

**PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES  
OF CRAWFISH CHITOSAN AS AFFECTED BY DIFFERENT  
PROCESSING PROTOCOLS**

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

in

The Department of Food Science

by  
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B.S., Seoul National University, 1991  
December 2004

## ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my major advisor, Dr. Witoon Prinyawiwatkul, for his guidance, encouragement, patience, and understanding throughout the course of this research.

I am also thankful to Dr. Hong Kyoon No for providing me the research topic and valuable suggestions.

I would like to thank Dr. Marlene E. Janes and Dr. Zhimin Xu for serving on my committee. Further acknowledgements are extended to Dr. Jack N. Losso, Dr. Joan M. King, Dr. Marlene E. Janes, and Dr. Zhimin Xu, not only for providing me the research facilities but also for imparting valuable knowledge that I used in this research.

Special thanks are also extended to Dr. Kayanush J. Aryana, Dr. Charles Boeneke, and Ms. Paula McGrew, from the Department of Dairy Science and also Dr. Lavry Khachatryan from the Department of Chemistry at LSU for their valuable technical assistance and constructive advice.

My sincere gratitude and appreciation goes to my dear colleagues and friends, Kandasamy Nadarajah and Janette Saidu who have assisted and supported me throughout the most rigorous phase of my research with their expertise, wisdom, and friendship.

Last but not least, I thank my husband, Christian, and my children, Kristi and Victor, who are the sunshine of my life for their encouragement and support throughout every endeavor of mine. It is to them that this work is dedicated.

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## ABSTRACT

Chitosan is made from chitin by a chemical process involving demineralization (DM), deproteinization (DP), decolorization (DC), and deacetylation (DA). Very little work has been done to demonstrate the effects of altering or excluding any of the processing steps on chitosan characteristics. The present study was undertaken to evaluate the effects of process modification during chitosan production on the physicochemical and functional properties of crawfish chitosans.

Five experimental chitosan samples (DCMPA, DMCPA, DMPCA, DMPAC, DAMPC) prepared with modified processing protocols and the control (DPMCA - traditional chitosan production process) were evaluated and compared with the two commercial crab chitosans. All samples were subjected to physicochemical (moisture, nitrogen, and ash contents, degree of deacetylation, molecular weight, viscosity, solubility, bulk density, and color) and functional (water binding capacity, fat binding capacity, emulsion capacity, and emulsion viscosity) characteristic analysis. Three experimental replicates were performed with a duplicate analysis of each sample.

Results indicated that process modification of crawfish chitosan production yielded some differences on each characteristic compared with the control and commercial chitosans. For instance, changing the sequence of DC for the production of crawfish chitosan affected its properties. DCMPA and DMCPA resulted in an increase in molecular weight and ash, respectively. In contrast, DMPCA led to lower viscosity. The most notable change observed with the DMPAC chitosan was a light brown degraded colored chitosan that exhibited properties of a weak polyelectrolyte. When chitosan process started with DA, a very poor of yield were obtained. When DM and DP were reversed during production, the results showed some

difference, e.g., the lower viscosity, higher fat binding capacity, and higher emulsion viscosity of DMPCA over DPMCA.

This study demonstrated that process modification of crawfish production affected physicochemical and functional properties. The optimal chitosan production may vary depending on the intended final usages in food systems as demonstrated by functional properties from this study.

## CHAPTER 1

### INTRODUCTION

Chitosan is a natural carbohydrate biopolymer derived by deacetylation (DA) of chitin, a major component of the shells of crustacea such as crab, shrimp, and crawfish. After cellulose, chitin is the second most abundant natural biopolymer found in nature (No and Meyers, 1989).

Like cellulose, chitosan is a fiber. However, unlike plant fiber, chitosan possesses unique properties including the ability to form films, optical structural characteristics and much more. Chitosan also possesses a positive ionic charge, which gives it the ability to chemically bind with negatively charged fats, lipids and bile acids (Sandford, 1992).

Chitosan is a non-toxic, biodegradable and biocompatible polymer. Over the last several years, chitinous polymers, especially chitosan, have received increased attention as one of the promising renewable polymeric materials for their extensive applications in the pharmaceutical and biomedical industries for enzyme immobilization and purification, in chemical plants for wastewater treatment, and in food industries for food formulations as binding, gelling, thickening and stabilizing agent (Knorr, 1984).

Chitosan is easily obtained from crab especially Dungeness crab (*Cancer magister*), shrimp particularly the Pacific shrimp (*Pandalus borealis*), lobster, or crawfish shells. These are the richest source of chitin and the major U.S. sources of crustaceans that are processed into chitin and chitosan (Knorr, 1991). Louisiana has the largest and oldest successful crustacean farming industry in the U.S, namely the red swamp crawfish (or crayfish) *Procambarus clarkii*. This aquaculture industry currently has an annual production capacity in excess of 100 million pounds, of which approximately 80% is consumed locally and 15% is marketed throughout the U.S. In addition, the processing plants annually generate as much as 80 million pounds of

peeling waste during recovery of edible tail meat that makes up 15% of the total product. The waste residue representing 85% of the biomass has traditionally been discharged in landfill dumping sites without pretreatment (Rout, 2001). While much research has been done with chitosan extraction from crab shell, limited information is available on the extraction possibilities with crawfish shell waste. The humongous waste from crawfish particularly in Louisiana represents an outlet economic potential for the state.

Previous studies demonstrated that crawfish and crustacean wastes, as well as organically-rich shellfish processing streams in general, can no longer be considered as disposable “waste” products with minimal economic value, but should be considered as profitable alternatives leading to valuable products of commerce (No et al., 1992). Similar research studies by Lee (1989) demonstrated that the astaxanthin-rich shell from crawfish waste is a valuable natural resource for commercially feasible pigment which is marketed as a fish food additive in aquaculture, especially for Salmon. Apart from the recoverable pigment, it has been shown that crawfish shell waste possesses a significant and renewable major resource for the biopolymer chitin (23.5% on a dry basis compared to 14-27% and 13-15% of the dry weight of shrimp and crab processing waste, respectively) and chitosan (No and Meyers, 1989,1992).

Therefore, the applications of crawfish shell wastes as a source of astaxanthin, chitin and chitosan represent a total byproduct utilization concept with realistic implications in other crustacean waste recovery industries (No and Meyers, 1989). Further significance can be seen in the utilization of astaxanthin pigment, chitin, and protein from crawfish shell as mentioned earlier in a variety of fields with different applications. However, this thesis study will focus on the isolation of chitosan with crawfish shell as a source.

Crawfish shell as well as crustacean shell waste, mainly consist of protein (30-40%), calcium carbonate (30-50%), and chitin (20-30%) on a dry basis (Johnson and Peniston, 1982). These proportions vary with species and seasons (Green and Kramer, 1984). Chitin represents one third of the shell composition, and is highly hydrophobic and insoluble in water and most organic solvents. Chitosan, the deacetylated product of chitin, is soluble in very dilute acids such as acetic acid or formic acid. Traditional isolation of chitin from crustacean shell waste consists of three basic steps: demineralization (DM-calcium carbonate and calcium phosphate separation), deproteinization (DP-protein separation), and decolorization (DC-removal of pigments). These three steps are the standard procedure for chitin production (No, 1989). The subsequent conversion of chitin to chitosan (DA, deacetylation) is generally achieved by treatment with concentrated sodium hydroxide solution (40-50%) at 100°C or higher to remove some or all of acetyl group from the chitin (No and Meyers, 1995).

Earlier studies by No et al. (2000b); Cho et al. (1998); Wu and Bough (1978) have demonstrated that the physicochemical characteristics of chitosan affect its functional properties, which also differ due to crustacean species and preparation methods. Several procedures have been developed and proposed by many researchers over the years for preparation of chitosan from different crustacean shell wastes. Some of these formed the basis of chemical processes for industrial production of chitosan. Few attempts have been made to compare functional properties of chitosans prepared from various processes with those of commercially available chitin and chitosan products. Rout (2001) evaluated the effects of reversing the first two steps (deproteinization or demineralization) or reducing the number of steps (deproteinization or decoloration or either both) on fat and water binding capacities of chitin and chitosan, and reported a high fat binding capacity with crab and crawfish chitosan when the processing step

was reduced from four to three steps (excluding decolorization). No et al. (2002) studied the effects of elimination of deproteinization (DP) step or reduction of alkali treatment time on the physicochemical and functional properties of chitosan products; they reported that chitosan prepared without DP has comparable N<sub>2</sub> content, and lower degree of acetylation, solubility, and water and fat binding capacity. However, the end product is higher in molecular weight and viscosity, and lower in dye binding capacity than traditional chitosan prepared with the DP treatment.

However, a comprehensive study to examine the effects of process alteration/modification of chitosan production on various physicochemical characteristics and functional properties of crawfish chitosan products has not yet been reported. Hence, the aim of this study was to evaluate physicochemical and functional properties of crawfish chitosan as affected by modification of process protocols from the traditional four basic processing steps (DP, DM, DC, and DA) used in the isolation of chitosan from crawfish shell waste. The specific objectives, therefore, were to:

1. Develop an optimum chitosan production process for our particular intended application;
2. Study the physicochemical and functional properties of crawfish chitosan, prepared from modified process protocols, and compare these properties with those of commercial crab chitosans;
3. Evaluate how decoloration (DC) affects physicochemical and functional properties of crawfish chitosans;
4. Determine the effects of reversing the steps such as demineralization (DM) and deproteinization (DP) during the production of chitosan from crawfish shell on their physicochemical and functional properties;

5. Investigate the effect of deacetylation (DA) when it was preceded other steps (i.e., the first step) during the chitosan production.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Definition and Composition of Chitosan

Chitosan is a fiber-like substance derived from chitin, a homopolymer of  $\beta$ -(1 $\rightarrow$ 4)-linked *N*-acetyl-D-glucosamine. Chitin is the second most abundant organic compound in nature after cellulose (Ruiz-Herrera, 1978). Chitin is widely distributed in marine invertebrates (Figure 1), insects, fungi, and yeast (Austin et al., 1981). However, chitin is not present in higher plants and higher animals. Generally, the shell of selected crustacean was reported by Knorr (1984a) to consist of 30-40% protein, 30-50% calcium carbonate and calcium phosphate, and 20-30% chitin. Chitin is widely available from a variety of source among which, the principal source is shellfish waste such as shrimps, crabs, and crawfish (Allan et al., 1979). It also exists naturally in a few species of fungi.

In terms of its structure, chitin is associated with proteins and, therefore, high in protein contents. Chitin fibrils are embedded in a matrix of calcium carbonate and phosphate that also contains protein. The matrix is proteinaceous, where the protein is hardened by a tanning process (Muzzarrelli, 1977). Studies of Asford and co-workers (1977) demonstrated that chitin represents 14-27% and 13-15% of the dry weight of shrimp and crab processing wastes, respectively.



Figure 1. Seafood Produces Considerable Amounts of Waste which are the Principal Source of Chitin and Chitosan

With regards to their chemical structure (Figure 2), chitin and chitosan have similar chemical structure. Chitin is made up of a linear chain of acetylglucosamine groups while chitosan is obtained by removing enough acetyl groups ( $\text{CH}_3\text{-CO}$ ) for the molecule to be soluble in most diluted acids. This process is called deacetylation. The actual difference between chitin and chitosan is the acetyl content of the polymer. Chitosan having a free amino group is the most useful derivative of chitin (No and Meyers, 1992).

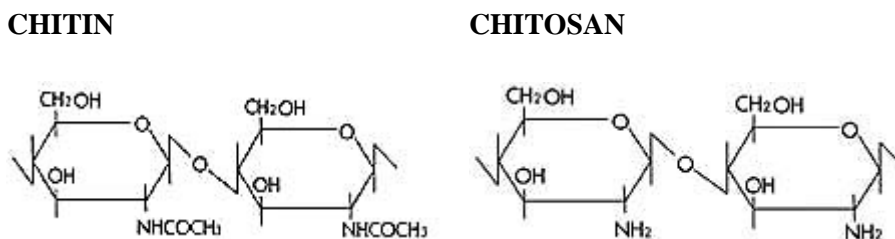


Figure 2. Structure of Chitin and Chitosan

## 2.2 Characteristics of Chitosan

Chitosan is a non toxic, biodegradable polymer of high molecular weight, and is very much similar to cellulose, a plant fiber (Figure 3).

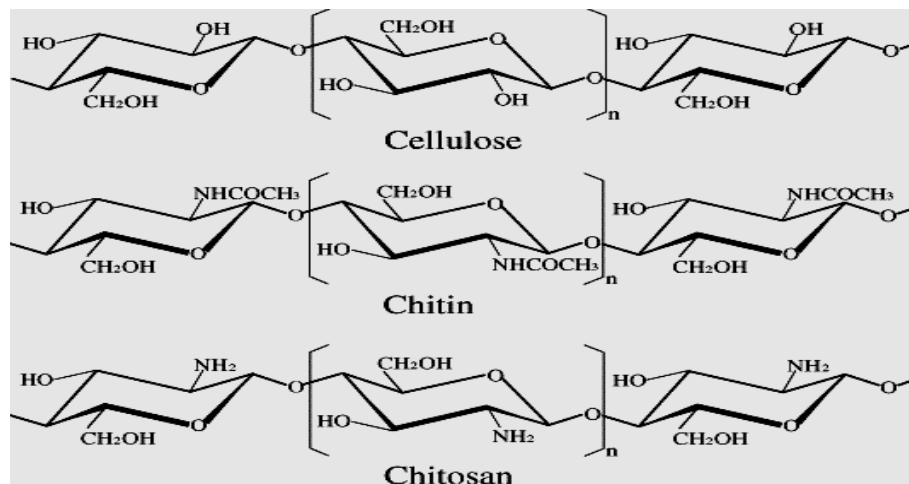


Figure 3. Structure of Cellulose, Chitin, and Chitosan

As seen in Figure 3, the only difference between chitosan and cellulose is the amine (-NH<sub>2</sub>) group in the position C-2 of chitosan instead of the hydroxyl (-OH) group found in cellulose. However, unlike plant fiber, chitosan possesses positive ionic charges, which give it the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules (Li et al., 1992). In this respect, chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility, biodegradability, adsorption, and ability to form films, and to chelate metal ions (Rout, 2001).

### **2.2.1 Degree of Deacetylation (DD)**

The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a compound (chitosan) with a high degree chemical reactive amino group (-NH<sub>2</sub>). This makes the degree of deacetylation (DD) an important property in chitosan production as it affects the physicochemical properties, hence determines its appropriate applications (Rout, 2001). Deacetylation also affects the biodegradability and immunological activity (Tolaimate et al., 2000).

A sharp nomenclature border has not been defined between chitin and chitosan based on the degree of *N*-deacetylation (Rout, 2001). In an earlier study by Rudall (1963), he reviewed evidences suggesting that approximately one in every six to seven residues in the chain has a proportion of free amino groups that manifests some histochemical properties. In any case, the degree of deacetylation can be employed to differentiate between chitin and chitosan because it determines the content of free amino groups in the polysaccharides. In fact there are two advantages of chitosan over chitin. In order to dissolve chitin, highly toxic solvents such as lithium chloride and dimethylacetamide are used whereas chitosan is readily dissolved in diluted

acetic acid. The second advantage is that chitosan possesses free amine groups which are an active site in many chemical reactions (Knaul et al., 1999).

The degree of deacetylation of chitosan ranges from 56% to 99% with an average of 80%, depending on the crustacean species and the preparation methods (No, 2000; No and Meyers, 1995). Chitin with a degree of deacetylation of 75% or above is generally known as chitosan (Knaul et al., 1999). Various methods have been reported for the determination of the degree of deacetylation of chitosan. These included ninhydrin test, linear potentiometric titration, near-infrared spectroscopy, nuclear magnetic resonance spectroscopy, hydrogen bromide titrimetry, infrared spectroscopy, and first derivative UV-spectrophotometry (Khan et al., 2002).

The IR spectroscopy method, which was first proposed by Moore and Roberts (1980), is commonly used for the estimation of chitosan DD values. This method has a number of advantages and disadvantages. First, it is relatively fast and unlike other spectroscopic methods, does not require purity of the sample to be tested nor require dissolution of the chitosan sample in an aqueous solvent (Baxter et al., 1992). However, the IR method utilizing baseline for DD calculation, and as such there may be possible argument for employment of different baseline which would inevitably contribute to variation in the DD values. Secondly, sample preparation, type of instrument used and conditions may influence the sample analysis. Since chitosan is hygroscopic in nature and samples with lower DD may absorb more moisture than those with higher DD, it is essential that the samples under analysis be completely dry (Khan et al., 2001; Blair et al., 1987).

The followings are some baselines proposed for the determination of the degree of deacetylation of chitosan: (1) Domszy and Roberts (1985),  $DD = 100 - [(A_{1655} / A_{3450}) \times 100 / 1.33]$ , (2) Sabnis and Block (1997),  $DD = 97.67 - [26.486 \times (A_{1655} / A_{3450})]$ , and (3) Baxter et al.

(1992),  $DD = 100 - [(A_{1655} / A_{3450}) \times 115]$  and (4) Rout (2001),  $DD = 118.883 - [40.1647 \times (A_{1655} / A_{3450})]$  (Rout, 2001).

### **2.2.2 Molecular Weight**

Chitosan is a biopolymer of high molecular weight. Like its composition, the molecular weight of chitosan varies with the raw material sources and the method of preparation. Molecular weight of native chitin is usually larger than one million Daltons while commercial chitosan products have the molecular weight range of 100,000 – 1,200,000 Daltons, depending on the process and grades of the product (Li et al., 1992). In general, high temperature, dissolved oxygen, and shear stress can cause degradation of chitosan. For instance at a temperature over 280°C, thermal degradation of chitosan occurs and polymer chains rapidly break down, thereby lowering molecular weight (Rout, 2001). Also, maximal depolymerization caused by utilization of high temperature or concentrated acids, such as hydrochloric acid followed by acetic acid and sulfurous acid, results in molecular weight changes with minimal degradation with the use of EDTA (Rout, 2001).

The molecular weight of chitosan can be determined by methods such as chromatography (Bough et al., 1978), light scattering (Muzzarelli, 1977), and viscometry (Maghami and Roberts, 1988).

### **2.2.3 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 in complex biological environments such as in the food system. Higher molecular weight chitosans often render highly viscous solutions, which may not be desirable for industrial handling. But, a

lower viscosity chitosan obtained from crawfish waste as shown in this thesis research may facilitate easy 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. 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. Bough et al. (1978) found that deproteinization with 3% NaOH and elimination of the demineralization step in the chitin preparation decrease the viscosity of the final chitosan products. Moorjani et al. (1975) also stated that it is not desirable to bleach the material (i.e., bleaching with acetone or sodium hypochlorite) at any stage since bleaching considerably reduces the viscosity of the final chitosan product.

Similarly, No et al.(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. Chitosan solution stored at 4°C is found to be relatively stable from a viscosity point of view (No et al., 1999).

The effect of particle size on the quality of chitosan products was investigated by Bough et al.(1978), who reported that smaller particle size (1mm) results in chitosan products of both higher viscosity and molecular weight than those of either 2 or 6.4 mm particle size. They further enumerated that a larger particle size requires longer swelling time, resulting in a slower deacetylation rate. But, in contrast, Lusena and Rose (1953) reported that the size of chitin

particle within the 20-80 mesh (0.841-0.177 mm) range had no effect on the viscosity of the chitosan solutions.

#### **2.2.4 Solubility**

While chitin is insoluble in most organic solvents, chitosan is readily soluble in dilute acidic solutions below pH 6.0. Organic acids such as acetic, formic, and lactic acids are used for dissolving chitosan. The most commonly used is 1% acetic acid solution at about pH 4.0 as a reference. Chitosan is also soluble in 1% hydrochloric acid but insoluble in sulfuric and phosphoric acids. Solubility of chitosan in inorganic acids is quite limited. Concentrated acetic acid solutions at high temperature can cause depolymerization of chitosan (Roberts and Domszy, 1982). Above pH 7.0 chitosan solubility's stability is poor. At higher pH, precipitation or gelation tends to occur and the chitosan solution forms poly-ion complex with anionic hydrocolloid resulting in the gel formation (Kurita, 1998).

The concentration ratio between chitosan and acid is of great importance to impart desired functionality (Mima, 1983). At concentrations as high as 50 percent organic solvent, chitosan still works as a viscosifier causing the solution to remain smooth. There are several critical factors affecting chitosan solubility including temperature and time of deacetylation, alkali concentration, prior treatments applied to chitin isolation, ratio of chitin to alkali solution, and particle size.

The solubility, however, is controlled by the degree of deacetylation and it is estimated that deacetylation must be at least 85% complete in order to achieve the desired solubility (No et al., 1995). The acid-soluble chitosans with >95% solubility in 1% acetic acid at a 0.5% concentration could be obtained by treatment of the original chitin with 45-50% NaOH for 10-30 min. Chitosans treated with 45% NaOH for only 5 min, and/or with 40% NaOH for 30 min, were

not deacetylated sufficiently to be soluble in 1% acetic acid. Insoluble particles were found in both solutions. According to Bough et al. (1978), a reaction time of 5 min with 45% NaOH may not be enough for chitin particles to be sufficiently swollen. A decrease of the NaOH concentration to 40% required increased time of >30 min to obtain a soluble chitosan (No et al., 2000).

### **2.2.5 Bulk Density**

The bulk density of chitin from shrimp and crab is normally between 0.06 and 0.17 g/ml, respectively (Shahidi and Synowiecki, 1991), indicating that shrimp chitin is more porous than crab chitin. Krill chitin was found to be 2.6 times more porous than crab chitin (Anderson et al., 1978). In a study conducted by Rout (2001), the bulk density of chitin and chitosan from crawfish shell, is very high (0.39 g/cm<sup>3</sup>); this was calculated as an unpacked bulk density of chitosan particles passed through a 0.5 mm mesh into a 25 ml measuring cylinder. This perhaps could be due to the porosity of the material before treatment. But once crawfish shell had been demineralized or deproteinized or both there seem to be very minor variations unpacked in bulk density between chitin and chitosan produced. A comparison of the bulk densities of crawfish and commercial chitin and chitosan indicated some variations, which can be attributed to crustacean species or sources of chitosan and the methods of preparation (Rout, 2001), as also stated earlier by Brine and Austin (1981). Rout (2001) reported that increased degree of deacetylation (DD) decreased bulk density.

### **2.2.6 Color**

The pigment in the crustacean shells forms complexes with chitin (4-keto and three 4, 4'-diketo- $\beta$ -carotene derivatives) (Rout, 2001). Chitosan powder is quite flabby in nature and its

color varies from pale yellow to white whereas starch and cellulose powder have smooth texture and white color.

### **2.2.7 Water Binding Capacity (WBC) and Fat Binding Capacity (FBC)**

Water uptake of chitosan was significantly greater than that of cellulose and even chitin (Knorr, 1982). Basically, WBC for chitosan ranges between 581 to 1150% with an average of 702%, according to Rout (2001). In his report, Rout (2001) also noted that reversing the sequence of steps such as demineralization (DM) and deproteinization (DP) had a pronounced effect on WBC and FBC. DP of demineralized shell also gives higher WBC compared to the process when DM of the deproteinized shell is conducted. Besides, the process of decoloration (DC) also causes a decrease in WBC of chitosan than those of unbleached crawfish chitosan.

The fat uptake of chitin and chitosan ranges from 315 to 170% with chitosan having the lowest and chitin the highest fat uptake (Knorr, 1982). In a study by Rout (2001) on this aspect, he reported that the average FBC of crawfish chitosans and commercial crab chitosans for soybean oil was 706% and 587%, respectively. The inclusion of decoloration step during the production of chitosan was found to decrease the fat binding capacity of crawfish chitosans, and decoloration (bleaching) had been shown to affect the viscosity of chitosan (Moorjani, 1975). The decreased viscosity as evidenced may be a cause for decrease in fat binding capacities among unbleached and bleached crawfish chitosan samples.

Rout (2001) also reported that changing the sequence of steps, i.e., when demineralization (DM) is conducted prior to deproteinization (DP), followed by deacetylation (DA), caused an increase in FBC compared with when deproteinization (DP) is performed prior to demineralization (DM), followed by deacetylation (DA).

### **2.2.8 Emulsification**

Even though chitosan alone does not produce emulsions, Cho et al. (1998) reported that emulsifying capacity of egg yolk increased with the addition of chitosan compared with the control. At 0.5% chitosan concentration, better emulsifying capacity was observed compared with at 0.1 or 0.3% chitosan. In general, chitosan emulsions tend to be very stable under temperature changes and aging. With viscosity, the degree of deacetylation is reported to be a determining factor in the emulsification properties of chitosan. The protein solution containing chitosan with intermediate DD produces less effective emulsion compared with that containing chitosan with higher DD. The optimum chitosan DD for sunflower oil emulsification is 81 and 89 as reported by Del Blanco et al. (1999) and Rout (2001), respectively.

### **2.2.9 Antimicrobial Properties**

Recent studies in antibacterial activity of chitosan have revealed that chitosan is effective in inhibiting growth of bacteria. The antimicrobial properties of chitosan depend on its molecular weight and the type of bacterium. For gram-positive bacteria, chitosan with 470 KDa was the most effective, except for *Lactbacillus sp.*, whereas for gram-negative bacteria, chitosan with 1,106 KDa was effective. Chitosan generally showed stronger bactericidal effects for gram-positive bacteria (*Listeria monocytogenes*, *Bacillus megaterium*, *B. cereus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *L. brevis*, and *L. bulgaris*) than for gram-negative bacteria (*E.coli*, *Pseudomonas fluorescens*, *Salmonella typhymurium*, and *Vibrio parahaemolyticus*) in the presence of 0.1% chitosan (No et al., 2002).

Koide (1998) reported that chitin and chitosan *in vitro* show antibacterial and anti-yeast activities. One of chitosan derivatives, i.e., N-carboxybutyl chitosan, was tested against 298 cultures of different pathogenic microorganisms that showed bacteriostatic and bactericidal

activities, and there were marked morphological alterations in treated microorganisms when examined by electron microscopy (Muzzarelli, 1990).

Conversely, growth inhibition and inactivation of mould and yeasts seem to depend on chitosan concentration, pH, and temperature (Rout, 2001). According to Cuero (1999), the antimicrobial action of chitosan is influenced by intrinsic and extrinsic factors such as the type of chitosan (e.g., plain or derivative), degree of chitosan polymerization, host nutrient constituency, substrate chemical and/ or nutrient composition, and environmental conditions such as substrate water activity.

In an extensive research by Tsai and Su (1999) on the antimicrobial activity of chitosan prepared from shrimp against *E.coli*, they found that higher temperature and acidic pH of foods increased the bactericidal effect of chitosan. They also explained the mechanism of chitosan antibacterial action involving a cross-linkage between polycations of chitosan and the anions on the bacterial surface that changes membrane permeability.

Chitosan has been approved as a food additive in Korea and Japan since 1995 and 1983, respectively (Weiner, 1992; KFDA, 1995; No, 2002). Higher antibacterial activity of chitosan at lower pH suggests that addition of chitosan to acidic foods will enhance its effectiveness as a natural preservative (No et al., 2002).

Chitosan coating have been shown to significantly delay fruit spoilage or decaying of fruits and vegetables such as tomatoes, strawberries, etc., at different temperatures. Chitosan coated fruits were not only firmer and higher in titratable acidity, but were slow to decay and exhibited less pigmentation than control samples at the end of storage (El Ghaouth et al., 1992). The low molecular weight chitosan has a greater inhibitory effect against phytopathogens than the high molecular weight chitosan (Hirano et al., 1989).

### **2.2.10 Formation of Film**

Chitosan has an ability to form film which makes it suitable for use as food preservation for control of psychotropic pathogen in fresh/ processed meat and fish products packaged under modified atmosphere (Smith et al., 1994). According to Charles et al. (1994), the most potential application of chitosan is as a coating agent in the area of fruit preservation. The biodegradability of chitosan is one of the most advantageous features for concern of the environmental damage occurring by improper disposal of petrochemical based plastics (Knorr, 1991).

N, O-carboxymethyl chitosan can form a strong film that is selectively permeable to such gases as oxygen and carbon dioxide. Apples coated with this material remain fresh for up to six months. The chitosan coating has been shown to delay ripening of banana for up to 30 days where as chitosan film manifests a slightly yellow appearance, with the color darkening as thickness increased (Setha et al., 2000).

### **2.3 Production of Chitin and Chitosan**

Chitosan as mentioned before is extracted from crustacean shell waste such as crab, shrimp, lobster, and crawfish. The shells contain approximately 30-40% protein, 30-50% calcium carbonate, and 20-30% chitin on a dry basis (Johnson and Peniston, 1982). These portions vary with crustacean species and seasons (Green and Mattick, 1979).

Isolation of chitosan from crawfish shell wastes involves four traditional steps (Figure 4): demineralization (DM), deproteinization (DP), decolorization (DC), and deacetylation (DA). However, the isolation of chitin specifically consists of only two steps: demineralization (DM) and deproteinization (DP), which involves the dissolution of calcium carbonate with 1.0 N HCl and the removal of proteins with 3% NaOH, respectively.

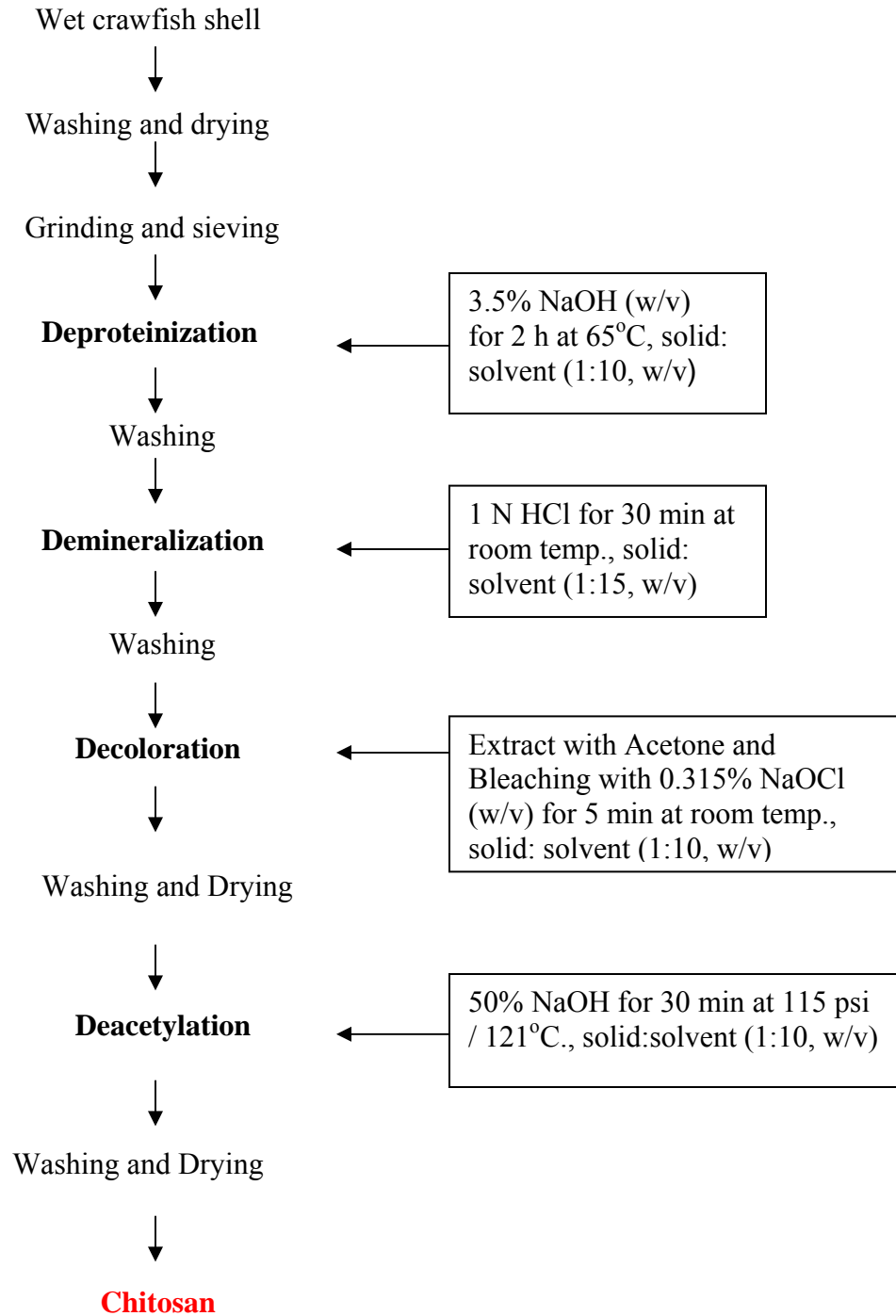


Figure 4. Traditional Crawfish Chitosan Production Flow Scheme (Modified from No and Meyers, 1995)

Fortunately, the sequence of demineralization and deproteinization steps can be reversed. In fact many authors have followed the procedure of acidic decalcification after removal of protein (Muzzarelli, 1977).

Though the process normally involves the use of dilute sodium hydroxide and dilute hydrochloric acid for deproteinization and demineralization, respectively, there have been reports indicating several variations of the characteristics of final chitosan products, but this also depends on the crustacean species from which chitin is isolated, and on the production sequence (Cho et al., 1998; No et al., 2000b; Wu and Bough, 1978).

The demineralized and deproteinized chitin has a light pink color due to the presence of astaxanthin pigment. When bleached product is desired, this pigment can be eliminated during the decolorization (DC) step. The resulting chitin is insoluble in most organic solvents; however, its deacetylated derivative chitosan is soluble in weak acids. The subsequent conversion of chitin to chitosan is generally achieved by treatment with concentrated sodium hydroxide solution (40-50%) at 100°C or higher for 30 minutes to remove some or all of the acetyl groups from the polymer (No and Meyers, 1995).

### **2.3.1 Deproteinization**

Chitin occurs naturally in association with protein (Chitinoprotein). Some of this protein can be extracted by mild methods, but other portion is not readily extracted, suggesting strong covalent bonding to chitin (Attwood and Zola, 1967). With regards to chemical structure, protein is bound by covalent bonds to the chitin through aspartyl or histidyl residues, or both, thus forming stable complexes such as glycoproteins.

Crustacean shell waste is usually ground and treated with dilute sodium hydroxide solution (1-10%) at elevated temperature (65-100°C) to dissolve the proteins present. Reaction time usually ranges from 0.5 to 12 hr depending on preparation methods. Prolonged alkaline

treatment under severe conditions causes depolymerization and deacetylation. To obtain uniformity in reaction, it is recommended to use relatively high ratios of solid to alkali solution of 1:10 or 1:15-20 with proper agitation because a minimum ratio of 1:4 (w/v) of shell weight to KOH solution, had only a minor effect on the DP efficiency of shells (No and Meyers, 1995). Optimal conditions for deproteinization involve treatment of the crawfish shells with 3.5% (w/w) NaOH solution for 2 hr at 65°C with constant stirring and a solid to solvent ratio of 1:10 (w/v) (No et al., 1989).

During the deproteinization process, foam formation can occur, but the foam is not as brisk and intense as that produced during demineralization. Shahidi and Synowiecki (1991) suggested that optimal deproteinization can be achieved using dilute potassium hydroxide solution. Generally, if maximizing protein yield and quality is the objective, then protein extraction before demineralization is recommended (Johnson and Peniston, 1982) or the pH of the aqueous solution must be reduced to the isoelectric point of protein for precipitation (Green and Mattick, 1979).

### **2.3.2 Demineralization**

Demineralization is usually accomplished by extraction with dilute hydrochloric acid (up to 10%) at room temperature with agitation to dissolve calcium carbonate as calcium chloride. A wide variation of the demineralization process has been reported in the literature. The use of HCl acid at higher concentration and also 90% formic acid to achieve demineralization has been reported. Optimum demineralization is achieved by constant stirring of the dried ground crawfish shell with 1N HCl for 30 min at ambient temperature and a solid to solvent ratio of 1:15 (w/v) (No et al., 1989). The ash content of the demineralized shell is an indicator of the effectiveness

of the demineralization process. Elimination of the demineralization resulted in products having 31-36% ash.

During the demineralization process excessive undesirable foams are produced due to the CO<sub>2</sub> generation ( $[\text{CaCO}_3 + 2\text{HCl} \rightarrow \text{CaCl}_2 + \text{CO}_2 (\uparrow) + \text{H}_2\text{O}]$ ). To control or reduce the foam, No et al.(1998) recommended the use of commercial antifoam comprising of 10% solution of active silicone polymer without an emulsifier. They also demonstrated that at 1.0ml of antifoam /L of 1N HCl, the performance of antifoam is more efficient during demineralization with smaller shell particle size (<0.425 mm and under a slightly faster stirring speed at 300 rpm). Furthermore, they recommended that deproteinization followed by demineralization is a favorable sequence in terms of the amount of antifoam required to control foaming.

### **2.3.3 Decoloration**

Acid and alkali treatments alone produce a colored chitin product. For commercial acceptability, the chitin produced from crustacean sources, needs to be decolorized or bleached to yield cream white chitin powder (No et al., 1989). The pigment in the crustacean shells forms complexes with chitin. In earlier research studies, Fox (1973) found one 4-keto-and three 4, 4'-diketo- $\beta$ -carotene derivatives firmly bound to the exoskeletal chitin of red kelp crab. The level of association of chitin and pigments varies from species to species among crustacean.

Several workers have used reagents to eliminate pigments from crustacean exoskeleton, usually crab. However, with crawfish shell the reagents alone do not seem as effective as the procedure developed currently. This suggests that carotenoids, are more strongly bound to the crawfish shell matrix than are those reported from other crustacea (No et al., 1989). Hence, the stronger the bond the more harsh treatment is required to prepare a white colored chitin. During the process of decoloration, it should be noted that the chemical used should not affect the

physicochemical or functional properties of chitin and chitosan. No et al. (1989) was able to prepare a near white colored crawfish chitin by extraction with acetone and dried for 2 hr at ambient temperature, followed by bleaching with 0.315 % (v/v) sodium hypochloride solution (containing 5.25% available chlorine) for 5 min with a solid to solvent ratio of 1:10 (w/v), based on dry shell. But, the color of chitin products varied from cream white to intermediate pink color (No et al., 1989). Without prior acetone extraction, bleaching for more than 1 hr was needed to obtain a commercially acceptable white product.

#### **2.3.4 Deacetylation**

Deacetylation is the process to convert chitin to chitosan by removal of acetyl group. It is generally achieved by treatment with concentrated sodium or potassium hydroxide solution (40-50%) usually at 100°C or higher for 30 min or longer to remove some or all of the acetyl groups from the polymer (No and Meyers, 1989). The N-acetyl groups cannot be removed by acidic reagents without hydrolysis of the polysaccharide, thus, alkaline methods must be employed for N-deacetylation (Muzzarelli, 1977).

Depending upon the production sequence, deacetylation can be achieved by reaction of demineralized shells or crawfish chitin with 50% NaOH (w/w) solution at 100°C for 30 min in air using a solid to solvent ratio of 1:10 (w/v) (No et al., 1989). There are several critical factors that affect the extent of deacetylation including temperature and time of deacetylation, alkali concentration, prior treatments applied to chitin isolation, atmosphere (air or nitrogen), ratio of chitin to alkali solution, density of the chitin, and particle size (Rigby, 1936). Considering all these as necessary conditions, the ideal purpose of deacetylation is to prepare a chitosan that is not degraded and is soluble in dilute acetic acid in minimal time.

## **2.4 Factors Affecting Production of Chitosan**

A number of processing factors affect chitosan's physicochemical characteristics.

### **1. Temperature of Deacetylation**

Higher temperature tends to increase the degree of deacetylation but reduces molecular size (Lusena and Rose, 1953). There is a substantially linear relationship between temperature (plotted along the abscissa as  $1/T$  in K) and the rate of deacetylation (plotted logarithmically along the ordinate) (Peniston and Johnson, 1980).

### **2. Time of Deacetylation and Alkali Concentration**

Wu and Bough (1978) suggested that deacetylation proceeds rapidly to about 68% during the first 1hr in 50% NaOH solution at 100°C. However, the reaction progresses gradually thereafter reaching about 78% in 5 hr. Thus, alkali treatment beyond 2 hr does not deacetylate chitin significantly, rather it degrades the molecular chain. In a concentration study with 35, 40, and 50% NaOH (Bough et al., 1978), as alkali concentration decreased, rates of decrease in both viscosity and molecular weight distribution also slowed. Bough et al. (1978) alluded that chitosan deacetylated for 5 min with 50% NaOH at 145-150°C had higher viscosities (1.7-16.4 fold) and molecular weight (1.1-1.8 fold) than did chitosans deacetylated for 15 min. Similarly, decrease in viscosity with increased reaction time was shown and confirmed.

### **3. Effect of Treatment Conditions Applied in Chitin Isolation**

Treatment conditions applied to chitosan isolation primarily affect viscosity of the product than any other property. Madhavan and Nair (1974) reported that the use of HCl at concentrations above 1.25N adversely affected the viscosity of the final chitosan product. In addition, chitosan viscosity tends to decrease with increased time of demineralization (Moorjani et al., 1975). On the other hand, Bough et al. (1978) found that deproteinization with 3% NaOH,

and elimination of the demineralization step in chitin preparation, decreased the viscosities of chitosan samples, where as Moorjani et al. (1975) indicated that it is not desirable to bleach the material at any stage since bleaching considerably reduces the viscosity of the final chitosan product.

#### **4. Atmosphere**

Many scientists have agreed that free access of oxygen to chitin during deacetylation has a substantial degrading effect on chitosan. Deacetylation in the presence of nitrogen yielded chitosan of higher viscosity and molecular weight distributions than did in air. However, little differences in nitrogen and ash compositions were observed (Bough et al., 1978; Lusena and Rose, 1953; Rigby, 1936).

#### **5. Ratio of Chitin to Alkali Solution**

Moorjani et al. (1978) emphasized that the ratio of chitin solids to alkali solution plays a significant role in determining the quality of chitosan, based on viscosity determination. The reported solid to solution ratios range from 1:10 to 1:100 on a wet basis, and 1:4 on a dry basis or when dry heating is used.

#### **6. Particle Size**

Particle size in chitosan productions has sparked controversial reports on its effect on chitosan quality. Some agree that small particle size is better than large particle size. According to Bough et al. (1978), smaller particle size (1mm) results in a chitosan product of both higher viscosity and molecular weight than that of larger particle size (above 2 to 6.4 mm). The larger particle sizes require longer swelling time resulting in a slower deacetylation rate. However, Lusena and Rose (1953) indicated that the size of chitin particle within the 20-80 mesh range

(0.841-0.177 mm) had no effect on the extent of deacetylation and viscosity of the chitosan solutions.

## **2.5 Some Examples of Alternative Techniques for Production of Chitosan**

The characteristics of the final chitosan products differ depending on the crustacean species from which chitin is isolated, and the production method or sequence (Cho et al., 1998; No et al., 2000). Various procedures have been developed and proposed by many researchers over the years for chitosan processing (No et al., 1989). There are numerous reviews on various and diverse preparation methods for recovery and evaluation of physicochemical properties of chitosan.

### **2.5.1 Deproteinization**

Deproteinization is conventionally accomplished by extraction with dilute sodium hydroxide solution (1-10%) at elevated temperature (65-100°C) for 1-6 hr (No et al., 1995). Bough et al. (1978) extracted protein from shrimp shells with 3% NaOH at 100°C for 1 hr and No et al. (1989) similarly treated crawfish shell waste with 3.5% NaOH at 65°C for 2hr.

Optimal deproteinization can be accomplished by treatment with dilute potassium hydroxide solution (Shahidi and Synowiecki, 1991). Cosio et al. (1982) and Chen et al. (1994) in their various studies accomplished deproteinization for shrimp shell at pH 11.5 at 30°C, and for prawn in 5N NaOH for 1 hr at 100°C, respectively. Removal of protein by enzymatic digestion for production of chitin and chitosan was attempted by Shimahara et al. (1982), Takeda and Abe (1962), and Takeda and Katsuura (1964) in an effort to minimize deacetylation. Bough et al.(1978) also extracted protein from shrimp shells with Rhyzome-62 concentrate at 60°C for 6 hr at pH 7 but complete removal of protein was not attained. Hackman (1954) and Whistler and BeMiller (1962) attempted to extract protein for several days but prolonged alkaline treatment

under severe conditions caused depolymerization and deacetylation. Shahidi and Synowiecki (1991) reported a 2 hr extraction period for removal of all proteins present in the shells. These workers also observed that relatively high ratios of solid to alkali solution, 1:10 or above is usually used to obtain uniformity in reaction with proper agitation.

Cho and No (1999) performed deproteinization by autoclaving under conditions of 15 psi/121°C with 3% NaOH for 15min and a solid: solvent ratio of 1:10. Their results showed that deproteinization can effectively be achieved by autoclaving conditions because no significant differences in nitrogen content and bulk density as well as water and fat binding capacity were observed. Rout (2001) investigated the effects of process modification of crawfish chitin and chitosan production by reversing the first two steps (deproteinization or demineralization) or reducing the number of steps (deproteinization or decoloration or both) on fat and water binding capacities of chitin and chitosans; he reported that simplifying the process affected both water and fat binding capacities. Among crab and crawfish chitosans, the DMPA crawfish chitosan had the highest fat binding capacity.

No et al. (2002) investigated the effects of elimination of deproteinization step or reduction of alkali treatment time on physicochemical and functional properties of chitosan products. Chitosan prepared without deproteinization had comparable nitrogen content, lower degree of deacetylation, solubility, water and fat binding capacity, and lower dye binding capacity, but higher molecular weight and viscosity than chitosans prepared with deproteinization treatment.

### **2.5.2 Demineralization**

Demineralization is conventionally accomplished by extraction with dilute hydrochloric acid at room temperature to dissolve calcium carbonate to calcium chloride. Among such

methods are those of Hackman (1954), Anderson et al. (1978), Bough et al. (1978), and No et al. (1989). But, reaction time varied with preparation methods from 30 min (No et al., 1989) to over 2 days (Hackman, 1954). However, Synowiecki et al. (1981) and Chen et al. (1994) accomplished demineralization with 22% HCl and 6N HCl, respectively, at room temperature. To avoid modifications such as depolymerization or deacetylation caused by harsh treatments, Austin et al. (1981) suggested the use of mild acids such as ethylenediaminetetra-acetic acid (EDTA) for decalcification.

Prolonged demineralization time for up to 24 hr results in only a very slight drop in the ash content but can cause polymer degradation (Brzeski, 1982) or decreased viscosity (Moorjani et al., 1975). Also it is important that the amount of acid be stoichiometrically equal to or greater than all minerals present in the shells to ensure complete reaction (Johnson and Peniston, 1982; Shahidi and Synowiecki, 1991).

### **2.5.3 Decoloration**

When bleached chitin is desired, pigments can be removed with reagents. Hackman (1954) obtained a cream-colored lobster chitin by washing with ethanol and ether, and Blumbeg (1951) extracted pigments with cold sodium hypochloride solution, containing 0.5% available chlorine, and Kamasastri (1961) with absolute acetone. Anderson (1978), Brine (1981), and Brzeski (1982) also accomplished decoloration of chitin with chloroform, H<sub>2</sub>O<sub>2</sub>, and ethyl acetate, respectively. No et al. (1989) prepared a white colored crawfish chitin by acetone extraction, followed by bleaching with 0.315% sodium hypochlorite solution. However, Moorjani et al. (1975) recommends not to bleach the material at any stage because the bleaching process considerably reduces the viscosity of the final chitosan product.

#### 2.5.4 Deacetylation

A new process for treating chitin under high concentrations of sodium hydroxide with microwave energy was proposed by Peniston and Johnson (1980) to accelerate the deacetylation of chitin within 18 min with 50% NaOH at a mean temperature under 80°C. Chitin was deacetylated with concentrated aqueous NaOH in the presence of water-miscible organic solvents such as 2-propanol, 2-methyl-2-propanol or acetone (Batista and Roberts, 1990).

Although it is difficult to prepare chitosan with a degree of deacetylation greater than 90% without chain degradation, Mima et al. (1983) developed a method for preparation of chitosan having a desired degree of deacetylation of up to 100%, without serious degradation of the molecular chain. This was achieved by intermittently washing the intermediate product in water two or more times during the alkali treatment for less than 5 hr in 47% NaOH at 110°C.

A simple and inexpensive technique for deacetylation of chitin has been developed in which Alimuniar and Zainuddin (1992) produced chitosan by treatment of prawn chitin with strong sodium hydroxide at ambient temperature (30°C) without heating in an inert atmosphere or without the addition of other additives to control the reaction. With 50% NaOH, the acid-soluble chitosan with 87% degree of deacetylation could be formed in a single day using 560 ml of the solution for 10 g of chitin, two days using 420 ml, three days using 280 ml and six days using 140 ml.

For a large-scale preparation of chitosan, the process of deacetylation needs to be optimized. No et al. (2000) used autoclaving conditions (15 psi/121°C) to deacetylate chitin to prepare chitosan under different NaOH concentration and reaction times. Effective deacetylation was achieved by treatment of chitin under an elevated temperature and pressure with 45% NaOH for 30 min with a solid: solvent ratio of 1:15. Treated chitosan showed similar nitrogen content,

degree of deacetylation, and molecular weight, but significantly higher viscosity value than those of commercial chitosan.

## 2.6 Applications of Chitosan

The poor solubility of chitin is the major limiting factor in its utilization. Chitosan is considered as a potential polysaccharide because of its free amino groups that contribute polycationic, chelating, and dispersion forming properties along with ready solubility in dilute acetic acid. Chitosan possesses exceptional chemical and biological qualities that can be used in a wide variety of industrial and medical applications. Some of these are listed below (Table 1) (Knorr, 1984; Muzzarelli, 1977).

### 2.6.1 In the Wastewater Treatment

The prime commercial applications for chitosan currently is in industrial wastewater treatment since chitosan carries a partial positive charge and binds to metal ions, thus makes the metal ions removal from waste streams or contamination sites easier (Asano, 1978). In terms of utilization, crawfish chitosan as a coagulant for recovery of organic compounds in wastewater was demonstrated to be equivalent, or superior to, the commercial chitosans from shrimp and crab waste shell and synthetic polyelectrolytes in turbidity reduction (No and Meyers, 1992).

Table 1. Applications of Chitosan

Wastewater Treatment	Removal of metal ions, flocculant/coagulant, protein, dye, amino acids
Food Industry	Removal of dye, suspended solids, preservative, color stabilization, food stabilizer, thickener and gelling agent, animal feed additive, etc.
Medical	Wound and bone healing, blood cholesterol control, skin burn, contact lens, surgical sutures, dental plaque inhibition, clotting agent, etc.
Agriculture	Seed coating, fertilizer, controlled agrochemical release
Cosmetics	Moisturizer, face, hand, and body creams, bath lotion, etc.
Biotechnology	Enzyme immobilization, protein separation, cell recovery, chromatography

The wastewater released from food processing plants typically seafood, dairy or meat processing industries contains appreciable amount of protein which can be recovered with the use of chitosan; this protein, after drying and sterilization, makes a great source of feed additives for farm animals (Rout, 2001).

The removal of dyes is difficult to achieve because of their high resistance to degradation by light, chemical, biological, and other exposures. However, chitin and chitosan have been found to have extremely high affinity for dyes which may contribute to aquatic toxicity (Rout, 2001). Asano et al. (1978) found that chitosan is effective for conditioning municipal and industrial sludge due mainly to their effectiveness in sludge conditioning, rapid biodegradability in soil environments, and economic advantages in centrifugal sludge dewatering.

### **2.6.2 In the Food Industry**

The food processing industry extensively uses polysaccharides in food product development and processing for the purpose of imparting desirable functional properties such as thickening, gelling, emulsifying, and whipping. Without exception, chitosan have been documented to possess several distinctive properties (Knorr, 1984). The good water uptake of chitosan has been found to be significantly higher than that of microcrystalline cellulose (Knorr, 1982).

Several studies have also demonstrated the effectiveness of chitosan for coagulation and recovery of suspended solids in processing wastes from poultry (Bough et al., 1975), eggs (Bough, 1976), seafood (Bough, 1976) and vegetable operations (Bough, 1975). These studies indicate that chitosan can reduce the suspended solids of various food processing wastes by 70 to 98%. Chitosan also is effective for dewatering activated sludge suspensions resulting from biological treatment of brewing and vegetable canning wastes (Bough, 1976)

### **2.6.3 In Medical**

In the study conducted by Kratz et al. (1997), their results showed that when positively charged chitosan binds with negatively charged heparin they produce a stable heparin-chitosan complex that stimulates re-epithelialisation of full thickness wounds in human skin.

### **2.6.4 In Cosmetics**

Chitosan has been used extensively in hair care, especially commercial shampoos and conditioners with chitosan as the main ingredient because of several advantages. Among these advantages, chitosan is physiologically safe as it contains no harmful monomers from any polymerization step. The other one is the ability to form films with proteins which is more stable at high humidity, less statically charged during brushing and combing than other traditional hair treated fixatives (Rout, 2001).

High purity grade chitosan is needed in many applications especially in food, cosmetics, and pharmaceuticals. At present, a standardized and reliable quality assessment system for chitosan is lacking mainly due to its complicated nature of this biopolymer (No, 1997).

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1 Crawfish Chitosan Production

##### 3.1.1 Raw Material

Cooked undersized crawfish shell waste was obtained from a commercial crawfish processor (Bayou Land Seafood, Beaux Bridge, Louisiana). Upon receipt, shells of tail and the head were separated, and placed separately into double black polyethylene bags and kept in labeled (parts of shell and date) carton boxes. These were then stored at -20°C until utilized.

Preceding preparation of crawfish chitosan, the frozen tail shells were thawed at ambient temperature, washed under running warm tap water to remove soluble organics, adherent proteins and other impurities. The tail shells were then dried in the oven (Model # E32-Bakbar Turbofan oven-Moffat Limited, Christchurch, New Zealand) at 70°C for a period of 24 hrs or longer until completely dried shells were obtained. To obtain a uniform size product, the dried shell was ground through a centrifugal grinding mill (Model # DR64857-Retsch/Brinkmann ZM-1, Westbury, NY) and sifted with 20-(0.841-mm) and 40-mesh (0.425 mm) sieves. Dried ground shell was placed in opaque plastic bottles and stored at ambient temperature until used.

##### 3.1.2 Isolation of Chitosan

###### 1. DP (Deproteinization)

Depending upon the production sequence, the crawfish shells or demineralized shells was deproteinized with 3.5% (w/w) NaOH solution for 2 hr at 65°C with constant stirring at a solid to solvent ratio of 1:10 (w/v) (No et al., 1989). Samples were then filtered under vacuum, and the filtrate was washed with tap water for 30 minutes and oven-dried.

## **2) DM (Demineralization)**

Depending upon the production sequence, the crawfish shells or deproteinized shells were demineralized with 1N HCl for 30 min at ambient temperature with a solid to solvent ratio of 1:15 (w/v) (No et al., 1989), then filtered under vacuum. The filtrate was washed for 30 min with tap water and oven-dried.

## **3) DC (Decoloration)**

Crawfish shells (also referred to as demineralized, deproteinized or crawfish chitin) were decolorized with acetone for 10 min and dried for 2 hr at ambient temperature, followed by bleaching with 0.315 % (v/v) sodium hypochloride (NaOCl) solution (containing 5.25% available chlorine) for 5 min at ambient temperature with a solid to solvent ratio of 1:10 (w/v), based on dry shell (No et al., 1989). Samples were then washed with tap water and dried under vacuum for 2-3 hrs until the powder was crispy.

## **4) DA (Deacetylation)**

Removal of acetyl groups from chitin was achieved by autoclaving at a pressure of 15 psi for 30 min at 121°C using 50% concentrated sodium hydroxide solution (NaOH) with a solid to solvent ratio of 1:10 (w/v) according to No et al.(1989). The resulting chitosans were washed to neutrality in running tap water, rinsed with distilled water, filtered, and dried at 60°C for 24 hr in the oven.

Six crawfish chitosans were prepared. The abbreviation (**DC**MPA, **DM**CPA, **DM**PCA, **DM**PAC, **D**PMCA, and **D**AMPC) denotes the sequential processes used to prepare crawfish chitosans: **DC**MPA = decolorized + demineralized + deproteinized + deacetylated; **DM**CPA = demineralized + decolorized + deproteinized + deacetylated; **DM**PCA = demineralized + deproteinized + decolorized + deacetylated; **DM**PAC = demineralized + deproteinized +

deacetylated + decolorized; **DPMCA** = deproteinized + demineralized + decolorized + deacetylated; **DAMPC** = deacetylated + demineralized + deproteinized + decolorized.

Commercial crab chitosans Sigma91 and Vanson75 were purchased from Sigma Chemical Co. (St. Louise, MO) and Vanson Inc. (Redmond, WA), respectively. They were used as controls to compare with the physicochemical and functional properties of the crawfish chitosans developed in this study.

### **3.2 Physicochemical and Functional Properties Measurements**

#### **3.2.1 Moisture Content**

Moisture content of the crawfish chitosan was determined by the gravimetric method (Black, 1965). The water mass was determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass (or weight) was the difference between the weights of the wet and oven dry samples. Procedures were as follows: weighed and recorded weight of dish, placed 1.0g of chitosan sample in duplicates in the metal aluminum dish, recorded weight of dish with sample, then placed the sample with the lid (filter paper to prevent or minimize contamination) in the oven. Adjusted the oven temperature to 60°C, and dried the samples for 24 hrs or overnight. Took the sample from the oven and placed it in a desiccator until it cools to room temperature. Weighed the sample, and recorded this weight as weight of dry sample. Calculated moisture content as:

$$\frac{(\text{wet weight, g} - \text{dry weight, g}) \times 100}{(\text{wet weight, g})} = \% \text{ of moisture content}$$

#### **3.2.2 Nitrogen**

The nitrogen of the crawfish chitosan was determined using a microprocessor-based, software-controlled instrument Model-TruSpec CN (Model # FP-428 Leco Corporation St. Joseph, MI. USA). There were three phases during an analysis cycle, i.e., purge, burn and

analyze. The encapsulated sample was purged of any atmospheric gases that had entered during sample loading. During the burn phase the sample was dropped into a hot furnace (850°C) and flushed with pure oxygen for a very rapid combustion. Finally, in the analyze phase, the remaining combustion product (nitrogen) was measured by the thermal conductivity cell. The final result was displayed as percent nitrogen (Theory of Operation Manual).

### **3.2.3 Ash**

Ash of the crawfish chitosan was calculated according to the standard method # 923.03 (AOAC, 1990). Placed 2.0g of chitosan (triplicate) into previously ignited, cooled, and tarred crucible. The samples were heated in a muffle furnace preheated to 600°C for 6 hr. The crucibles were allowed to cool in the furnace to less than 200°C and then placed into desiccators with a vented top. Allowed them to cool and weighed crucible and ash.

Calculation:  $\frac{(\text{Weight of residue, g})}{(\text{Sample weight, g})} \times 100 = \% \text{ Ash}$

### **3.2.4 Degree of Deacetylation**

Chitosan samples prepared in the form of film were studied for the degree of deacetylation (DD). The chitosan films were prepared by casting 1.0% w/v chitosan in 1% acetic acid solution, followed by drying in a vacuum air for 12 hr. The chitosan films were deprotonated by washing 2-3 times with methanol. The chitosan films were kept in desiccators for 12hr and then placed in sealed plates before scanning. The DD of chitosan was established using a FTIR (Fourier Transform Infrared Spectroscopy) instrument (Model # M2000, Midac Corp, Irvine, CA. USA) with frequency of 4000-400  $\text{cm}^{-1}$ . The degree of deacetylation (DD) of the chitosan was calculated using the baseline by Domszy and Roberts (1985). The computation equation for the baseline is given below:

$$DD = 100 - [(A_{1655} / A_{3450}) \times 100 / 1.33]$$

where  $A_{1655}$  and  $A_{3450}$  were the absorbance at  $1655\text{ cm}^{-1}$  of the amide-I band as a measure of the N-acetyl group content and  $3450\text{ cm}^{-1}$  of the hydroxyl band as an internal standard to correct for film thickness. The factor '1.33' denoted the value of the ratio of  $A_{1655} / A_{3450}$  for fully N-acetylated chitosan.

### **3.2.5 Molecular Weight**

For the determination of viscosity-average molecular weight (Dalton), the chitosan was dissolved in a mixture of 0.1 M acetic acid with 0.2 M NaCl, then the automated solution viscometer (Relative Viscometer Model Cat #9721-R56, Cannon instrument Corp., State College, PA. USA) was used to measure the intrinsic viscosity ( $\eta$ ). The Mark-Houwink equation relating to intrinsic viscosity with empirical viscometric constants  $K=1.81 \times 10^{-3}\text{ cm}^3/\text{g}$  and  $a=0.93$  (No et al., 2003) for chitosan was used to calculate the molecular weight using this equation:  $[\eta]=KM^a$ . Six or eight different dilute solutions were used to do this experiment (see Appendix B).

### **3.2.6 Viscosity**

Viscosity of chitosan was determined with a Brookfield viscometer (Model DV-II + Brookfield Engineering Laboratories, Inc., Stoughton, MA.). Chitosan solution was prepared in 1% acetic acid at a 1% concentration on a dry basis. Measurement was made in duplicate using a No. 5 spindle at 50 rpm on solutions at  $25^\circ\text{C}$  with values reported in centipoises (cPs) units.

### **3.2.7 Solubility**

Crawfish chitosan powder (0.1 g in triplicate) were placed into a centrifuge tube (known weight) then dissolved with 10 ml of 1% acetic acid for 30 min using an incubator shaker operating at 240 rpm and  $25^\circ\text{C}$  (C25KC, New Brunswick Scientific Co., Inc. NJ). The solution was then immersed in a boiling water bath for 10 minutes, cooled to room temperature ( $25^\circ\text{C}$ )

and centrifuged at 10,000 rpm for 10 min. The supernatant was decanted. The undissolved particles were washed in distilled water (25ml) then centrifuged a 10,000 rpm. The supernatant was removed and undissolved pellets dried at 60°C for 24hr. Finally, weighed the particles and determined the percentage solubility. Calculation:

$$\frac{(\text{Initial weight of tube + chitosan}) - (\text{Final weight of tube + chitosan})}{(\text{Initial weight of tube + chitosan}) - (\text{Initial weight of tube})} \times 100 = \% \text{ solubility}$$

### **3.2.8 Bulk Density**

The bulk density of crawfish chitosan was determined using the following procedure. Each of the chitosan samples (20-40 mesh particle size) were placed into a 25 ml graduated cylinder tube until reaching the marked line of 25 ml without tapping the tube and recorded the volume of the sample. The procedure was repeated five times for each sample. On the other hand, each of chitosan samples was placed in the same cylinder tube but this time tapping the tube and recorded the volume of the sample. The procedure was also repeated five times for each sample. The bulk density was computed as grams per milliliter of the sample.

### **3.2.9 Color**

The color of chitosan powder, expressed in L\*, a\*, b\*, c\*, h\*, and whiteness values, was measured (five readings) using a Minolta Spectrophotometer CM-508d (Minolta Co, Ltd. Japan). The whiteness was calculated using a formula from NFI (1991); whiteness =  $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$ .

### **3.2.10 Water Binding Capacity (WBC)**

WBC of chitosan was measured using a modified method of Wang and Kinsella (1976). WBC was initially carried out by weighing a centrifuge tube containing 0.5 g of sample, adding 10 ml of water, and mixing on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with intermittent shaking for 5 s every 10 min and

centrifuged (Model # Z383K, HERMLE-National Labnet Company, Woodbridge, NJ. USA) at 3,500 rpm (6,000 x g) for 25 min. After the supernatant was decanted, the tube was weighed again. WBC was calculated as follows:  $\text{WBC (\%)} = [\text{water bound (g)} / \text{initial sample weight (g)}] \times 100$ . All experiments were triplicated.

### **3.2.11 Fat Binding Capacity (FBC)**

FBC of chitosan was measured using a modified method of Wang and Kinsella (1976). FBC was initially carried out by weighing a centrifuge tube containing 0.5 g of sample, adding 10 ml of oil (five types of oil: soybean oil (Pure Wesson® Congra Foods, Irvine, CA. USA), canola (Pure Wesson®), corn (Pure Wesson®), sunflower (Pure Wesson®), and olive (San Marc' Can-America Inc. Tampa, FL. USA)) and mixing on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with shaking for 5 s every 10 min and centrifuged (Model # Z383K, HERMLE-National Labnet Company, Woodbridge, NJ. USA) at 3,500 rpm (6,000 x g) for 25 min. After the supernatant was decanted, the tube was weighed again. FBC was calculated as follows:  $\text{FBC (\%)} = [\text{fat bound (g)} / \text{initial sample weight (g)}] \times 100$ . All experiments were triplicated.

### **3.2.12 Emulsion Capacity at Various pHs**

The effect of chitosan on the emulsifying capacity of soy protein (Isolated soy protein, 90% of protein, PRO FAM 892, ADM Protein Specialties Decatur, IL) was determined in triplicate at various pH values by modifying the method of Prinyawiwatkul et al.(1993). Initially, 0.5g of chitosan was dissolved in 100 ml of 1% acetic acid to prepare 0.5% chitosan solution (pH 3.14). An emulsion was prepared by blending 9 ml of 0.5% chitosan solution and 38 ml of 1% soy protein solution, later they were adjusted to pH 2, 4, 6, 8, and 10 using either 0.5N NaOH or 1N HCl. Soy bean oil (Wesson vegetable oil - Hunt-Wesson, Inc. Fullerton, CA) supplemented

with 0.03% Oil-Red-O biological stain (Aldrich Chemical Co., Inc., Milwaukee, WI) was dispensed from a 50 ml burette through a 2 cm diameter hole at the bottom of an inverted blender jar which contained chitosan and protein solutions adjusted previously to a certain pH. To this was added soybean oil drop wise at a speed of 0.5 ml/s while the mixture was blended at low speed in an Osterizer blender (Model #6698, Oster Division of Sunbeam Products, Inc., Boca Raton, FL.) until the emulsion broke. The breakpoint or endpoint can be described when visible viscosity of emulsion disappeared and the mixture became oil-like in appearance. Phase inversion (coalescence) occurred was considered as the emulsion capacity of soy protein suspensions. Emulsifying capacity was expressed as milliliters of soybean oil emulsified per gram of soy protein.

### **3.2.13 Emulsion Capacity with Different Concentrations of Chitosan**

The effect of various concentrations of chitosan on the emulsifying capacity of soy protein (Isolated soy protein, 90% of protein, PRO FAM 892, ADM Protein Specialties Decatur, IL) was determined in triplicate by modifying the method of Borton et al. (1968). Initially, chitosan was dissolved in acetic acid (1%, v/v) at concentrations of 0% (control), 0.1%, 0.5%, and 1.0%. Emulsion was prepared by blending 38ml protein solution (1%) and 9ml of chitosan (0.1%, 0.5%, 1.0%) solution, while soybean oil (Wesson vegetable oil - Hunt-Wesson, Inc. Fullerton, CA), which was supplemented with 0.03% Oil-Red-O biological stain (Aldrich Chemical Co., Inc., Milwaukee, WI) was dispensed drop wise from a 50 ml buret through a 2 cm diameter hole at the bottom of an inverted blender jar at a speed of 0.5 ml/s while the mixture was blended at low speed in an Osterizer blender (Model #6698, Oster Division of Sunbeam Products, Inc., Boca Raton, FL.) until the emulsion broke. Emulsion capacity was expressed as milliliters of soybean oil emulsified per gram of soy protein.

### **3.2.14 Emulsion Viscosity**

Emulsions containing 80% of the amount of oil needed to reach the breakpoint were used for emulsion viscosity measurements (Prinyawiwatkul et al., 1993). Emulsion viscosity (EV) was determined at 25°C using a Brookfield viscometer (Model DV-II+ (Brookfield Engineering Laboratories, Inc., Stoughton, MA.) and a Helipath Stand equipped with a T-B spindle operated at 2.5 rpm. The emulsion viscosity was computed as cps. Two readings were recorded on duplicate samples of emulsions.

### **3.3 Statistical Analysis**

All experiments were carried out in triplicate, except for duplicate determinations of nitrogen content, moisture content, and emulsion viscosity. Average values (means) and standard deviations were reported. Mean separations were analyzed using the ANOVA (SAS) and Tukey's studentized range tests at  $\alpha = 0.05$ .

## CHAPTER 4

### RESULTS AND DISCUSSION

Results of the proximate analysis and physicochemical properties of various chitosan samples are presented in Tables 2-7.

#### 4.1 Yield

Yield was calculated as the dry weight of chitosan obtained from 400-600 g of dried crawfish shell powder. Chitosan yield ranged from 16.7-18.8%. The highest yields were obtained from DMPAC, followed by DCMPA, DMCPA, DPMCA (control) and DMPCA.

Table 2 - Crawfish Chitosan Production Yield (dry weight basis)

Sample	Yield (%)
DCMPA	18.3
DMCPA	17.8
DMPCA	16.7
DMPAC	18.8
DPMCA (control)	16.8
DAPMC	0.34

Results (Table 2) showed that the DMPCA had the lowest yield (16.7%), but was not different from the control DPMCA (16.8%). Brzeski (1982) reported about 14 % yield of chitosan from krill and 18.6% from prawn waste (Alimuniar and Zainuddin, 1992). The % yield of chitosan obtained (16.7-18.8%, Table 2) is lower than that (approximately 23%) of chitin reported in the literature (No and Meyers, 1989). This is due to loss of sample mass/weight from excessive removal of acetyl groups from the polymer during deacetylation (i.e. the conversion of chitin to chitosan). On the other hand, the yield of DAPMC was 0.34% which was quite an insignificant amount. Therefore, only five samples were considered for further analysis.

From this study, when chitosan process begins with deacetylation (DA), very poor yield (0.34%) was obtained due to depolymerization of the chitosan polymer. Depolymerization is the

process of converting a polymer into a monomer or a mixture of monomers as defined by the (IUPAC Compendium of Chemical Terminology 2nd ed., 1997). Kurita (1998) noted that chitosan solubility's stability is poor above pH 7 and at a higher pH, precipitation or gelation occurs. In addition, chitosan solution forms poly-ion complex with anionic hydrocolloid, giving rise to a gel formation, which was also observed for DAPMC in this study. Though high temperature over 280°C causes thermal degradation of chitosan and thus polymer chains rapidly break down, this was not the case in this study as very low temperature (121°C) was utilized. The probable explanation of what observed in this study is the rapid depolymerization of chitosan hydrochloride. Since chitosan polymer contains hydroxyl and amino, polar groups in its molecular structure, it possesses thermodynamic instability. For DAPMC, after harsh reaction of deacetylation, the shell was reacted with HCl, presuming that hydrogen chloride liberated in the probe attacks the  $\beta$ -glycosidic links between the monomer units, causing gelation and then depolymerization.

#### 4.1.1 Formation to Gel-like Mass

After deacetylation (DA) and demineralization (DM) step, the shell was immediately washed using the tap water to remove HCl. The pink colored wet shell (DAPM) suddenly transformed into a viscous gel as seen in Figure 5.



Figure 5. Non-Powdered (Gel-like) Chitosan

The shell was almost impossible to be washed because the filter mesh's holes were all clogged by the transformed gel-like product.

#### **4.1.2 Formation of a White Unidentified Mass**

When the gel-like mass was transferred to a beaker containing acetone for decoloration, it immediately transformed into a white mass of cotton ball (Figure 6).



Figure 6. Formation of White Unidentified Mass

#### **4.1.3 Disappearance and Poor Yield**

When the mass was washed with tap water, it dissolved swiftly leaving very small amounts of mass (0.34g out of 100g shell powders) with varying particle size (Figure 7). Thus, it can perhaps be assumed that this unknown mass is a product of depolymerization.



Figure 7. Poor Production Yield

## 4.2 Moisture Content

Results of the moisture, nitrogen and ash content of chitosan samples are presented in Table3.

Table 3. Proximate Analysis of Crawfish and Commercial Chitosans (dry weight basis)

Sample	Moisture (%)	Nitrogen (%)	Ash (%)
DCMPA	0.3 (0.35) c	8.23 (0.02) b	0.2 (0.07) a
DMCPA	0.6 (0.21) c	8.09 (0.01) b	0.9 (0.99) a
DMPCA	0.6 (0.21) c	8.01 (0.05) b	0.4 (0) a
DMPAC	0.6 (0.21) c	6.91 (0.05) d	1.6 (1.98) a
DPMCA (control)	0.7 (0.42) c	8.03 (0.06) b	0.3 (0.07) a
Vanson 75	4.5 (0) a	7.53 (0.14) c	1.4 (0.05) a
Sigma 91	3.5 (0.07) b	8.50 (0.21) a	1.8 (0.05) a

Numbers in parentheses are standard deviations. Means with different letters in each column are significantly different ( $p < 0.05$ ). DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Vanson75 and Sigma91 are commercial crab chitosans.

The crawfish chitosan samples had a moisture content ranging from 0.3% to 0.7%. Commercial crab chitosans, Vanson75 and Sigma91, had a relatively higher moisture content, 4.5% and 3.5%, respectively, than the experimental samples. Chitosan is hygroscopic in nature (Khan et al., 2001), hence it is very possible that the two commercial samples were affected by moisture absorption during storage. According to Li (1992), commercial chitosan products contain less than 10% moisture content.

## 4.3 Nitrogen Content

The nitrogen content of the crawfish chitosan samples varied between 6.91% and 8.23% on a dry basis compared with 7.53% to 8.50% for Vanson75 and Sigma91, respectively. With the exception of DMPAC (6.91%), DCMPA, DMCPA, DPMCA, and DMPCA (8.23%, 8.09%, 8.03%, and 8.01%, respectively) showed no significant differences ( $P > 0.05$ ) in nitrogen content,

but the values were slightly higher than that (7.06% to 7.97%) reported by No and Meyers (1995) for chitosan from crab and shrimp shell on a dry basis. This probably is due to the presence of protein residues as mentioned by Rutherford and Austin (1978). Protein is bound by covalent bonds forming stable complex with chitin and chitosan. Thus, it is very difficult to achieve 100% deproteinization. Even with complete DP, nitrogen (7.06 to 7.97%; No et al., 1995) is still remained as chitosan has the amino (-NH<sub>2</sub>) group.

#### **4.4 Ash**

Ash measurement is an indicator of the effectiveness of the demineralization (DM) step for removal of calcium carbonate. Elimination of the demineralization resulted in products having 31 – 36% ash (Bough et al., 1978). The ash content in chitosan is an important parameter. Some residual ash of chitosans may affect their solubility, consequently contributing to lower viscosity, or can affect other more important characteristics of the final product. A high quality grade of chitosan should have less than 1% of ash content (No et al., 1995). An ash content of less than 1% from crab chitosans has been reported by No and Meyers (1995). Table 3 also shows the ash content of crawfish chitosans compared with that of the commercial crab chitosans. Our crawfish chitosans contained less than 1% ash with a range of 0.2% to 0.9%, except for DMPAC with 1.6%. Commercial chitosan products contained less than 2.0% ash.

#### **4.5 Degree of Deacetylation**

The degree of deacetylation of our crawfish chitosan samples ranged from 68% to 73% with an average of 71% (Table 4). According to No and Meyers (1995), DD of chitosan ranges from 56% to 99% with an average of 80%. Sample DCMPC (73%) had the highest DD, followed by DMPCA, DMCPA and DPMCA (71%, 70%, and 68%, respectively). The FTIR absorption

spectra pattern (see Appendix C) of all crawfish chitosan samples (DCMPA, DMCPA, DMPCA, and DPMCA) and the commercial samples was quite similar.

Table 4. Molecular Weight and Degree of Deacetylation of Crawfish and Commercial Chitosans

Sample	Molecular Weight (Daltons)	Degree of Deacetylation (%)
DCMPA	10,596.62	73
DMCPA	9,639.34	70
DMPCA	6,984.29	71
DMPAC	674.49	-*
DPMCA (control)	6,476.40	68
Vanson 75	6,531.99	70
Sigma 91	7,194	71

DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial chitosan (Vanson75 and Sigma91). \* Not able to determined.

For the DMPAC sample, it was improbable to determine its DD value because of its very low viscosity (Table 4), thus did not allow us to prepare film properly for the DD measurement. As in the Table 4 and 5, DMPAC had a very low molecular weight and viscosity, which are very important characteristics of chitosan.

Commercial crab chitosans, Vanson75 and Sigma91, had similar %DD compared with crawfish chitosan samples. However, we believed that Sigma91 (71%DD) should have higher DD but the value obtained was lower than expected. Among several methods to determine DD mentioned earlier in the literature review (Chapter 2), we chose the IR spectroscopic method. According to Khan et al. (2002), the IR spectroscopic method is commonly used for the estimation of chitosan DD values for its advantages: it is relatively fast and does not require dissolution of the chitosan sample in an aqueous solvent. However, its disadvantage is that utilizing different baselines to calculate DD values would inevitably contribute to variation in

%DD values. Thus, accurate determination of the degree of deacetylation of chitosan is needed. DD values are not only highly dependent on the source and method of purification (No et al., 1989) but also on the type of analytical methods employed, sample preparation, and type of instrument used, and other conditions may influence the analysis of DD (Khan et al., 2002). The anomaly of Sigma91 was maybe due to one of these different protocols in the manufacturing process or the presence of impurities.

#### **4.6 Molecular Weight (MW)**

Molecular weight of chitosan varied with the sources and also the methods of preparation. The MW of native chitin is usually larger than one million Daltons while commercial chitosan products fall between 100,000 to 1,200,000 Daltons (Li et al., 1992). No and Meyers (1995) reported an average MW of  $0.12 \sim 1.5 \times 10^6$  Da., and the chitosan extracted from the crawfish shell waste had a MW of 46,000 Da. The molecular weight of our crawfish chitosan samples ranged from 674.49 to 10,596.62 Da. (Table 4). When chitosan process started with decoloration (DC), the highest molecular weight (10,596.62 Da.) was shown. Acetone may have tightened the molecular structure, which caused less accessible surface for the next steps. Our crawfish chitosans were likely underwent more depolymerization which resulted in lower molecular weight compared to the literature. In general and as previously mentioned, high temperature, dissolved oxygen, and shear stress can cause degradation of chitosan.

#### **4.7 Viscosity**

The viscosity of chitosan solutions reported in the literature generally ranges from 60 to 780 cP (Alimuniar and Zainuddin, 1992; Anderson et al., 1978). These ranges of viscosity have also been observed by Cho et al. (1998) with five commercially available chitosans.

Table 5 shows results of viscosity, solubility, and bulk density of our crawfish chitosans.

Table 5. Viscosity, Solubility, and Bulk Density of Crawfish and Commercial Chitosans

Sample	Viscosity (cP)	Solubility (%)	Bulk Density (g/ml)	
			tapped	untapped
DCMPA	563.7 (68.07) a	93.3 (0.61) a	0.23 (0.008) bc	0.19 (0.008) b
DMCPA	444.9 (78.59) ab	94.2 (0.81) a	0.23 (0.007) c	0.18 (0.007) b
DMPCA	131.8 (15.96) c	94.3 (0.80) a	0.20 (0.004) d	0.16 (0.006) c
DMPAC	1.0 (1.48) c	94.0 (0.69) a	0.24 (0.004) b	0.19 (0.003) b
DPMCA (control)	403.3 (25.23) ab	93.9 (0.33) a	0.23 (0.009) bc	0.19 (0.006) b
Vanson 75	144.9 (21.38) c	93.9 (1.27) a	0.17 (0.005) e	0.14 (0.003) d
Sigma 91	384.5 (24.08) b	87.8 (2.34) b	0.31 (0.003) a	0.24 (0.004) a

Numbers in parentheses are standard deviations. Means with different letters in each column are significantly different ( $p < 0.05$ ). DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial samples (Vanson75 and Sigma91).

Bough et al. (1978) stated that viscosity of chitosans varied considerably from 60 to 5,110 cP depending on the species. When shrimp and krill were utilized the products had a high viscosity up to 5,110 cP and 5,074 cP, respectively.

Our crawfish samples had viscosity ranging from 1.0 to 563.7 cP. DCMPA had the highest viscosity (563.7 cP) but comparable to that of DMCPA and DPMCA (444.9 cP and 403.3 cP, respectively), whereas DMPAC had a very low viscosity (1.0 cP)(Table 5). The two commercial crab chitosans showed lower viscosity values than our crawfish samples. Some residual ash may have affected their solubility, consequently contributing to a lower viscosity. When molecular weight is lower, viscosity also tends to decrease (No et al., 2000). On the basis of these composite observations, it is apparent that DMPAC is the case. DMPAC yielded 1.6% ash, which was relatively higher than other samples, and had the lowest MW, thus contributed to the lowest viscosity of 1.0 cP. From this study, it is more than likely that when the deacetylation process changes its order from the standard method (i.e., DMPAC in this study), significant

degradation of the chitosan structure occurs. The DD process is a very harsh treatment with concentrated sodium hydroxide (40-50%) usually at 100°C or higher for 30 min.

On the other hand, comparing DMPCA and DPMCA, the DMPCA showed viscosity of 131.8 cP whereas 403.3 cP for DPMCA. One is almost tempted to believe that reversing demineralization (DM) and deproteinization (DP) process during the chitosan production seems to have no effect on the characteristics of crawfish chitosan, but the results (Table 5) otherwise showed significant differences between the two samples (DMPCA vs. DPMCA).

There are some factors affecting viscosity during the production of chitosan such as the degree of deacetylation, molecular weight, concentration, ionic strength, pH, and temperature, etc. Moorjani et al. (1975) reported that chitosan viscosity decreased with increased time of demineralization. The viscosity of chitosan in acetic acid tends to increase with decreasing pH but decrease with decreasing pH in HCl. Intrinsic viscosity of chitosan is a function of the degree of ionization as well as ion strength (Bough et al., 1978). Deproteinization with 3% NaOH, and elimination of the demineralization step in chitin preparation, decreased the viscosities of the final chitosan samples (Bough et al., 1978). Moorjani et al. (1975) stated that it is not desirable to bleach the material at any stage since bleaching considerably reduces the viscosity of the final chitosan product.

#### **4.8 Solubility**

All five crawfish chitosan samples and the commercial chitosan, Vanson75, demonstrated an excellent solubility ranging from 93.3 to 94.3% with no significant difference (Table 5), while the commercial chitosan, Sigma91, showed slightly lower solubility (87.8%). Brine and Austin (1981) noted that lower solubility values suggest incomplete removal of protein. Since the chemical basis of this method is based on the reaction with the amino group, the presence of

protein contaminants remaining in the sample during the analysis process could adversely interfere with the results. The commercial chitosan, Sigma91, had the highest N-residue (8.5%) (Table 3). This implies that the deproteinization process on our five samples might have been nearly complete but Sigma91 still had some protein remaining or other impurities.

#### **4.9 Bulk Density**

According to Cho et al. (1998) and Brine and Austin (1981), the bulk density of crawfish and commercial chitin and chitosan varies, and this can be attributed to species or sources of chitosan and the methods of preparation.

The bulk density reported for chitin from shrimp and crab is 0.06 and 0.17 g/ml, respectively (Shahidi and Synowiecki, 1991), indicating that shrimp chitin is more porous than crab chitin. Krill chitin was found to be 2.6 times more porous than crab chitin (Anderson et al., 1978). In the study of No et al. (1995), the bulk density of commercial crab chitosan ranges from 0.18 – 0.33 g/ml, indicating up to 1.8 times difference in porosity.

Rout (2001) reported the bulk density of chitin and chitosan from crawfish shell to be very high (0.39 g/cm<sup>3</sup>). This may be due to the particle size and porosity of the material before treatment. But once crawfish shell had been demineralized or deproteinized or both there were minor variations in bulk density among chitin and chitosan produced.

The untapped bulk density of crawfish chitosan samples was in the range of 0.16 – 0.19 g/ml and those of the tapped samples were between 0.20- 0.24 g/ml (Table 5). This indicates that our crawfish chitosan samples are not that significantly different among themselves and are in the range reported by No et al. (1995). But Vanson75 had a lower bulk density of 0.17 g/ml and was more porous than the others. Cho and No (1999) noted that lower bulk density may indicate that the chitosan is more porous and may have been subjected to a lower alkali concentration

treatment for deproteinization. In contrast, Sigma91 had the highest tapped bulk density of 0.31 g/ml. The commercial chitosans (Vanson75 and Sigma91) varied in their bulk density even though they were prepared from crab shells.

#### **4.10 Color**

The color of chitosan samples was expressed in L\*, a\*, b\*, chroma, hue angle, and whiteness (Table 6) values. Chitosan powder is quite flabby in nature and its color varies from pale yellow to white (No et al., 1995). Rout (2001) noted that the pigment in the crustacean shell forms complexes with chitin (4-keto and three 4, 4'-diketo- $\beta$ -carotene derivatives). Based on visual observation, the color of our crawfish chitosan samples varied from white to extremely pink or yellow. As seen in Table 6, the highest L\*(lightness) values among our crawfish samples was observed for DMPCA (33.3), followed by DPMCA, DMCPA, DMPAC, and DCMPA (32.6, 30.9, 25.8, and 25.2, respectively). Commercial crab chitosan Vanson75 showed the highest brightness (48.4), while Sigma91 (24.4) showed the lowest value.

In a\* (redness) analysis, DMPAC demonstrated the highest intensity of red color (7.9) which may have been attributed to the degradation of chitosan. In contrast, commercial crab chitosan Vanson75 showed a negative value in redness (-0.6). The b\* (yellowishness) analysis showed DMPAC to have the highest value (25.1) while Sigma91 had the lowest yellow color value (7.7). The C\* (Chroma) value of DMPAC was the highest due to the highest a\* and b\* values. The DMPAC process seems to be the least effective in removal of color pigments.

The hue angle of the two commercial crab chitosans Vanson75 and Sigma91 were higher (93.4 and 88.0) than our crawfish chitosan samples. The hue angle of 0° and 90° indicates redness and yellowness, respectively. DMPAC had the lowest hue angle of 72.4, due to the highest a\* value.

With regards to their whiteness, data were analyzed using the formula from NFI (1991),

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

Vanson75 showed the highest whiteness value (47.3) and our crawfish chitosan samples DMPCA and DPMCA showed comparable values (31.9 and 31.1, respectively).

Table 6. Color Characteristics of Crawfish and Commercial Chitosans

Sample	L*	a*	b*	C*	h*	Whiteness
DCMPA	25.2 (1.76) d	1.7 (0.18) c	11.7 (0.28) c	11.8 (0.29) c	81.8 (0.84) d	24.2 (0) e
DMCPA	30.9 (0.31) c	1.1 (0.09) d	11.4 (0.34) c	11.5 (0.34) c	84.3 (0.33) c	29.9 (0) d
DMPCA	33.3 (1.16) b	2.6 (0.16) b	13.5 (0.64) b	13.7 (0.65) b	79.1 (0.36) e	31.9 (0) b
DMPAC	25.8 (0.82) d	7.9 (0.32) a	25.1 (0.63) a	26.3 (0.67) a	72.4 (0.48) f	21.3 (0) g
DPMCA (control)	32.6 (0.66) bc	1.3 (0.05) d	14.0 (0.38) b	14.1 (0.38) b	84.8 (0.17) c	31.1 (0) c
Vanson 75	48.4 (0.38) a	-0.6 (0.05) f	10.4 (0.23) d	10.4 (0.24) d	93.4 (0.27) a	47.3 (0) a
Sigma 91	24.4 (0.90) d	0.3 (0.10) e	7.7 (0.18) e	7.7 (0.17) e	88.0 (0.75) b	24.0 (0) f

Numbers in parentheses are standard deviations. Means with different letters in each column are significantly different ( $p < 0.05$ ), L\*=lightness, a\*=redness, b\*=yellowishness, C\*=chroma, and h=Hue angle. DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial samples (Vanson75 and Sigma91).

DMPAC gave a very low value of whiteness (21.3). According to Anderson et al. (1978), the tan color of chitosan may have been caused by degradation of pigments present in the chitin during deacetylation (DA) step. The DMPAC sample was demonstrated significantly different color values, indicating that more color remained in the sample. Our visual observation of all crawfish chitosan samples indicated the zone of light yellow hue angle. Among all crawfish

chitosan samples, we arbitrarily determined that DMPCA was the most efficient to remove color than others, based on visual observation.

#### 4.11 Water Binding Capacity (WBC)

Water binding capacity of crawfish and commercial chitosans is shown in Table 7. WBC differed among crawfish chitosan samples, ranging from 660.6 % to 745.4%, excluding the DMPAC (274.2%). These values were in agreement with those reported by Rout (2001) where WBC for chitosans ranged from 581 to 1,150% with an average of 702%.

Table 7. Water Binding Capacity of Crawfish and Commercial Chitosans

Sample	WBC (%)
DCMPA	660.6 (9.97) c
DMCPA	673.8 (17.68) c
DMPCA	745.4 (17.99) b
DMPAC	274.2 (4.04) e
DPMCA (control)	694.4 (14.06) c
Vanson 75	941.5 (20.87) a
Sigma 91	548.7 (11.99) d

Numbers in parentheses are standard deviations. Means with different letters in each column are significantly different ( $p < 0.05$ ). DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial chitosan sample (Vanson75 and Sigma91).

Cho and No (1998) reported the WBC range of 458% to 805% for five commercial chitosans from shrimp and crab shell. There was no difference in WBC of DCMPA, DMCPA, and DPMCA. DMPCA had a slightly higher WBC (745.4 %) than that of DPMCA, DMCPA, and DCMPA (694.4 %, 673.8 %, and 660.6 %, respectively). DMPAC showed poor WBC of 274.2 %. Vanson75 revealed a higher WBC (941.5%) than that of Sigma91 (548.7%) and our crawfish chitosan samples.

Reversing the sequence of steps such as demineralization (DM) and deproteinization (DP) caused a pronounced effect on WBC of chitosan (745.4% vs. 694.4%). Deproteinization (DP) of demineralized shell showed higher WBC compared to the process when demineralization (DM) of the deproteinized shell was conducted (Table 7). Besides, the decoloration (DC) step also led to decrease in WBC of chitosan when it was done after deacetylation.

#### **4.12 Fat Binding Capacity (FBC)**

Fat binding capacity (FBC) of five crawfish and two crab commercial chitosans was measured using five types of oils including soybean, canola, corn, sunflower, and olive oil. The results are shown in Table 8.

FBC differed among chitosan products, ranging from 370.2% to 665.4%. Vanson75 had the highest FBC, regardless of the type of oil used. Among our crawfish chitosan samples, DMPCA showed the highest FBC values: 533.4% with soybean oil, 526.3% with canola oil, 578.1% with corn oil, 573.3% with sunflower oil, and 574.9% with olive oil, although DMPCA had low viscosity (131.76 cp). DCMPCA and DMCPA showed no significant difference in FBC. The sample DMPAC showed the lowest FBC (445.3% - 495.9%) as it had very low viscosity (1.0 cP).

Moorjani (1975) advocated that changing the sequence of steps, when demineralization (DM) is conducted prior to deproteinization (DP) and finally deacetylation (DA), results in an increase in FBC than when deproteinization (DP) is conducted prior to demineralization (DM) and finally deacetylation (DA). Thus, the decreased fat binding capacity of DPMCA as evidenced in this study may be due to the reverse of DM and DP steps.

Amongst the five types of oil used, olive oil generally demonstrated more FBC with crab and crawfish chitosan samples, whereas canola oil showed the least FBC. Previous study by Rout (2001) showed that the average FBC of commercial crab chitosans and crawfish chitosans for soybean oil was 587% and 706%, respectively.

Table 8. Fat Binding Capacity of Crawfish and Commercial Chitosans

Sample	Fat Binding Capacity (%)				
	Soybean	Canola	Corn	Sunflower	Olive
DCMPA	519.7(17.99) bc	499.3(22.41) bc	539.6(11.57) bc	519.8(25.08) cd	545.6 (21.28) bc
DMCPA	511.8(27.83) bc	499.9(17.52) bc	505.8(40.82) cd	533.3(12.61) bc	545.7(4.98) bc
DMPCA	533.4(16.97) b	526.3 (27.79) b	578.1(6.88) b	573.3(22.15) b	574.9(23.83) b
DMPAC	470.8(21.42) c	445.3(16.82) de	464.3(18.78) de	469.1(10.24) e	495.9(11.03) cd
DPMCA (control)	491.9(5.35) bc	467.2(2.26) cd	514.3(12.88) cd	488.4(15.51) de	505.4(12.61) cd
Vanson 75	650.5(20.48) a	608.1(14.63) a	634.8(8.14) a	638.2(6.22) a	665.4(30.65) a
Sigma 91	393.4(16.75) d	399.6(21.03) e	413.4(16.86) e	370.2(9.95) f	459.1(4.38) d

Numbers in parentheses are standard deviations. Means with different letters in each column are significantly different ( $p < 0.05$ ). DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial chitosans (Vanson75 and Sigma91).

In comparison with cellulose as the control, cellulose demonstrated only 314% FBC (Rout, 2001). Cellulose and chitosan share similar chemical structure except that chitosan has –NH<sub>2</sub> groups instead of –OH groups in the polymeric structure.

Regardless of the type of vegetable oils, the five modified crawfish chitosan samples showed desirable FBC range from 445.3% (with canola) to 578.1% (with corn) which is in

agreement with those (314 to 535% with an average of 417%) reported by No et al. (1998).

#### 4.13 Emulsion Capacity (EC)

The effect of chitosan on the emulsifying capacity of soy protein was evaluated with five crawfish chitosan samples, two commercial chitosans (Vanson75 and Sigma91), and three controls (Figure 8). Results indicate that the capacity of soy protein to form emulsion was enhanced in the presence of chitosan.

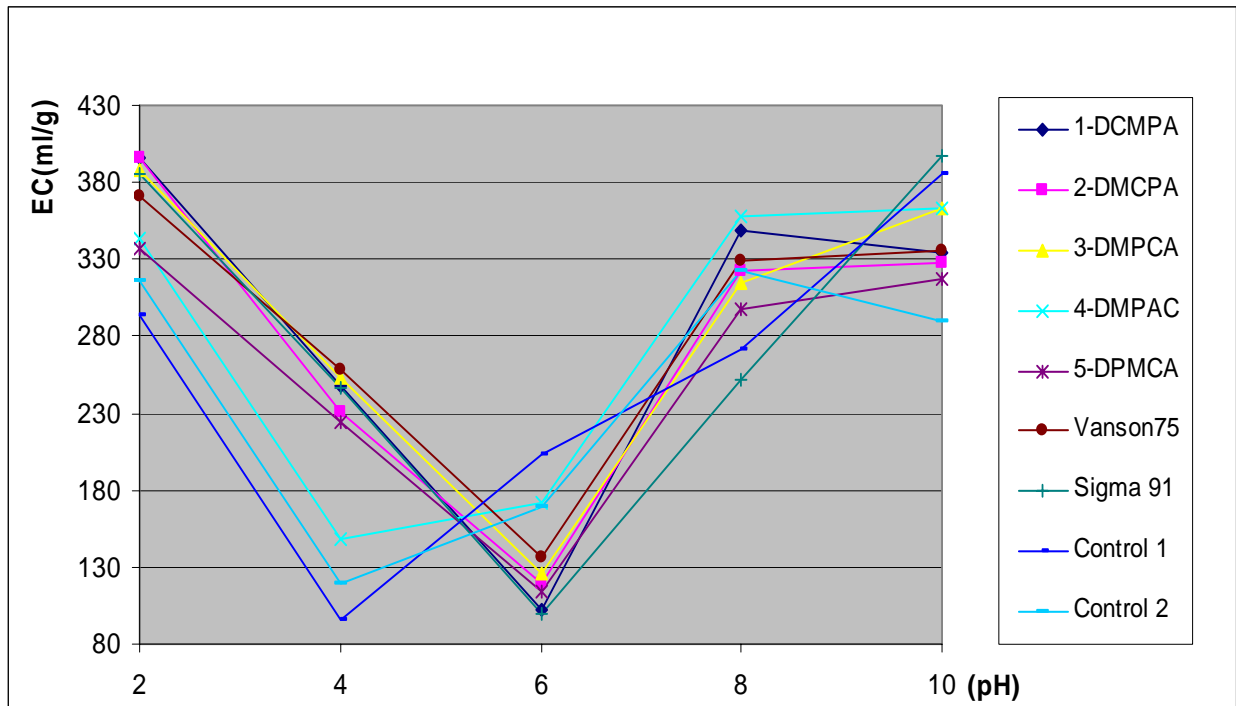


Figure 8. Emulsion Capacity Measurement of Crawfish and Commercial Chitosans at Various pHs

DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial chitosans (Vanson75 and Sigma91). Control 1 = 1% protein solution only; Control 2 = 1% acetic acid and 1% protein solution with no chitosan; and Control 3 = 0.5% chitosan solution only.

The three controls used in evaluating this property include; Control 1 - 1% protein solution only; Control 2 - 1% acetic acid and 1% protein solution with no chitosan; and Control 3

- 0.5% chitosan solution only. Even though chitosan alone does not produce emulsion (Knorr, 1982; in Cho et al., 1998), the emulsifying capacity of soy protein in the presence of chitosan was greatly enhanced (Figure 8).

According to Belitz and Grosch (1999), emulsion is basically a disperse system of one or more immiscible liquids, and emulsifiers are compounds which form interface films and thus prevent the disperse phases from flowing together. Proteins are amphoteric, meaning they behave as dipolar ions carrying both positive and negative charges. Depending on pH, they can exist as polyvalent cations, anions or zwitterions. They can possess the same number of positive and negative charges resulting in the net charge of zero at the isoelectric point. If the pH value is higher than the isoelectric point ( $pI$ ), the protein will have a negative charge, whereas a positive charge if the pH value is lower than the  $pI$ .

Soy protein has a pronounced minimum solubility in the pH range of 3.0 to 6.0 (Belitz and Grosch, 1999). Its isoelectric point is variable depending on the ions present and their concentration. In our study, at approximately near pH 4.0 (Brooks and Morr, 1984), protein was at its isoelectric point, was the least soluble, and precipitated ("isoelectric precipitation").

Unlike other polysaccharides, chitosan possesses a positive ionic charge, and has both reactive amino and hydroxyl groups, which give it the ability to chemically bond with negatively charged protein (Li et al., 1992). Chitosan is pH-dependent. When pH is less than 6.5, chitosan in solution carries a positive charge along its backbone, thus makes it possible for its use as emulsifier in many application (Rout, 2001). Because of its polar groups, chitosan also provides additional stabilization due to hydration forces (Del Blanco et al., 1999). According to Filar and Wirick (1978), chitosan functions only in acid systems to show possible utility as a thickener, stabilizer, suspending agent or film former.

At pH 4.0 (*pI*), the presence of chitosan (which possesses a positively charged molecule) in soy protein solution enhanced emulsion capacity; this may have been due to addition effect of positively charged molecules from chitosan. The decrease in emulsion capacity between protein *pI* and pH 6.0 was probably due to charge cancellation between chitosan (+) and protein (-). Between pH 4.0 - 6.0, the positive charge on chitosan is neutralized by the negative charge on soy protein because above pH 4.0, protein becomes negatively charged. Above pH 6.0, emulsion capacity increased because the solution had more negatively charged. Starting at pH 2.0 (Figure 8), the increase in emulsifying capacity was notable with the addition of our modified processed chitosan in soy protein solution. Though no significant differences were observed, DMCPA had the highest EC (395.8 ml/g), followed by DCMPA (395.3 ml/g), DMPCA (387.4 ml/g), and DPMCA (337.8 ml/g).

At pH 4.0, although there were no significant differences among the treatment samples, i.e., DMPCA (253.5 ml/g), DCMPA (247.6 ml/g), DMCPA (230.7 ml/g), and DPMCA (224.3 ml/g), but they were significantly different from the two controls. Emulsion capacity of control - 1 was 96.2 ml/g; control - 2 was 119.0 ml/g; but control - 3 showed no emulsion. All modified processed chitosans enhanced the emulsion capacity by at least 2.6 times, except for DMPAC whose emulsion capacity was 148.2 ml/g. The EC of DMPAC value was very similar to those of the controls; this means that DMPAC does not affect how protein behaves in solution.

At pH 6.0, chitosan samples showed relatively lower emulsion capacity ranging between 102.4 ml/g and 126.3 ml/g compared to control - 1(203.4 ml/g) and control - 2 (168.6 ml/g). This is probably due to the fact that at pH 6.0, soy protein /chitosan solution approached its isoelectric point. At pH 8.0, DCMPA showed higher emulsion capacity (348.7 ml/g) than DMCPA, DMPCA, and DPMCA (321.9 ml/g, 315.2 ml/g, and 298.2 ml/g) respectively, while control - 1

showed 271 ml/g. All modified chitosan samples did not show any significant difference among themselves at pH 8.0. At pH 10.0, DMPCA (362.4 g/ml) showed the highest EC value among our chitosan samples, followed by DCMPA (334.8 ml/g) and DMCPA (328.3 ml/g), but lower than all controls. However, the overall pH results indicated that DMPCA and DMCPA enhanced emulsion capacity more than other modified chitosan samples.

Del Blanco et al. (1999) stated that the degree of deacetylation is a determining factor in the emulsifying properties of chitosan, and chitosan with intermediate DD is a less effective emulsifier while chitosan with higher DD tends to produce poor emulsification. The optimum %DD of chitosan for sunflower oil emulsification is 81 and 89. In our study, the DD of samples ranged from 68% to 73% yet they still had an affect on emulsion. Comparing our modified chitosan samples to commercial samples (Figure 8), Vanson75 showed high emulsion capacity at pH 4.0 with 258.9 ml/g compared with our highest DMPCA at 253.5 ml/g. At pH 6.0, Vanson75 had an EC of 136.3 ml/g compared to 126.3 ml/g of DMPCA. Similarly, Sigma91 showed high EC at pH 10.0 with 396.8 ml/g, compared to 362.4 ml/g of DMPCA. Control - 1 and 2 showed similar pattern but had lower EC than our modified chitosans.

#### **4.14 Emulsion Capacity Measurement with Different Concentrations of Chitosan**

For comparison of the emulsion capacity as affected by different concentrations of chitosan, the pH of the solution was unadjusted with a range of 3.8 and 4.0. The solution consisting of 9ml of 1% acetic acid + 38 ml of 1% soy protein solution without chitosan was used as the control. The effect of chitosan at different concentrations (0.1%, 0.5%, and 1.0%) on the emulsion capacity of soy protein was evaluated with the five process modified crawfish chitosan samples - DCMPA, DMCPA, DMPCA, DMPAC, and DPMCA, and two commercial samples Vanson75 and Sigma91, and the control (Figure 9). Due to its viscosity, the

concentration of chitosan solution cannot be more than 1.0% because when the solution is too concentrated it becomes very thick.

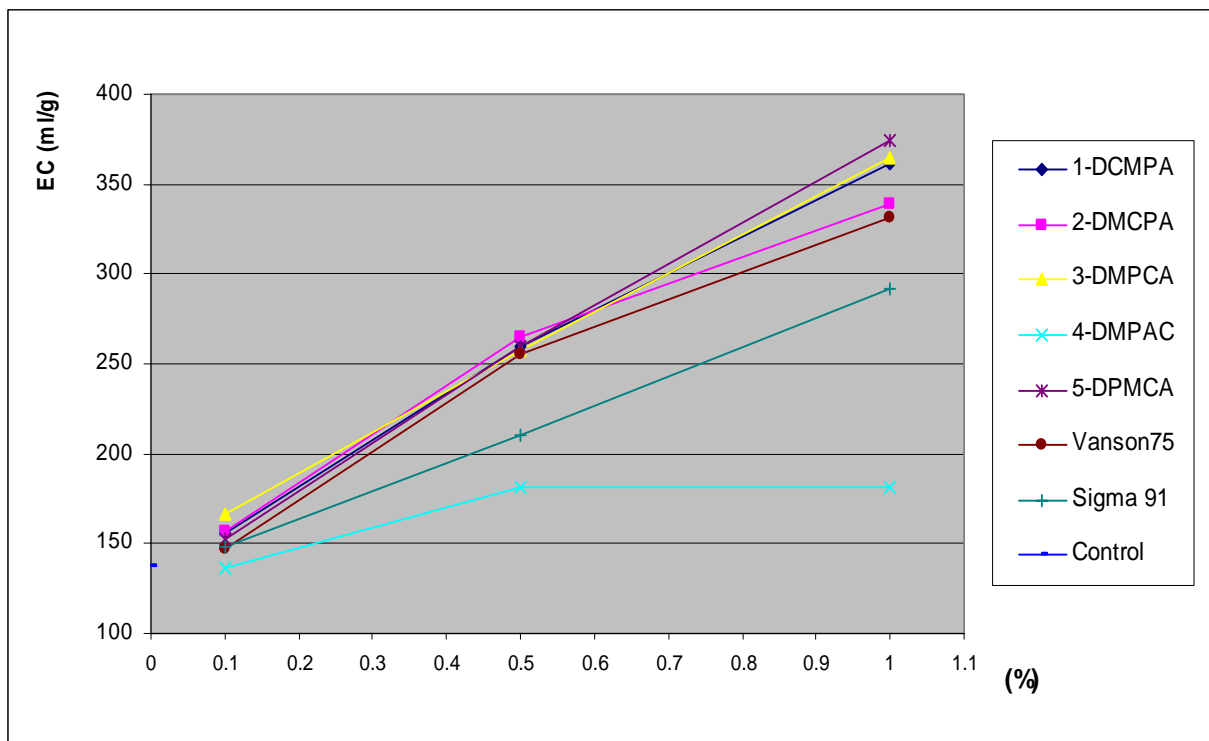


Figure 9. Emulsion Capacity Measurement at Various Concentrations of Crawfish and Commercial Chitosans

DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial chitosans (Vanson75 and Sigma91). Control= 9ml of 1% acetic acid + 38 ml of 1% protein solution without chitosan.

An increase in emulsifying capacity was more notable with 1.0% chitosan than with 0.1% or 0.5% chitosan (Figure 9). No significantly differences ( $p > 0.05$ ) in emulsion capacity among the modified crawfish chitosan samples were observed at each concentration, except DMPAC which showed very low values of 135.8 ml/g at 0.1%, 181.5 ml/g at 0.5%, and 181.0 ml/g at 1.0%.

At 0.1% concentration of chitosan, DMPCA showed the highest emulsion capacity of 166.4 ml/g, followed by DMCPA, DCMCPA, and DPMCPA with 156.9 ml/g, 155.7 ml/g, and 152.8 ml/g, respectively. At 0.5% concentration, DMCPA had EC of 265.5 ml/g, followed by DMCPA (259.7 ml/g) and DPMCPA (257.5 ml/g), but the latter two samples were not significantly different. At 1.0% chitosan concentration, DPMCPA demonstrated higher emulsion capacity of 374.2 ml/g, compared with that of DMPCA (364.3 ml/g). DMPCA, DMCPA and DCMCPA and the control DPMCPA demonstrated a better enhancer for emulsion capacity than the commercial crab chitosans. Vanson75 had 146.8 ml/g in 0.1%, 254.9 ml/g in 0.5%, and 331.9 ml/g in 1.0% chitosan solution. Sigma91 had 147.8 ml/g in 0.1%, 209.8 ml/g in 0.5%, and 291.6 ml/g in 1.0% chitosan solution. The control containing no chitosan had 137 ml/g of emulsion capacity compared with chitosan solutions that gave 166.3 ml/g in 0.1% of DMPCA, 265.5 ml/g in 0.5% of DMCPA, and 374.2 ml/g in 1.0% of DPMCPA (Figure 9).

#### **4.15 Emulsion Viscosity (EV)**

Emulsion viscosity (EV) of the soy protein containing modified crawfish chitosans was evaluated and compared with Mayonnaise (pH=4.4) (Kraft Mayo, Kraft North America, Inc. Glenview. IL) as the control sample (Figure 10).

Viscosity of mayonnaise (control) was 116,000 cP. This was relatively higher than the viscosity of emulsions prepared from soy protein and modified chitosan samples. The modified samples DMCPA showed high EV at pH 2.0 with 43,400 cP; at pH 4.0, DMPCA showed EV of 36,100 cP; at pH 6.0, DCMCPA showed EV of 4,300 cP; at pH 8.0, DMCPA had EV of 26,300 cP; and DMPCA had EV of 33,100 cP at pH 10.0.

The results shown at different pHs with the different chitosan modified samples indicated that almost all the modified processed chitosan exhibited high emulsion viscosity, except

DMPAC which showed very low EV (0.648 cP) at pH 4.0 (Figure 10). The decrease in EV of DPMCA may relate to low viscosity (1.0 cP) of chitosan itself at unadjusted pH.

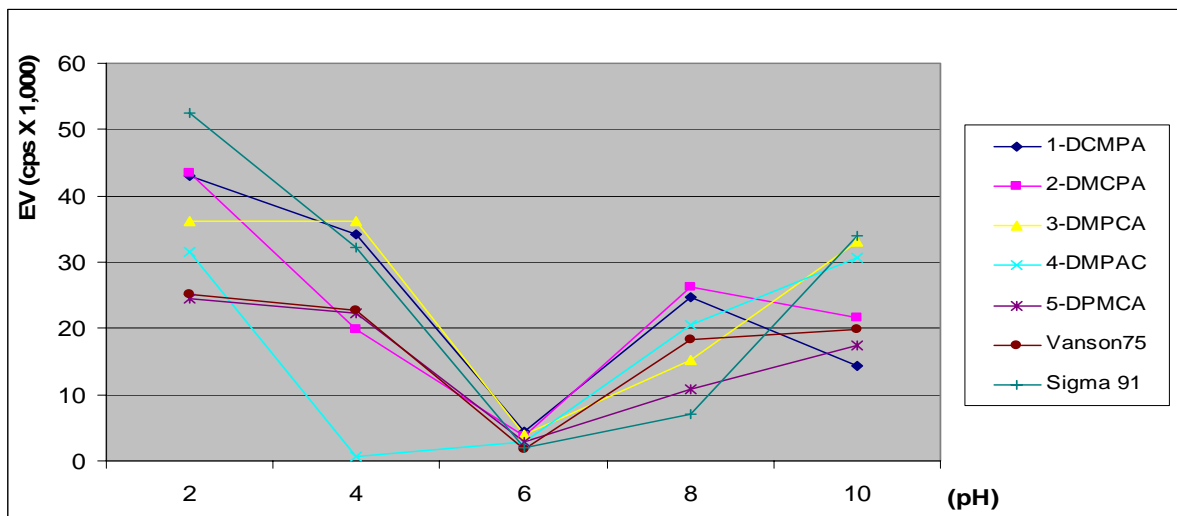


Figure 10. Emulsion Viscosity Measurement of Crawfish and Commercial Chitosans  
 DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial chitosans (Vanson75 and Sigma91).

Sigma91 showed high emulsion viscosity at pH 2.0 with 52,400 cP and at pH 10.0 with 34,000 cP. Vanson75 showed relatively lower emulsion viscosity than all the modified chitosan samples. At pH 10.0, DCMPA and DMCPA demonstrated decreased emulsion viscosity from 24,642 cP to 14,233 cP and 26,320 cP to 21,518 cP, respectively. This probably can be attributed to the denaturation of protein occurrence when pH was adjusted from 8.0 to 10.0.

## CHAPTER 5

### SUMMARY AND CONCLUSIONS

Throughout the literature on chitosan, the main emphasis is on its quality and physicochemical properties which vary widely with crustacean species and preparation methods. Upon this emphasis, this research study was attempted to prove or dispute these views by conducting similar studies and monitoring the modification of processing protocols of the chitosan production using crawfish shell waste, and to determine whether such modifications had any effect on the various physicochemical and functional properties of chitosans. From our results, we found that specific physicochemical and functional properties of chitosan have affected by process protocol alteration/modification.

Change/modification of decoloration (DC) step among four steps for the production of crawfish chitosan affected the physiochemical and functional properties. DCMPA and DMCPA resulted in an increase in molecular weight and ash, respectively. In contrast, DMPCA yielded chitosan with low-viscosity. The most notable change observed with DMPAC was a light brown degraded colored chitosan that exhibited properties of a weak polyelectrolyte. When the process of deacetylation changed its order from the standard method, significant degradation of the chitosan structure occurred, because the process was a very harsh treatment with concentrated sodium hydroxide (40-50%) usually at 100°C or higher for 30 min. Similarly, when chitosan process started with Deacetylation (DA), the sudden formation of gel, an unknown white polymer with very poor yield was obtained and the process considered unsuccessful. Thus, it is suggested not to conduct this process step for chitosan production.

In a similar manner, when demineralization (DM) and deproteinization (DP) were reversed during production, the results did not show much difference except for the low

viscosity, high fat binding capacity, and high emulsion viscosity of DMPCA over DPMCA. Our studies agreed with many other research studies that have shown that the sequence of demineralization and deproteinization steps can be reversed to give improved functional property. In fact many authors have followed the procedure of acidic decalcification after removal of protein.

Proximate analysis values for moisture, ash and nitrogen were much lower for all samples and control, compared to commercial chitosans. Similarly, functional properties (e.g., WBC, FBC, emulsion capacity, and emulsion viscosity) were much improved by process modification (DMPCA) over traditional process methods (DPMCA).

Overall, the results indicated that process modification in crawfish chitosan production yielded some differences on each characteristic over the control and commercial products. However, it will be very ambiguous to conclude that only one modified process is the optimum for the production of chitosan because the interests of applications may vary from one study to another and even from one industry to another, and as seen in our study. In view of the foregoing, it is our recommendation that for the purpose of achieving uniformity and proper product quality control for particular usage of chitosan, the relationship between the process protocols/conditions and the resulting specific characteristics of chitosan products must be monitored constantly and properly.

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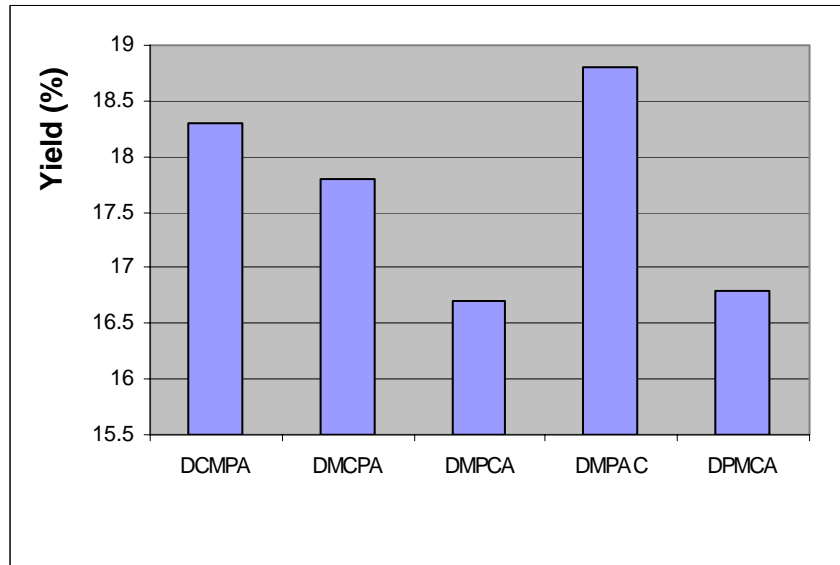
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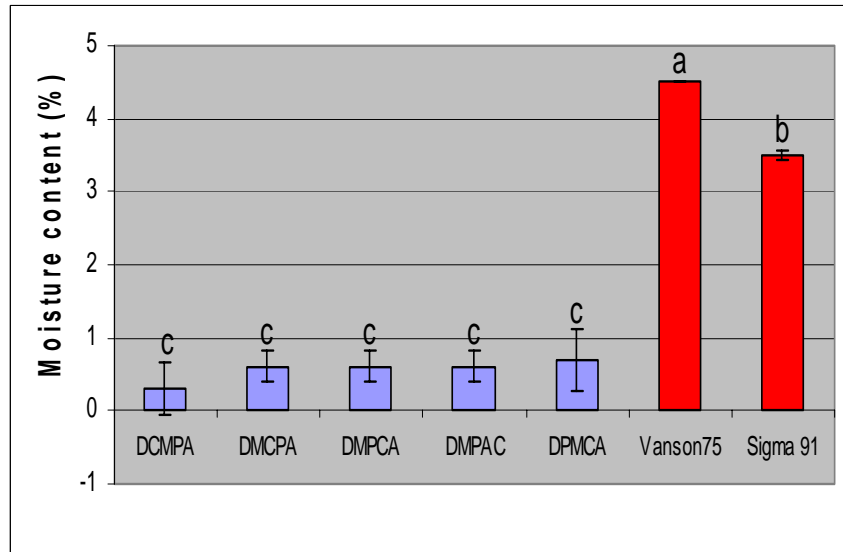
**APPENDIX A. DATA OF PHYSICOCHEMICAL ANALYSIS (TABLE OR FIGURE)**

APPENDIX 1. Crawfish Chitosan Production Yield



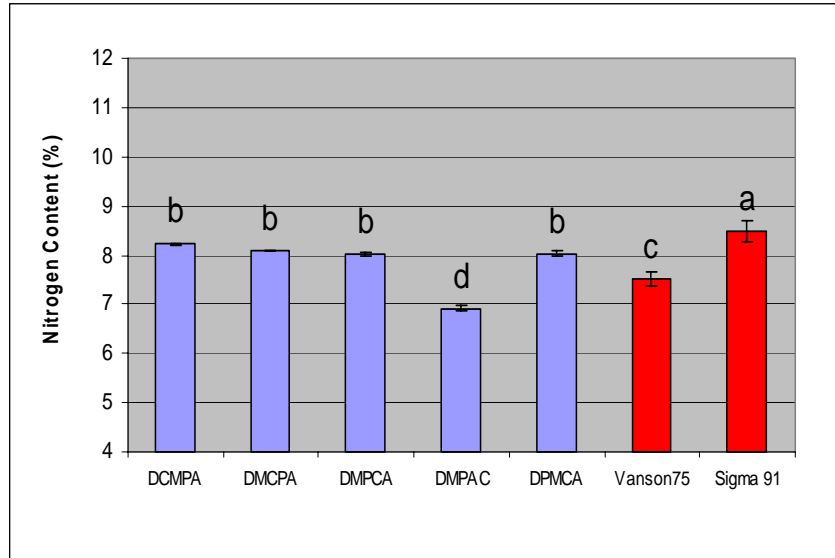
DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated.

APPENDIX 2. Moisture Content Measurement of Crawfish and Commercial Chitosans



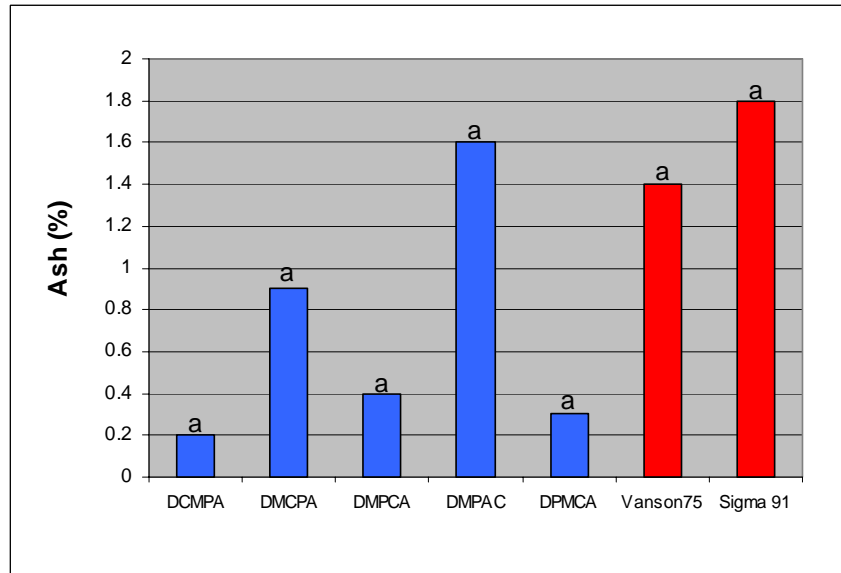
DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

APPENDIX 3. Nitrogen Content measurement of Crawfish and Commercial Chitosans



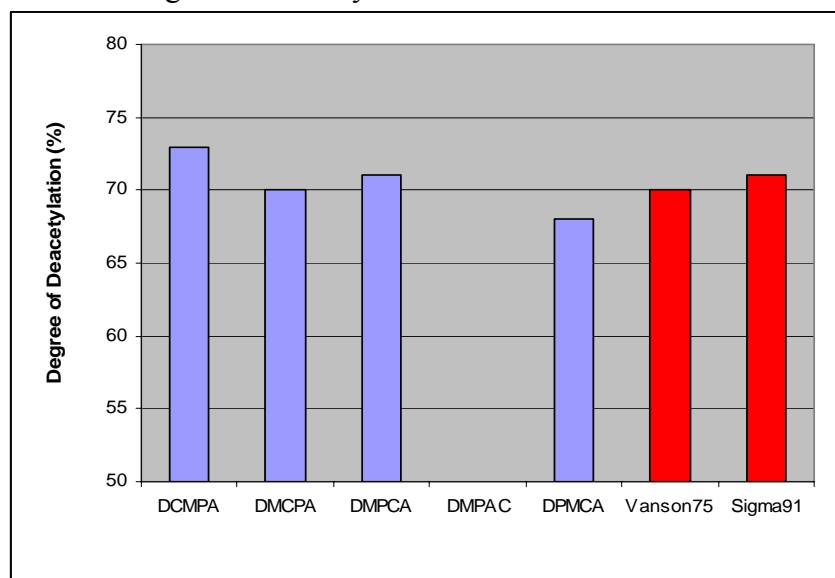
DCMCPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

APPENDIX 4. Ash Content measurement of Crawfish and Commercial Chitosans



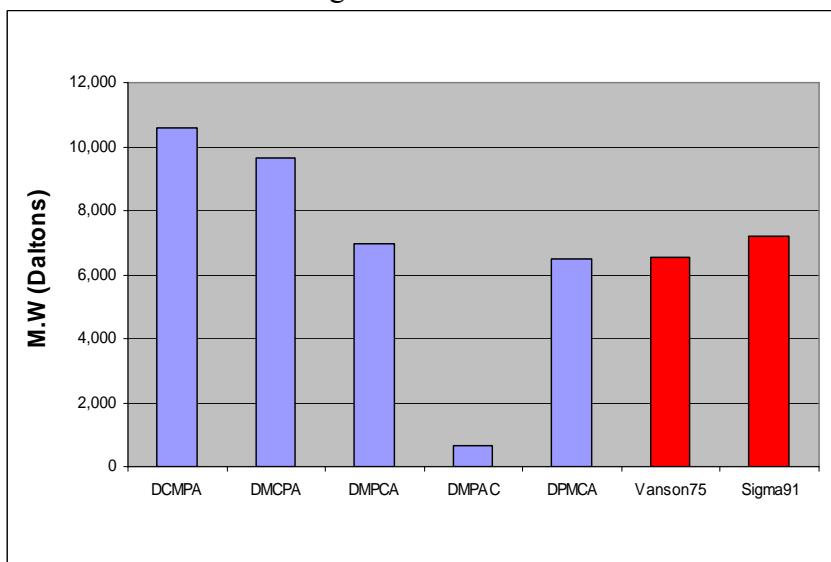
DCMCPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

## APPENDIX 5. Degree of Deacetylation of Crawfish and Commercial Chitosans



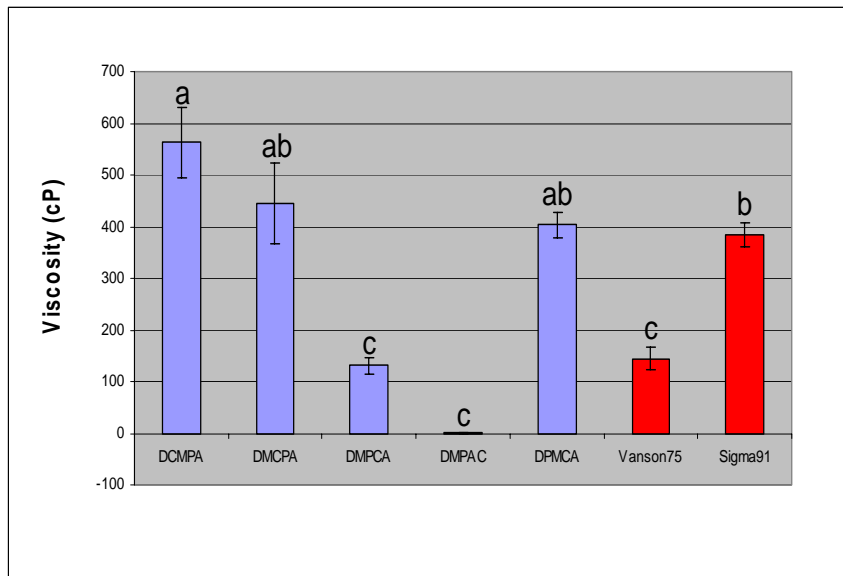
DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated DMPAC= demineralized, deproteinized, deacetylated decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

## APPENDIX 6. Molecular Weight of Crawfish and Commercial Chitosans



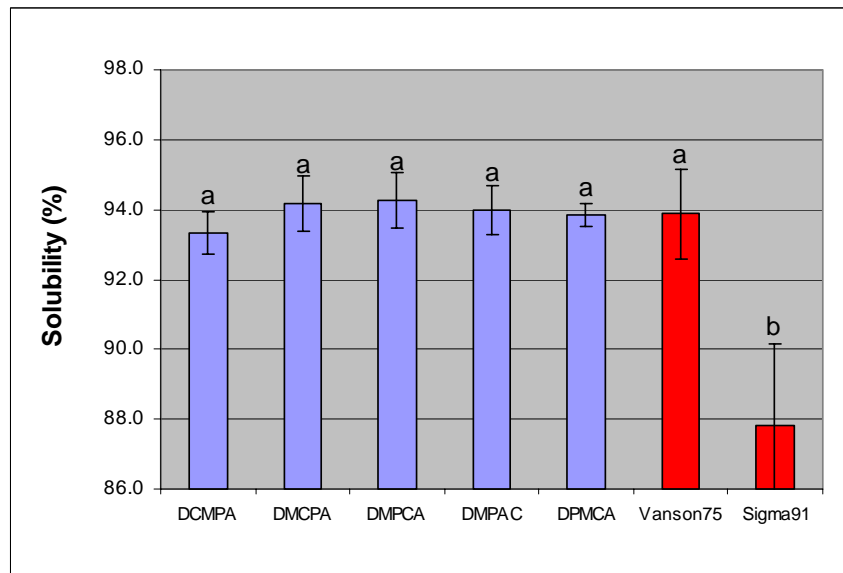
DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

## APPENDIX 7. Viscosity Measurement of Crawfish and Commercial Chitosans



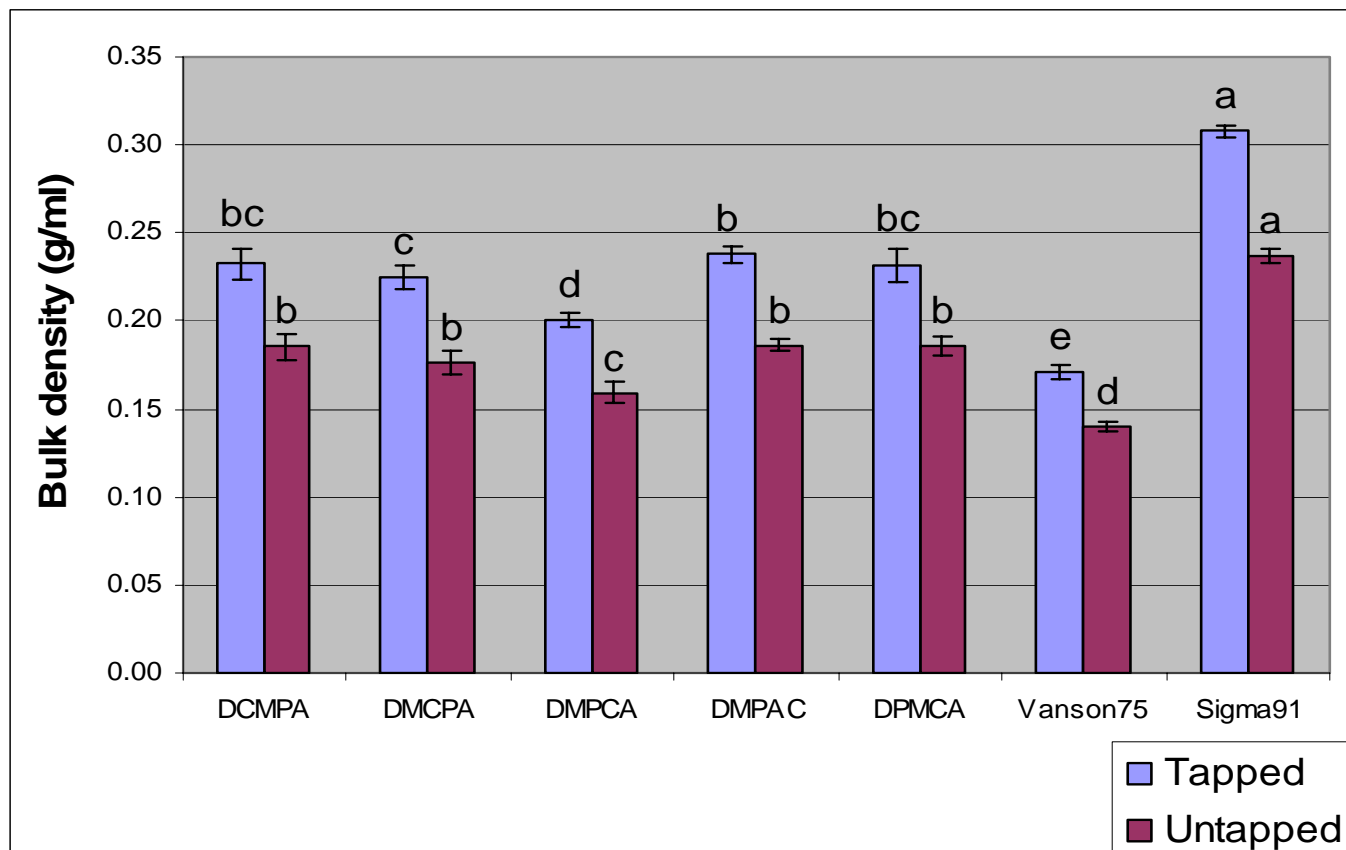
DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

## APPENDIX 8. Solubility Measurement of Crawfish and Commercial Chitosans



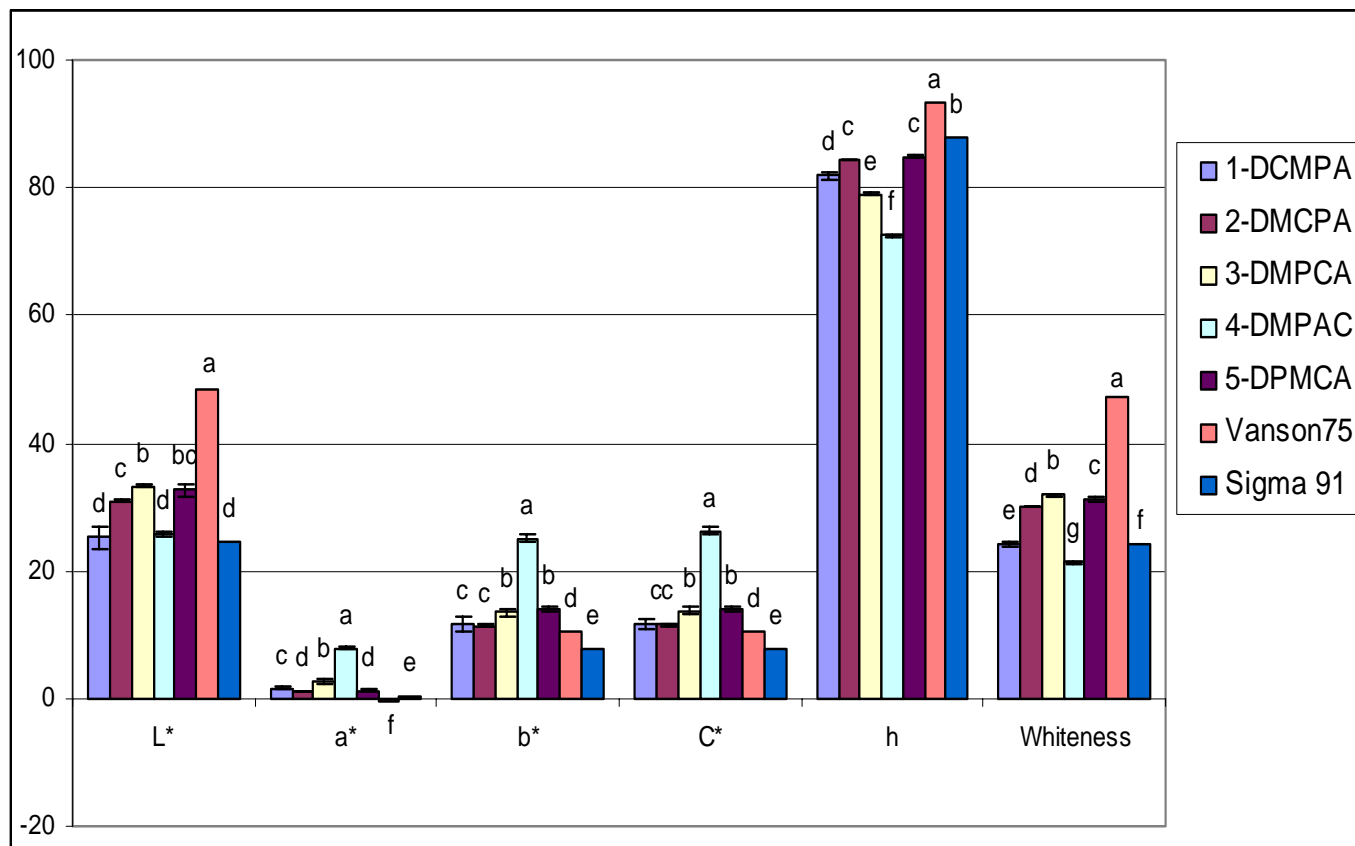
DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

APPENDIX 9. Bulk Density Measurement of Crawfish and Commercial Samples



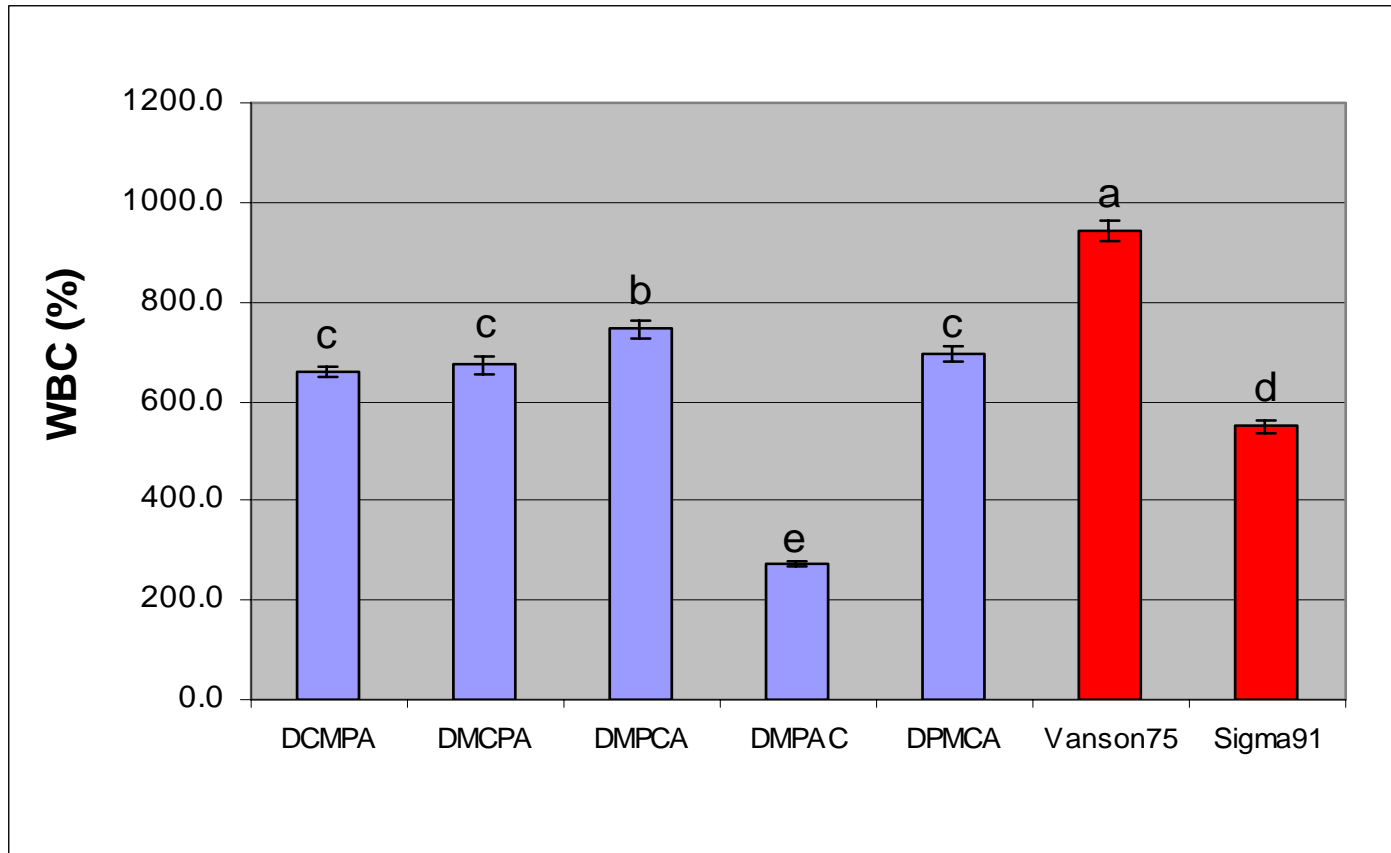
DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

APPENDIX 10. Color Characteristics of Crawfish and Commercial Chitosans



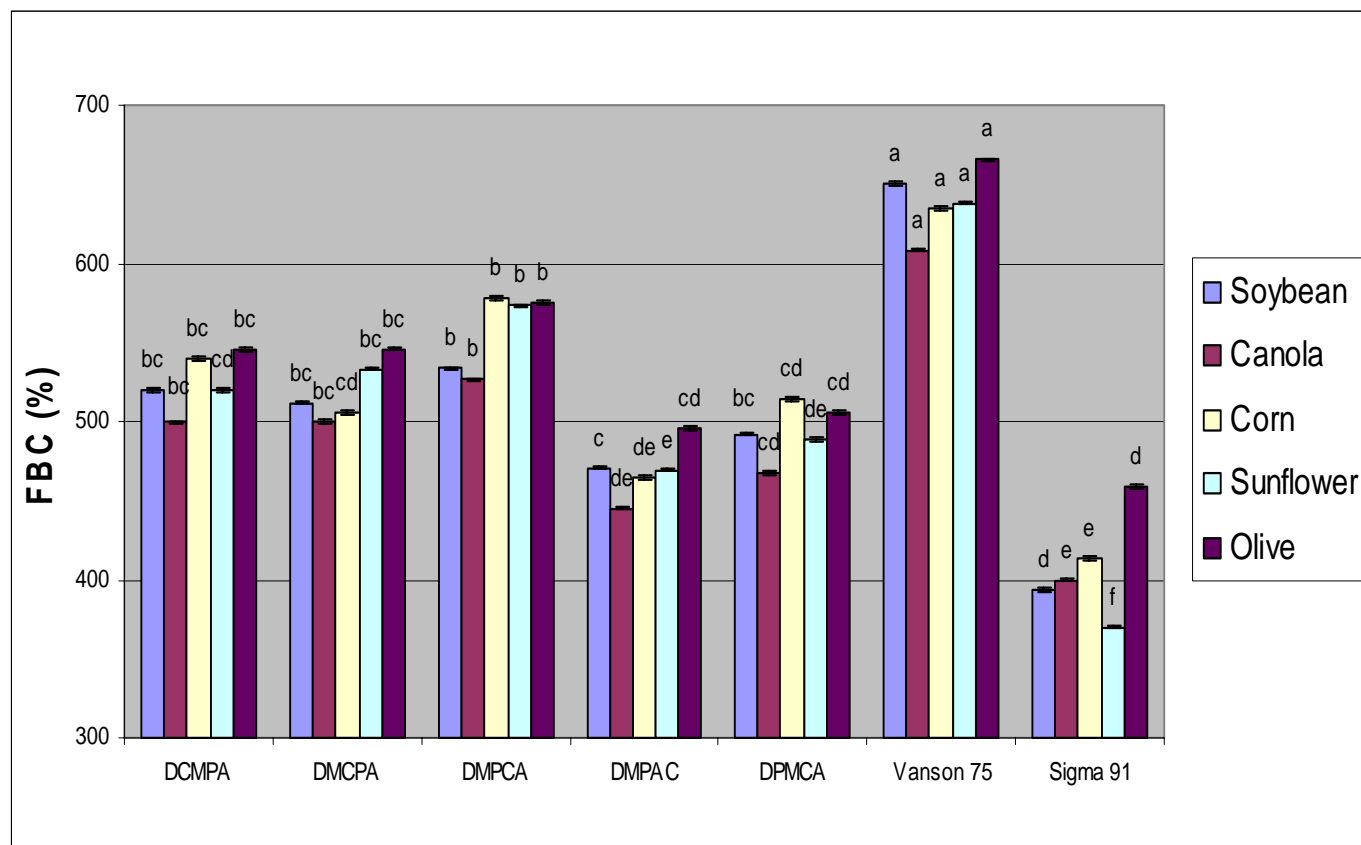
(L\*=Brightness, C\*=Intensity of Color, h=Hue angle,  $\Delta E^*$ =Difference of color, a\*=redness, b\*=yellowness) DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

APPENDIX 11. Water Binding Capacity of Crawfish and Commercial Chitosans



DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

APPENDIX 12. Fat Binding Capacity of Crawfish and Commercial Chitosans



DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

APPENDIX 13. Emulsion Capacity Measurement (ml/g) of Crawfish and Commercial Chitosans with pH Variations

Sample	pH				
	2	4	6	8	10
DCMPA	395.3 (5.37) a	247.6 (2.47) a	102.4 (7.85) d	348.7 (5.16) a	334.8 (0) cd
DMCPA	395.8 (25.38) a	230.7 (37.19) a	118.7 (11.60) cd	322.0 (34.29) a	328.3 (5.16) d
DMPCA	387.4 (15.70) a	253.5 (0.42) a	126.3 (7.07) bcd	315.2 (5.37) a	362.4 (2.69) bc
DMPAC	343.2 (15.49) abc	148.3 (4.17) b	171.7 (18.17) ab	358.5 (10.32) a	363.8 (7.42) b
DPMCA	337.8 (2.47) abc	224.3 (18.17) a	113.8 (1.20) d	298.7 (9.33) a	317.3 (13.22) de
Vanson 75	371.4 (23.55) ab	258.9 (19.66) a	136.3 (22.27) bcd	329.7 (15.49) a	335.8 (2.69) cd
Sigma 91	385.1 (9.05) a	245.9 (2.12) a	100.3 (16.55) d	252.3 (48.37) a	396.8 (0.85) a
Control 1	294 (22.06) c	96.1 (8.06) b	203.4 (14.64) a	270.9 (20.51) a	384.7 (13.08) ab
Control 2	315.6 (13.44) bc	119.0 (17.82) b	168.6 (5.16) abc	322.3 (57.49) a	290.1 (0.78) e
Control 3	0	0	0	0	0

Numbers in parentheses are standard deviations, Means with different letters are significantly different ( $p < 0.05$ ), DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91). Control 1=38 ml soy protein solution (1%) only; Control 2= 9ml Acetic acid (1%) + 38 ml soy Protein solution (1%); Control 3=9 ml Chitosan solution (0.5%) only.

APPENDIX 14. Emulsion Capacity Measurement (ml/g) of Crawfish and Commercial Chitosans with Different Concentrations of Chitosans

Sample	Concentration			
	0 %	0.1%	0.5%	1.0%
DCMPA		155.7 (0.99) ab	259.7 (13.65) a	361.4 (8.63) a
DMCPA		156.9 (2.69) ab	265.5 (13.65) a	339.0 (2.26) a
DMPCA		166.3 (5.59) a	257.5 (18.46) a	364.3 (6.65) a
DMPAC		135.8 ( 3.11) c	181.5 (0.21) b	181.0 (4.95) c
DPMCA		152.8 (1.06) ab	259.7 (1.20) a	374.2 (22.13) a
Vanson 75		146.8 (6.22) bc	254.9 (0.64) a	331.9 (2.05) ab
Sigma 91		147.8 (5.94) bc	209.8 (3.11) b	291.6 (14.07) b
Control	137.1			

Numbers in parentheses are standard deviations, Means with different letters are significantly different ( $p < 0.05$ ), DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated, Commercial crab chitosans (Vanson75 and Sigma91). Control = 9ml Acetic Acid (1%) + 38ml soy protein solution (1%) without chitosan solution; pH in general were ranged between 3.8-4.0

APPENDIX 15. Emulsion Viscosity Measurement (cP) of Crawfish and Commercial Chitosans with pH Variations

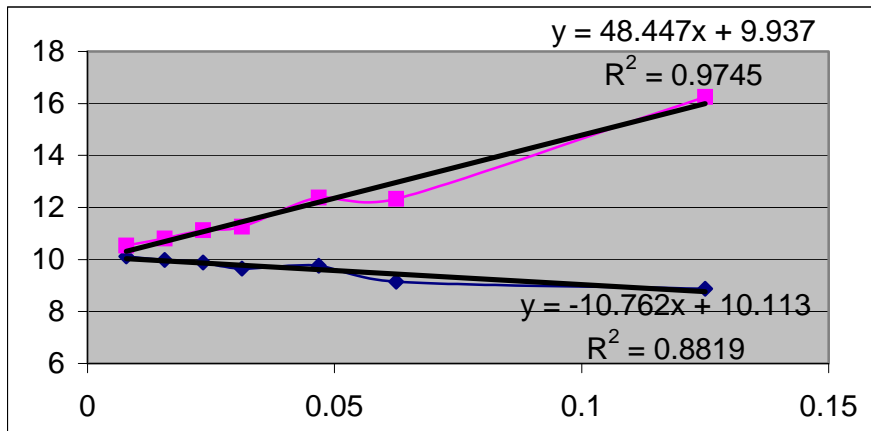
Sample	pH				
	2	4	6	8	10
DCMPA	42.9 (12.86) a	34.1 (0.87) ab	4.3 (0.35) a	24.6 (4.62) ab	14.2 (0.53) d
DMCPA	43.4 (7.91) a	19.7 (8.27) c	3.8 (0.63) abc	26.3 (4.83) a	21.5 (5.27) bcd
DMPCA	36.1 (0.43) a	36.1 (2.76) a	4.0 (0.12) ab	15.2 (0.25) bcd	33.1 (1.00) ab
DMPAC	31.6 (3.28) a	0.6 (0.04) d	2.8 (0.09) abc	20.5 (0.31) abc	30.6 (2.79) abc
DPMCA	24.4 (1.62) a	22.3 (0.53) bc	2.9 (1.12) abc	10.7 (2.34) cd	17.3 (2.00) d
Vanson 75	25.2 (0.82) a	22.8 (1.24) abc	1.7 (0.42) c	18.3 (0.62) abc	19.9 (2.46) cd
Sigma 91	52.4 (16.03) a	32.1 (2.34) abc	1.9 (0.04) bc	7.0 ( 0.04) d	34.0 (4.64) a

Actual mean values are the results from original value divided by 1000. Numbers in parentheses in each column are standard deviations. Means with different letters are significantly different ( $p < 0.05$ ). DCMPA=decolorized, demineralized, deproteinized, deacetylated; DMCPA= demineralized, decolorized, deproteinized, deacetylated; DMPCA= demineralized, deproteinized, decolorized, deacetylated; DMPAC= demineralized, deproteinized, deacetylated, decolorized; and DPMCA= deproteinized, demineralized, decolorized, deacetylated. Commercial crab chitosans (Vanson75 and Sigma91).

## APPENDIX B. DATA OF MOLECULAR WEIGHT CALCULATION

### APPENDIX 16. DCMPA

% or g/dL	$\eta_{inh}$	$\eta_{red}$
0.007813	10.12148	10.53244
0.015625	9.982784	10.80346
0.0234	9.890371	11.1285
0.03125	9.650955	11.26432
0.0468	9.764404	12.37795
0.0625	9.144637	12.33587
0.125	8.872194	16.25119



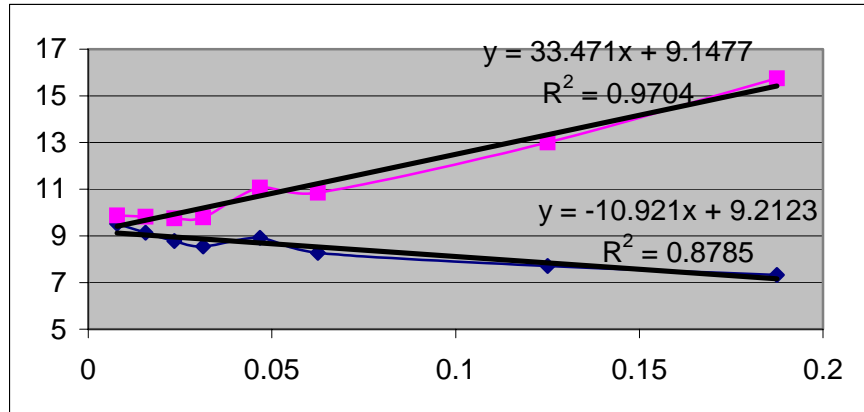
$$(9.937 + 10.113) / 2 = \eta = 10.025$$

$$10.025 = 1.81 \times 10^{-3} M^{0.93}$$

$$M = 10,596.62 \text{ g/mol} = 10,596.62 \text{ Daltons}$$

### APPENDIX 17. DMCPA

% or g/dL	$\eta_{inh}$	$\eta_{red}$
0.007813	9.51824	9.881096
0.015625	9.141293	9.82637
0.0234	8.782156	9.749675
0.03125	8.54813	9.798675
0.0468	8.921737	11.07305
0.0625	8.278867	10.84336
0.125	7.718437	12.99419
0.1875	7.329885	15.74677



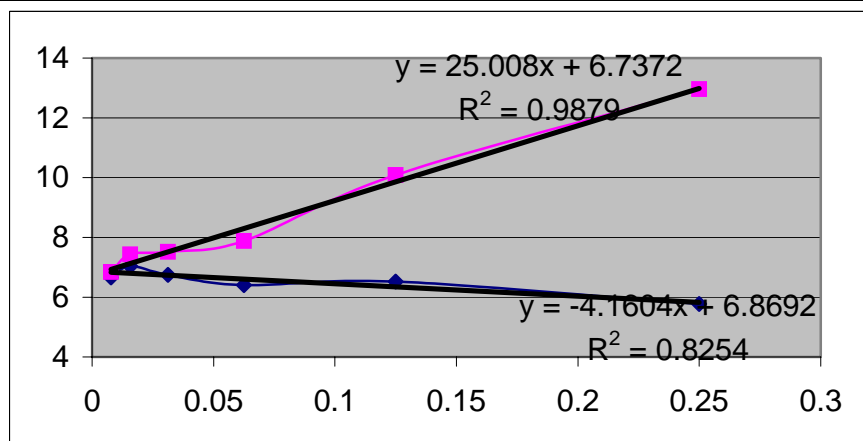
$$(9.1477+9.2123) / 2 = \eta = 9.18$$

$$9.18 = 1.81 \times 10^{-3} M^{0.93}$$

$$M = 9,639.34 \text{ g/mol} = 9,639.34 \text{ Daltons}$$

#### APPENDIX 18. DMPCA

% or g/dL	$\eta_{inh}$	$\eta_{red}$
0.007813	6.6632	6.83968
0.015625	7.034549	7.435725
0.03125	6.753286	7.518778
0.0625	6.410178	7.884384
0.125	6.527239	10.08977
0.25	5.779069	12.96346



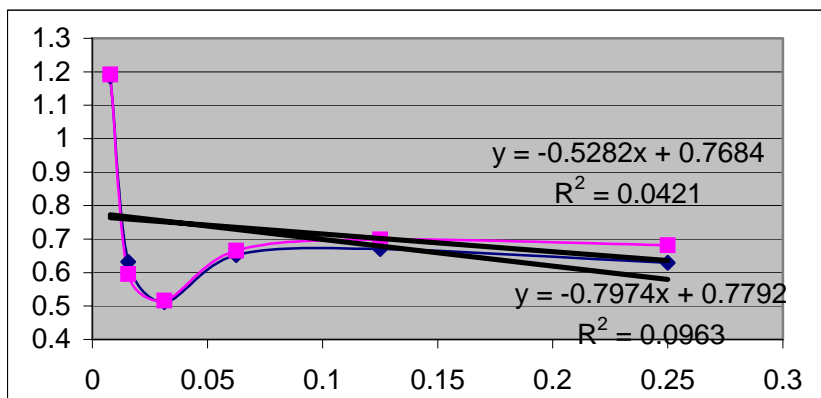
$$(6.7372+ 6.8692) / 2 = \eta = 6.8032$$

$$6.8032 = 1.81 \times 10^{-3} M^{0.93}$$

$$M = 6,984.29 \text{ g/mol} = 6,984.29 \text{ Daltons}$$

APPENDIX 19. DMPAC

% or g/dL	$\eta_{inh}$	$\eta_{red}$
0.007813	1.186535	1.192051
0.015625	0.632467	0.596026
0.03125	0.512104	0.516224
0.0625	0.651775	0.665242
0.125	0.670162	0.699032
0.25	0.629666	0.681933



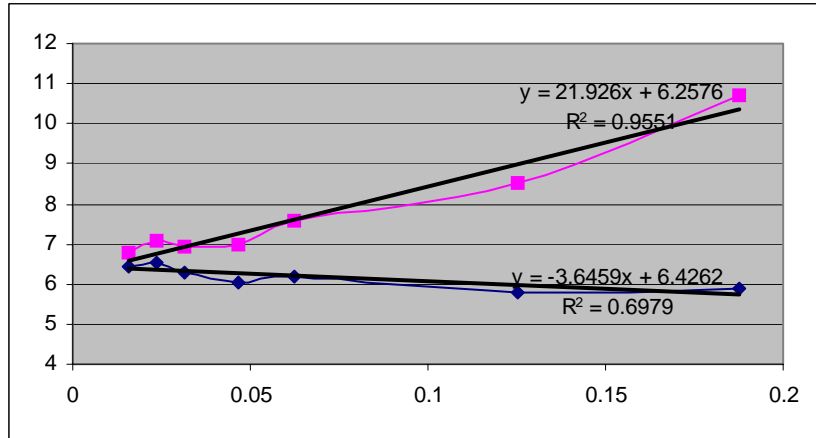
$$(0.7684 + 0.7792) / 2 = \eta = 0.7738$$

$$0.7738 = 1.81 \times 10^{-3} M^{0.93}$$

$$M = 674.49 \text{ g/mol} = 674.49 \text{ Daltons}$$

APPENDIX 20. DPMCA

% or g/dL	$\eta_{inh}$	$\eta_{red}$
0.015625	6.448283	6.78432
0.0234	6.540846	7.065811
0.03125	6.265636	6.92112
0.0468	6.03831	6.977885
0.0625	6.209283	7.586368
0.125	5.812025	8.542697
0.1875	5.875075	10.71417



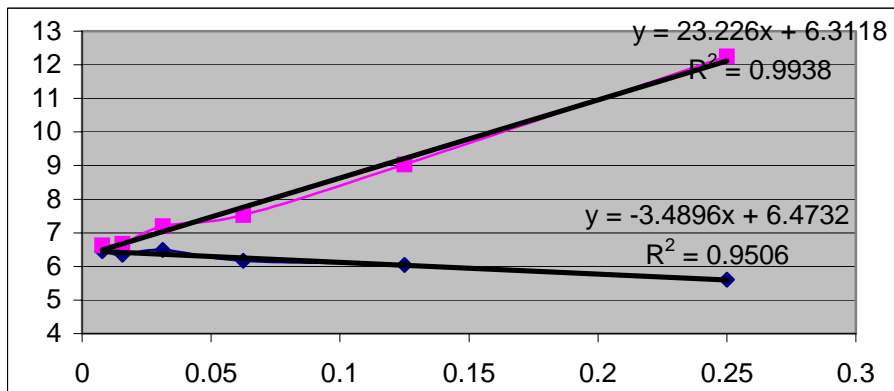
$$(6.2576 + 6.4262) / 2 = \eta = 6.3419$$

$$6.3419 = 1.81 \times 10^{-3} M^{0.93}$$

$$M = 6,476.40 \text{ g/mol} = 6,476.40 \text{ Daltons}$$

APPENDIX 21. Vanson 75

% or g/dL	$\eta_{inh}$	$\eta_{red}$
0.007813	6.459027	6.62473
0.015625	6.351052	6.676845
0.03125	6.488464	7.193078
0.0625	6.172229	7.531806
0.125	6.044866	9.031248
0.25	5.605785	12.24427



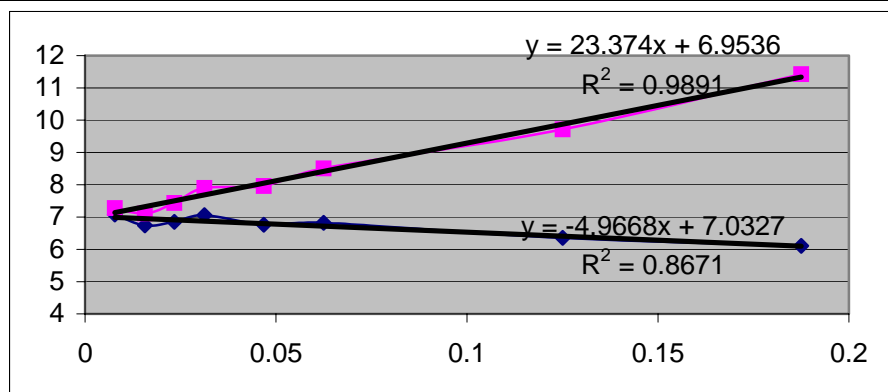
$$(6.3118 + 6.4732) / 2 = \eta = 6.3925$$

$$6.3925 = 1.81 \times 10^{-3} M^{0.93}$$

$$M = 6,531.99 \text{ g/mol} = 6,531.99 \text{ Daltons}$$

APPENDIX 22. Sigma 91

% or g/dL	$\eta_{inh}$	$\eta_{red}$
0.007813	7.076382	7.275656
0.015625	6.742087	7.110016
0.0234	6.85152	7.431325
0.03125	7.059056	7.898208
0.0468	6.763595	7.956538
0.0625	6.823169	8.508912
0.125	6.357467	9.70992
0.1875	6.105286	11.42202



$$(6.9536 + 7.0327) / 2 = \eta = 6.99315$$

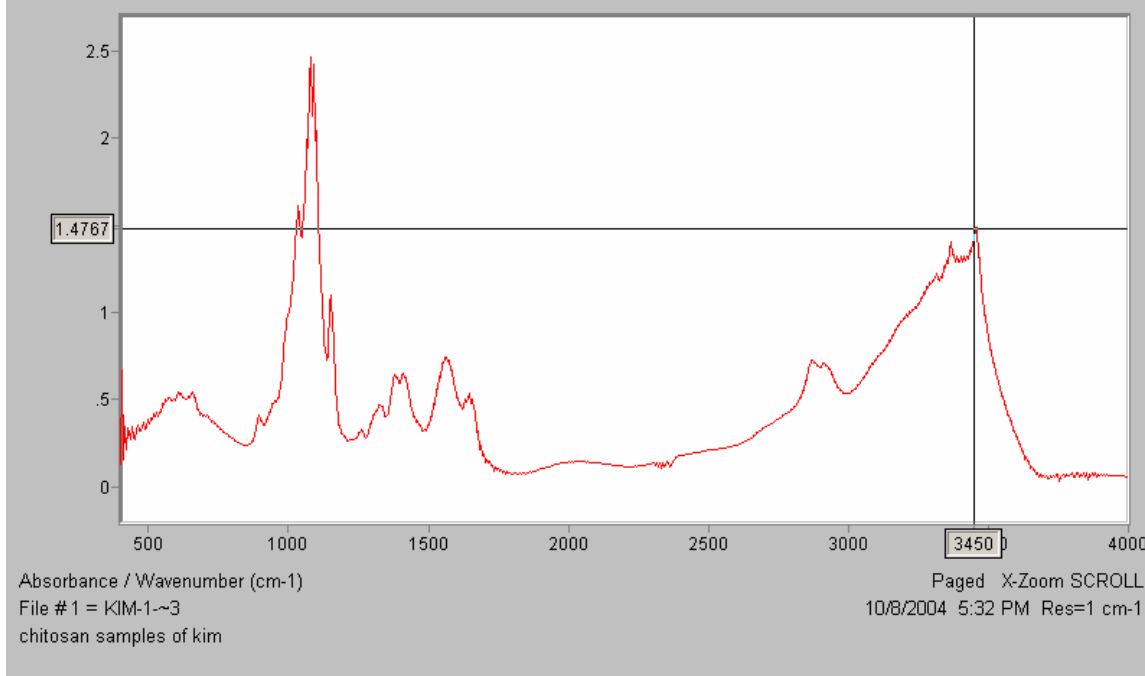
$$6.99315 = 1.81 \times 10^{-3} M^{0.93}$$

$$M = 7,194.21 \text{ g/mol} = 7,194 \text{ Daltons}$$

## APPENDIX C. DATA OF DEGREE OF DEACETYLATION

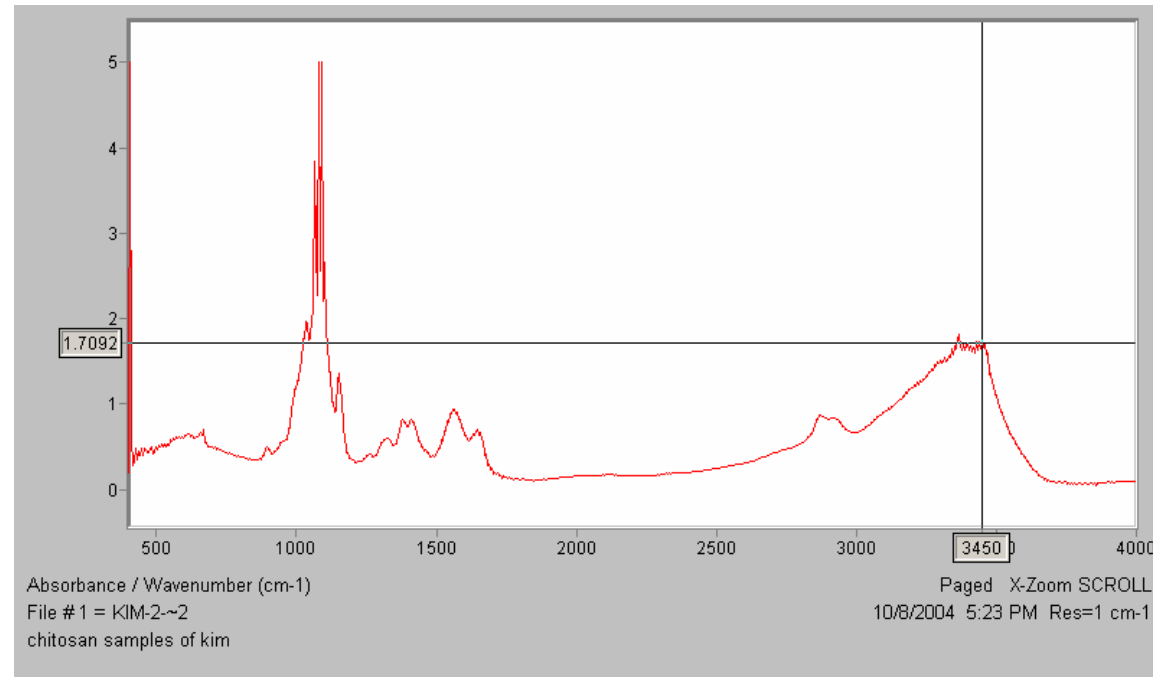
$DD = 100 - [(A_{1655} / A_{3450}) \times 100 / 1.33]$ ..... (by Domszy and Roberts (1985))

### APPENDIX 23. DCMPA



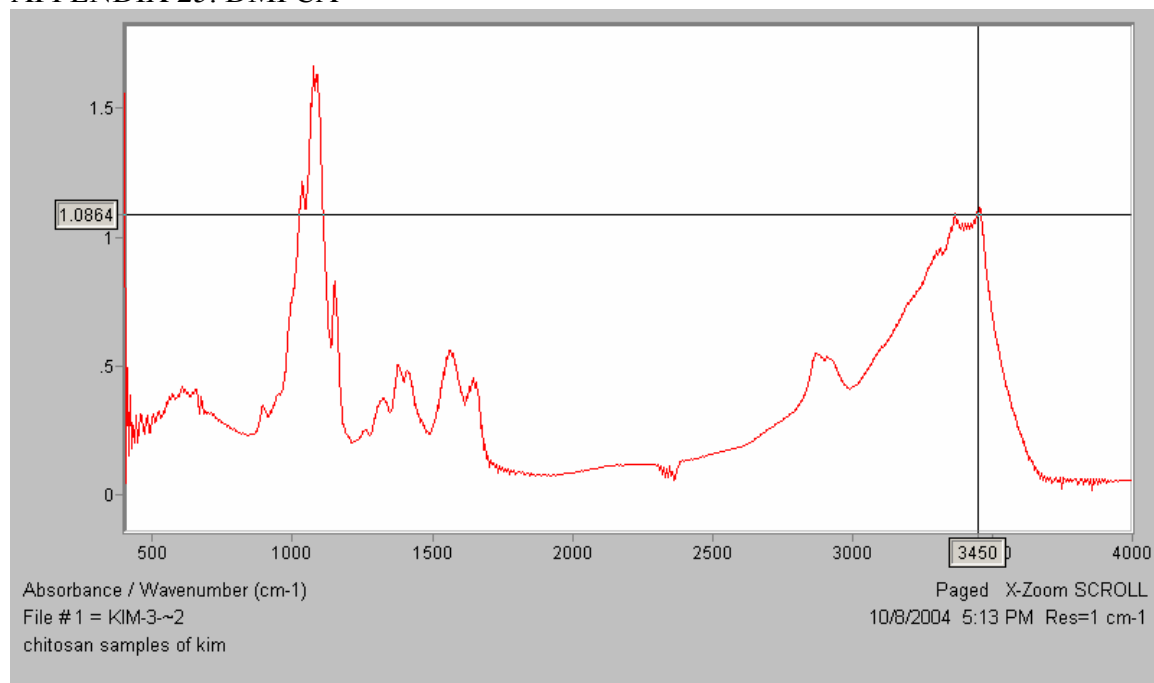
$A_{1655} = 0.525$ ,  $A_{3450} = 1.477$ , and  $DD = 73.3\%$

### APPENDIX 24. DMCPA



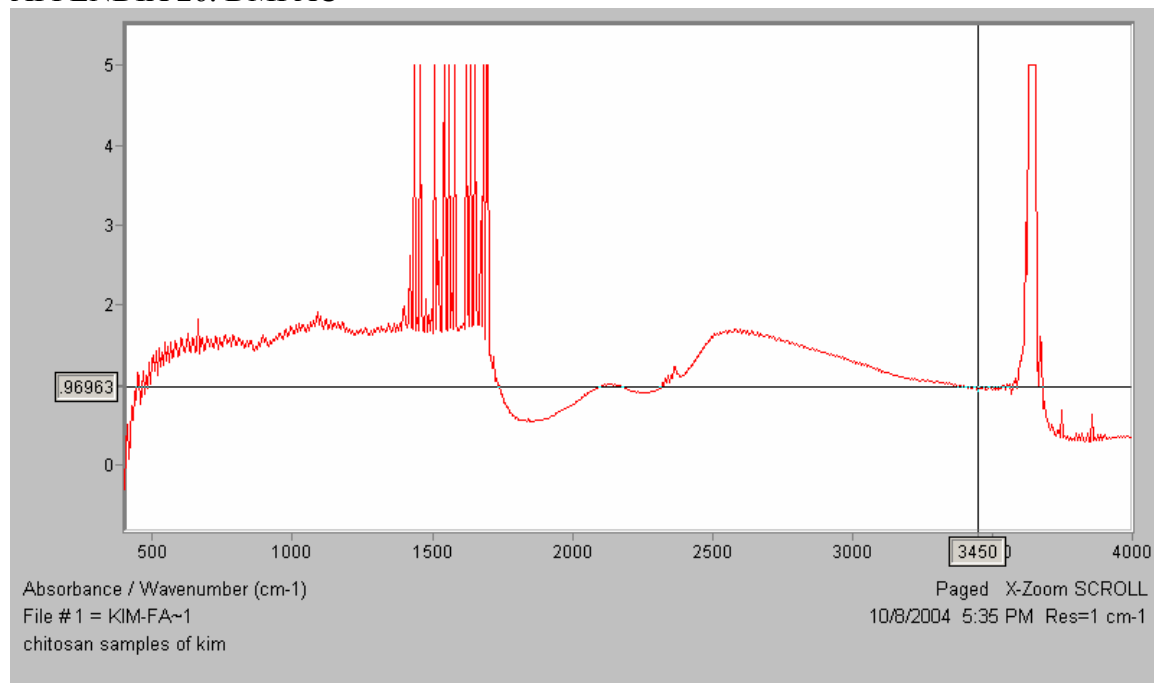
$A_{1655} = 0.679$ ,  $A_{3450} = 1.709$ , and  $DD = 70.1\%$

## APPENDIX 25. DMPCA



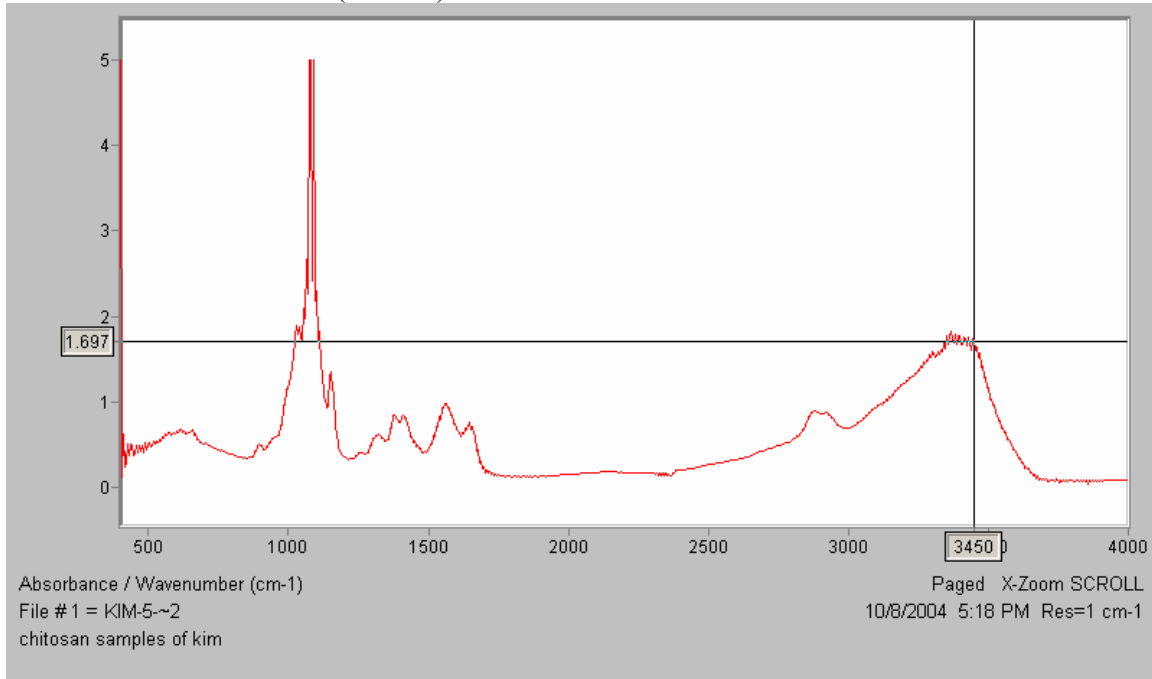
$A_{1655} = 0.419$ ,  $A_{3450} = 1.086$ , and  $DD = 71.0\%$

## APPENDIX 26. DMPAC



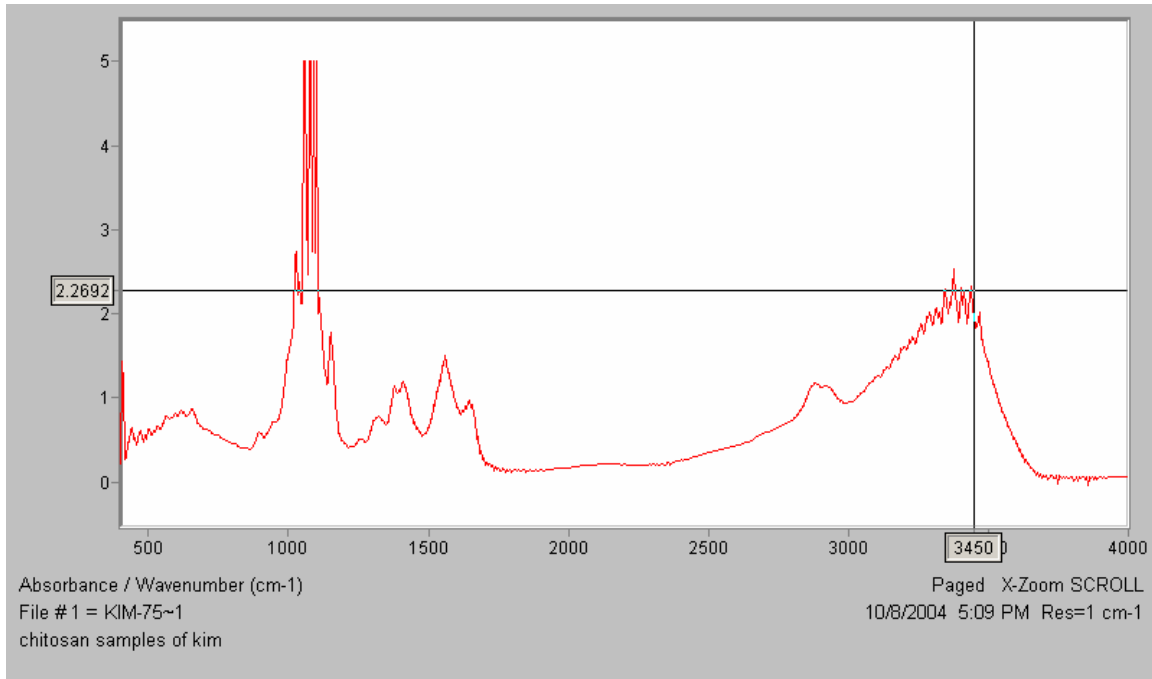
$A_{1655} = X$ ,  $A_{3450} = X$ , and  $DD = 0\%$

### APPENDIX 27. DPMCA(control)



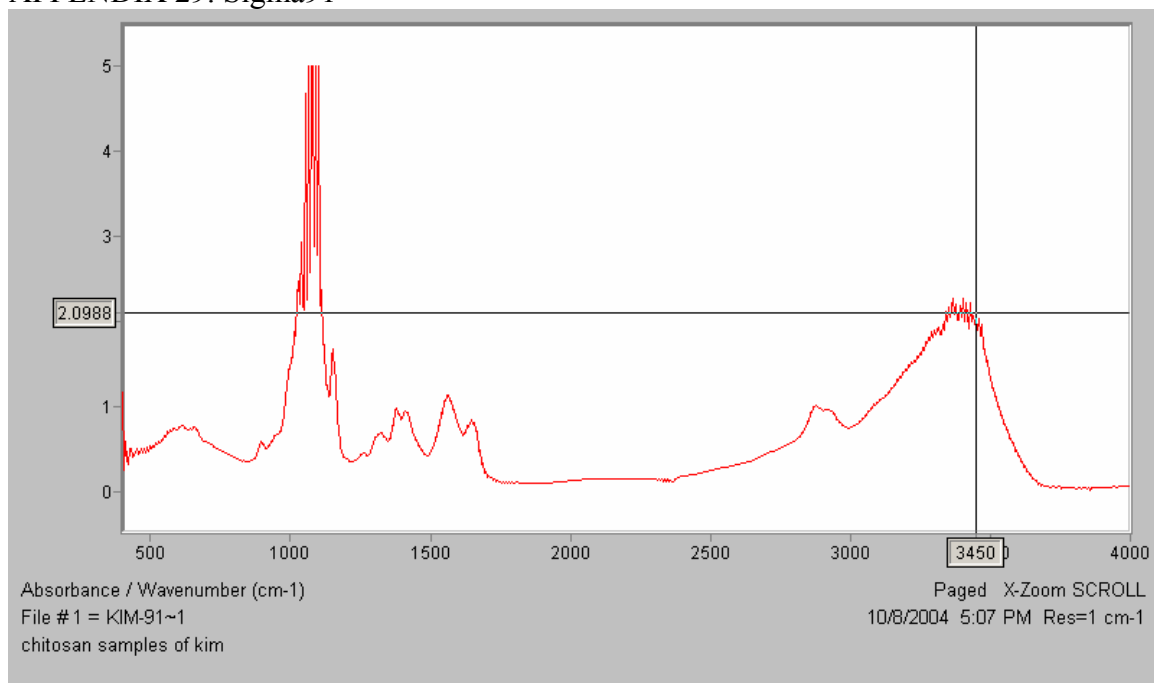
$A_{1655} = 0.731$ ,  $A_{3450} = 1.697$ , and  $DD = 67.6\%$

### APPENDIX 28. Vanson75



$A_{1655} = 0.911$ ,  $A_{3450} = 2.269$ , and  $DD = 69.8\%$

## APPENDIX 29. Sigma91



$A_{1655} = 0.808$ ,  $A_{3450} = 2.098$ , and  $DD = 71.1\%$

**APPENDIX D. DATA OF AMOUNTS OF OIL CONSUMED FOR EMULSIFICATION**

APPENDIX 30. Amount of Oil Consumed for Emulsion Capacity (EC) with pH Variations.

[9 ml chitosan (0.5%) + 38 ml protein solution (1%)] / with Soybean Oil (ml)

	pH 2	pH 4	pH 6	pH 8	pH 10
DCMPA	135.2	84.7	35.0	119.3	114.5
DMCPA	135.4	78.9	40.6	110.1	112.3
DMPCA	132.5	86.7	43.2	107.8	124.0
DMPAC	117.4	50.7	58.7	122.6	124.4
DPMCA	115.3	76.7	39.0	102.0	108.5
Sigma91	131.7	84.1	34.3	86.3	135.7
Vanson75	127.0	88.5	46.6	112.8	114.9
Control 1 (Protein solution only (1%))	100.6	32.9	69.6	92.7	131.6
Control 2 (Acetic acid (1%)+ Protein solution (1%))	108.0	40.7	57.7	110.2	99.2
Control 3 (Chitosan solution only (0.5%))	0	0	0	0	0

APPENDIX 31. Amount of Oil Consumed for Emulsion Capacity (EC) with Different Concentrations of Chitosan Solutions, w/o pH Adjustment.

[9ml chitosan (0.1%, 0.5%, 1.0%) + 38 ml protein solution (1%)---with Soybean Oil (ml)]

Chitosan Concentrations	0.1%			0.5%			1%		
	a	b	mean	a	b	mean	a	b	mean
DCMPA	53	53.5	53.3	92.1	85.5	88.8	125.7	121.5	123.6
DMCPA	53	54.3	53.7	87.5	94.1	90.8	115.4	116.5	116.0

DMPCA	58.2	55.5	56.9	83.6	92.5	88.1	126.2	123.0	124.6
DMPAC	47.2	45.7	46.5	62.1	62.0	62.1	63.1	60.7	61.9
DPMCA	52.5	52	52.3	88.5	89.1	88.8	122.6	133.3	128.0
Sigma 91	49.1	52	50.6	71	72.5	71.8	96.3	103.1	99.7
Vanson 75	48.7	51.7	50.2	87.3	87.0	87.2	113.0	114.0	113.5

\*Control (containing 9 ml Acetic Acid (1%) + 38 ml Protein Solution (1%) without chitosan solution) = 46.9. The pH of all these solutions oscillated approximately from 3.8 to 4.1.

APPENDIX 32. Amount of Oil Consumed for Emulsion Viscosity (EV) Measurement with Chitosan. These values were obtained from calculating 80% of EC (Emulsion Capacity)

[9 ml chitosan (0.5%) + 38 ml protein solution (1%)] / with Soybean Oil (ml)

	pH 2	pH 4	pH 6	pH 8	pH 10
DCMPA	108.2	67.7	28.0	95.4	91.6
DMCPA	108.3	63.2	32.5	88.1	89.8
DMPCA	106.0	69.4	34.6	86.2	99.2
DMPAC	93.9	40.6	47.0	98.1	99.5
DPMCA	92.2	61.3	31.2	81.6	86.8
Sigma91	105.4	67.3	27.4	69.0	108.6
Vanson75	101.6	70.8	37.3	90.2	91.9

## VITA

The author was born in Taegu, South Korea, on January 7, 1961. At the age of 14, her family immigrated to Paraguay, South America, and she graduated from the Faculty of Chemical Science and Pharmacy at the Universidad Nacional de Asuncion in December 1987. She returned to her country on January 1988, to enter the college of pharmacy at Seoul National University and received a Bachelor of Science degree in pharmacy in January 1991. In 1992, she got married and the next year she and her family moved to USA. She joined the Department of Food Science at Louisiana State University in January 2001. She conducted research under Dr. Witoon Prinyawiwatkul's supervision. She is currently a candidate for a Master of Science degree in food science.