FINAL REPORT

RESEARCH MANAGEMENT CENTRE
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ANTIMICROBIAL SPECTRUM ACTIVITY OF LAURIC ACID AND CHITOSAN

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ABSTRACT

Future work will focus on the use of biologically active derived antimicrobial compounds bound to biopolymers. However many natural antimicrobials have a limited spectrum of activity and are effective only at very high concentrations. The need for new antimicrobials with wide spectrum activity and low toxicity is increasing. The objectives of this research include to study the behaviour of lauric acid (LA) and chitosan as an antimicrobial agent, to determine the antimicrobial effects of LA and chitosan against food spoilage microorganisms and to investigate the inhibition growth of combined antimicrobial agent on food spoilage microorganisms. The antimicrobial activity testing was based on the agar diffusion method and liquid culture test based on standard serial broth dilution assay. The present test emphasizes on the efficacy of chitosan and LA to inhibit the growth of selected Gram-positive food pathogenic bacteria i.e. *Bacillus subtilis* and Gram-negative food pathogenic bacteria *i.e. Escherichia coli.* The result shows that chitosan and LA have good inhibitory effects against *E. coli* and *B. subtilis* respectively. It is also suggested that the combination of chitosan and LA is insufficient to retard the growth of Gram-negative bacteria, *E. coli.* However, it shows a good inhibition effect on Gram-positive bacteria, *B. subtilis* as can be seen on the area of zone inhibition in agar diffusion method. The liquid culture test shows that chitosan is not effective in inhibiting the microbial growth in an aqueous condition compared to agar plate test. In contrast, lauric acid gave a better inhibition effect in liquid culture test. Combination of chitosan and LA showed obvious effects towards inhibition of *B. subtilis* and *E. coli* indicated that they had synergistic antimicrobial effect in solid or liquid condition.

Keywords: Chitosan; Lauric acid; Antimicrobial; *Escherichia coli*; *Bacillus subtilis*; spectrum activity

ABSTRAK

Kajian masa depan akan bertumpu kepada penggunaan bahan anti mikrob dari bahan aktif biologi bergabung dengan bio-polimer. Walaubagaimanapun, kebanyakan bahan anti mikrob asli mempunyai spektrum aktiviti yang terhad dan efektif hanya pada kepekatan yang sangat tinggi. Keperluan terhadap bahan anti mikrob baru yang mempunyai aktiviti spektrum yang lebih besar dan kesan toksik yang rendah akan meningkat. Antara tujuan kajian ini adalah untuk mengkaji sifat asid laurik dan 'chitosan' sebagai agen anti mikrob, untuk menentukan kesan anti mikrob oleh asid laurik dan 'chitosan' terhadap mikroorganisma perosak makanan dan untuk menyiasat kesan gabungan kedua-dua bahan anti mikrob tersebut terhadap perencatan pertumbuhan mikro organisma perosak makanan. Ujian aktiviti anti mikrob adalah berdasarkan kaedah peresapan agar dan ujian kultur yang berdasarkan kepada siri pencairan kaldu piawai. Ujian yang dijalankan bertumpu kepada keberkesanan 'chitosan' dan asid laurik untuk merencat pertumbuhan bakteria perosak makanan Gram-positif terpilih iaitu '*Bacillus subtilis*' dan bakteria perosak makanan Gram-negatif iaitu '*Escherichia coli*'. Keputusan menunjukkan 'chitosan' dan asid laurik masing-masing mempunyai kesan perencatan yang baik terhadap *E. coli* dan *B. subtilis*. Keputusan juga menunjukkan gabungan 'chitosan' dan asid laurik tidak cukup untuk memperlahankan pertumbuhan bakteria Gram-negatif, *E. coli*. Walaubagaimanapun, gabungan tersebut menunjukkan kesan perencatan yang baik terhadap bakteria Gram-positif, *B. subtilis* seperti yang dilihat pada kawasan zon perencatan dalam kaedah peresapan agar. Ujikaji kultur menunjukkan 'chitosan' tidak efektif dalam merencat pertumbuhan mikrob dalam keadaan akuas berbanding ujian agar. Manakala, asid laurik pula memberi kesan perencatan yang baik di dalam keadaan cecair. Gabungan 'chitosan' dan asid laurik telah menunjukkan kesan yang jelas terhadap perencatan *B. subtilis* dan *E. coli* dimana, ini menunjukkan ia mempunyai kesan anti mikrob yang 'synergistic' dalam keadaan pepejal atau cecair.

Kata kunci: Chitosan; Asid Laurik; anti mikrob; *Escherichia coli*; *Bacillus subtilis*; aktiviti spektrum

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LIST OF ABBREVIATIONS

AM	Antimicrobial
BSI	British International Standard
CFU	Colony forming units
LA	Lauric acid

LIST OF SYMBOLS

M_i	initial weight
M_f	final weight
$X(t)$	cell concentration of microorganism
μ	specific growth rate

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CHAPTER 1

INTRODUCTION

1.0 Background

A widespread trend world wide is the movement towards “natural” food products. In effort to meet this demand, there has been increased interest in the food industry in using antimicrobial preservatives that are perceived as more “natural”. / A possible solution may be using combination of antimicrobials (Sofos *et al.*, 1998). Instead of concentrating on development of new antimicrobial, it is more practical if combine the antimicrobial agents that already being research.

Lauric acid, a medium length – long chain fatty acid is found in the form of glycerides in a number of natural’s fats, coconut oil and palm kernel oil. It offers advantages in food processing as it acts as a kind of preservative, staving off oxidation and spoiling. Lauric acid has been shown to have an antimicrobial effect against gram positive bacteria and yeast (Beuchat and Golden, 1989; Kabara, 1993).

Chitosan, polysaccharides of β-1-4 linkage and a deacetylated form of chitin, appears as a natural antimicrobial candidate fort he incorporation because it can inhibit the growth of a wide variety of fungi, yeast and bacteria (Rhoades and Roller, 2000).

Combination of both antimicrobial agent chitosan and lauric acid will enhance the effectiveness inhibition growth for food spoilage microorganisms (*E. coli, S.aureus, L. monocytogenes* and *B.cereus*).

1.1 Objectives of Research

The main objectives of this study are:

1. To study the behaviour of lauric acid and chitosan as an antimicrobial agent.
2. To determine the antimicrobial effect of lauric acid and chitosan against food spoilage microorganisms.
3. To investigate the inhibition growth of combine antimicrobial agent on food spoilage microorganisms.

1.2 Scopes of Research

Scopes of this research are:

1. Determination of the antimicrobial effect of lauric acid and chitosan against food spoilage microorganisms.
2. Investigation of the inhibition growth of combine antimicrobial agent on food spoilage microorganisms.

CHAPTER 2

LITERATURE REVIEW

2.0 Antimicrobial Agents

There are numerous AM agents that exist and widely used in a variety of applications in the food, pharmaceutical and cosmetic industries. Veterinary Dictionary defined AM agent as an agent which capable to kill microorganisms or suppresses their multiplication or growth.

Han (2003) affirmed that to be able to use AM agents in the food, pharmaceuticals and cosmetics products, the industry must follow the guidelines and regulations of the country that they are going to use them in, for example, FDA and/or EPA in the United States. This implies that new AM packaging materials maybe developed using only agents which are approved by the authorization agencies for example from FDA approved or notified-to-use within the concentration limits' for food safety enhancement or preservation (Han, 2003). Lysozyme is well known as an AM protein and has attracted considerable interest as a natural food preservative and was approved by FDA; therefore the incorporation of lysozyme into food packaging material will become more popular in Malaysia.

2.1 Types and Applications of AM Agents

There are various types of AM agents that could be incorporated into the packaging systems which are chemical AMs, antioxidants, biotechnology products,

AM polymers, natural AMs and gas (Ming *et al*., 1997; Scannel *et al*., 2000, Yogesh, 2006; Suppakul *et al*., 2003; Hotchkiss, 1997). Table 2.1 lists some typical natural and synthetic AM agents that are used in food packaging.

Table 2.1: Examples of typical AM agents used in food packaging

Class of AM agents	**Examples**
Antibiotics	Natamycin
Bacteriocin	Nisin, pediocins
Chelating agents	Ethylene diamine tetra acetate, purophosphate, citrates
Enzymes	Lactoperoxidase, lysozyme, lactoferrin
Essentioal oils	Eugenol, thymol, salicylaldehyde, cinnamic acid
Fatty acids and esters	Monolaurin
Fungicides	Benomyl, imazalil
Gas	Ethanol, Hinokithiol, $C10_2$
Inorganics	Sulphites, sulfur dioxide
Isothiocynanates	Allyl isothiocyanate, hypothuiocyanate
Metals	Silver, copper
Mineral acids	Phosphoric acid
Organic acids	Propionic, benzoic, sorbic, acetic, lactic, malic, succinic, tartaric
Oxygen absorber	BHT
Parabens	Methyl, propylparaben
Phenolic antioxidants	Butylatedhydroxyanisole, Butylatedhydroxytoluene 2-terbutylhydroquinone
Proteins	Conalbumin, cathepsin
Others	Reuterin (3-hydroxypropionaldhyde), hydrogen peroxide, ozone, sulfur dioxide

Source: Adapted from Hotchkiss (1997) and Han (2000)

The most widespread use of AM agent in the industry is chemical substances which include alcohol, antibiotics, fungicides and organic acid. Organic acids and their mixture possess strong AM activity and have been used as food preservatives, food contact substance and food contact material sanitizers (Han, 2003). The use of antibiotics as package additives is not approved for the purpose of AM functions and is also controversial due to the development of resistant microorganism. However, antibiotics may be incorporated for short-term used in medical devices and other non-food product (Han, 2003).

Chlorine oxide, allyl isothiocynates and ozone are examples of the gaseous AM agents that have been successfully incorporated into packages which offer

protection in the headspace of food packaging (Yogesh, 2006). Ethanol vapor generation consists of ethanol absorbed or encapsulated in carrier materials and enclosed in polymer packets. The ethanol permeates the selective barrier and is released into the headspace within the package (Appendini and Hotchkiss, 2002).

Various AM agents could be incorporated into the packaging systems which are chemical AMs, antioxidants, biotechnology products, AM polymers, natural AMs and gas (Ming *et al*., 1997; Scannel et al., 2000, Yogesh, 2006; Suppakul *et al*., 2003; Hotchkiss, 1997). Each antimicrobial agent has its own unique characteristics which differentiate it from others (Table 2.2).

Table 2.2: Few types of AM agents and their mode of action.

Types of AM agents	Mode of action
penicillins, cephalosporins and bacitracin	Synthesis of the bacterial cell wall which results in cell lysis because the contents of the bacterial cell are hypertonic and therefore under high osmotic pressure. A weakening of the cell wall causes the cell to rupture, spill its contents, and be destroyed.
griseofulvin, fluoroquinolones and rifampicin.	Synthesis of nucleic acids. Without DNA and RNA synthesis a microorganism cannot replicate or translate genetic information.
amphotericin B and polymyxin B	Alteration the permeability of the cell membrane, causing a leakage of metabolic substrates essential to the life of the microorganism. Their action can be either bacteriostatic or bactericidal
sulfonamides, aminosalicylic acid (PAS) and isoniazid (INH)	Metabolic processes within the microorganism. They are structurally similar to natural metabolic substrates, but since they do not function normally, they interrupt metabolic processes. Most of these agents are bacteriostatic
macrolides, tetracyclines, chloramphenicol	Translation of proteins by the ribosome. This action may be bacteriocidal, if errors in translation are induced (aminoglycosides) or bacteriostatic, if translation is inhibited.

Source: Adapted from Veterinary Dictionary.

The example of inhibition of microbial growth can be observed by the clear zones around the film on the plate agar test (Figure 2.1). Table 2.3 shows a few of AM agents that have been applied in food packaging.

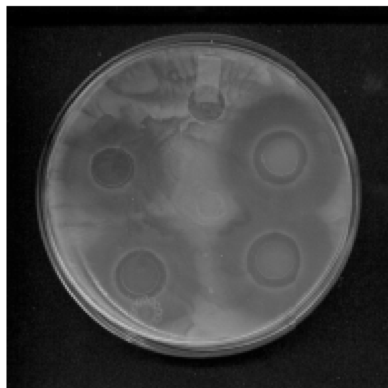


Figure 2.1 Clear zones of inhibition of the spoilage bacteria *Brochothrix thermosphacta* around discs of whey protein film containing lysozyme after 48 hr at room temperature (Han, 2000).

Table 2.3: Application of AM Food Packaging (Han, 2000)

Antimicrobial Agent	**Packaging Material**	**Food**	**References**
Organic Acid			
Potassium Sorbate	LDPE	Cheese	Han (1996)
	LDPE	Culture Media	Han and Floros (1997)
	MC/Palmitic Acid	Culture Media	Rico-Pena and Torres (1991)
	MC/HPMC/Fatty Acid	Culture Media	Vojdani and Torres (1990)
	MC/Chitosan	Culture Media	Chen *et al.* (1996)
	Starch/Glycerol	Chicken Breast	Baron and Sumner (1993)
Calcium Sorbate	CMC/Paper	Bread	Ghoshh *et al.* (1973,1977)
Propionic Acid	Chitosan	Water	Ouattara *et al.* (1999)
Acetic acid	Chitosan	Water	Ouattara *et al.* (1999)
Benzoic acid	PE-co-MA	Culture Media	Weng *et al.* (1997)
Sodium Benzoate	MC/Chitosan	Culture Media	Chen *et al.* (1996)
Sorbic acid anhydride	PE	Culture Media	Weng and Chen (1997); Weng and Hotchkiss (1993)
Benzoic acid anhydride	PE	Fish fillet	Huang *et al.* (1997)

Fungicide/Bacteriocin			
Benomyl	Ionomer	Culture Media	Halek and Garg (1989)
Imazalil	LDPE	Bell paper	Miller *et al.* (1984)
	LDPE	Cheese	Weng and Hotchkiss (1992)
Nisin (peptide)	Silicon coating	Culture Media	Daeschel *et al.* (1992)
	SPI, corn zein film, SS	Culture Media	Padgett *et al.* (1998)
Peptide/Protein/Enzyme			
Lysozyme	PVOH, Nylon, Cellulose Acetate	Culture Media	Appendini and Hotchkiss (1996)
	SPI film, corn zein film	Culture Media	Padgett *et al.* (1998)
Glucose oxidase	Alginate	Fish	Field *et al.* (1986)
Alcohol oxidase	-	-	Brody and Budny (1995)
Alcohol/thiol			
Ethanol	Silica gel sachet	Culture Media	Shapero *et al.* (1978)
	Silicon oxide sachet (Ethicap^{TM})	Bakery	Smith *et al.* (1987)
Hinokithiol	Cyclodextrin/Plastic (Seiwa^{TM})	-	Gontard (1997)
Oxygen absorber/antioxidant			

Reduced iron complex	Sachet (Ageless™)	Bread	Smith *et al.* (1986)
BHT	HDPE	Breakfast cereal	Hoojjat *et al.* (1987)
Gas			
CO_2	Calcium hydroxide sachet	Coffee	Labuza (1990)
	-	Fruit/Vegetable	Sacharow (1988)
SO_2	Sodium metabisulfite	Grape	Gontard (1997)
Other			
UV Irridiation	Nylons	Culture Media	Paik and Kelly (1995); Hagelstein *et al.* (1995)
Silver zeolite	LDPE	Culture Media	Ishitani (1995)
Grape fruit seed extract	LDPE	Lettuce, Soybean sprouts	Lee *et al.* (1998)

2.2 Lauric acid

Lauric acid is colorless, needle-like crystal and slight odor of Bay Oil. Lauric acid or dodecanoic acid is part of the class of organic compounds known as lipids, which are vital in the construction of cellular membranes and act as a source of food under starvation conditions. The molecular weight of the lauric acid is $C_{12}H_{24}O_2$. Contrary to popular beliefs, natural coconut and coconut milk are good for the health, mostly because of their high lauric acid content. Figure 2.2 shows the 3D structure of lauric acid.

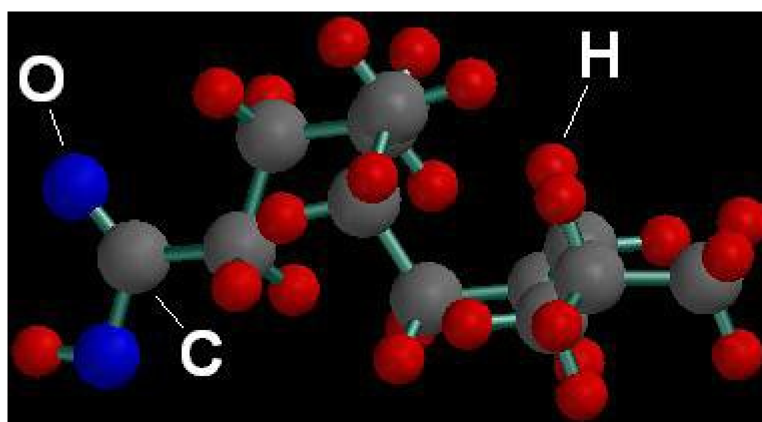


Figure 2.2 Lauric acid molecular 3D structure

2.2.1 Uses of Lauric Acid

Lauric acid is potential to use as the antimicrobial agent because it sources are from local sources, coconut. Besides, it is inexpensive, has a long shelf-life, and is non-toxic and safe to handle and suitable used for incorporate in packaging. It is widely used as a lubricant and as an additive in industrial preparations. It is used in the manufacture of metallic stearates, pharmaceuticals, soaps, cosmetics, and food packaging. It is also used as a softener, accelerator activator and dispersing agent in rubbers. Because of Lauric acid is insoluble in water, ethanol can be useful to solute the Lauric acid.

2.2.2 Health Effects

Lauric acid is able to raise metabolism, believed to be due to its activation of 20% of thyroidal hormones, which otherwise lie dormant. This is supposed from lauric acid's release of enzymes in the intestinal tract which activate the thyroid. This could account for the metabolism-raising properties of coconut oil.

2.2.3 Lauric Acid Intake in Selected Asian Countries

Based on the per capita intake of coconut oil in 1985 as reported by Kaunitz, the per capita daily intake of lauric acid can be approximated. For those major producing countries such as the Philippines, Indonesia, and Sri Lanka, and consuming countries such as Singapore, the daily intakes of lauric acid were approximately 7.3 grams (Philippines), 4.9 grams (Sri Lanka), 4.7 grams (Indonesia), and 2.8 grams (Singapore). In India, intake of lauric acid from coconut oil in the coconut growing areas (e.g., Kerala) range from about 12 to 20 grams per day , whereas the average for the rest of the country is less than half a gram. An average high of approximately 68 grams of lauric acid is calculated from the coconut oil intake previously reported by Prior et al in 1981 for the Tokelau Islands. Other coconut producing countries may also have intakes of lauric acid in the same range.

2.2.4 Lauric Acid Intake in the U.S.

In the United States today, there is very little lauric acid in most of the foods. During the early part of the 20th Century and up until the late 1950s many people consumed heavy cream and high fat milk. These foods could have provided approximately 3 grams of lauric acid per day to many individuals. In addition, desiccated coconut was a popular food in homemade cakes, pies and cookies, as well as in commercial baked goods, and 1-2 tablespoons of desiccated coconut would have supplied 1-2 grams of lauric acid. Those foods made with the coconut oil based shortenings would have provided additional amounts. Until two years ago, some of the commercially sold popcorn, at least in movie theaters, had coconut oil as the oil.

This means that for those people lucky enough to consume this type of popcorn the possible lauric acid intake was 6 grams or more in a three(3) cup order.

Some infant formulas (but not all) have been good sources of lauric acid for infants. However, in the past 3-4 years there has been reformulation with a loss of a portion of coconut oil in these formulas, and a subsequent lowering of the lauric acid levels. Only one U.S. manufactured enteral formula contains lauric acid (e.g., Impact®); this is normally used in hospitals for enteral tube feeding; it is reported to be very effective in reversing severe weight loss in AIDS patients , but it is discontinued when the patients leave the hospital because it is not sufficiently palatable for continued oral use (D.P. Kotler, private communication, 1995) The more widely promoted enteral formulas (e.g., Ensure®, Nutren®) are not made with lauric oils, and, in fact, many are made with partially hydrogenated oils.

There are currently some candies sold in the US that are made with palm kernel oil, and a few specialty candies made with coconut oil and desiccated coconut. These can supply small amounts of lauric acid. Cookies such as macaroons, if made with desiccated coconut, are good sources of lauric acid, supplying as much as 6 grams of lauric acid per macaroon (Red Mill Farm's Jennies Macaroons is apparently the only brand in the U.S. that supplies this amount). However, these cookies make up a small portion of the cookie market. Most cookies in the United States are no longer made with coconut oil shortenings; however, there was a time when many U.S. cookies (e.g., Pepperidge Farm) were about 25% lauric acid.

Originally, one of the largest manufacturers of cream soups used coconut oil in the soup formulations. Many popular cracker manufacturers also used coconut oil as a spray coating. These products supplied a small amount of lauric acid on a daily basis for some people.

2.2.5 Probable Levels of Lauric Acid Required For Antimicrobial Effect

Based on the amount of lauric acid found in human milk, which is known to be effective in its role as an antimicrobial component for the infant, the percent of calories that would be appropriate can be determined. For example, human milk

provides at least 3.5% of calories as lauric acid for the human infant. Mature human milk has been noted to have up to 12% of the total fat as lauric acid (approximately 6.6% of calories. The upper end of this range represents approximately twice the amount of calories as lauric acid (i.e., 7% of calories) as does the minimum.

When developing lauric-rich diets for adults, one can use this range as the starting point for calculating the amount of lauric fat to be consumed. Based on the upper end of the range, we see that this would entail providing an adult consuming 3000 kilocalories a day with 52 grams of coconut oil (approximately 24 grams of lauric acid). This could be accomplished by use, for example, of two 250 ml cans of a calorically dense enteral formula (e.g., Carnation Nutren 2.0) *if* that product was made with full coconut oil. As it is, that product is made with MCT oil and corn oil and provides no lauric acid.

Lauric acid-rich diets can be developed readily for infants and children. For infants, a formula made with coconut oil that supplies at least 7% of the calories as lauric acid would be needed. When infants progress to solid food, these foods can be enriched with added coconut oil. Cereals and strained baby foods make ideal bases for 2-5 gram additions coconut oil (0.5-1.0 teaspoons). This would add approximately 1-2 grams of lauric acid. Children can utilize the same protocol as outlined for adults with alterations in the portions of food depending on the caloric needs of the child.

2.3 Chitosan

Chitosan is a modified natural carbohydrate polymer derived from chitin which has been found in a wide range of natural sources such as crustaceans, fungi, insects and some algae (Tolaimate and others 2000). The primary unit in the chitin polymer is 2-acetamido-2-deoxy-β-D-glucose. These units are combined by 1-4 glycosidic linkages, forming a long chain linear polymer without side chains. Chitin is chemically identical to cellulose, except that the secondary hydroxyl group on the alpha carbon atom of the cellulose molecule is substituted with acetoamide groups (Figure 2.1). Removal of most of the acetyl groups of chitin by treatment with strong

alkali yields chitosan (Peniston and Johnson, 1980) which is 2-amino-2-deoxy-β-D-glucose. A sharp nomenclature with respect to the degree of *N*-deacetylation has not been defined between chitin and chitosan (Muzzarelli 1977). In general, chitin with a degree of deacetylation of above 70% is considered as chitosan (Li and others 1997a).

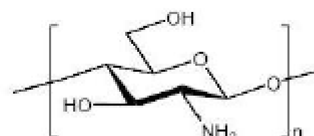


Figure 2.3 Chemical structure of chitosan

Chitosan is insoluble in water but soluble in acidic solvents below pH 6. Organic acids such as acetic, formic and lactic acids are used for dissolving chitosan, and the most commonly used solvent is 1% acetic acid solution. Solubility of chitosan in inorganic acid solvent is quite limited. Chitosan is soluble in 1 % hydrochloric acid but insoluble in sulfuric and phosphoric acids. Chitosan solution's stability is poor above pH 7 due to precipitation or gelation that takes place in alkali pH range. Chitosan solution forms a poly-ion complex with anionic hydrocolloid and provides gel.

2.3.1 Characterization of Chitosan

Chitosan can be characterized in terms of its quality, intrinsic properties such as purity, molecular weight, viscosity, and degree of deacetylation and physical forms (Sanford 1989). The quality and properties of chitosan product may vary widely because many factors in the manufacturing process can influence the characteristics of the final chitosan product (Li and others 1992).

2.3.1.1 Degree of Deacetylation of Chitosan

Among many characteristics, the degree of deacetylation is one of the more mportant chemical characteristics, which influences the performance of chitosan in many of its applications (Muzzarelli 1977; Li and others 1992; Baxter and others 1992). In addition, the degree of deacetylation, which reveals the content of free

amino groups in the polysaccharides (Li and others 1992), can be used to differentiate between chitin and chitosan. In general, chitin with a degree of deacetylation of above 70% is considered as chitosan (Li and others 1997a). In the process of deacetylation, acetyl groups from the molecular chain of chitin are removed to form amino groups (Figure 2.4).

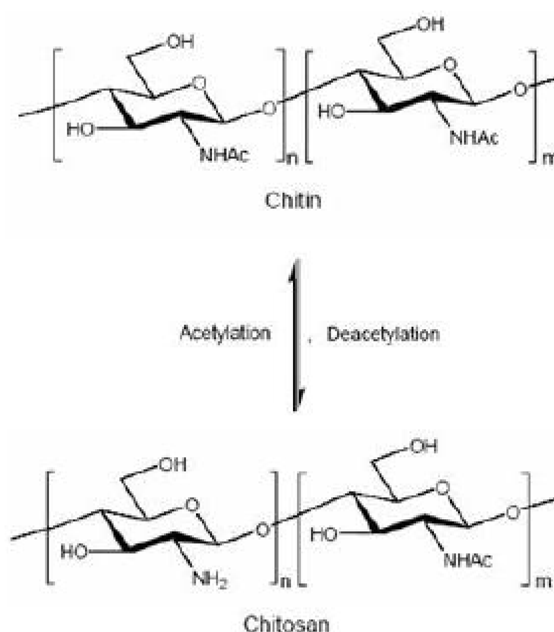


Figure 2.4 Conversion of chitin into chitosan

Variables such as temperature or concentration of sodium hydroxide solution affect the removal of acetyl groups from chitin, resulting in a range of chitosan molecules with different properties and hence its applications (Baxter and others 1992; Mima and others 1983). Since the degree of deacetylation depends mainly on the method of purification and reaction conditions (Baxter and others 1992; Li and others 1997), it is essential to characterize chitosan by determining its degree of deacetylation prior to its utilization.

A number of methods have been used to determine the degree of deacetylation, such as linear potentiometric titration (Ke and Chen 1990), infrared spectroscopy (Baxter and others 1992), nuclear magnetic resonance spectroscopy (Hirai and others 1991), pyrolysis-mass spectrometry (Nieto and others 1991), first derivative UV-spectrophotometry (Muzzarelli and Rocchetti 1985), and titrimetry (Raymond and others 1993). Some of the methods are either too tedious, too costly for routine analysis (e.g., nuclear magnetic resonance spectroscopy), or destructive to

the sample (Khan and others 2002). From the literature, the degree of deacetylation values of chitosan appear to be highly associated with the analytical methods employed (Khan and others 2002). However, one of the most frequently used methods is infrared spectroscopy because of its simplicity.

2.3.1.2 Molecular Weight

The molecular weight of chitosan varies depending on the raw material sources and preparation methods (Li and others 1992). Most commercial chitosans have a degree of deacetylation that is greater than 70% and a molecular weight ranging between 100,000 Da and 1.2 million Da (Li and others 1997; Onsoyen and Skaugrud 1990). Various factors, such as temperature, dissolved oxygen concentration, and shear stress can cause degradation of chitosan. The molecular weight of chitosan can be determined by methods such as chromatography (Bough and others 1978), light scattering (Muzzarelli 1977), and viscometry (Maghami and Roberts, 1988). Among many methods, viscometry is a simple and rapid method for the determination of molecular weight. The intrinsic viscosity of a polymer solution is related to the polymer molecular weight according to the Mark-Houwink equation:

$$[n] = KM^{a}$$

where [η] is the intrinsic viscosity, M the viscosity-average molecular weight, and K and a are constants for a given solute-solvent system and temperature.

2.3.1.3 Viscosity

As with molecular weight and degree of deacetylation, viscosity is an important characteristic of chitosan. Viscosity of chitosan is highly dependent on the degree of deacetylation, molecular weight, concentration of solution, ionic strength, pH, and temperature. The processes involved in the extraction of chitosan also affect the viscosity of chitosan. For instance, chitosan viscosity decreases with an increased time of demineralization (Moorjani and others 1975). Bough and others (1978) found that elimination of the demineralization step in the chitin preparation decreased the viscosity of the final chitosan products. Moorjani and others (1975) reported that bleaching chitosan with acetone or sodium hypochlorite at any stage of the extraction

process leads to considerable reduction in viscosity. No and others (1999) demonstrated that chitosan viscosity is considerably affected by physical (grinding, heating, autoclaving, ultrasonication) and chemical (ozone) treatments, except for freezing, and decreases with an increase in treatment time and temperature.

2.3.2 Sources of Chitosan

Chitosan is converted from chitin, which is a structural polysaccharide found in the skeleton of marine invertebrates, insects and some algae. Chitin is perhaps the second most important polysaccharide after cellulose and is an abundantly available renewable natural resource. The aquatic species that are rich in chitinous material (10-55 % on a dry weight basis) include squids, crabs, shrimps, cuttlefish and oysters. Mucoraceous fungi, which are known to contain chitin and the deacetylated derivate, chitosan, in cell walls (22 to 44%), have been used for commercial chitin production (Muzzarelli 1977; Muzzarelli and others 1994). However, in comparison with marine sources, which yield more than 80,000 metric tons of chitin per year (Muzzarelli 1977; Subasingle 1995), chitin production from fungal waste is negligible.

Depending on the sources, the physicochemical properties and functionalities of chitosan differ (Rhazi and others 2004). For example, chitosan prepared from squid contains β-chitin (amine group aligned with the OH and CH_2OH groups) and those prepared from crustaceans contain α-chitin (anti-parallel chain alignment) (Shepherd and others 1997; Felt and others 1999). Despite a wide range of available sources, chitosan is commercially manufactured only from crustaceans (crab, shrimp, krill, and crayfish) primarily because a large amount of crustacean exoskeleton is available as a byproduct of food processing. Disposal of crustacean shell waste has been a challenge for seafood processors. Therefore, production of value-added products, such as chitin, chitosan and their derivatives, and utilization of these value added products in different fields are of utmost interest to food industries. Continual use of new raw materials as a source of chitin would enable production to be significantly increased. Major progress is being made in the development of profitable technology for isolation of chitin and its derivatives (Rashidova and others 2004). However, commercial extraction has been hampered by the corrosive nature

of the strong acids and bases used in the manufacture of chitosan, which destroys equipment, requires careful handling by workers, and presents potential environmental hazards (Peniston and Johnson 1980; Leffler 1997).

CHAPTER 3

MATERIALS AND METHODS

3.0 Microbiological Study

The present sub-topic discussed the method to determine the efficacy of chitosan and lauric acid incorporated into starch-based film to inhibit the growth of microorganism. The antimicrobial activity testing was based on the agar diffusion method and liquid culture test based on standard serial broth dilution assay.

3.1 Agar Diffusion Method (Zone Inhibition Assay)

The agar diffusion test was carried out using the method described by Dawson *et al.* (1995). The strain selection represented typical spoilage organism groups commonly occurring in various kinds of food products. The strains were as follows: (1) *Escherichia coli*, a conventional hygiene indicator organism, a Gram-negative rod belonging to the same family of *Enterobacteriaceae* as for example *Salmonella*. (2) *Bacillus subtilis*, a Gram-positive rod capable of forming heat-resistant spores. Spores and vegetative cells of *Bacillus* species are widely distributed in nature and are common for example in cereals. For the agar plate test, the starch-based films containing chitosan and lauric as AM agent were cut into six squares (0.5 cm x 0.5 cm). Six sample squares were then placed onto the plate which was spreaded with bacteria (0.1 mL per plate). The agar plates were incubated at 37°C for 48 hours.

3.2 Liquid Culture Test

The liquid culture test was used to determine the antimicrobial activity of the test compounds by optical density and viable count and provides information on microbial growth kinetics, thus being more sensitive than the agar diffusion method (Han, 2003; Mann and Markham 1998). The liquid culture test will be divided into 2 categories; optical density measurement ($O.D_{600nm}$) and cfu/mL measurement. The optical density measurement also known as the turbidity measurement employs a variety of instrument to determine the amount of light scattered by a suspension of cells. Particulate objects such as bacteria scatter light in proportion to their numbers.

CFU/mL measurement (viable cell counts) also called plate counts, involve plating out (spreading) a sample of a culture on a nutrient agar surface. The sample or cell suspension can be diluted in a nontoxic diluent (e.g. water or saline) before plating. If plated on a suitable medium, each viable unit grows and forms a colony. Each colony that can be counted is called a colony forming unit (cfu) and the number of cfu's is related to the viable number of bacteria in the sample.

3.2.1 Optical Density Measurement ($O.D_{600nm}$)

For the liquid culture test (Chung *et al.* 2002), each film was cut into squares (1 cm x 1 cm). Three sample squares were immersed in 20 mL nutrient broth (Merck, Germany) in a 25 mL universal bottle. The medium was inoculated with 200µL of *Escherichia coli* in its late exponential phase, and then transferred to an orbital shaker and rotated at 37°C at 200 r.p.m. The culture was sampled periodically (0, 2, 4, 8, 12, 24, 28, 32, and 48 hours) during the incubation to obtain microbial growth profiles. The same procedure was repeated for the control starch-based film. The optical density ($O.D._{600nm}$) was measured at $\lambda = 600_{nm}$ using a spectrophotometer (figure 3.1) (Model UV-160, Shimadzu, Japan). For comparison purposes, the specific cell growth rates during the exponential growth phase were calculated as follows;

$$dX(t)dt = \mu X(t) \tag{3.3}$$

Whereas; *X (t)* is the cell concentration of microorganism in the medium O.D.$_{600nm}$, μ is the specific growth rate of *E.Coli* (h^{-1}) and t is the time (h).

Figure 3.1 Spectrophotometer Model UV-160

3.2.2 CFU/mL Measurement

The test method was slightly modified from the procedure by Hoffman *et al.* (2001). *Bacillus subtilis* and *Escherichia coli* was grown in nutrient broth and incubated aerobically for 16 hours at 37°C. Two 10 mL tubes of each strain were then centrifuged for 20 min at 1500 rpm decanted, washed with 0.1% nutrient broth (Merck, Germany), centrifuged for 20 min and decanted. Each of the two pellets of organisms was placed into 99 mL of peptone water to obtain an inoculum of approximately 10^8 CFU/mL. The 10^8 CFU/mL count was verified by serial dilution of the inoculum suspended in peptone water and plating in nutrient agar (Merck, Germany). 15 mL of the inoculum were added to each of the petri plates containing

the films. The film with no added AM was used as control. The plates were put on a shaker and rotated at room temperature (23°C) at 50 rpm. After 0, 2, 4, 8, 12, 24 and 48 hours, 0.1 mL samples were taken out from the petri dishes, diluted and plated in duplicate in nutrient agar. The plates were incubated at 37°C in an oven for 24 hours. The number of colonies on each plate was counted and reported as CFU/mL.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.0 Microbiological Study of Lauric acid and Chitosan Incorporated in AM Starch-based Film

The present sub-topic discussed the effectiveness of chitosan and lauric acid incorporated in starch-based film in various tests. The present test will focus on the efficacy of chitosan and lauric acid to inhibit the growth of selected Gram positive food pathogenic bacteria i.e. *Bacillus subtilis* and Gram negative food pathogenic bacteria *i.e. Escherichia coli.*

4.1 Agar Plate Test

Agar plate test also known as zone inhibition assay was performed as a preliminary step to screen the antibacterial activity of all films formulations incorporated with antimicrobial agents. The inhibitory zone in agar diffusion test can be affected by the solubility and diffusion rate of the test compounds in agar medium, thus agar diffusion test does not accurately reflect the antimicrobial effectiveness of the test compounds (Kim *et al*., 1995; Han, 2003).

4.1.1 Effect of Chitosan on *Escherichia coli* and *Bacillus subtilis* in Agar Plate Test

Inhibition effect of chitosan against *E. coli* and *B. subtilis* was expressed in figure 4.1. It is shown that, chitosan is 4.45% more effective in inhibiting the growth of *E. coli* compared to *B. subtilis.* Chitosan has been reported to have inhibitory activity against many bacterial species, including *L. monocytogenes* and *E. coli* (Lee *et al*, 2003).

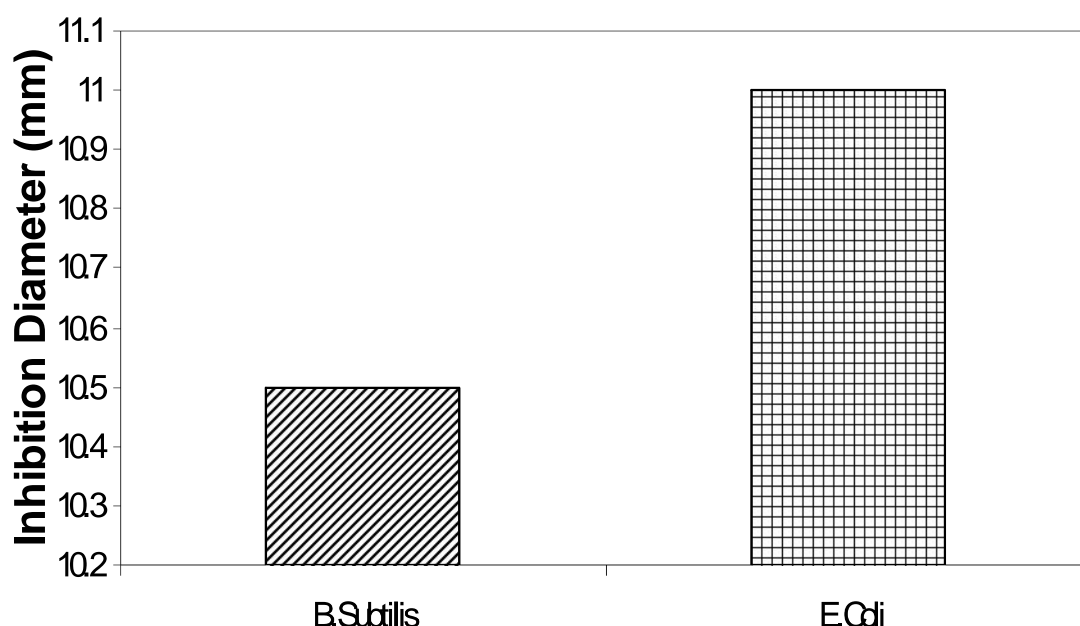


Figure 4.1 Inhibition of *B. subtilis* and *E. coli* on agar plates figure based on average zone diameter expressed as an area (mm) of inhibition zone

4.1.2 Effect of Lauric acid on *Escherichia* in Agar Plate Test

Figure 4.2 shows that inhibition area is proportional to the concentration of Lauric acid in the film. However, for a 0.07g/mL Lauric acid-incorporated film, no inhibition was observed. Lauric acid alone only has antimicrobial effect against Gram-positive bacteria and yeasts (Beuchat & Golden 1989; Kabara 1993).

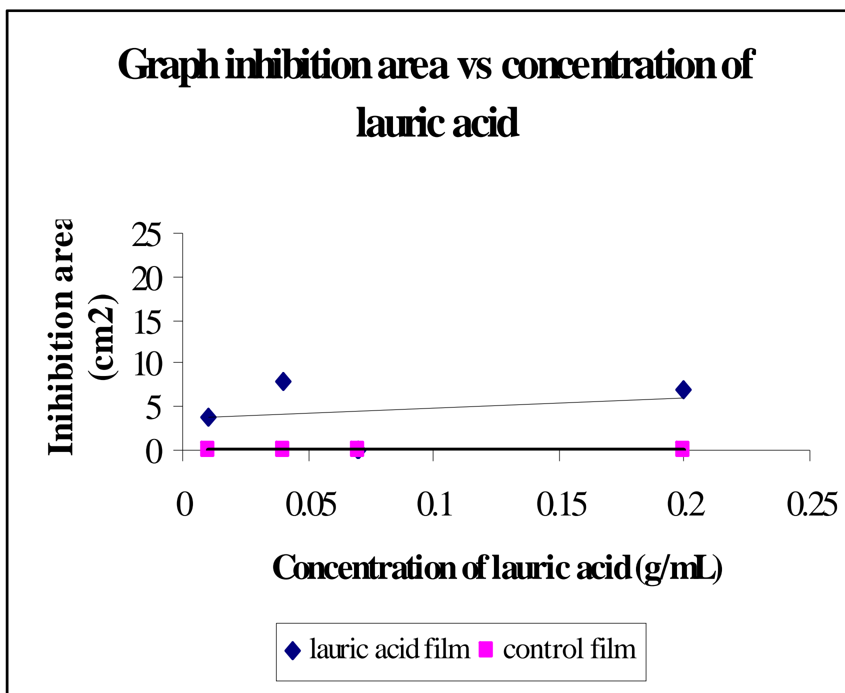


Figure 4.2 Inhibition of *E. coli* on agar plates figure based on average zone diameter expressed as an area (cm^2) of inhibition zone

4.1.3 Effect of Chitosan and Various Concentration of Lauric Acid on *Escherichia coli* and *Bacillus subtilis* in Agar Plate Test

Antibacterial activity of lauric acid against two pathogenic bacteria was expressed in terms of zone inhibition. All samples were examined for possible inhibition zones after incubation at 37°C for 24 hours. The inhibitory activity was measured based on the average diameter of the clear inhibition zone. If there was no clear zone surrounding as revealed in figure 4.3a-4.4a, it was assumed that there was no inhibitory effect (Salleh *et al*, 2007). Figure 4.3-4.4 shows the inhibition area for each plate test. The control films showed no inhibition area and colonies were formed all over the plate (Figure 4.3a and 4.4a). However, LA chitosan-based film successfully inhibited the growth of both type of Gram-staining bacteria *B. subtilis* and *E. coli* (Figure 4.3b and 4.4b).

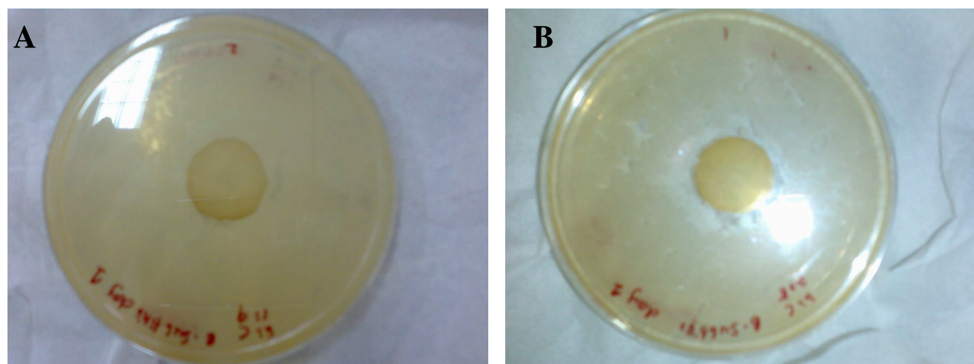


Figure 4.3 Comparison of inhibition zones between a) control film and b) LA chitosan-based film on plate containing *B. subtilis*

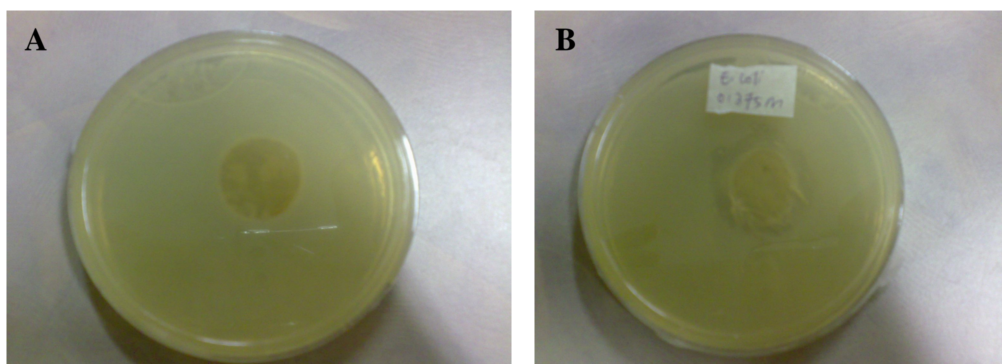


Figure 4.4 Comparison of inhibition zones between a) control film and b) LA chitosan-based film on plate containing *E. coli.*

For this test, a measurement of inhibition zones on/around film squares on inoculated bacteria was determined. Figure 4.5 shows the plotted graph for calculated inhibition area for each plate test. It is shown that clear zone expended is less than 5 mm diameter for inhibition of *E. coli* compared to *B. subtilis*. The result suggested that lauric acid is insufficient to retard the growth of gram negative bacteria, *E. coli*. However, it shows a good inhibition effect on gram positive bacteria, *B. subtilis* where as the concentration of lauric acid increase the area of zone inhibition increase.

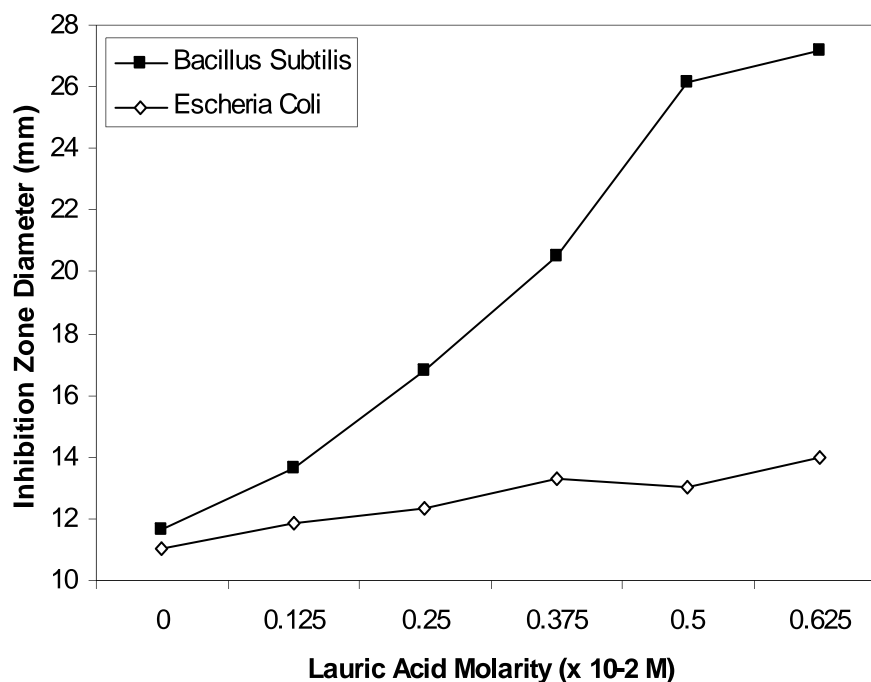


Figure 4.5 Comparison inhibition zone between *E. coli* and *B. subtilis*

4.1.4 Effect of Lauric Acid and Various Concentration of Chitosan on *Escherichia coli* and *Bacillus subtilis* in Agar Plate Test

Antibacterial activity of various concentration of incorporated in starch-based film against two pathogenic bacteria was expressed in terms of zone inhibition. The details of antimicrobial effectiveness of starch-based film incorporated with chitosan and lauric acid are shown in figure 4.6 and table 4.1. The inhibitory activity was measured based on the average diameter of the clear inhibition zone. If there was no clear zone surrounding as revealed in figure 4.6, it was assumed that there was no inhibitory effect, and was assigned as NI with a value of 0.00.

After 24 hours incubation at 37°C for control film (S), there was no inhibition occurred for both *B. subtilis* and *E. coli.* Bacteria colonies also occurred at the top of film sample. This is due to starch do not have antimicrobial activity. On the contrary, antimicrobial film incorporated with chitosan and lauric acid showed a very wide clear zone of inhibition indicated that an effective inhibition towards those bacteria.

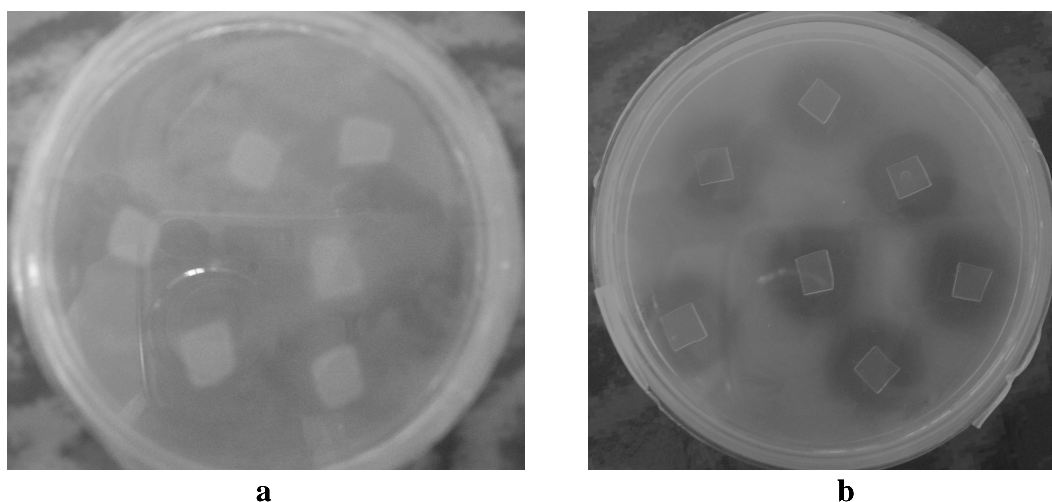


Figure 4.6 Comparison of inhibition area of (a) control film and (b) AM incorporated film

Table 4.1: Inhibition of *B. subtilis* and *E. coli* on Agar Plates Based on Average Zone Diameter Expressed as an Area (cm) of Inhibition Zone

Film	*B. subtilis*	*E. coli*	Remarks
Control (S)	NI	NI	NI = No inhibitory effect (all area on plates and film covered by bacteria)
S:C ratio 1:9	2.423	2.825	
S:C ratio 2:8	2.55	2.8	
S:C ratio 3:7	2.488	2.95	
S:C ratio 4:6	2.5	1.7	
S:C ratio 5:5	2.631	1.7	
S:C ratio 6:4	2.7	1.7	
S:C ratio 7:3	2.825	1.7	
S:C ratio 8:2	3.125	3.00	
S:C ratio 9:1	3.075	3.05	

Liquid Culture Test ($O.D_{600\ nm}$ Measurement)

Figure 4.7 shows Inhibition of *B. subtilis* and *E. coli* on agar plates figure based on average zone diameter expressed as an area (cm) of inhibition zone. It was found that Starch and chitosan different mixing ratio (S: C) 8:2, revealed the best inhibition on *B. subtilis* which is Gram- positive bacteria compared to other S: C ratios. In contrast, S: C ratio 9:1 showed a very good inhibition on *E. coli* (Gram-negative bacteria). From figure 4.7, the results indicated that S: C ratio 8:2 is the best to inhibit both *B. subtilis* and *E. coli* effectively followed by S: C ratio 9:1. S: C ratio from 1:9-3:7 obviously more effective towards inhibition of *E. coli* than S: C 4:6-7:3.

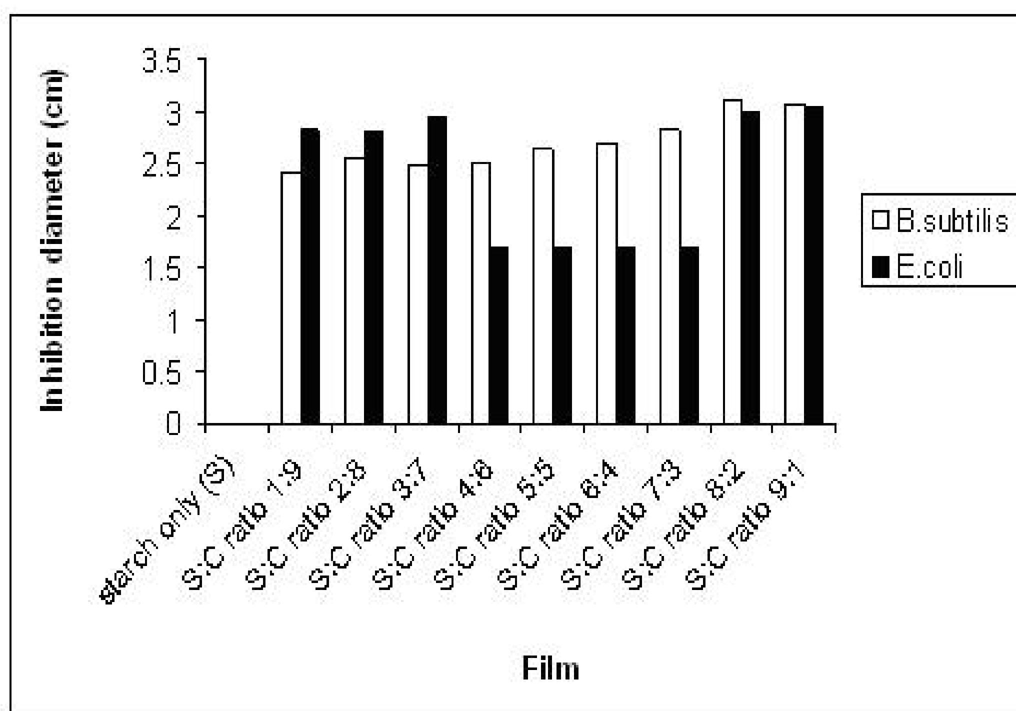


Figure 4.7 Inhibition of *B. subtilis* and *E. coli* on agar plates figure based on average zone diameter expressed as an area (cm) of inhibition zone

4.2 Liquid culture test

Liquid culture test had been done to support the result of agar plate test. The liquid culture test determines the antimicrobial activity of the test compounds by viable count and provides information on microbial growth kinetics, thus being more sensitive than the agar diffusion method (Han, 2003; Mann and Markham 1998).

4.2.1 Effect of Chitosan on *E. coli* and *B. subtilis* in Liquid Culture Test

Figure 4.8 shows the survival of microorganisms in liquid culture at 37°C that were in contact with chitosan. It is shown that chitosan does not effective in inhibiting the microbial growth in an aqueous condition compared to agar plate test. It maybe due to the low migration of chitosan from the film to aqueous medium (Lee *et al*, 2003).

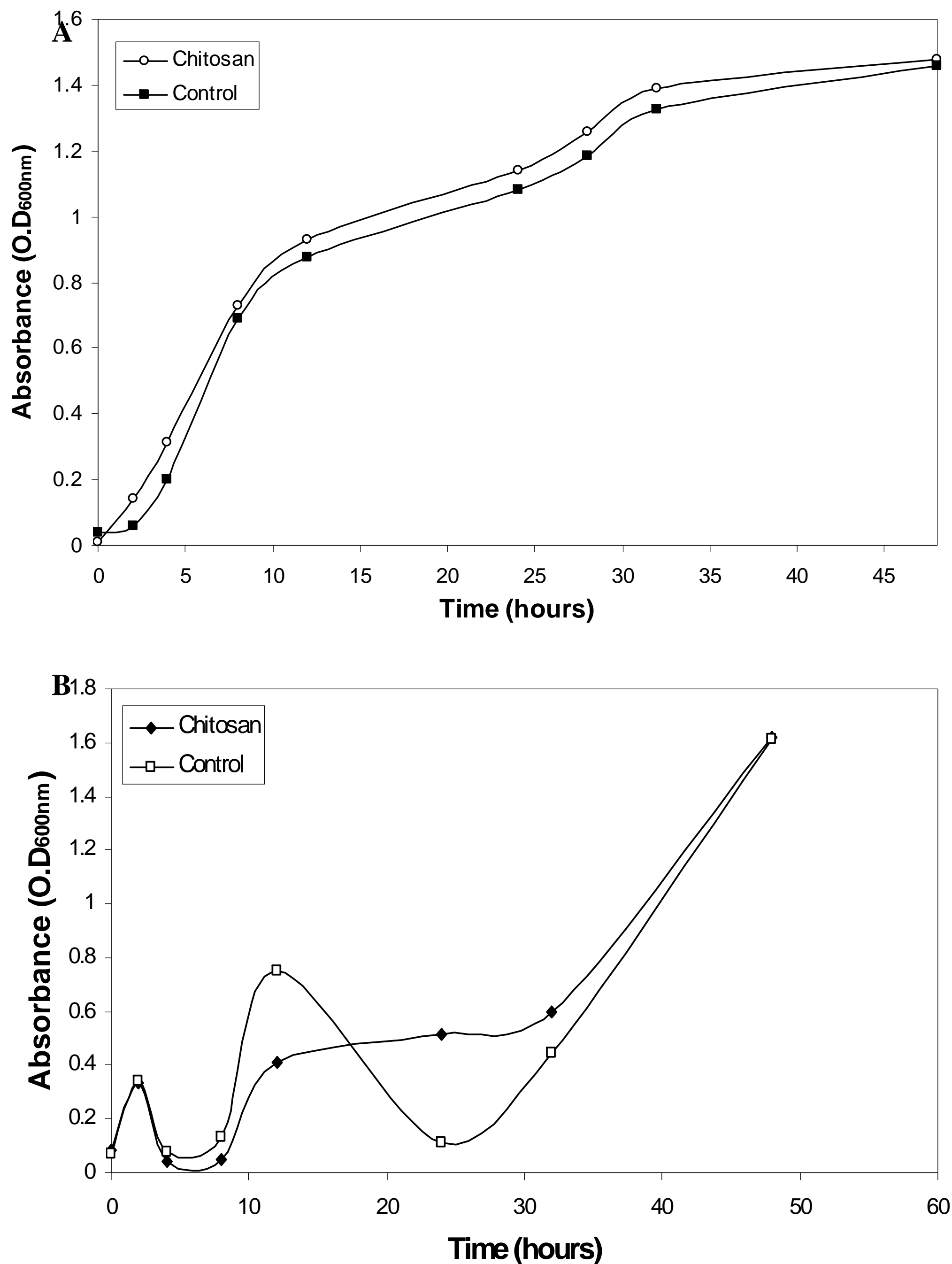


Figure 4.8 Inhibition of microbial growth by chitosan in liquid culture medium containing (a) *E. coli* and (b) *B. subtilis* at 37°C

4.2.2 Effect of Lauric Acid on *E. coli* in Liquid Culture Test

Similarly, the effect of various concentration of lauric acid on the inhibition of microorganisms is distinguishable much more clearer in figure 4.9. It shows that when the concentration of Lauric acid in a film increased, the inhibition effects towards *E.coli* growth also increased. This is also observed for 0.07 g/mL lauric acid-incorporated film which is different with the agar diffusion test. This might be because of the differences in the degree of lauric acid insolubility.

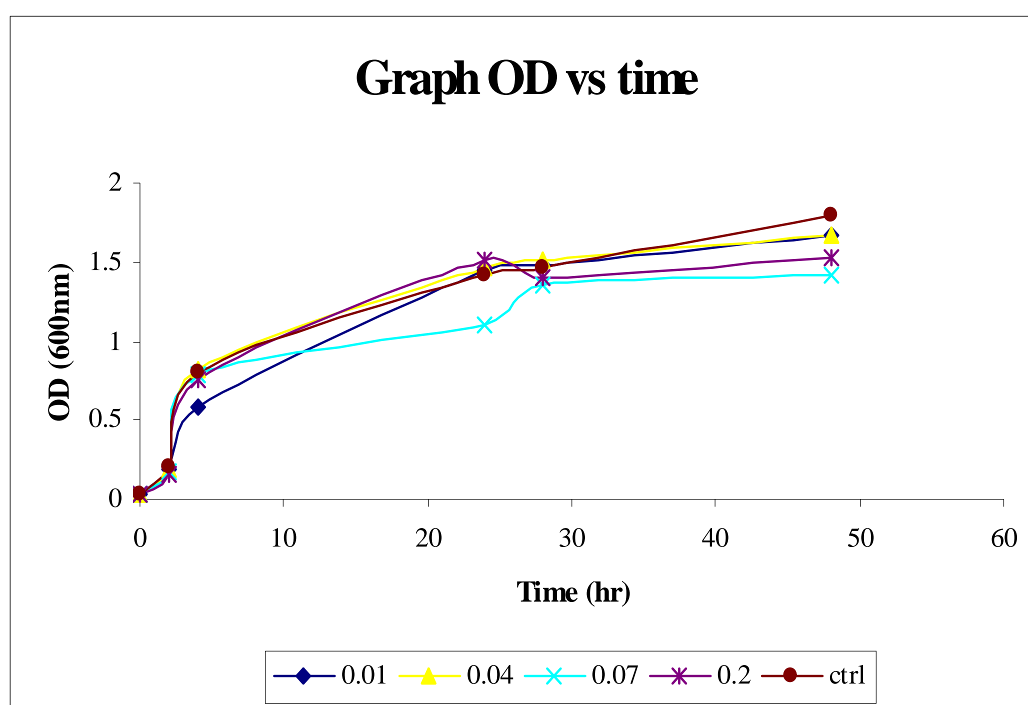


Figure 4.9 Inhibition of microbial growth by the lauric acid in a liquid culture medium containing *E. coli* at 37°C

4.2.3 Effect of Chitosan and Various Concentration of Lauric Acid on *E. coli* and *B. subtilis* in Liquid Culture Test

Figure 4.10 and 4.11 shows the inhibition of *E. coli* and *B. subtilis* by the LA chitosan-based films in liquid culture broth at 37°C respectively. It is shown that 0.625×10^{-2}M is the most effective concentration to inhibit *E. coli*. Lauric acid inhibition with the liquid culture test but lack of inhibition with the zone assay (agar

plate test) may be due to the difference in the mobility of the bacterial cells within the two systems. The zone of inhibition assay allows little or no mobility of the non-motile *E. coli*, whereas the liquid culture test uses a liquid broth under constant agitation which facilitates cell movement and exposure to the film despite the non-motile characteristic of the strain. The fluid nature and agitation of the broth may be have increased mobility of the bacteria and diffusion of the lauric acid resulting in a bacteriocidal activity not seen in the zone test on solid media (Padgett *et al*, 2000).

Similarly, 0.625×10^{-2}M is also the most effective concentration of LA incorporated in chitosan-based film which inhibited the growth of *B. subtilis* (figure 4.11). Although there were inhibition for both *E. coli* and *B. subtilis*, the LA chitosan-based film were more effective against Gram-positive bacteria than the Gram-negative bacteria studied. Lauric acid has been found to have an antimicrobial effect against gram-positive bacteria while the incorporation of chitosan as film based help inhibit gram-negative bacteria (Salleh *et al*, 2007; Padgett *et al*, 2000). In fact, one of the reasons for the antimicrobial character of chitosan is the positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms (Shahidi *et al*, 1999).

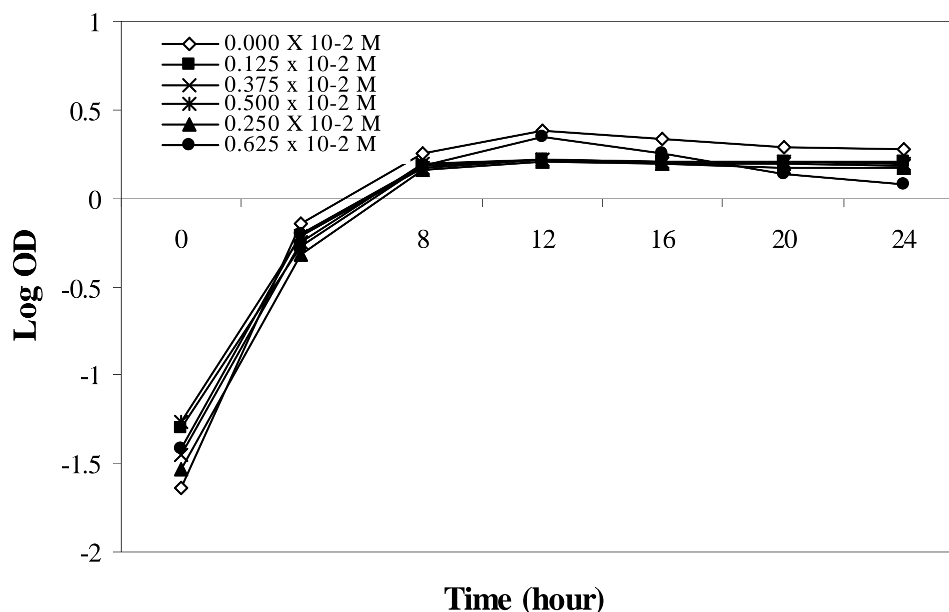


Figure 4.10 Inhibition of microbial growth by the LA chitosan-based film in a liquid culture medium containing *E. coli* at 37°C

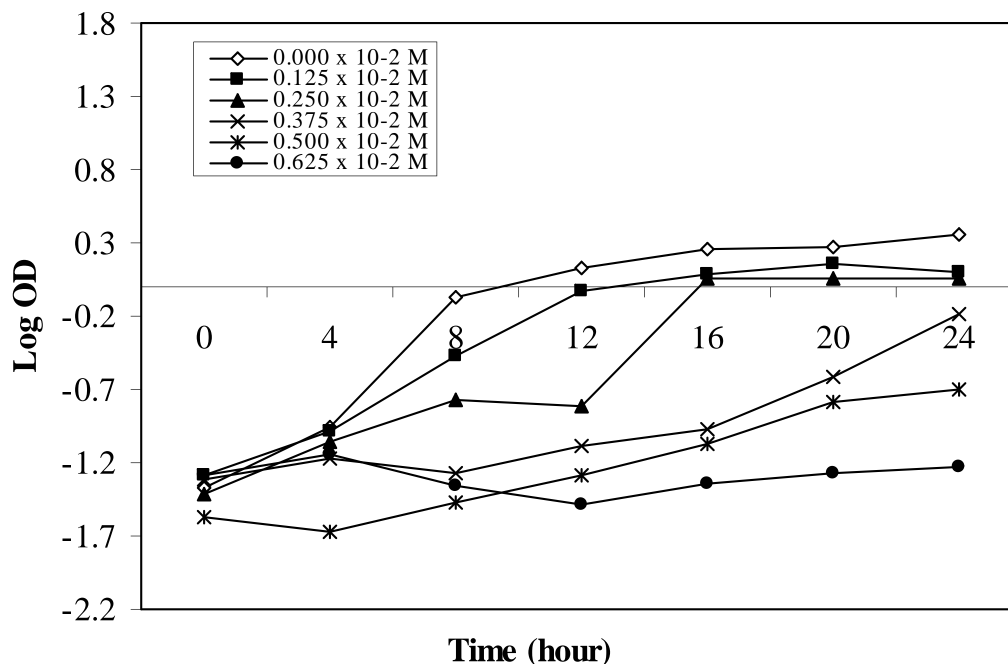


Figure 4.11 Inhibition of microbial growth by the LA chitosan-based film in a liquid culture medium containing *B. subtilis* at 37°C

4.2.4 Effect of Lauric acid and Various Concentration of Chitosan on *E. coli* and *B. subtilis* in Liquid Culture Test (O.D$_{600\ nm}$ Measurement)

Figure 4.12 shows the inhibition of developed AM film on *B. subtilis* whereas figure 4.13 shows that on *E. coli* in liquid culture test. It was clearly observed that S: C ratio 8:2 is the most effective formulation to inhibit *B. subtilis* as can be seen in figure 4.12. Meanwhile, S: C ratio 9:1 is the best formulation to inhibit *E. coli* (figure 4.13). Although there were inhibition for both *B. subtilis* and *E. coli*, the antimicrobial starch-based film incorporated with lauric acid and chitosan were more effective against Gram-positive bacteria than the Gram-negative bacteria studied.

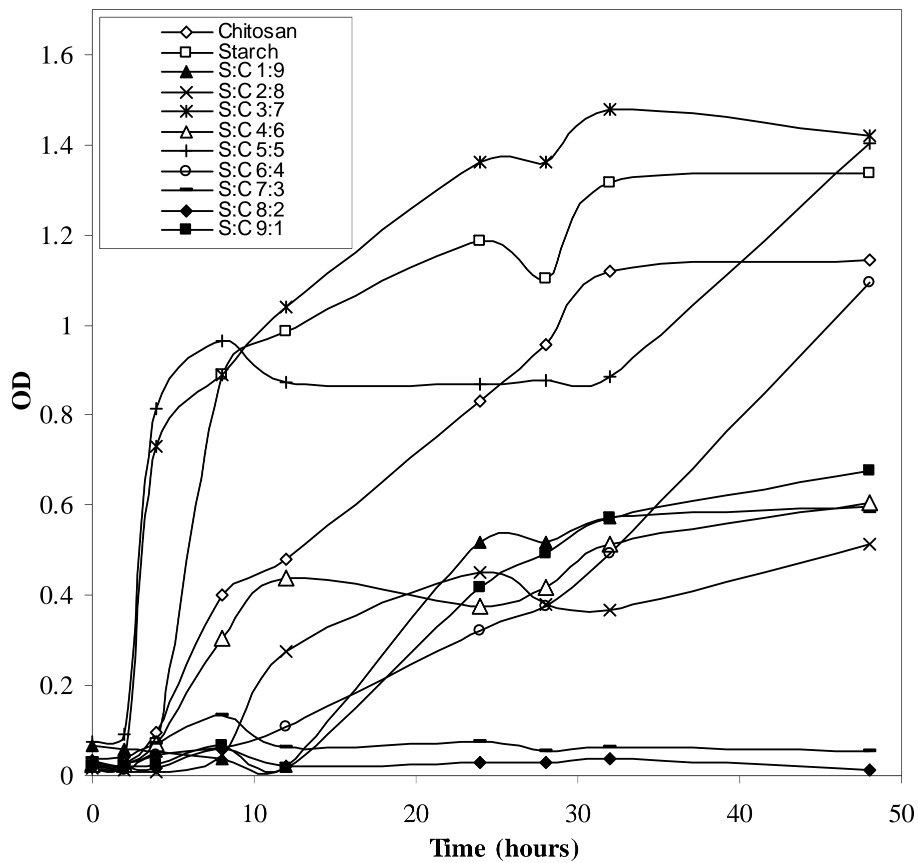


Figure 4.12 Inhibition of controls (starch only and chitosan only) and starch (S): chitosan (C) at different ratios on *B. subtilis* in liquid culture test

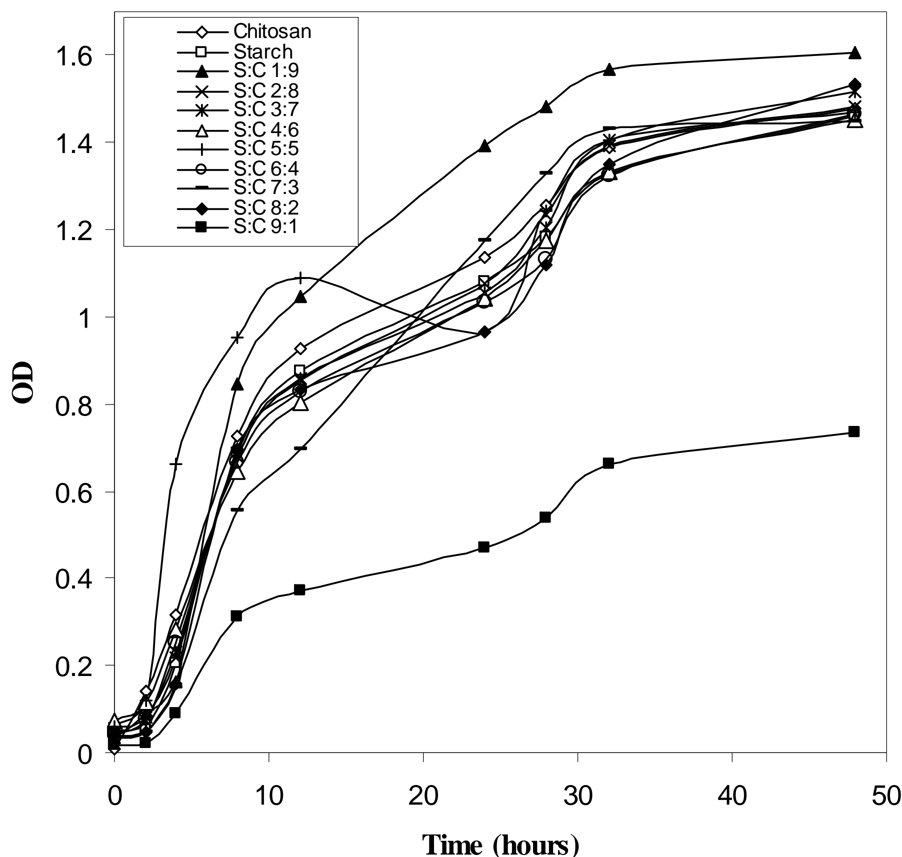


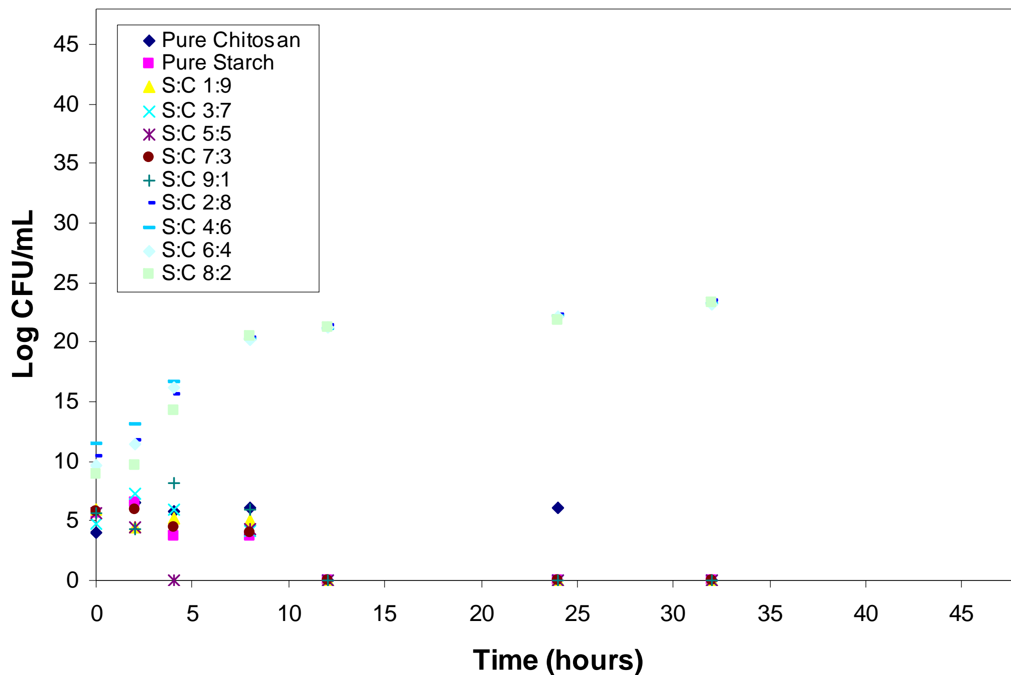
Figure 4.13 Inhibition of controls (starch only and chitosan only) and starch (S): chitosan (C) at different ratios on *E. coli* in liquid culture test

In fact, one of the reasons for the antimicrobial character of chitosan it's positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms (Shahidi *et al*, 1999). In the Gram-positive bacteria, the major constituent of its cell wall is peptidoglycan and there is very little protein. The cell wall of Gram-negative bacteria also has an outer membrane, which constitutes the outer surface of the wall (Zheng & Zhu, 2003). Study from Jiang et al, (1997), observed that from electron micrographs for Gram-positive and Gram-negative bacteria in the presence of chitosan show the cell membrane of Gram-positive bacteria was weakened or even broken, while the cytoplasma of Gram-negative bacteria was concentrated and the interstice of the cell were clearly enlarged. This study indicated that the mechanisms of the antimicrobial activity of chitosan were different between Gram-positive and Gram-negative bacteria. The

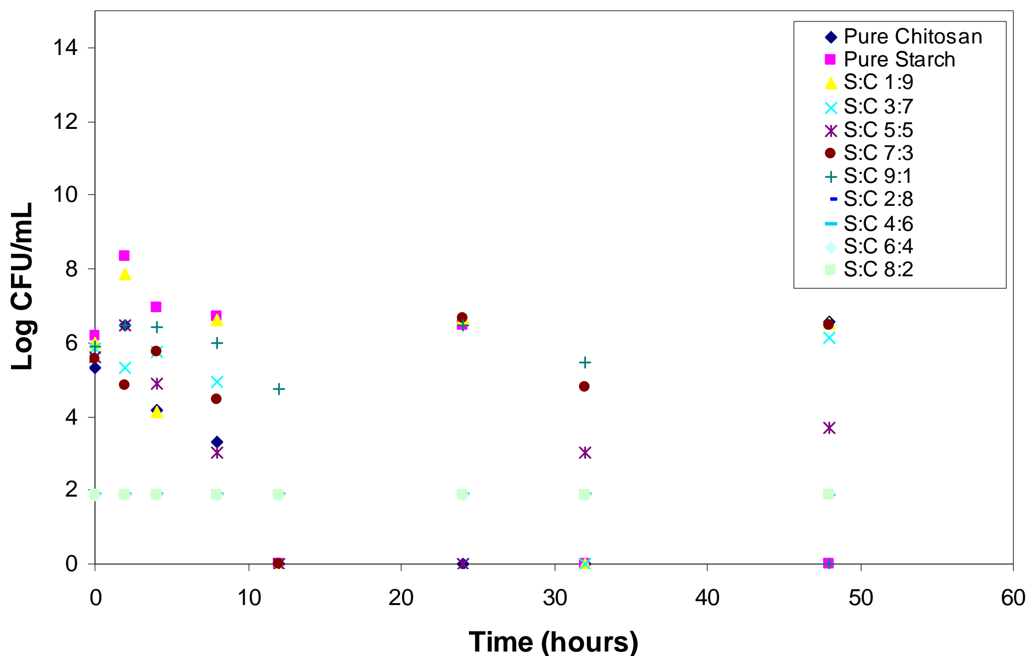
mechanism seems much more difficult and complex in the Gram-negative to eliminate the bacteria. Additionally, the antimicrobial mechanism of chitosan might differ from that of other polysaccharides because there are positives charges on the surface of chitosan (Jiang et al., 1997).

4.2.5 Effect of Lauric Acid and Various Concentration of Chitosan on *Escherichia* **coli** and *Bacillus* **s***ubtilis* in Liquid Culture Test (CFU/mL)

The CFU/mL measurements were conducted to verify the results in optical density measurement. Although there a re several studies in the existing literature that make use of optical density measurements to determine bacterial growth, which do not seems have considered the artifact of the additional turbidity effect caused by the release of compound from the films (Fernandez-Saiz et al, 2009; Coma et al, 2002; Liu et al, 2006; Liu et al, 2001). Figure 4.14 and 4.15 shows the log reduction of the bacterial count for *E. coli* and *B. subtilis* respectively. It is shown that the combination of various concentration of chitosan with lauric acid is more effective against B. subtilis. This is in agreement with previous results in OD measurement test in previous section 4.2.4.



Figures 4.14 CFU results inhibitory on *E. coli* on (a) chitosan film (b) starch film (c) S:C ratio 1:9 (d) S:C ratio 3:7 (e) S:C ratio 5:5 (f) S:C ratio 7:3 (g) S:C ratio 9:1



Figures 4.15 CFU results inhibitory on *B. subtilis* on (a) chitosan film (b) starch film (c) S:C ratio 1:9 (d) S:C ratio 3:7 (e) S:C ratio 5:5 (f) S:C ratio 7:3 (g) S:C ratio 9:1

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.0 Introduction

Previous chapter discussed all the result obtained from this study. The findings of the present study emphasize the promising potential and feasibility of lauric acid and chitosan in controlling the growth of selected bacteria.

5.1 Conclusions

The progress presented in this thesis began with formulating the AM starch based film incorporated with lauric acid and chitosan in order to monitor the effectiveness of both antimicrobial agents towards inhibiting the growth of selected microorganisms.

Lauric acid and chitosan alone gives a poor inhibition effect on bacterial growth. Lauric acid and chitosan may be combined with other antimicrobial substances for enhancing its antimicrobial efficacy. Since chitosan dissolves in slightly acidic solutions, a simple and economical way of enhancing antimicrobial properties of chitosan films would be to dissolve it in organic acids that possess antimicrobial properties (Begin and Calsteren 1999). Various organic acids that naturally occur in fruits and vegetables and possess general antimicrobial activity such as acetic, lactic, malic, and citric, sorbic, benzoic and succinic acids can be used for this purpose (Beuchat 1998).

LA-chitosan-based film exhibited good film forming property due to the presence of high density of amino groups and hydroxyl groups and inter and intra molecular hydrogen bonding. The chitosan and lauric acid showed interesting qualities in the field of antimicrobial packaging, due to antimicrobial activities of chitosan and lauric acid. Combination of chitosan and lauric acid as an active film showed obvious effects towards inhibition of *B. subtilis* and *E. coli* indicated that the film had synergistic antimicrobial effect. The LA chitosan-based film demonstrates more effective antimicrobial ability against *B. subtilis* than *E. coli*. $0.625x10^{-2}$M is the most effective concentration of LA which had greater inhibition on both selected microbe than other solutions as revealed in agar plate test and liquid culture test.

5.1 Recommendations for Future Works

- ❖ Testing with real foods to understand the controlled release kinetics of active compound from the packaging material into various foodstuffs.

- ❖ Growth inhibition study is expanded to more variety of microorganism for a wider use of Lauric acid.

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APPENDIX A

Proceedings of The 2nd SEATUC Symposium, 26-27 February 2008. Institut Teknologi Bandung, Indonesia

PHYSICAL AND MECHANICAL STUDY OF CHITOSAN AND LAURIC ACID AS ANTIMICROBIAL AGENTS IN STARCH-BASED FILMS

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ABSTRACT. This study aimed at the development of food packaging based on wheat starch incorporated with chitosan and lauric acid as antimicrobial agents. Starch-based film incorporated with chitosan and lauric acid was prepared by casting method. Chitosan has a widely been used in antimicrobial films, to provide edible protective coating, dipping and spraying for the food products due to its antimicrobial properties and good mechanical properties. Incorporation of chitosan and lauric acid as antimicrobial agents into starch-based films affected the physical and mechanical properties of starch-based films. The starch-based films also having antimicrobial properties that can extend shelf-life of the food packed. The effect of addition of lauric acid and chitosan into starch-based films on physical and mechanical properties were characterized and FTIR analysis was also performed to determine functional groups interactions between film and added antimicrobial agent. The incorporation of chitosan and lauric acid into starch-based film enhanced the physical and mechanical properties of starch-based films. Adding chitosan and lauric acid as antimicrobial agents in starch-based film indicated that there were interactions present with the functional groups of starch as measured by FTIR.

Keywords: Chitosan, Lauric Acid, Antimicrobial agent, Starch-based film, Food Packaging

APPENDIX B

Proceedings of Polymer Advanced Technologies (PAT2007), 22-25 October 2007. Shanghai, China

MECHANICAL PROPERTIES AND ANTIMICROBIAL ANALYSIS OF ANTIMICROBIAL STARCH-BASED FILM

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ABSTRACT. Antimicrobial (AM) packaging is one of the most promising active packaging systems. Increase demand in food safety, quality, convenience and environmental concerns associated with the handling of plastic waste has emphasized the importance in developing biodegradable and edible films from natural polymers, such as starch. Starch-based film is considered an economical material for antimicrobial packaging. This study aimed at the development of food packaging based on wheat starch incorporated with chitosan and lauric acid as antimicrobial agents. Chitosan has a widely been used in antimicrobial films, to provide edible protective coating, dipping and spraying for the food products due to its antimicrobial properties. Incorporation of chitosan and lauric acid as antimicrobial agent into starch-based film enhance physical and mechanical properties of starch-based film. The starch-based film also having antimicrobial properties that can extend shelf-life of the food packed. The antimicrobial effect of antimicrobial starch-based (AM) film was tested on B. substilis and E. coli. Inhibition of bacterial growth was examined using two methods, i.e. zone of inhibition test on solid media and liquid culture test (optical density measurements). The control (pure wheat starch) and AM film (incorporated with chitosan and lauric acid) were produced by casting method. From the observations, AM films exhibited inhibitory zones. Interestingly, a wide clear zone on solid media was observed for B. substilis growth inhibition whereas inhibition for E. coli was not as effective as B. substilis. From the liquid culture test, the AM films clearly demonstrated a better inhibition against B. substilis than E. coli.

Keywords: Biodegradable, Edible, Antimicrobial starch-based film, Chitosan, Lauric acid

APPENDIX C

Proceedings of 3rd International Symposium Food and Agricultural Products: Processing and Innovations, 24-26 September 2007, Naples, Itali.

INHIBITION OF *BACILLUS SUBTILIS* AND *ESCHERICHIA COLI* BY ANTIMICROBIAL STARCH-BASED FILM INCORPORATED WITH LAURIC ACID AND CHITOSAN

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ABSTRACT. Food safety is one of key issues of public health. One option is to use packaging to provide an increased margin of safety and quality. Active packaging technologies are being developed as a result of these driving forces. Active packaging is an innovative concept that can be defined as a mode of packaging in which the package, the product and the environment interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the product (Suppakul, Miltz, Sonneveld & Bigger, 2003). Antimicrobial (AM) packaging is one of the most promising active packaging systems. Starch-based film is considered an economical material for antimicrobial packaging. This study aimed at the development of food packaging based on wheat starch incorporated with lauric acid and chitosan as antimicrobial agents. The purpose is to restrain or inhibit the growth of spoilage and/or pathogenic microorganisms that are contaminating foods. The antimicrobial effect was tested on B. substilis and E. coli. Inhibition of bacterial growth was examined using two methods, i.e. zone of inhibition test on solid media and liquid culture test (optical density measurements). The control and AM films (incorporated with chitosan and lauric acid) were produced by casting method. From the observations, only AM films exhibited inhibitory zones. Interestingly, a wide clear zone on solid media was observed for B. substilis growth inhibition whereas inhibition for E. coli was only revealed underneath the film discs. From the liquid culture test, the AM films clearly demonstrated a more effective inhibition against B. substilis than E. coli.

Keywords: Antimicrobial packaging, Lauric acid, Chitosan, *B. subtilis*, *E. coli*.

APPENDIX D

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PREPARATION, CHARACTERIZATION AND ANTIMICROBIAL ANALYSIS OF ANTIMICROBIAL STARCH-BASED FILM INCORPORATED WITH CHITOSAN AND LAURIC ACID

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ABSTRACT. Antimicrobial (AM) packaging is one of the most promising active packaging systems. Increase demand in food safety, quality, convenience and environmental concerns associated with the handling of plastic waste has emphasized the importance in developing biodegradable and edible films from natural polymers, such as starch. Starch-based film is considered an economical material for antimicrobial packaging. This study aimed at the development of food packaging based on wheat starch incorporated with chitosan and lauric acid as antimicrobial agents. Chitosan has a widely been used in antimicrobial films, to provide edible protective coating, dipping and spraying for the food products due to its antimicrobial properties. Incorporation of chitosan and lauric acid as antimicrobial agent into starch-based film enhance physical and mechanical properties of starch-based film. The starch-based film also having antimicrobial properties that can extend shelf-life of the food packed. The antimicrobial effect of antimicrobial starch-based (AM) film was tested on *B. substilis* and *E. coli*. Inhibition of bacterial growth was examined using two methods, *i.e.* zone of inhibition test on solid media and liquid culture test (optical density measurements). The control (pure wheat starch) and AM film (incorporated with chitosan and lauric acid) were produced by casting method. From the observations, AM films exhibited inhibitory zones. Interestingly, a wide clear zone on solid media was observed for *B. substilis* growth inhibition whereas inhibition for *E. coli* was not as effective as *B. substilis*. From the liquid culture test, the AM films clearly demonstrated a better inhibition against *B. substilis* than *E. coli*.

Keywords: Biodegradable, Edible, Antimicrobial starch-based film, Chitosan, Lauric acid