CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Statement of problems

Chitosan, a waste product from food industries, is a deacetylated derivative of chitin. It is a nontoxic and biodegradable polysaccharide based polymer. Nowadays chitosan is attracting a lot of attention in medical, agriculture, cosmetic, and food industries. Researches on chemical modification of chitosan have also been reported. This includes quaternary ammonium chitosan, which was proved to impart antimicrobial activities. Other quaternary ammonium derivatives have also been proposed.

Here we focus our attention in the chemical modifications of chitosan by synthesis of quaternized *N*-alkyl chitosan derivatives. Then they were tested for solubility and ability to delay ripens or decays of the fruit, banana in this case. This is in fact the first study to use quaternized *N*-alkyl chitosan in fruit coating application. It was hypothesized that the quaternization of *N*-alkyl chitosan can be achieved. This derivative should be water soluble and can extend the shelf-life of the fruits. The outcome of this study should provide fundamental information that can lead to the development of chitosan derivatives for agricultural application in Thailand.

1.2 Objectives

1. To determine an optimum condition for synthesis of *N*-alkylated derivatives of ammonium chitosan derivative.
2. To investigate the possibility to use quaternary ammonium chitosan and its *N*-alkylated derivatives for banana coating.

1.3 Scope of this research

The thesis was outlined as follows:

1. Synthesis of *N*-alkyl chitosan using butylaldehyde and benzaldehyde via Schiff 's base intermediate
2. Synthesis of *N*-alkyl quaternary ammonium chitosan by reaction with methyl iodide
3. Determination of %*N*-alkylation and %methylation by NMR spectroscopy
4. Solubility determination of the quaternized *N*-alkyl chitosan
5. Study of banana coating.

1.4 Theory and Literature review

1.4.1 Chitosan

Chitosan is a natural nontoxic biopolymer derived by deacetylation of chitin, a polysaccharide found in the exoskeleton of crustaceans. Chitosan mainly consists of 2-amino-2-deoxy-D-glucose (GlcN) repeating unit with a small amount of 2-acetyl-2-deoxy-D-glucose residue. The amount of GlcN unit in chitosan is generally referred to the percent degree of deacetylation or %DD. It has been observed that the degree of acetylation of chitosan influences physical and chemical properties as well as biological activities of chitosan, tensile strength of chitosan films, enzyme binding and immunological activity [1]. Various techniques are used for determination of %DD such as IR [2], NMR [3] and metachromatic titration [4]. The structure of chitosan and chitin are shown in Fig. 1.1.

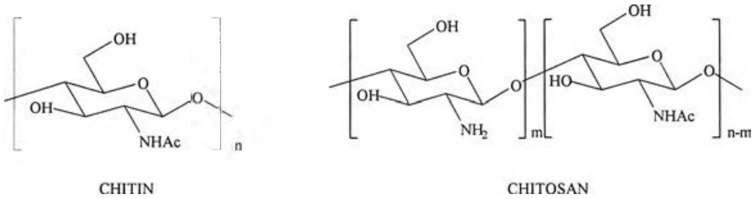


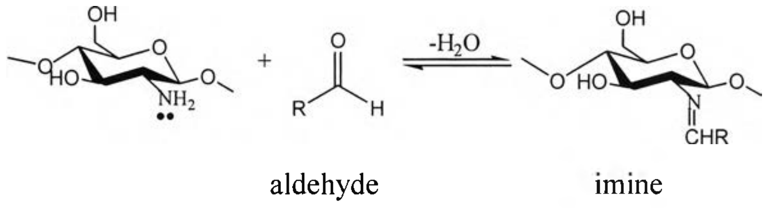
Figure 1.1 Structure of chitin and chitosan

The intriguing properties of chitosan have been known for many years. It has been used in various fields: agriculture, industry, and medicine. Especially in medicine, chitosan is used extensively as a biomaterial owing to anticoagulant properties, antibacterial and antifungal action [5]. To improve the properties of chitosan, the chemical reaction at the amino group was studied.

1.4.2 Chemical modification of chitosan

Chitin and chitosan can both be considered to be weak bases, and as such, they undergo the typical neutralization reaction of alkaline compounds. In these reactions the nonbonding pair of electrons on the primary amine groups of the glucosamine units performs the role of accepting protons. Thus the primary amine groups become cationically charged.

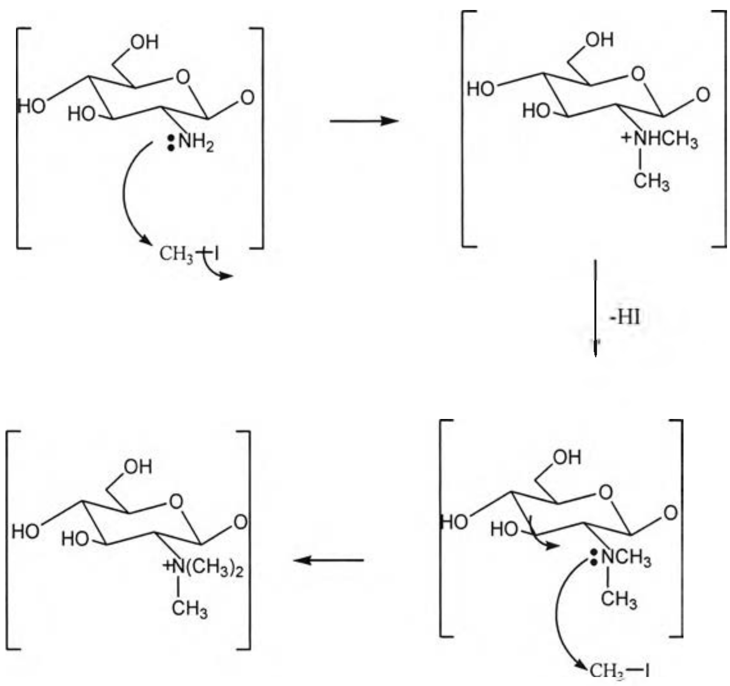
Chitosan carries a number of hydroxyl (-OH) and amino ($-NH_2$) groups. This prompts many researchers to find ways of modifying its properties either by physical blending or chemical reactions. The nonbonding pair of electrons on the primary amine groups also makes chitosan a potent nucleophile, reacting readily with most aldehydes to form imines as Scheme 1.1.



Scheme 1.1 Nucleophilic reaction of chitosan.

Beside the reductive alkylation, methylation at the amino groups was also explored by many researchers, because the resulting product is quaternary ammonium chitosan (QAC) (Scheme 1.2). The presence of positive charge along the polymer chain helps increase its solubility in water [6].

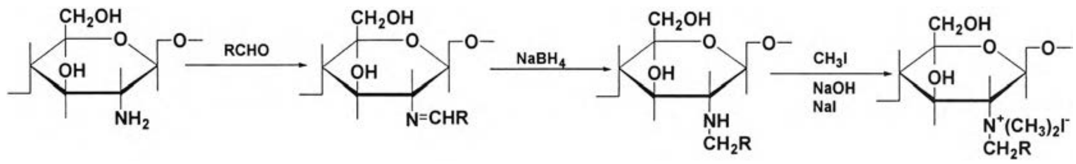
Domard and coworkers [6] reported the optimal method to produce QAC by doing the reaction in NMP and sodium hydroxide at 1.4 M concentration. After adding methyl iodide, the reaction was kept at 36 °C. To increase the degree of methylation, Sieval and et al. [7] had modified the reaction by adding methyl iodide in two steps, resulting in an increase of degrees of quaternization to 70%. Nevertheless, methylation at hydroxyl groups cannot be avoided, as reported by Sieval and et al.[7] and Sarisuta [17].



Scheme 1.2 Synthesis of quaternary ammonium chitosan (QAC)

Other quaternized derivatives were also suggested. Kim and coworkers [8] were interested in antimicrobial activity of quaternary ammonium derivatives of chitosan. They synthesized *N*-alkyl chitosan derivatives by introducing alkyl groups into the amine groups of chitosan via Schiff's base intermediates then quaternized these derivatives by reaction with methyl iodide (Scheme 1.3). The best reducing agent of Schiff's base for the *N*-alkylation step is sodium cyanoborohydride as reported by Desbrieres and coworkers [9].

Others have show that QAC and its *N*-alkylated derivatives could also be used as antistatic materials, especially for textile [10].



Chitosan Schiff's base N-alkyl chitosan Quaternized products

Scheme 1.3 Synthesis of quaternary ammonium salt of *N*-alkyl chitosan

1.4.3 Antimicrobial activities of chitosan and their derivatives

Chitosan has been shown to have antimicrobial potential. Chitosan acts more quickly on fungi and algae than on bacteria. However, like other properties of chitosan, this activity is dependent on the type of chitosan, degree of deacetylation, and molecular weight. The antimicrobial activity of chitosan and their derivatives was reported by Kim and coworkers [8]. They studied the antibacterial activities against *S. aureus* with their QAC derivatives. The antibacterial activities increased with increasing the chain length of the alkyl substituent. As well as the study by Jia and coworkers [11], they determined minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of QAC in water to be 0.25 and 0.50% in acetic acid medium. They found that the antibacterial activities of quaternized chitosan against *E. coli* are related to its molecular weight. It was also found that the antibacterial activity of quaternized chitosan against *E. coli* is stronger than that of chitosan.

In another study by Hong and coworkers [12], the antibacterial activities of six chitosan derivatives and six chitosan oligomers with different molecular weights were examined against four gram-negative and seven gram-positive bacteria. The high molecular weight chitosan showed a higher antibacterial activity than chitosan oligomers. QAC exhibit antibacterial activity on both gram-positive and gram-negative bacteria. The exact mechanism of the antimicrobial action of chitosan and their derivatives is still unknown, but different mechanism has been proposed [13].

- Interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leads to the leakage of proteinaceous and other intracellular constituents.
- It acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of toxins and microbial growth.
- It activates several defense processes in the host tissue, acts as a water binding agent and inhibits various enzymes.
- Binding of chitosan with DNA and inhibition of mRNA synthesis occurs via chitosan penetrating the nuclei of the micro-organisms and interfering with the synthesis of mRNA and proteins.

1.4.4 Chitosan in the edible film industry

The use chitosan as edible film and coating to extend shelf life and improve freshness of foods have been examined during the past few years due to its eco-friendly and biodegradable nature. The common packaging material, polyethylene film, is used to protect foods from fermentation, but it cannot protect fungal growth. It is due to water condensation during storage that promotes fungal growth. There are many mechanisms involved in extending shelf life of food by coating film. These include controlled moisture transfer between food and surrounding environment, controlled release of chemical agents like antimicrobial substances, antioxidants, reduction of oxygen partial pressure in the package that results in decreased rate of metabolism, and controlled rate of respiration. Oxygen, carbon dioxide and water vapor permeability of edible and conventional films are presented in Table 1.1 [14]. The oxygen permeabilities of most edible coating were lower than the conventional plastic film.

Table 1.1 Gas permeability of edible coatings

Film	Permeability		
	O_2	CO_2	H_2O Vapor
Chitosan	0.0041	-	0.49
Zein	0.36$\pm$ 0.16	2.67$\pm$ 1.09	0.116 $\pm$ 0.019
HPC	3.57 $\pm$ 0.03	143.9 $\pm$ 3.76	0.110 $\pm$ 0.004
PE	8.30	26.1	-
PP	0.55 + 0.005	-	0.00065 $\pm$ 0.06
PVC	0.09-17.99	1.35-26.98	0.00071

*Zein is protein extract from rice; HPC is hydroxypropyl cellulose; PE is Polyethylene; PP is Polypropylene; PVC is polyvinyl chloride

Thus the film-forming properties of chitin and chitosan were studied for the food wraps. Due to its ability to form semi-permeable film, chitosan coating can be expected to modify the internal atmosphere as well as decreases the transpiration loss and delay the ripening of fruits. Juang and coworkers [15] reported the application of chitosan coating reduced respiration rate and weight loss, delayed the increase in PPO activity and the changes in color, eating quality and partially inhibited decay of fruit during storage.

1.5 Statement of hypotheses

The primary hypothesis was that quaternization of *N*-butyl chitosan and *N*-benzyl chitosan would produce water soluble chitosan derivatives. The secondary hypothesis was that the soluble quaternized *N*-alkyl chitosan could be used to coat banana in order to delay its browning.