size of 10 x 8 cm^2 was tested 6 times. It was found that the film prepared from 1% propionic acid with 1:1 ratio of chitosan to gelatin gave the highest tearing resistance, followed by that prepared from 5% propionic acid with the same chitosan to gelatin ratio (Figure 4.10). The film prepared from citric acid became crispier in relation to the increase of gelatin in the ratio, resulting in films with very low tearing resistance, especially when compared to the citric acid- film without gelatin.

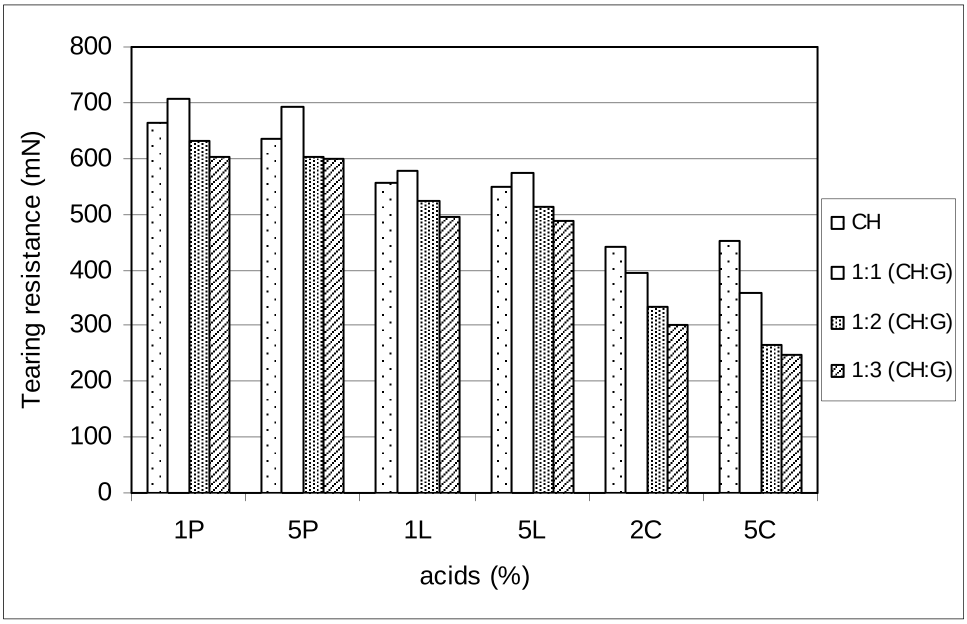

Figure 4.10 Relationship between different chitosan:gelatin ratios and tearing resistance of the prepared film (CH = films contained 1 part chitosan; 1:1 CH:G = the ratio of chitosan:gelatin was 1:1; 1:2 CH:G = the ratio of chitosan:gelatin was 1:2; 1:3 CH:G = the ratio of chitosan:gelatin was 1:3; 1P = films prepared from 1% propionic acid; 5P = films prepared from 5% propionic acid; 1L = films prepared from 1% lactic acid; 5L = films prepared from 5% lactic acid; 2C = films prepared from 2% citric acid; 5C = films prepared from 5% citric acid)

The chitosan-gelatin film’s thickness tended to increase significantly ($p < 0.05$) in accordance with increase in gelatin content (Figure 4.11). This finding agreed well with that of Wirathikowit (2001). The films’ thickness obtained from the present study ranged between 0.027-0.076 mm. The film prepared from 5% lactic acid with 1:1 of chitosan to gelatin ratio was the thinnest film, followed by that prepared from 5% propionic acid with similar chitosan to gelatin ratio.

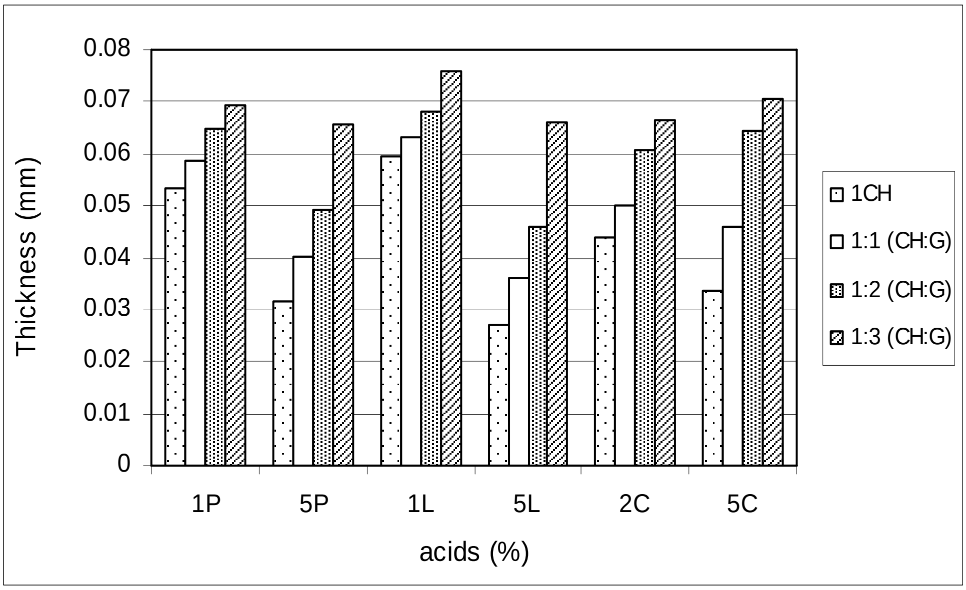

Figure 4.11 Relationship between different chitosan:gelatin ratios and thickness of the prepared film (CH = films contained 1 part chitosan; 1:1 CH:G = the ratio of chitosan:gelatin was 1:1; 1:2 CH:G = the ratio of chitosan:gelatin was 1:2; 1:3 CH:G = the ratio of chitosan:gelatin was 1:3; 1P = films prepared from 1% propionic acid; 5P = films prepared from 5% propionic acid; 1L = films prepared from 1% lactic acid; 5L = films prepared from 5% lactic acid; 2C = films prepared from 2% citric acid; 5C = films prepared from 5% citric acid)

Similar to thickness, the films' weight also increased significantly ($p<0.05$) in relation to the increase in gelatin content (Figure 4.12). The weight of each film ranged between 1.217-2.99 g. Each film prepared from 5% propionic acid with 1:3 chitosan to gelatin ratio was the heaviest film, followed by that prepared from 5% lactic acid with similar chitosan to gelatin ratio.

The chitosan-gelatin film's water vapor permeability also tended to increase along with the increase of gelatin content (Figure 4.13). This probably was the results of hydrophilic properties of chitosan and gelatin (Park and Chinnan, 1996). Higher gelatin content should also result to higher water vapor permeability.

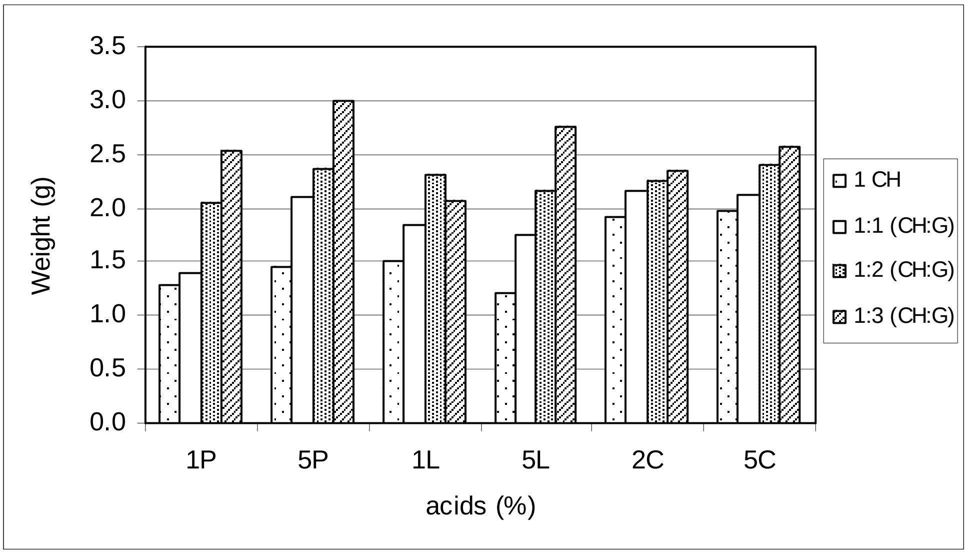

Figure 4.12 Relationship between different chitosan:gelatin ratios and weight of the prepared film (CH = films contained 1 part chitosan; 1:1 CH:G = the ratio of chitosan:gelatin was 1:1; 1:2 CH:G = the ratio of chitosan:gelatin was 1:2; 1:3 CH:G = the ratio of chitosan:gelatin was 1:3; 1P = films prepared from 1% propionic acid; 5P = films prepared from 5% propionic acid; 1L = films prepared from 1% lactic acid; 5L = films prepared from 5% lactic acid; 2C = films prepared from 2% citric acid; 5C = films prepared from 5% citric acid)

At the present stage a conclusion could be made that the ratio of chitosan to gelatin at 1:1 could yield the most satisfactory properties of the chitosan-gelatin films. Therefore, 1:1 chitosan and gelatin ratio was chosen for preparing film for later experiments. Since the films prepared from citric acid yielded unsatisfactory properties (not transparent and fragile) and very low mechanical properties (tearing resistance, tensile strength and elongation at break) comparable to films prepared from lactic and propionic acids, citric acid was not used for further experiments involving effects of plasticizer on film properties..

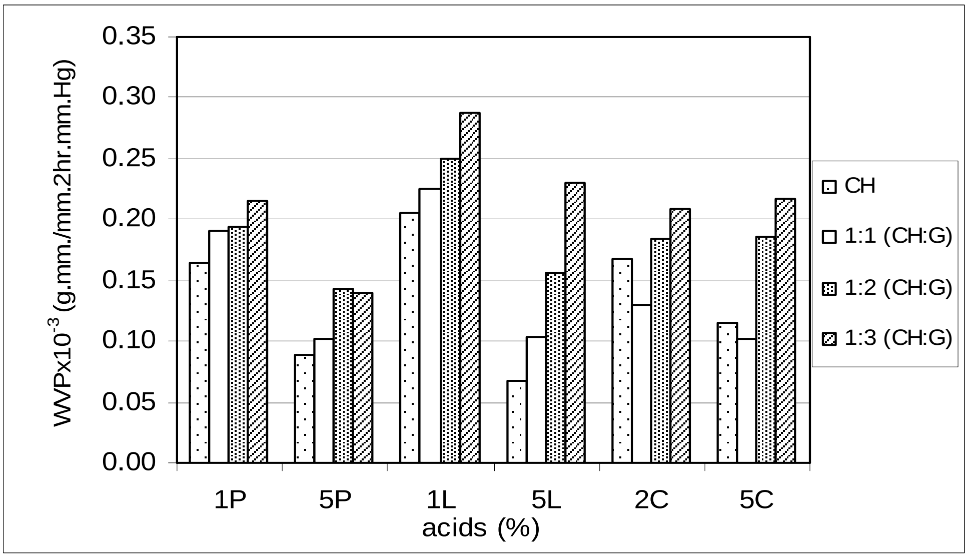

Figure 4.13 Relationship between different chitosan:gelatin ratios and water vapor permeable (WVP) of the film (1 CH means 1 part of chitosan; 1:1 CH:G means the ratio of chitosan to gelatin being 1:1; 1:2 CH:G means the ratio of chitosan to gelatin being 1:2; 1:3 CH:G means the ratio of chitosan to gelatin being 1:3; 4P means 1% propionic acid; 5P means 5% propionic acid; 1L means 1% lactic acid; 5L means 5% lactic acid; 2C means 2% citric acid; 5C means 5% citric acid)

4.2.5 Effects of plasticizer on properties of chitosan-gelatin film

Effects of two different plasticizers, glycerol and sorbitol, on properties of chitosan-gelatin (1:1) film prepared from 1 and 5% of 150 ml lactic, as well as propionic acids were determined (Table 4.11). The film was found to be transparent, slightly yellowish, tough and difficult to tear off. The film that contained plasticizer had smoother surface and were more flexible than that without it. Acid smell was noticeable, especially from the film that prepared from propionic acid. The film was also found to be water soluble.

After neutralized, the films prepared from both acids were not water soluble (Table 4.12). They were quite soft, flexible and easy to tear off. Both sides of the film, including side that exposed to air and to plastic surface, were smooth and glassy. Acid smell became non discernible. After heated dry in an oven, the films lost their moisture, as well as their flexibility.

The films' tensile strength tended to decrease as elongation at break and vapor permeability increased, especially with higher plasticizer concentration (Figure 4.14). As suggested by Lieberman and Gilbert (1973), a plasticizer reduces hydrogen bonding so that molecular space increased and as such leads to the decrease in films stiffness and the increase in their flexibility and vapor permeability. With the addition of 0.25 ml glycerol/g chitosan and 0.25 ml sorbitol/ g chitosan, the tensile strength of the films prepared from both acids decreased in relation to that of films without plasticizer. The results showed that the films containing 0.25 g of glycerol/ g chitosan that prepared from 5% lactic acid yielded the highest tensile strength, followed by the films containing 0.25g of sorbitol/ g chitosan that prepared also from 5% lactic acid, while the films containing 0.5g sorbitol/ g chitosan that prepared from 1% propionic acid yielded the lowest tensile strength.

Table 4.11 Characteristic of chitosan-gelatin film (1:1) containing plasticizer that prepared from lactic and propionic acid before neutralization

Acid	**Concentration (%)**	**Chitosan: Gelatin ratio**	**Plasticizer**	**Characteristics**					
				Transparency	**Acid smell**	**Flexibility**	**Coarseness of film surface**		**Water solubility**
							Exposed to air	**Contacting plastic**	
Lactic	**1**	**1:1**	**Glycerol**	/	S1	F3	/	-	/
		1:1	**Sorbitol**	/	S1	F4	/	-	/
	5	**1:1**	**Glycerol**	/	S2	F4	/	-	/
		1:1	**Sorbitol**	/	S2	F5	/	-	/
Propionic	**1**	**1:1**	**Glycerol**	/	S4	F2	/	-	/
		1:1	**Sorbitol**	/	S4	F3	/	-	/
	5	**1:1**	**Glycerol**	/	S5	F3	/	-	/
		1:1	**Sorbitol**	/	S5	F4	/	-	/

Notes:

/ = Showing characteristics

- = Not showing characteristics
S = Acid smell (S1<S2<S3<S4<S5)
F = Flexibility (F1<F2<F3<F4<F5)

Table 4.12 Characteristic of chitosan-gelatin film (1:1) containing plasticizer that prepared from lactic and propionic acid after neutralization

Acid	Concentration (%)	Chitosan: Gelatin ratio	Plasticizer	Characteristics					
				Transparency	Acid smell	Flexibility	Coarseness of film surface		Water solubility
							Exposed to air	Contacting plastic	
Lactic	1	1:1	Glycerol	/	-	F2	-	-	-
		1:1	Sorbitol	/	-	F3	-	-	-
	5	1:1	Glycerol	/	-	F3	-	-	-
		1:1	Sorbitol	/	-	F3	-	-	-
Propionic	1	1:1	Glycerol	/	-	F2	-	-	-
		1:1	Sorbitol	/	-	F1	-	-	-
	5	1:1	Glycerol	/	-	F3	-	-	-
		1:1	Sorbitol	/	-	F2	-	-	-

Notes:

/ = Showing characteristics

\- = Not showing characteristics
S = Acid smell (S1<S2<S3<S4<S5)
F = Flexibility (F1<F2<F3<F4<F5)

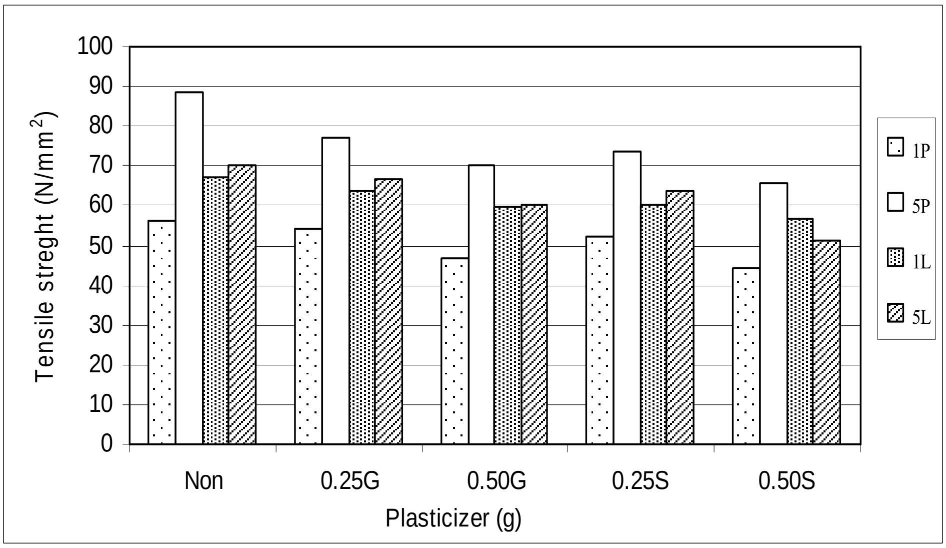

Figure 4.14 Effects of plasticizer on tensile strength of chitosan-gelatin film (1:1) (Non = without plasticizer; 0.25G = 0.25 g glycerol/ g chitosan; 0.50G = 0.50 g glycerol/ g chitosan; 0.25S = 0.25 g sorbitol/ g chitosan; 0.50S = 0.50 g sorbitol/ g chitosan; 1P = 1% propionic acid; 5P = 5% propionic acid; 1L =1% lactic acid; 5L = 5% lactic acid)

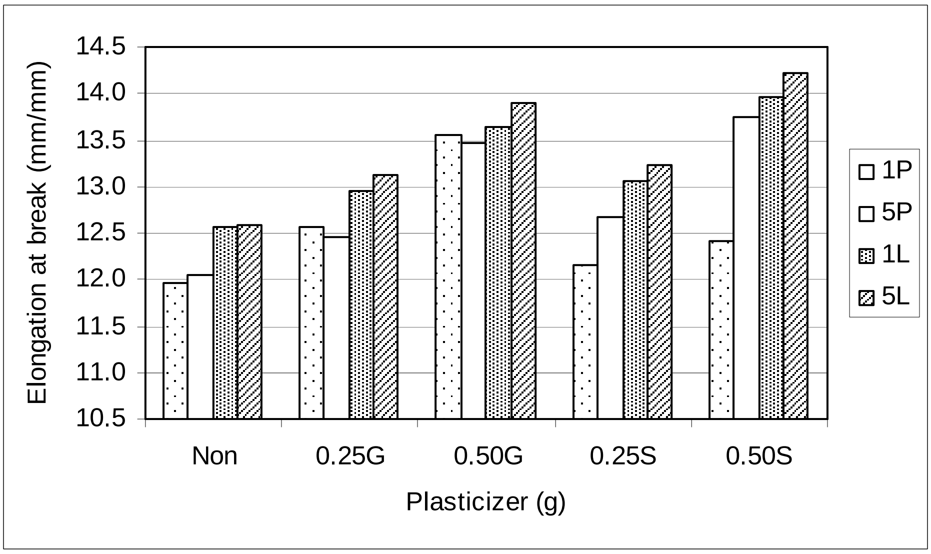

Figure 4.15 Effects of plasticizer on elongation at break of chitosan-gelatin film (1:1) (Non = without plasticizer; 0.25G = 0.25 g glycerol/ g chitosan; 0.50G = 0.50 g glycerol/ g chitosan; 0.25S = 0.25 g sorbitol/ g chitosan; 0.50S = 0.50 g sorbitol/ g chitosan; 1P = 1% propionic acid; 5P = 5% propionic acid; 1L =1% lactic acid; 5L = 5% lactic acid)

The elongation at break of chitosan-gelatin film tended to increase in relation to the increase in the amount of added plasticizer (Figure 4.15). This could explain from the ability of plasticizers that reduces the molecular force, which then leaves wider molecular space in the film (Banker, 1966; Arvanitoyannis and Biliaderis. 1999). As a result, the films become more elastic but not as strong as those without plasticizer. Chitosan-gelatin films containing 0.50 g sorbitol/ g chitosan that were prepared from 5% lactic acid showed the highest degree of the elongation at break, following by films containing 0.50 g sorbitol/ g chitosan that were prepared from 1% lactic acid. Films containing 0.25g sorbitol/ g chitosan that prepared from 1% propionic acid yielded the lowest degree of elongation at break.

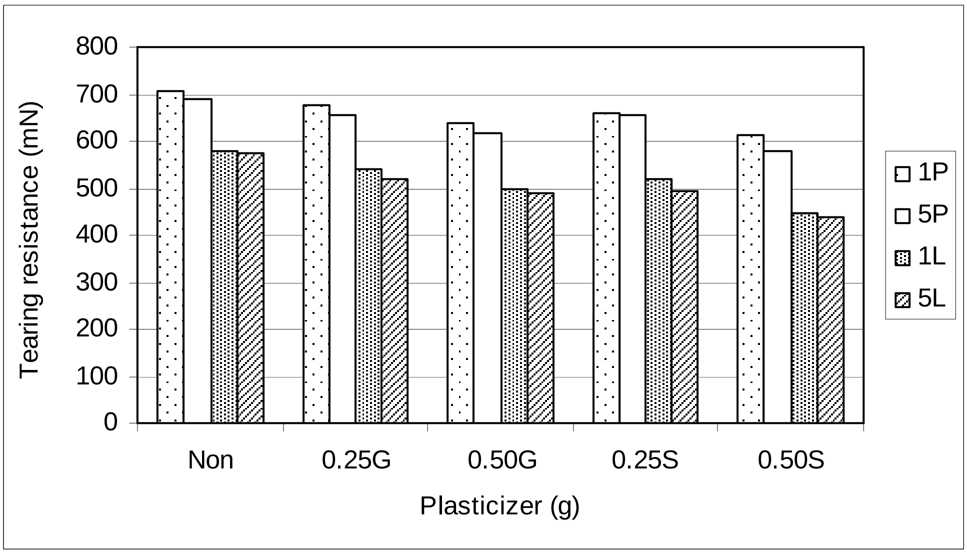

Figure 4.16 Effects of plasticizer on tearing resistance of chitosan-gelatin film (1:1) (Non = without plasticizer; 0.25G = 0.25 g glycerol/ g chitosan; 0.50G = 0.50 g glycerol/ g chitosan; 0.25S = 0.25 g sorbitol/ g chitosan; 0.50S = 0.50 g sorbitol/ g chitosan; 1P = 1% propionic acid; 5P = 5% propionic acid; 1L =1% lactic acid; 5L = 5% lactic acid)

For the tearing resistance of chitosan-gelatin film tended to decrease with the increase in the amount of plasticizer (Figure 4.16) because molecular force has reduced by plasticizer (Banker. 1966). The resulted in this study that chitosan-gelatin films containing 0.25 g glycerol/ g chitosan that were prepared from 1% propionic acid showed the highest degree of tearing resistance, following by films containing 0.25 g glycerol / g chitosan that were prepared from 5% propionic acid. Films containing 0.50g sorbitol/ g chitosan that prepared from 5% lactic acid yielded the lowest degree of tearing resistance.

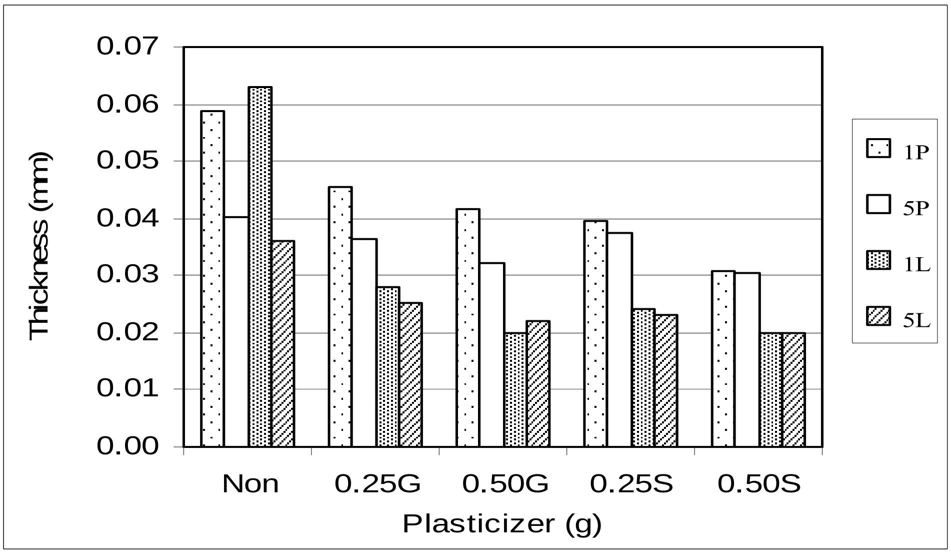

Figure 4.17 Effects of plasticizer on thickness of chitosan-gelatin film (1:1) (Non = without plasticizer; 0.25G = 0.25 g glycerol/ g chitosan; 0.50G = 0.50 g glycerol/ g chitosan; 0.25S = 0.25 g sorbitol/ g chitosan; 0.50S = 0.50 g sorbitol/ g chitosan; 1P = 1% propionic acid; 5P = 5% propionic acid; 1L =1% lactic acid; 5L = 5% lactic acid)

Thickness of chitosan-gelatin film decreased with increasing amount of plasticizer (Figure 4.17) similar to study of Wirathikowit (2001). Increasing of plasticizer concentration might reduce interaction between films molecules so addition of plasticizer to film result in thickness were 0.02-0.063 mm. Although, the chitosan-gelatin films prepared from 5% lactic acid and 0.5g sorbitol/ g chitosan were the thinnest film, weight of the prepared films tended to rise with the increase in the amount of plasticizer. This was the result of the added plasticizer to make increase mass of chitosan-gelatin film (Figure 4.18).

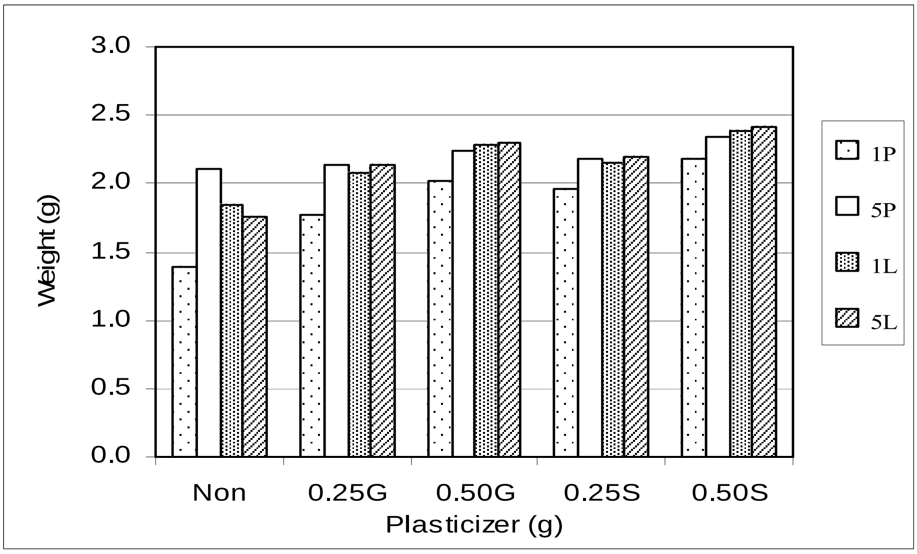

Figure 4.18 Effects of plasticizer on weight of chitosan-gelatin film (1:1) (Non = without plasticizer; 0.25G = 0.25 g glycerol/ g chitosan; 0.50G = 0.50 g glycerol/ g chitosan; 0.25S = 0.25 g sorbitol/ g chitosan; 0.50S = 0.50 g sorbitol/ g chitosan; 1P = 1% propionic acid; 5P = 5% propionic acid; 1L =1% lactic acid; 5L = 5% lactic acid)

The film’s vapor permeability tended to increase in accordance with increasing plasticizer concentrations (Figure 4.19). The addition of glycerol as plasticizer resulted to a significant increase in the film’s vapor permeability ($p<0.05$). The highest vapor permeability was found in the film prepared from 1% propionic acid, followed by that prepared from 5% propionic acid. The film prepared from 5% lactic acid had the least vapor permeability. Sorbitol had less effect on the film’s vapor permeability than glycerol. The film produced from 1% propionic acid yielded the highest vapor permeability and that produced from 5% lactic acid yielded the lowest vapor permeability

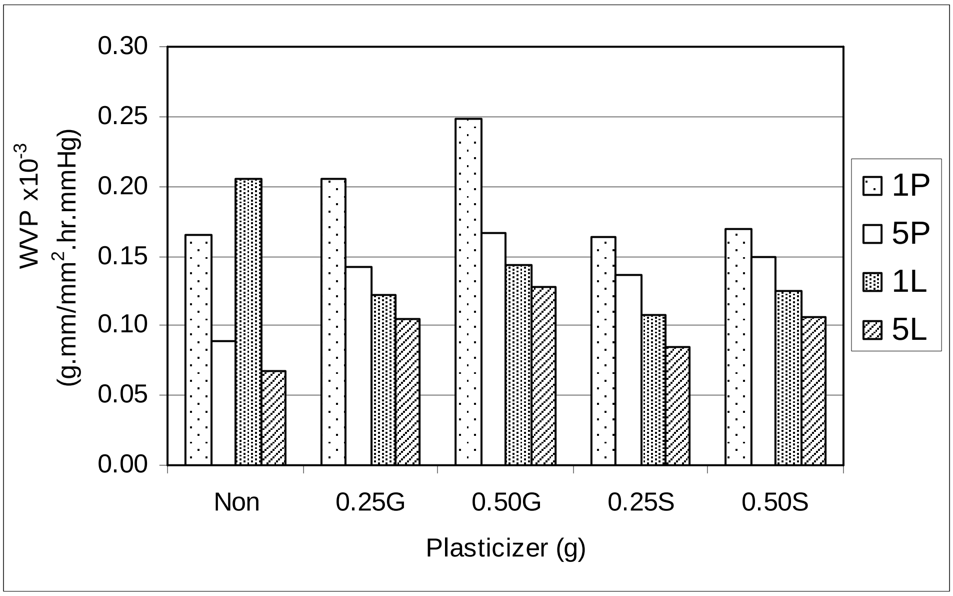

Figure 4.19 Effects of plasticizer on water vapor permeable (WVP) of chitosan-gelatin film (1:1) (Non = without plasticizer; 0.25G = 0.25 g glycerol/ g chitosan; 0.50G = 0.50 g glycerol/ g chitosan; 0.25S = 0.25 g sorbitol/ g chitosan; 0.50S = 0.50 g sorbitol/ g chitosan; 1P = 1% propionic acid; 5P = 5% propionic acid; 1L =1% lactic acid; 5L = 5% lactic acid.)

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The study also showed that at the amount, the plasticizer with less molecular weight yielded a higher rate of vapor permeability since the molecular weight is in direct relation to the solubility of a substance, which, in turn, affects the film’s vapor permeability due to its better vapor-combining capability (Banker, 1966). Molecular weight of Glycerol is 92.10, which is about half that of sorbitol 182.17 (Merck, 2000), glycerol is, thus, better dissolve than sorbitol. As a result, the film with glycerol as plasticizer yielded higher vapor permeability than with sorbitol.

When concentrations of plasticizers increased, tensile strength decreased, while elongation at break and WVP increased. These was the results of the addition of plasticizer, which reduced the hydrogen bond and increase the space between molecule causing the produced films to be more flexible (Lieberman and Gilbert, 1973; Banker, 1966).

Table 4.13 Properties comparison between different chitosan films and PE film

Film types	**Tensile Strength (N/mm^2)**	**Elongation at break (mm)**	**Tearing Resistance (mN)**	**10^{-3}WVP ($g.mm/mm^2.hr.mm.Hg$)**
1L-CH	60.583	13.408	556.25	0.2056
5P-CH	61.141	12.8	635	0.0889
1L-CHG	67.169	12.574	577.5	0.2257
5P-CHG	88.711	12.04	691.25	0.1013
1L-CHG-0.50s	56.521	13.967	447.65	0.1244
5P-CHG-0.50gl	70.385	13.463	616.7	0.1667
PE*	10-32	150-400	-	0.09

* from Bristan and Katan, (1974); Crawford, (1987); Grass, (1993)

Notes:	
1L-CH	Chitosan film contained 1% chitosan, prepared from 1% lactic acid
1L-CHG	Chitosan-gelatin film, ratio 1:1, prepared from 1% lactic acid
1L-CHG-0.50gl	Chitosan-gelatin film containing 0.50 g glycerol/ g chitosan
5P-CH	Chitosan film contained 1% chitosan, prepared from 5% propionic acid
5L-CHG	Chitosan-gelatin film, ratio 1:1, prepared from 5% lactic acid
5P-CHG-0.50s	Chitosan-gelatin film containing 0.50 g of sorbitol/ g chitosan
PE	Commercially available polyethylene film

The results showed that all the produced chitosan-gelatin films were stronger than polyethylene film in terms of tensile strength and tearing resistance. This probably due to since chitosan film is produced from polysaccharide, which gives greater strength because molecular structure has strong than petroleum polymer (Whistler, 1993). Since chitosan is considered to be polysaccharide, which possesses a higher rate of vapor permeation (Kester and Fennema, 1986; Weber, 2000; Butter et.al., 1996), it had found to have also higher vapor permeability than the PE film. In reported by Thai industry standard (1993), the assigned standards for cling film from polyethylene and polyvinyl chloride were that tensile strength is not less than 4.0 N/ mm^2**,** elongation at break is not less than 60-190, while tearing resistance is not more than 150-800 mN. In the present study, elongation at break of the produced chitosan-gelatin based film was much less than that of the Thai industry standard (1993). This indicated the unsuitability of the produced film to use as cling film. Its properties, especially elongation at break, still required the improvement.

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The present study was focused on production and quality improvement of chitosan films using varying concentrations of 3 different organic acids, i.e. lactic, citric and propionic acids, as well as one additive, i.e. gelatin and 2 different plasticizers, i.e. glycerol and sorbitol. Effects of chitosan content on properties of chitosan film were also observed.

1) Chitosan used in the production of the film was extracted from black tiger shrimp waste. The obtained chitosan was found to be yellowish white. It composed of 6.34 % nitrogen, 0.1 % fat, 0.25 % ash, and 6.85 % moisture. Deacetylation percentage was found to be 86.7 %. Viscosity of chitosan solution containing 1 % chitosan in 1% lactic acid solution was 230 cp.

2) The results showed that films prepared from 150 ml of 1 % chitosan in 1% lactic acid solution had the most suitable properties for further experiments. Physical and mechanical properties of the film was as follows: weight 1.5020 mg/ film sheet (size 20 x 25 cm), thickness 0.0593 mm, tensile strength 60.5833 N/ mm^2, elongation at break 13.408 mm/mm and vapor permeability 0.1564 x 10^{-3} g.mm/ mm^2 hr.mm.Hg.

3) Addition of gelatin to chitosan solution tended to improve tensile strength of the prepared films, especially at the 1:1 ratio of chitosan and gelatin. All physical and mechanical properties of the films stronger than that than the ratios of 1:2 and 1:3 (chitosan: gelatin), that is to say the film’s tearing resistance and elongation at break obtained from the three (lactic, propionic and citric) acids were high. However, the addition of gelatin to chitosan solution turned the film crispier and harder than pure chitosan film.

4) The film became softer when plasticizer was added and its strength decreased along with the increase in the amount of plasticizer. It was also found that, at the same

concentration, the film with glycerol as plasticizer yielded higher tensile strength than that with sorbitol as plasticizer. The film's vapor permeability tended to increase along with the increase in the amount of the plasticizer.

5) By comparison, the film obtained from this study yielded less flexibility than polyethylene film. So, it is currently not yet suitable for use as food wrapper due to its low flexibility, but it can be used for other activities instead.

5.2 Recommendations

1) Since the film produced could absorb water to a certain extent, further experiment should focus on the most suitable type and concentration of fatty acid in order to improve and diversify the film's properties.

2) The morphological study of the film's surface using SEM is another interesting point of study since the film's morphological appearance can be the indicator of absorption and arrangement of chitosan and gelatin's molecules, which can tell the film's strength. Besides, X-ray diffraction can be used in the study of arrangement of those molecules, acid solvents and plasticizers to test the impact of the acid solvents on chitosan crystallinity.

3) Chitosan film can be applied to lamination or wrapping products. Particularly, it will give dry products a bright shine due to its shiny surface, and thus help increase their value-added and attractiveness.

4) The study on the total cost of the complete process of chitosan film production is highly recommended so that it can be used as the baseline data in the decision-making process and in the selection of optimal methods of chitosan film production.

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APPENDIX

APPENDIX A

THE CHEMICAL ANALYSIS

1. Protein analysis

1.1 Chemical reagent

- Catalyst Mixture of $CuSO_4$.$5H_2O$, K_2SO_4 and TiO_2
- Concentrated sulfuric acid (H_2SO_4)
- 45% solution sodium hydroxide (NaOH)
- 4% Boric acid
- Bromcrysol green and methyl red indicator
- Standard 0.100 HCl solution

1.2 Procedure:

Digestion

1. Weigh approximately 1 g ground sample into digestion flask, recording weight (W) to nearest 0.1 mg. Include reagent blank and high purity lysine HCl as check of correctness of digestion parameters. Weigh a second subsample for laboratory dry matter determination.
2. Add 15 g K_2SO_4, 0.04 g $CuSO_4$.$5H_2O$, 0.5 to 1.0 g alundum granules, or add 16.7 g K_2SO_4, 0.01 $CuSO_4$.$5H_2O$, 0.6 g TiO2 and 0.3 g pumice. Then add 20 mL sulfuric acid. (Add additional 1.0 mL sulfuric acid for each 0.1 g fat or 0.2 g other organic matter if sample weight is greater than 1 g.)
3. Place flask on preheated burner (adjusted to bring 250 mL water at 25oC to rolling boil in 5 min).

4. Heat until white fumes clear bulb of flask, swirl gently, and continue heating for 90 min for copper catalyst or 40 min for CuSO4/TiO2 mixed catalyst.
5. Cool, cautiously add 250 mL distilled water and cool to room temperature (less than 25^{o}C). **Note**: If bumping occurs during distillation, volume of water may be increased to ca. 275 mL.

Distillation

1. Prepare indicator by Methyl red/bromocresol green indicator solution. Dissolve 0.2 g methyl red and dilute to 100 mL in 95% ethanol. Dissolve 1.0 g bromocresol green and dilute to 500 mL in 95% ethanol. Mix 1 part methyl red solution with 5 parts bromocresol green solution (combine all of both solutions).
2. Prepare titration flask by Boric acid solution. 4%, with indicator. Dissolve 40 g H_3BO_3 and dilute to 1 L in water and add 3 mL methyl red/bromocresol green indicator solution, (1). Solution will be light orange color.
3. Slowly down side of flask, add sufficient 45% sodium hydroxide solution (approximately 80 mL) to make mixture strongly alkali. (Do not mix until after flask is connected to distillation apparatus or ammonia will be lost.)
4. Immediately connect flask to distillation apparatus and distill at about 7.5 boil rates (temperature set to bring 250 mL water at 25oC to boil in 7.5 min) until at least 150 mL distillate is collected in titrating flask.
5. Remove digestion flask and titrating flask from unit, rinsing the condenser tube with distilled water as the flask is being removed.

Titration

1. Titrate H_3BO_3 receiving solution with standard 0.1000N HCL solution to first trace of pink. Lighted stir plate may aid visualization of end point. Record mL HCl to at least nearest 0.05 mL.

1.3 Calculation: Percent Nitrogen (N)

Calculate results as follows:

$$\text{Nitrogen, \%} = \frac{1.4007 \times (\text{mL HCL, sample} - \text{mL HCL, blank}) \times \text{normality HCL}}{\text{g sample}}$$

Calculation: Percent Crude Protein (CP)

CP = % N x F

- F = 6.25 for all forages and feeds except wheat grains
- F = 5.70 for wheat grains

2. Fat Content Analysis

2.1 Chemical reagent

- Anhydrous Diethyl Ether, purified for fat extraction Mallinkrodt #0844 or equivalent. To prevent ether from absorbing water, purchase it in small containers and keep containers tightly closed.

2.2 Procedure

Sample drying

1. Weigh 1.5 to 2 g of ground sample into a thimble recording the weight to nearest 0.1 mg (W1). Weigh a second subsample for dry matter determination.
   - Or -
   1A) If the sample contains large amounts of carbohydrates, urea, glycerol, lactic acid or water-soluble components, weigh 2 g sample to nearest 0.1 mg (W1) into a small filter cone. Extract with five 20 mL portions of deionized water allowing each portion to drain, and then insert the paper and sample into thimble.
2. Dry for 5 hr at 100oC.

3. Dry beakers to be used for fat determination for at least 1 hr at 100oC. Cool the appropriate number of fat beakers in a desiccator. Weigh and record the weight to the nearest 0.1 mg (W2).
4. When the drying period is over, remove the samples from the oven to a desiccator. (This is a convenient stopping point. The samples should be stored in a desiccator if not immediately extracted.)

Extraction

1. Line the fat beakers up in front of the extractor and match the thimbles with their corresponding fat beakers.
2. Slip the thimble into a thimble holder and clip the holder into position on the extractor.
3. Add about 40 mL of diethyl ether (one glass reclaiming tube full) to each fat beaker.
4. Wearing white gloves slip the beaker into the ring clamp and tightly clamp the beaker onto the extractor. If the clamp is too loose, insert another gasket inside the ring.
5. Raise the heaters into position. Leave about a 1/4 inch gap between the beaker and the heating element.
6. Turn on the heater switch, the main power switch, and the condenser water.
7. After the ether has begun to boil, check for ether leakage. This can be detected by sniffing around the ring clamp. If there is leakage, check the tightness of the clamp and if necessary replace the gasket(s).
8. Extract for minimum of 4 hr on a Hi setting (condensation rate of 5 to 6 drops per second), or for 16 hr on a Low setting (condensation rate of 2 to 3 drops per sec).
9. After extraction, lower the heaters, shut off the power and water, and allow the ether to drain out of the thimbles (about 30 min). This is a good stopping point.

Ether Distillation and Weighing of Fat Residue

1. Remove the thimble from the holder, and rinse the holder with a small portion of diethyl ether from the washbottle. Clip an ether reclaiming tube in place and reattach the fat beaker.
2. Reposition the heaters and turn on the electricity and water. Proceed to distill the ether using a Hi setting. Watch Closely.
3. Distill until a thin layer of ether remains in the bottom of the beaker, and then lower the heater. Do not allow beakers to boil dry. Overheating will oxidize the fat. When the last beaker has finished, shut off the power and water.
4. Wipe the exterior of the beaker clean with a Kimwipe as it is being removed from the extractor.
5. Empty the reclaiming tubes into the "USED" diethyl ether container.
6. Place the tray of beakers in an operating hood to finish evaporating the ether. If there is no hurry, air moving through the hood will be sufficient without heat. A steam-bath may be used to speed up the evaporation. Beakers should remain in the hood until all traces of ether are gone. Carefully sniff each beaker to determine if any ether remains.
7. Place the beakers in a 102^{o}C gravity convection oven. **Warning**: If a beaker containing ether is placed in the oven an explosion may occur.
8. Dry for 1/2 hr. No longer. Excessive drying may oxidize the fat and give high results.
9. Cool in a desiccator and weigh and record weight to the nearest 0.1 mg (W2).
10. The fat beakers are best cleaned by warming on a steambath or on a hot plate on a low setting. Add some used ether to dissolve the fat. The use of a rubber policeman is helpful. After soaking the beakers in Alconox detergent, wash those using hot water and vigorous brushing. The thimbles are best cleaned by blowing out with air.

2.3 Calculations: Percent Crude Fat (Ether Extract)

$$\textbf{\% Crude Fat} = (W3 - W2) \times 100 / W1 / 100$$

- W1 = initial sample weight in grams
- W2 = tare weight of beaker in grams
- W3 = weight of beaker and fat residue in grams

3. Ash analysis

3.1 Equipment:

- Crucibles, porcelain, low wide form, 30 mL, with covers numbered with furnace-proof ink
- Muffle furnace with pyrometric controller
- Analytical balance, sensitive to 0.1 mg
- Desiccator, with vented lid
- Drying oven

3.2 Procedure:

1. Remove crucibles with cover which have been dried for at least 2 hr at 100oC from oven, to desiccator. Cool and record weight of crucibles with cover to the nearest 0.1 mg (W1).
2. Weigh 1.5 to 2.0 g of sample into the crucible, recording weight of crucible with cover and sample to the nearest 0.1 mg (W2).
3. Ash in furnace at 600oC for 2 hr after the furnace reaches temperature.
4. Allow crucibles to cool in furnace to less than 200oC and place crucibles with cover in desiccator with vented top. Cool and weigh crucible with cover and ash to the nearest 0.1 mg (W3).

3.3 Calculation: Percent Ash

$$\% \text{ ASH (DM basis)} = (W3 - W1) \times 100 / (W2 - W1) / 100$$

- W1 = tare weight of crucible in grams
- W2 = weight of crucible and sample in grams
- W3 = weight of crucible and ash in grams

4. % Deacetylation

4.1 Chemical reagent

- 0.2M Hydrobromide salt
- 9M Hydrobromide salt
- Methanol
- 0.1 M Sodium Hydroxide
- Phenolpthalein indicator

4.2 Procedure

1. Prepared the chitosan hydrobromide salt by mix 0.5g of chitosan in 0.2M hydrobromide 100 ml.
2. Then add 50 ml of 9M hydrobromide to the solution with vigorous stirring to precipitate.
3. Centrifuge and remove the supernatant.
4. Wash the product with methanol until neutral and then allow being air dried.
5. Chitosan- hydrobromide salt products dissolve in 100 ml distilled water.
6. Thesolution is titraed with standardized 0.1 M Sodiun hydroxide and phenolpthalein as an indicator. Colorless of solution has change to pink as an end point.

4.3 Calculations

$$\%\text{deacetylation} = [0.1 \text{x} N \text{x} V_{NaOH} \text{x} MW]/m$$

N = normality of Sodium hydroxide

V_{NaOH} = volume of sodium hydroxide used at the end point

MW = molecular weight of monomer unit of chitosan

m = weight of starting chitosan

APPENDIX B
THE PHYSICAL ANALYSIS

1. Moisture Content Analysis

1.1 Apparatus

- Moisture can
- Hot air oven (Binder)
- Dessicator
- Weighing machine ()

1.2 Procedure

1. Place moisture can in a hot air oven (103°C) for 30 min. and cool down in dessicator before weighing and recording the weight.
2. Weigh sample 5.0000 g (4 digits) in aluminum cans (W1) and record.
3. Dry in a hot air oven (103°C) for 3 hr. or until weight is constant.
4. Cool down in dessicator (W2).
5. Weigh W2 and record results.
6. Calculate percentage of moisture content.

1.3 Calculation

% Moisture content = [W1-W2]/W1/100

2. Viscosity of chitosan solution

2.1 Apparatus

- Brookfield Viscometer (RVDV-II)

2.2 Procedure

1. Prepare chitosan solution by dissolving 5.0 g chitosan with 500 ml 1% acetic solution (w/v) for 24 hr in beaker. Undissolved particles are removed by filtration.
2. Warm solution in water bath (30° C) for 30-40 min or temperature constant.
3. Measure the viscosity of chitosan solution by using a Brookfield viscometer with needle No.2 and run velocity until percentage accuracy near 100.
4. Repeat 3 times and record data

3. Water vapor permeability

3.1 Apparatus

- Dessicator
- Plastic cup
- Paraffin
- Wax
- Weighing machine

3.2 Procedure

1. Prepared the film in round shape which surface area is 18 cm^2.
2. Put on the top of plastic cup that content 3 grams silica gel.
3. Seal the eage of film and plastic cup with wax and paraffin.
4. Weigh the cup and record

5. Put the cup into dessicator (temp25 ºC, %RH 65)
6. Weigh the cup every 12 hr until the different weight equal to 0.0001 gram
7. Repeat 4 times and calculate the average
8. Plot curve and then calculate the slope
9. Calculate the result

3.3 Calculation.

WVTR= [G/t]/A

G/t = Ratio of weigh change

A = Ares of sample (cm^2)

WVP = Permeance x film thickness

Permeance = WVTR/ Δp = WVTR/ S (R_1-R_2)

S = 23.73 mm.Hg

R_1 = %RH in dessictor (65% RH)

R_2 = %RH in cup (0% RH)

4. Tensile Testing

4.1 Apparatus

- Tensile testing machine, Universal testing machine Lloy Ltd.

4.2 Procedure

1. Prepare films to size 5.0 x 10.0 cm.
2. Put sample in dessicator (temp25 ºC, %RH 65)
3. Test sample in specific condition (grip length 5 cm, load cell 10 kg, speed 20mm/min)
4. Repeat 4 times and calculate the average

4.3 Calculation

TS (N/mm^2) = Data reading (N) / surface area (mm^2)

Elongation at break= [length of film after break- grip length] x100 /grip length

5. Tearing testing

5.1 Apparatus

- Tearing resistance , Elmendorf Tear ETS

5.2 Procedure

1. Four samples each are cut from the film in the machine direction and in the transverse direction
2. A sample is positioned in the tester and clamped in place
3. A cutting knife in the tester is used to create a slit in the sample which ends 43mm from the far edge of the sample
4. The pendulum is released to propagate the slit through the remaining 43 mm.
5. The energy loss by the pendulum is used to calculate an average tearing force.

6. Weighing testing

6.1 Apparatus

- Weighing balance

6.2 Procedure

1. Prepare the film size 20 x 25 cm
2. Put film in weighing balance
3. Record the data

7. Thickness testing

7.1 Apparatus

- Digital Micrometer, Mar Ltd.

7.2 Procedure

1. Prepare film size 10x10 cm
2. Measure thickness value at 12 points on each film and calculate the average
3. Record the data.

BIOGRAPHY

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