8-2011

Depolymerization of Chitosan by High-Pressure Homogenization and the Effect on Antimicrobial Properties

Deidra Shannon Lyons
dlyons3@utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Deidra Shannon Lyons entitled "Depolymerization of Chitosan by High-Pressure Homogenization and the Effect on Antimicrobial Properties." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Federico M. Harte, Major Professor

We have read this thesis and recommend its acceptance:

Michael P. Davidson, Dr. Lana Zivanovic

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

DEPOLYMERIZATION OF CHITOSAN BY HIGH - PRESSURE HOMOGENIZATION AND THE EFFECT ON ANTIMICROBIAL PROPERTIES

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Deidra S. Lyons
August 2011

DEDICATION

I dedicate this work to my family who has supported me through the ups and downs in life. Your unconditional love and support has created a foundation that propels me to go after my dreams. I would also like to thank my son who motivates me to work hard and persevere no matter what.

ACKNOWLEDGEMENTS

I would like to thank my professor Dr. Harte for your encouragement and endless patience. You have an incredible way of teaching and seeing the strengths and weakness of your students, this creates a unique learning experience that I am grateful to have had. I can only hope my future boss will be as wonderful as you. I would also like to pay tribute to my committee for your support and willingness to teach at a moment's notice; you have given me a role model to strive for.

ABSTRACT

The focus of this study was to look at relationship between polydispersity caused by high pressure homogenization and molecular weight dependent antimicrobial activity of chitosan. It has been shown that chitosan has antimicrobial activity against bacteria, fungi, and viruses. Chitosan is obtained by partial de-N-acetylation of chitin which, consists of a ß 1-4 copolymer of glucosamine and N-acetylglucosamine residues. In this experiment we compared chitosan of sixteen different molecular weights after being processed through a high pressure homogenizer. Processed chitosan (420 kDa average molecular weight, 30% of acetylation) was dissolved in a 1% (v/v) acetic acid in water to a final concentration of 1% (w/v) and apparent viscosity of 183 MPa. The chitosan solution was passed through a high pressure homogenizer with 0-5 passes at pressure levels 0, 100, 200, and 300 MPa. After processing, the chitosan acetate was investigated to determine the effect on polydispersity in terms of molecular weight and the antimicrobial properties of chitosan at different molecular weights. All compounds were tested against *Escherichia coli* K-12 to determine antimicrobial activity. There is growing interest in the application of chitosan in food industry due to its wide range of desirable properties including being non-toxic and biodegradability. However, as a hydrophobic material, it is very challenging to work with. Though chitosan is a challenge to work my findings indicated a strong antimicrobial relationship with chitosan at 1% concentration with a molecular weight of 200 kDa and lower

against *E. coli*. In conclusion chitosan has viable application with a variety of foods and can be used as a preservative that decrease bacterial activity below detection level's and helps to prolong shelf-stable products.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Factors Affecting Food Quality

Loss in food quality may be caused by a wide range of reactions, including some that are predominantly physical (e.g., movement of moisture, change in texture, evaporation of low molecular mass flavor compounds, and damage induced by freezing and thawing), chemical (e.g., oxidative rancidity, color loss, and non-enzymatic Mallard browning reactions), enzymatic (e.g., lipolytic rancidity, proteolysis, and enzymatic browning reactions), and microbiological reactions (e.g., food spoilage)[1]; [2, 3]. The actions of microorganisms are the main cause for food spoilage and food poisoning. Thus, food preservation procedures are generally targeted towards the inhibition of microbial growth or microbial inactivation.

Several food preservation methods have been used to inhibit microbial growth, including chilling, freezing, acidification, reduced water activity, modified atmosphere packaging, chemical preservatives, etc. These methods slow the growth of microorganisms, but will not necessarily inactivate them. Preservation methods that inactivate microorganisms include heat, ionizing irradiation, ultrasound under pressure, hydrostatic pressure and pulse electric field[4, 5] [6, 7] [8]. Heat treatment is the most effective method for food preservation.

However, this preservation method could cause undesirable changes to food quality.

At this time, consumers are demanding less processed food which also has maximum sensory properties and nutritional value. Ensuring food safety and at the same time maintaining the nutrition and quality attributes has resulted in an increased interest in alternative preservation techniques. Use of natural preservatives has attracted great attention, as they may be able to inhibit or inactivate microorganism without causing adverse effects on food quality. For example, chitosan is a natural, non-toxic, biodegradable polymer obtained by the deacetylation of chitin from the exoskeleton of crustaceans [9]. It is well known for its antimicrobial activity against bacteria, viruses and fungi [10-13]. The antimicrobial properties of chitosan combined with its non-toxic nature, make it a promising candidate to be used as food preservative.

In the US it is estimated that there are 9.4 million cases of foodborne illness, 55,961 hospitalizations, and 1,351 deaths attributable to foodborne illness each year [14]. Even though there have been major improvements that have been incorporated into industry standards, including technologies of production, distribution, hygiene standards, and consumer education during the last few decades, food poisoning continues to increase in most countries[15] [16] [17]. Food poisoning is responsible for human suffering through illness and death, as well as huge economic losses; this is seen in developing as well as developed

countries. According to a published article " Foodborne Diseases Active Surveillance Network (FoodNet) reports showed that *Salmonella*, *Campylobacter*, *Shigella*, *Cryptosporidium*, and Shiga toxin *Escherichia coli* (STEC) O157 continue to be leading causes of both the number and incidence of laboratory-confirmed foodborne infections in the United States" [18].Despite the increase in attention to prevent foodborne cases, it still remains a consistent challenge.

Factor Affecting Food Safety

Antibacterial property of chitosan

Chitosan has been shown to have antibacterial activity against both Gram-positive and Gram-negative bacteria. Chitosan also does not cause toxicity to mammalian cells [19, 20]. The compound has been shown that chitosan to be effective against *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Staphylococcus aureus* [12, 21].
Li et al. [22] found that chitosan acetate at 0.15% (w/v) completely inhibited the growth of *E. coli* (Gram negative) and *S. aureus* (Gram positive). Jung et al. [23] studied the effect of chitosan against eight Gram-negative bacteria (*Pseudomonas fluorescens*, *Proteus vulgaris*, *Erwinia carotovora*, *Serratia marcescens*, *E. coli, Vibrio parahaemolyticus*, *V. vulnificus*, and *Salmonella* Typhimurium) and six Gram-positive bacteria (*L. monocytogenes*, *S. aureus*,

Bacillus subtilis, *B. cereus*, *Lactobacillus curvatus*, and *L. plantarum*) [24]. Results showed that acid-soluble chitosans at 0.1% (V/V) nearly completely or completely inhibited growth of all bacteria compared to the control. Water-soluble chitosan inhibited bacterial growth by 1 to 8 logs at 0.1%. Both acid-soluble and water-soluble chitosan showed greater antimicrobial activity against Gram-positive bacteria than Gram-negative bacteria. Similar results were obtained by No et al. [11]. They studied the antibacterial activity of chitosan and chitosan oligomers against four Gram-negative (*E. coli*, *Pseudomonas fluorescens, Salmonella* Typhimurium*, and V. parahaemolyticus*) and seven Gram-positive bacteria (*L. monocytogenes, Bacillus megaterium, B. cereus, S. aureus, L. plantarum, L. brevis, and L. bulgaricus*). Inhibition of bacterial growth was observed in all cases. Chitosans of 1671 kDa to 28 kDa showed higher antibacterial activities than chitosan oligomers with molecular weights of 22 kDa to 1 kDa. Chitosan (0.1% v/v) generally showed stronger bactericidal effects with Gram-positive bacteria than Gram-negative bacteria, which was consistent with findings by Jung et al. [23]. However, Tsai et al. [25] showed that low molecular weight chitosan (12.0 kDa) had similar antibacterial activities against *L. monocytogenes* and *E. coli* [26].

The antimicrobial activity of chitosan in food systems has also been investigated. Sagoo et al. [27] studied the effect of chitosan in the preservation of chilled, comminuted pork products. Results showed that dipping of standard and skinless pork sausages in chitosan solutions (1.0%) reduced the native microflora (total

viable counts, yeasts and molds, and lactic acid bacteria) by approximately 1-3 log cfu/g for 18 days at 7ºC. Chitosan treatment also increased the shelf-life of chilled skinless sausages from 7 to15 days. The addition of 0.3 and 0.6% chitosan to an unseasoned minced pork mixture reduced total viable counts, yeasts and molds, and lactic acid bacteria by up to 3 log CFU/g for 18 days at 4ºC compared with the untreated control. Thus, chitosan was effective in reducing microbial growth in chilled comminuted pork products. Darmadi and Izumimoto also studied the effect of chitosan in meat preservation. They found that during incubation at 30ºC for 48 h or storage at 4ºC for 10 days, 0.5-1.0% chitosan inhibited the growth of spoilage bacteria, reduced lipid oxidation, and resulted in better sensory attributes [28].

The antibacterial property of chitosan depends on molecular weight, pH and concentration of chitosan used. Kim et al. [29] evaluated the effects of the molecular weight (MW), type of chitosan and pH of chitosan solution on antibacterial activity against *Salmonella* Enteritidis [30] and on the shelf life of eggs. Two types of chitosan were used for this study: α-chitosan (MW = 282, 440, 746, and 1110 kDa) and β-chitosan (MW = 577 kDa). The 282 kDa α-chitosan had strongest bactericidal effects and increased the shelflife of eggs by almost 3 wk at 25°C compared with non-coated eggs. α-chitosan (282 kDa) with a pH of 4.5 to 5.5 offered a protective coating against *Salmonella* Enteritidis while preserving the internal quality of eggs. Liu et al. also studied the effect of MW and concentration of chitosan on antibacterial activity against *E. coli*. All of the

chitosan samples with MW from 5.5 to 15.5 kDa had antimicrobial activity at higher than 200 ppm. The antibacterial activity of low MW chitosan was higher than that of the high MW samples.

Antiviral property of chitosan

Chitosan have demonstrated antiviral activities, especially against plant viruses. Iriti et al. showed that treatments with 0.1% chitosan enhanced tobacco inducible defenses against tobacco necrosis necrovirus, reducing the virus-induced necrotic lesions by 32% to 83% [31]. Kulikov et al. [32] studied the effect of the molecular weight of chitosan on its ability to suppress systemic infection of bean mild mosaic virus in bean (*Phaseolus vulgaris* L.) plants. Four chitosan fractions with an average molecular weight of 1.2 to 40.4 kDa were used. It was shown that treatment of bean plants with chitosan at 10 or 100 µg/ml inhibited virus accumulation and systemic propagation. The degree of chitosan-induced antiviral resistance increased as the molecular weight of chitosan decreased [33]. Chitosan was also shown to inhibit the systemic propagation of potato virus X, alfalfa mosaic virus, peanut stunt virus, and cucumber mosaic virus [34, 35]. In a separate study with potato plants and potato X virus, Chirkov et al. [36] indicated that higher molecular weight chitosan (120 kDa) had stronger antiviral effect than a lower molecular chitosan (3 and 36 kDa), showing that the antiviral activity depended not only on the structure of the chitosan but also on molecular weight. Umemra et al. studied the inhibitory activity of a sialylglycopolymer prepared from

chitosan against influenza virus infection and demonstrated that the sialylglycopolymer could be an better candidate for a safe and effective anti-influenza drug [37].

The antiviral effect of chitosan against foodborne viral surrogates was also investigated by Su et al. [38]. Chitosan oligosaccharide lactate (MW= 5 kDa) and water-soluble chitosan (MW=53 kDa) at 0.7, 0.35%, and 0.17% were tested against feline calicivirus (FCV-F9), murine norovirus (MNV-1), and bacteriophage MS2. It was shown that water-soluble chitosan at 0.7% decreased the titer of FCV-F9 and MS2 by ~4.2 and 1.6 log PFU/ml, respectively, from an initial titer of ~5 log PFU/ml. Chitosan oligosaccharide lactate at 0.7% decreased the titer of FCV-F9 and MS2 by ~1.4 and 1.0 log PFU/ml, respectively, from initial titer of ~5 log PFU/ml. Neither type of chitosan's had an effect on MNV-1.

Antifungal Properties of Chitosan

Major postharvest losses of fruits are due to fungal infection. Chitosan has shown antifungal properties against many fungi and is effective in controlling post-harvest quality losses of fruits and vegetables and in extending the shelflife of produce. Recently, Meng et al. [39], studied the effects of chitosan (350 kDa) and oligochitosan (6 kDa) on growth of *Alternaria kikuchiana* or *Physalospora piricola* on pear fruit. Pear fruit inoculated with the two fungal pathogens were treated with chitosan or oligochitosan at 0.1, 0.5, 1.0, 1.5, 5.0 and 10 g/L at 25°C. Both

chitosan and oligochitosan at 5.0 g/L completely inhibited spore germination of the two fungi after 6 h and completely inhibited mycelial growth after 6 days. Thus, Meng et al. [40] concluded that chitosan and oligochitosan could reduce the disease incidence caused by *A. kikuchiana* and *P. piricola* and inhibited the lesion expansion of the two fungi in pear fruit.

The antifungal activity of chitosan was also examined against *Penicillium digitatum, Penicillium italicum, Botrydiplodia lecanidion* and *Botrytis cinerea* [41]. Chitosans (92.1 kDa and 357.3 kDa, with 94.2% N-deacetylation) at 0.05, 0.1 and 0.2% were found to cause 25.0–90.5% growth inhibition on test organisms after 5 days of cultivation at 24ºC. Chitosan treatment significantly reduced the percentage decay of Tankan fruit during storage at 24ºC. After 42 days of storage at 13ºC, chitosan-coated Tankan fruits were firmer, exhibited less decay and weight loss than the control fruit.

The effect of chitosan coating (1.0 and 1.5% w/v) in controlling decay of strawberries was also investigated [42]. Chitosan coating significantly reduced decay of strawberries as compared to the control at 13°C. Chitosan-coated strawberries stored at 4°C were firmer, had higher titratable acidity, and synthesized anthocyanin at a slower rate than non-treated berries. Chitosan coating decreased respiration rate of the berries with a greater effect at higher concentration.

Roller and Covill studied the antimicrobial properties of chitosan glutamate against 15 yeasts and molds in laboratory media and apple juice [43]. It was shown that chitosan at 5 g/L completely prevented growth of *Mucor racemosus* and three strains of *Byssochlamys* spp. on agar plates incubated at 25 ºC for 3 weeks, but had no effect on *A. flavus*, *C. cladosporioides* or *P. aurantiogriseum*. Chitosan reduced the growth rate of *M. racemosus* by ~20% at 1 g/L, and by ~50-70% at 2 g/L. Chitosan in apple juice (pH 3.4) at levels ranging from 0.1 to 5 g/L inhibited growth at 25ºC of *Zygosaccharomyces bailii*, *Saccharomycodes ludwigii*, *S. cerevisiae, S. pombe, and S. exiguus*. *Zygosaccharomyces bailii* was the most sensitive strain to chitosan treatment, which was completely inactivated by 0.1 and 0.4 g/ L for 32 days at 25ºC. The most resistant strain was *S. ludwigii*; a concentration of 5 g/L of chitosan was required to inactivate this strain and to maintain yeast-free conditions in apple juice for 14 days at 25ºC. It was concluded that growth inhibition and inactivation of filamentous molds and yeasts was concentration-, pH- and temperature-dependent.

MICROORGANISM EFFECT ON FOOD QUALITY

There are three types of food spoilage microorganisms: yeast, mold and bacteria. Yeasts and molds are major food spoilage organisms for low water activity, low pH processed foods such as jams, syrups or dried-cured meats [44]. Bacteria generally spoil high water activity, high pH, protein rich foods, such as meat, poultry, fish and milk and some dairy products. Food sources that offer a nutrition

source and are have a high amount of available water source support the growth of a wide range of microorganisms [45].

Raw perishable foods tend to contain a variety of microorganisms. Over time one particular organism or type of organism tends to dominate, and in so doing ultimately causes spoilage to food [46]. Decline in food quality in storage can occur as a consequence of the activities of microorganisms as they multiply in the food causing undesirable sensory changes including off odors, off flavors, discoloration, texture changes, gas and slime [47]. These changes lead to product loss for the consumers and economic losses for the food industry.

CHITIN TO CHITOSAN

Chitin is composed of ß (1-4) linked D- glucosamine with a varying degree of N-acetylation. The annual amount of chitin produced from crustacean, mollusks, insects and fungi is about 100 billion tons [48]. The major sources of chitin are shrimp, crab, squids, insects, mushrooms, and filamentous fungi.

Chitosan is a general name for a group of partially or fully deacetylated chitin compounds. The properties of chitosan depend on the manufacturing process used, which influences the purity, viscosity, deacetylation, molecular weight, and polymorphous structure [49]. Basic chitin structures are often linear but may contain various degrees of branching. Chitin itself is water- insoluble due to

extensive crystallization. It comes in two allomorphic forms. The first, α-chitin, is an antiparallel arrangement that has strong intermolecular hydrogen bonding and is the most abundant chitin in nature (shrimp, crabs). The second, ß- chitin, is a parallel arrangement which is associated with the protein found in squid pens [50]. Chitin is closely associated with proteins, minerals, lipids and pigments [51]. All of these components must be removed while preserving the highly polymeric chitin for chitosan to be produced. Thus, chitin must undergo various processing steps in order to obtain the final useable material called chitosan. One method for chitosan preparation uses HCl (solid/liquid ratio 1:10) for demineralization followed by 3% (w/v) NaOH treatment with application of heat for protein removal. This is followed by a discoloration step using 0.5% $KMnO_4$ (w/v) and oxalic acid both in aqueous solution [52]. The last step which is removal of acetyl groups can be performed using the Horowitz technique which consists of treating chitin with solid KOH for 30 min at 180°C, resulting in the removal (95%) of acetyl groups.

Chitosan is obtained by alkaline deacetylation of chitin (Fig. 1 and Fig. 2). The degree of deacetylation usually ranges from 30% to 95% depending on the methods used and causes change in chemical characteristics of chitosan by creating free amino groups in the polysaccharide. In several previous studies, chitosan with a high degree of deacetylation had a higher antimicrobial activity than chitosan with a low degree of deacetylation [53]. These results suggest that the free amino groups play a pivotal role in the antibacterial activity

observed in chitosan. The amino group in chitosan has a pKa value of ~6.5, which leads to protonation in acidic to neutral solutions; this chemical modification makes chitosan water-soluble, a property that facilitates its use in the food industry.

Chitosan is insoluble in water but soluble in weak organic acids such as acetic, formic, succinic, lactic, and malic. It is a polycationic compound. This property enables chitosan to interact readily with negatively charged substances such as proteins, anionic polysaccharides (e.g., alginate, carrageenan, pectin), fatty acids, bile acids, and phospholipids as a result of the high density of amino groups present in the polymer [54]. It also allows for chitosan to readily bind to negatively charged surfaces such as the surface of bacteria [55].

Recently, chitosan and its derivatives have received great attention due to the fact that chitin is an abundant renewable resource, is non-toxic, biodegradable and has antimicrobial properties. This allows for many applications ranging from pharmaceuticals, cosmetics , food [56, 57] as well as agricultural application [58], and wastewater treatment [59]. The most important potential food application areas are shown in Table1. The applications include antimicrobial agent (bactericidal and fungicidal) [60], edible film (controlled transfer of moisture, release of antimicrobial substances and antioxidant, reverse osmosis membranes) [54], clarification, de-acidification of fruits and beverages, emulsifying agent, thickening and stabilizing agents, color stabilization, [61],

nutritional quality (dietary fiber), enzyme immobilization, and encapsulation of nutraceuticals [62, 63].

Table 1 Food application of chitin, chitosan and their derivatives in the food industry[61]

Area of Application	Examples
Antimicrobial Agent	Bactericidal Fungicidal Antiviral
Edible Film Industry	Controlled moisture transfer between food and surrounding environment Controlled release of antimicrobial substances Controlled release of antioxidants Controlled enzymatic browning in fruits and vegetables
Other Applications	Enzyme immobilization Encapsulation of nutraceuticals

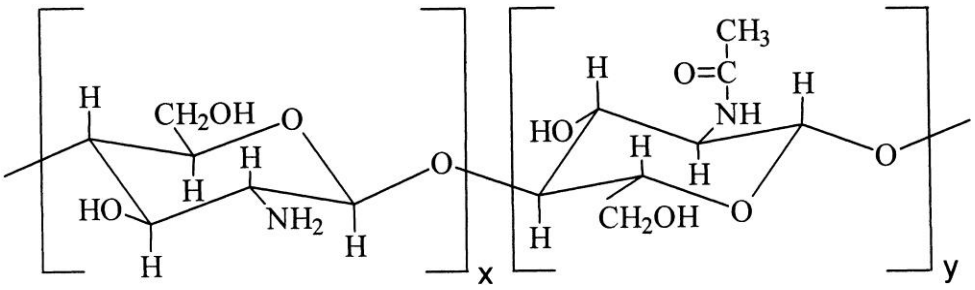

Figure 1.Repeat residues for chitin and chitosan. Chitin is composed predominantly of GlcNAc (y) unit; Chitosan is composed predominantly of GlcN (x) unit. [64]

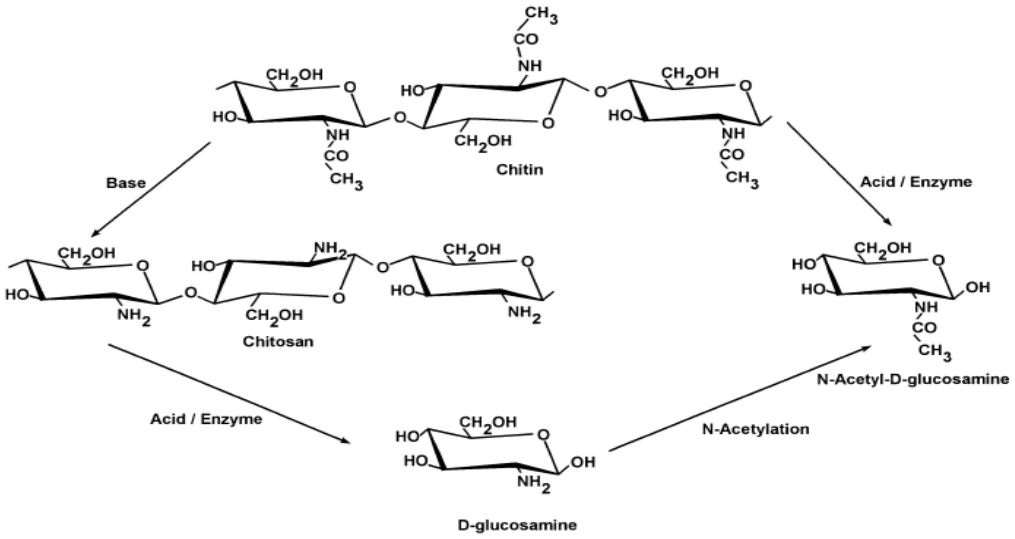

Fig.

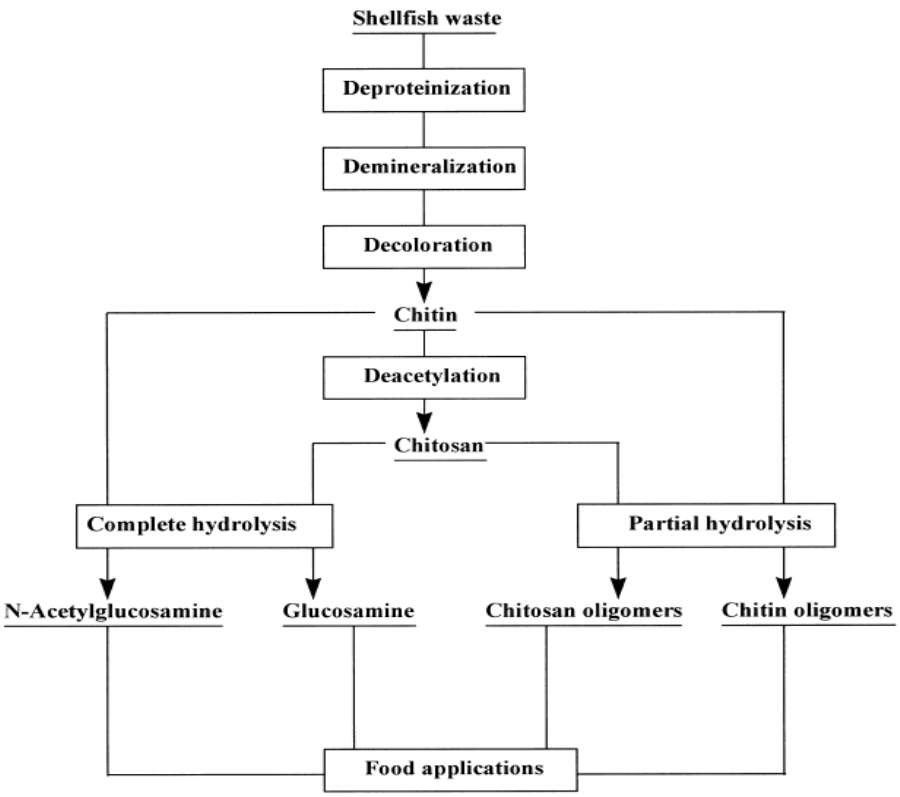

Figure 2. Preparation of chitin derivatives from chitin [61]

CHITOSAN FOOD APPLICATIONS

With the increasing consumer awareness and concerns about food safety and security there is greater need for use of naturally sourced preservatives in foods. These include plant essential oils, microbial metabolites and similar compounds. Chitosan which has natural antimicrobial properties is a potential new natural antimicrobial. It also has been shown that chitosan minimizes a variety of negative post-harvest conditions that influence shelf life such as enzymatic reactions. No et al. (2007) applied chitosan as a coating on fresh fruits and vegetables resulting in a reduction in post-harvest browning due to polyphenol oxidase activity, anthocyanin hydrolysis, and non-enzymatic polymerization of O-quinones into melanins [65]. A chitosan coating on litchi (*Litchi chinensis* Sonn) was investigated to reduce post-harvest browning Caro and Joas 2005. Fruits were dipped in a 1% (w/w) chitosan combined with either citric acid or tartaric acid. The control was non-treated fruit. Results indicated a decrease in enzymatic activity. Thus use of organic acid in combination with chitosan prevented post-harvest browning of litchi fruit [66] [67] investigated the effects of chitosan coating for logan fruit in stored up to 30 days. Fruits were coated with aqueous solutions of 0.5, 1.0 and 2.0% (w/w) chitosan and stored in 2^{o}C and 90% relative humidity. Fruit treated with 2% (w/w) chitosan remained a bright color for up to 30 days and 95% plus it did not rot. Also, increasing concentrations of chitosan eliminated any off flavors during storage [67]. Zhang and Quantick [68] reported that chitosan coating, irrespective of concentration

(1% (w/w) and 2% (w/w) dissolved in 2%(w/w) glutamic acid), delayed changes in anthocyanins, flavonoids, and total phenolics, all which could lead to browning.

Parks et al. 2005 and El Ghaouth et al. 1991, 1992 investigated methods for application of chitosan to protect fresh fruits from fungal activity during storage. Results showed there is evidence that chitosan coating has the potential to prolong the storage life and control decay of fruits. Strawberry is among the most perishable fruits and is vulnerable to physical injuries and fungal infection caused by *Botrytis cinerea* and *Rhizopus* sp. [69, 70]. El Ghaouth and others (1991, 1992a) investigated the effect of chitosan coating on decay and quality of strawberries at 13^{o}C. Strawberry fruits were inoculated with a spore suspension of *Botrytis cinerea* [71] or *Rhizopus stolonifer* [69] and subsequently dipped in chitosan solutions (1.0% and 1.5% w/v in 0.25 N HCl). In both studies, chitosan coating significantly reduced the decay of strawberries compared to the control. However, there was no prevention of decay by increasing concentration of chitosan from 1.0% to 1.5%. During storage at 4^{o}C, chitosan-coated berries were firmer, had higher titratable acidity, and synthesized anthocyanin at a slower rate than the control and the fungicide -treated berries [69]. Chitosan coating decreased the respiration rate of strawberries with a greater effect at higher concentration [70]. The improved storability of fresh strawberries by chitosan-based coatings also has been documented by Reddy

and others [72], Han and others [73], Park and others [70], Hernandez-Muńoz and others [74], and Vargas and others [75].

Chitosan has been extensively studied as an antimicrobial agent in food packaging due to its ability to form films, coatings and be used as a carrier of other additives. Ouattara et al. [76] looked at the effects of chitosan combined with acetic acid or propionic acid. Propionic acid was found to be more effective then acetic acid. These authors studied vacuum packages that were coated with 2% and 2.5% chitosan (w/w) and used to store grilled pork in refrigerated conditions. The results showed that pork in vacuum packages without the coating had a final count of 6 log CFU aerobic plate count while those with the coating were 3.75 and 3.61 log CFU/g for 2% and 2.5% chitosan, respectively [77].

RELATION BETWEEN MOLECULAR WEIGHT AND ANTIMICROBIAL PROPERTIES OF CHITOSAN

Chitosan is being looked at as a preservative in a variety of applications for antimicrobial activity, antifungal activity, packaging, improvements in controlling movement of moisture and enzymatic reactions. It is known that chitosan antimicrobial ability is connected to molecular weight, degree of acetylation, concentration and type of target microorganism.

Mechanism of Inactivation

The exact mechanism of inhibition or inactivation of microorganisms by chitosan is still unknown. Two potential mechanisms have been suggested for Gram-positive and Gram-negative bacteria. Gram-negative bacteria have an outer membrane that is complex and contains various polysaccharides, proteins, lipids and peptidoglycans. The outer membrane plays a major role determining how Gram-negative bacteria react to chitosan. According to Liu et al. (2004) [78], electron micrographs of chitosan-treated *E. coli* showed an altered outer membrane, which was disrupted and covered by an additional tooth-like layer while the inner membrane was unaffected [78] .

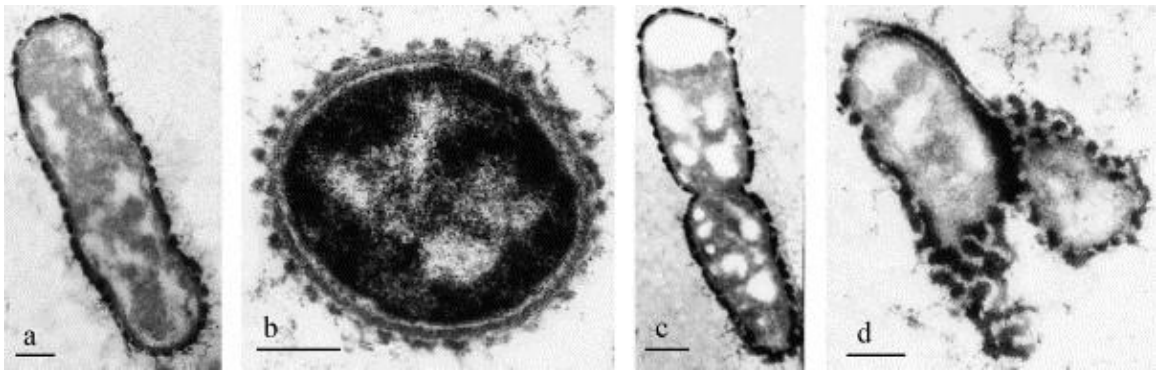

Figure 3. Electron microphotographs of *E. coli* after treatment with the 0.5% chitosan acetate solution for 20 min Bar=200 nm [77]

According to Young et al. 1982 [79], since Gram-negative bacterial cell surface is negatively charged, due to teichoic acids and lipopolysaccharides, an

electrostatic interaction occurs between the chitosan and the bacteria that produces antimicrobial activity.

Results have also shown that, as the molecular weight of chitosan decreases, the antimicrobial ability increases [80]. On a molecular level and due to electrostatic interactions, the chitosan disrupts the outer membrane, breaking down the cytoplasmic membrane barrier, and is involved in cell lysis. Furthermore, since chitosan is a chelating agent it may remove trace metals that are crucial for microorganism growth [81, 82]. Research by Tharanathan and Kittur showed that chitosan binds to DNA therefore preventing transcription [48, 83] and may develop an outer layer or “shell” on the surface of the microbial cell preventing the transport of nutrients [83].

Again, the actual mechanism for Gram-positive bacteria is still unknown. The cell wall of Gram-positive bacteria is peptidoglycan. The cell membrane is a lipid bilayer with proteins. What was produced by Liu et al. 2004 due to electron microphotographs of Gram- positive S. aureus, showed a breakdown of cellular membrane and a deformation of newly divided cells when exposed to chitosan (see Figure 4). It is suggested that these two factors combined eventually cause leakage of intracellular constituents and ultimately, bacterial lysis [77, 82, 84, 85].

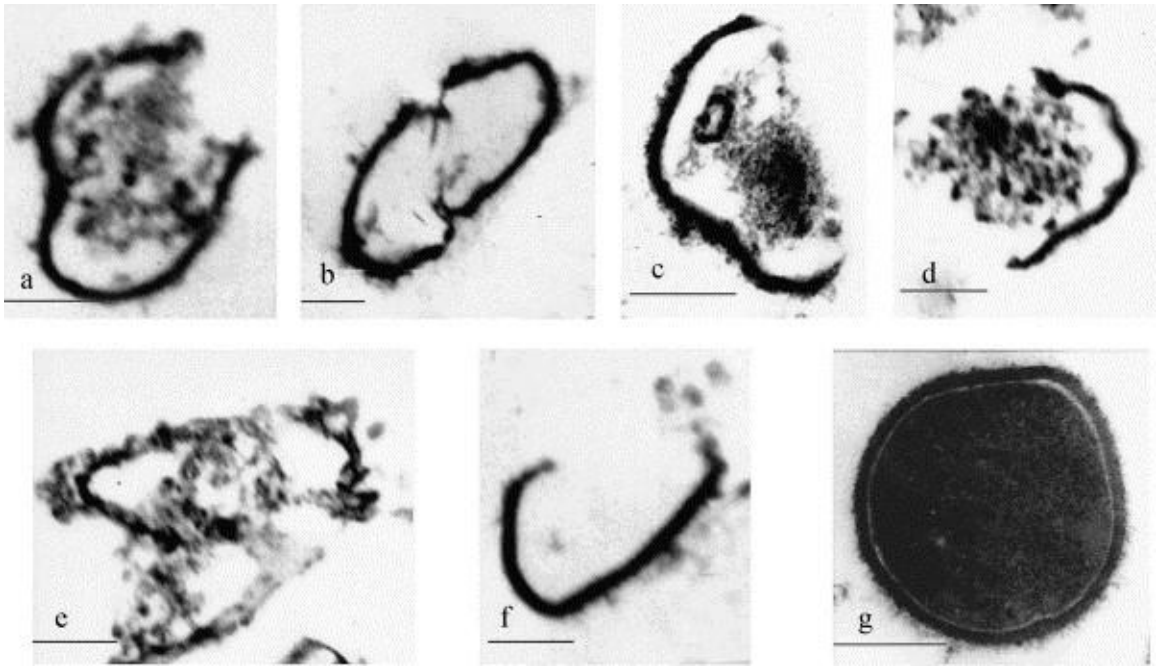

Figure 4. Transmission electron microphotographs of *S. aureus* cells after treatment with the 0.5% chitosan acetate solution for 20 min. Bar=200 nm [77]

Factors

As stated above, there is a relationship between the antimicrobial properties, molecular weight, concentration, and the degree of deacetylation of chitosan [86] .Chitosan produced commercially has a molecular weight ranging from 100 to 1200 kDa [87]. However, commercial chitosan has limited application due to insolubility at a pH above 6.3 [88, 89]. It is thus common practice to convert chitosan into chitosan oligomers (COS) with a range of 1 to 22 kDa to achieve greater solubility. There is disagreement regarding the effectiveness of chitosan polymers versus oligomers. Most investigators use the uncertain term “low

molecular weight" or "high molecular weight" in reference to chitosan without indicating the exact molecular weight. There is a wide range of data on the bactericidal activity of chitosan for various bacteria tested, experimental conditions, and chitosan molecular weight, but results do not always agree. For example, according to Jeon et al. 2001 [90], chitosan polymers are more effective at inhibiting Gram-negative bacteria such as *E. coli* while chitosan oligomers show no significant inactivating effect. According to [91], chitosan polymers generally show a stronger bactericidal effect towards Gram-positive bacteria than towards Gram-negative bacteria at 0.1% (w/v) [91]. Other authors suggested that an increase in MW decreases antimicrobial activity of chitosan, i.e., chitosan oligomers exhibit higher antimicrobial activity against *E. coli* than water–insoluble chitosan polymers [80, 92]. It was observed by [90] that 746 kDa chitosan was most effective against Gram-negative bacteria such as *E. coli* and *P. fluorescens*, versus 470 kDa chitosan applied to other Gram-negative bacteria such as against *S.* Typhimurium and *V. parahaemolyticus [90]*. In contrast, No et al. 2002 [93] stated that chitosan with MW of 1106 and 224 kDa showed little or no antibacterial activity against *S.* Typhimurium. An investigation done by Zheng et al. [80], looked at antimicrobial activity of chitosan with MW < 305 kDa. Results showed that, with Gram-negative bacteria, as the MW decreased the antimicrobial activity increased while with Gram-positive bacteria, the opposite occurred, i.e., as the MW increased the antimicrobial activity increased. Similarly, Uchida et al. (1989) found that COS of 1 kDa had the greatest inhibitory effect against Gram-negative bacteria while

chitosan oligomers of 4 and 2 kDa were most effective against Gram-positive bacteria in compassion with other MWs [94]. For lower MW chitosan, 9.3 kDa chitosan restricted growth of *E. coli* while 2.2 kDa chitosan promoted growth of the bacterium [83]. Prior studies comparing chitosan to chitosan oligomers reported chitosan and its enzymatic hydrolysates suppressed growth of *E. coli*, *P. aeruginosa*, *B*. *subtilis* and *S. aureus* while chitosan oligomers showed weak or no antibacterial activity with levels as high as 0.5 to 1.0% (w/v) [78, 94]. Seo et al. (2007) [89] showed that 28 kDa chitosan at 0.1% (v/v) had weak or no antibacterial effect against *S.* Typhimurium [89].

The relationship between molecular weight and antimicrobial activity has produced a variety of results by various researchers. At present, it has been commonly recognized that the biological activity of chitosan depends on its deacetylation degree, degree of depolymerization, pH and the target organism. Simply put, there is still room for clarity to ascertain the relationship between the antimicrobial activity of chitosan and its MW and concentration.

DEPOLYMERIZATION OF CHITOSAN

Chitosan and its derivatives have been used in a wide variety of applications, but the effectiveness of these materials has been found to be dependent upon

their molecular size. The purpose of depolymerization is to alter the molecular weight of chitosan and produce polydispersity. Two common methods for depolymerization are enzyme and acid–hydrolysis and a new method for depolymerization of chitosan is high pressure homogenization.

Enzyme-hydrolysis of chitosan

One method of depolymerization of chitosan uses enzyme hydrolysis. Chitosan is susceptible to the hydrolytic activities of nonspecific enzymes such as papain, chitinase and lysozyme. These non-specific enzymes are found to catalyze the cleavage of glycosidic linkages in chitosan [95, 96]. However, there are difficulties in hydrolysis due to physicochemical properties of chitosan, such as chain flexibility in solution [97] [98], rheological properties crystal size and crystallinity which all depend on intrinsic factors such as the degree of deacetylation, distribution of the acetyl groups, molecular weight and molecular weight polydispersity. The properties affect the affinity the between enzyme and chitosan and have an impact on the hydrolytic process. The exact mechanism of non-specific enzyme catalysis remains unknown and a controlled enzymatic hydrolysis of chitin to low molecular weight chitosan and chito-oligosaccharides with specific lengths is a challenge *[59].*

Chemical depolymerization of chitosan

In one method, using acid-hydrolysis, chitosan is dissolved in a 0.1 M HCl for times ranging from 10-103 h and the reaction is stopped by increasing the pH to around 4.5 using a base such as NaOH. The kinetics of the acid depolymerization reactions is followed based on the reduction of apparent viscosity and/or intrinsic viscosity over time. However, the lack of control over parameters during the reaction makes it difficult to generate polymers of defined molecular weight [100]. An example of this is the challenge in determining the rate of de-N-acetylation relative to the rate of depolymerization. In the acid-hydrolysis reaction the “hydrolysis of the N-acetyl linkage (de-N-acetylation) may occur in addition to hydrolysis of the O-glycosidic linkage (depolymerization)” [101]. For this reason though acid-hydrolysis is a common method for altering MW of chitosan it has many faults.

Depolymerization by High-pressure homogenization (HPH)

HPH is a novel approach for the depolymerization of chitosan polysaccharides that are dispersed in water and display Newtonian or pseudoplastic (Power Law) flow properties [102]. In most cases, chitosan polysaccharide dispersions are time independent shear-thinning liquids, meaning that as the shear rate increases, the apparent viscosity decreases. This non-linear relationship is used to describe non-homogeneous solutions that may have coiled, entangled,

or agglomerated particles [103]. After HPH processing of chitosan solution, an exponential decay in viscosity was observed due to homogenization pressure and the number of homogenization cycles. Also, for a given polysaccharide, once the critical MW is reached the equilibrium viscosity reaches equilibrium when processed at a fixed homogenization pressure and number of homogenization cycles [102]. The advantage of high pressure homogenizers is ability to predetermine molecular weight ranges of chitosan while processing and to potentially have smaller scale in polydispersity.

CHAPTER II

DEPOLYMERIZATION OF CHITOSAN USING HIGH PRESSURE HOMOGENIZATION

This paper was produced by Deidra Lyons and Federico M. Harte. The use of "our" in this chapter refers to my co-authors and myself. My primary contributions to this paper include (1) the experimental work, (2) most of the collection and analysis of data, (3) most of the gathering and interpretation of literature, and (4) most of the writing.

ABSTRACT

Chitosan consists of a ß 1-4 copolymer of glucosamine and N-Acetylglucosamine residues obtained by partial depolymerization and de-N-acetylation of chitin. It has been shown that chitosan has antimicrobial activity against bacteria, fungi, and viruses. The objective of this study was to establish the relationship between molecular weight and antimicrobial activity of chitosan after processed by high pressure homogenization (HPH). In these experiments, chitosan was evaluated at 16 different molecular weights for its ability to inactivate *Escherichia coli* K-12. Chitosan (420 kDa average molecular weight, 30% of acetylation) was dissolved in a 1% (v/v) aqueous acetic acid to a final concentration of 1% (w/v) and 183 MPa Newtonian viscosity. The chitosan solution was passed through a high pressure homogenizer (0 to 300 MPa) up to five homogenization cycles. After processing, the chitosan (CS) acetate solution was used to study the effect of molecular weight on antimicrobial activity. All chitosan solutions were tested against *Escherichia coli* K-12. The results showed HPH to be an effective tool in depolymerization of chitosan for a molecular weight range from 168 kDa to 650 kDa and smaller molecular weights were more effective against *E. coli* K-12. Results indicated the antimicrobial activity increased as the MW decreased. In comparison with the larger MW's we saw an almost complete suppression for 236 kDa to 168 kDa. This study demonstrates that molecular weight plays a role in antibacterial activity. Chitosan generally showed a stronger bactericidal effect for Gram-negative bacteria when applying lower molecular weights. The

concentration of chitosan did not seem to affect activity. There is an interest in application of chitosan in food industry due to wide range of possible applications and non-toxic biodegradable features.

Introduction

Chitin is ß (1-4) -2- amino-2-deoxy-2-amino -D-glucopyranose that is a biopolymer. Chitosan is a nontoxic biopolymer derived by deacetylation of chitin, a major component of crustaceans. In working with chitosan there are several applications being applied either alone or in combination with other natural polymers (starch, gelatin, alginates) in the food and pharmaceutical industries mainly due to its high biodegradability and antimicrobial properties [91, 104-107]. Microbiological activity of chitosan had been detected for many bacteria, viruses and fungi. However, there is a relationship between molecular weight, concentration, degree of depolymerization and the degree of deacetylation [86] on the antimicrobial activity of chitosan. The objective of this study was to establish the relationship between molecular weight and antimicrobial activity of chitosan after being processed through high pressure homogenization (HPH).

General production of chitin to chitosan involves four steps, deproteinization, demineralization, decolorization and deacetylation. There are two critical steps that affect the antibacterial activity in chitosan the first is degree of deacetylation the second is depolymerization. Degree of deacetylation is a term that describes the removal of acety groups from the molecular chain of chitin, leaving behind a complete amino group (-NH2). With respect to antibacterial activity, deacetylation is one of the critical step that produces this activity due to polycationic amines which interact with the negatively charged residues at the cell surface of bacteria

[108] thus inhibiting the growth of bacteria.Chitosan versatility depends mainly on this high degree chemical reactive amino group.

One method of preparation for deacetylation of chitin is the suspension of chitin in a 40% aqueous sodium hydroxide solution to obtain alkali chitin, this is dissolved with crushed ice at 0°C under vigorous stirring. The solution is warmed up to 25°C with stirring for a specified time and then neutralized with aqueous hydrochloric acid solution. The solution is then precipitated and washed with an acetone/ water mixture and dried under vacuum [21].

The second critical step is depolymerization with this step we are able to determine relationship between MW and antimicrobial activity. Today there are several common methods used such as acid-hydrolysis and enzyme-hydrolysis. However for this paper we examined a novel method using high-pressure homogenization. Looking at the first example using acid-hydrolysis, chitosan is dissolved in a 0.1 M HCl for times ranging from 10-103 h and the reaction is stopped by increasing the pH to around 4.5 using a base such as NaOH. The disadvantage of using this method is the reaction is relatively uncontrolled, producing polydispersed chitosans [109].

The second example involves enzyme-hydrolysis using papain, chitinase or lysozyme to alter the molecular weight of chitosan. The enzyme solution is combined with chitosan and the suspension is stirred overnight under mild conditions. The chitin-enzyme dispersion is filtered and dissolved into a 3 M NaCl

solution for a few hours, then it is washed with water to remove salt and set aside as a suspension in distilled water [110]. The water is removed through filtration. The disadvantage of enzymatic methods is that it is not possible to predetermine molecular weights and not possible to remove the entire enzyme from the finished product.

In this study we used high-pressure homogenizer to depolymerize chitosan. The intent of this study is to focus on several parameters dealing with antimicrobial activity of chitosan. The first focus was using the HPH as an alternative to alter the molecular weight, to determine the validity of using this method to depolymerize the chitosan polysaccharide. The second was continuing with prior research to determine the relationship between MW and antimicrobial activity. Preparation of chitosan in HPH involves passing chitosan acetate solution through HPH 1 to 5 times at pressure of 0, 100, 200, and 300 MPA, respectively. The advantage of high pressure homogenizers is ability to target molecular weight ranges of chitosan while potentially producing a small polydispersity range.

Materials and Methods

Chitosan Preparation

Different molecular weights of chitosan samples A to O were previously obtained by high pressure homogenization (Table 2). Briefly, chitosan (average molecular weight 702 kDa and 30% deacetylation; Primex, (Iceland) was dispersed in a 1% (v/v) aqueous acetic acid to a final concentration of 1% (w/v) and 183 MPa apparent viscosity. The chitosan solutions were then passed through a high pressure valve homogenizer (model FPG 12500, Stansted Fluid Power, Essex, UK) operated at 100, 200, or 300 MPa for 1 to 5 homogenization cycles.

Microbial assay

Peptone, standard methods agar, tryptic soy broth (TSB), and trypticase soy agar (TSA) were obtained from Fisher Scientific Co. (Fair Lawn, NJ). Double-distilled deionized water was used for all solutions and media preparation. Measurements of pH were performed using a pH meter (Model 250, Denver Instrument, Bohemia, NY). The chitosan polysaccharide dispersions with different molecular weights were stirred overnight at room temperature and then filtered using Miracloth (EMD Bioscience, Inc., San Diego, CA) to remove potential impurities.

For preparation of the microbial suspension, the bacterium was inoculated into 55 ml tryptic soy broth. It was incubated in shaker (150 rpm; Lab-Line Instruments, Inc. Melrose Park, IL) at 37°C for 12 h. The culture was diluted to a final concentration of 10^6 CFU/ml. Chitosan samples (3.6 ml) were transferred to sterile culture tubes and inoculated with 0.4 ml of the microbial suspension to achieve 10^5 CFU/ml. Samples were mixed and incubated at 37°C. Samples were then plated after 0 min, 30 min, 1 h, 1.5 h and 2 h of incubation. Two individual samples were analyzed for each sampling point. Enumeration was performed by stepwise dilutions followed by plating on standard methods agar and incubation for 24 h at 35 °C for *E. coli*. Enumeration of the initial inoculum was performed as well.

Each experiment was replicated three times in a completely randomized design. Analysis of variance was done on the variable inactivation of *E. coli* K-12 after 2 h of exposure to chitosan with different molecular weights.

RESULTS AND DISCUSSION

Effect of High Pressure Homogenizer on Depolymerization of Chitosan

High pressure homogenization has been shown to be a valid tool to reduce molecular weight of polysaccharides. Results also suggest that this technology may potentially reduce polydispersity and allow for the manufacture of polysaccharides with target molecular weights. Comparing results from 100 MPa (0-5 passes) to results produced under 300 MPa (0-5 passes). One could deduce increasing the pressure to 300 MPa may be the most efficient method when using HPH as a way of conserving energy.

Table 2. Effect of homogenization pressure and number of passes on viscosity and molecular weight of all chitosan.

Pressure (MPa)	Passes	Viscosity (MPa)	MW (kDa)	Identification
0	0	183.8	702	Control
100	1	85.2	650	E
200	1	38.01	410	F
300	1	23.73	293	K
100	2	62.97	621	D
200	2	26.14	340	G
300	2	16.27	236	L
100	3	55.38	571	C
200	3	21.37	308	H
300	3	12.93	203	M
100	4	49.23	525	B
200	4	18.47	278	I
300	4	11.38	182	N
100	5	41.41	487	A
200	5	16.76	261	J
300	5	9.95	168	O

Table 1 shows the effect of homogenization pressure and number of passes on viscosity and molecular weight of all chitosan samples processed through high pressure homogenizer.

Antibacterial activity of chitosan as affected by MW

In all samples tested chitosan was at a concentration of 1%, this concentration appeared to be sufficient to show antimicrobial activity. The effect of molecular weight on the antibacterial activity of chitosan against E*. coli* is shown in Figures 5-9 for homogenization pressure 100, 200, and 300 MPa, respectively. Molecular weight relationships of antibacterial activity by chitosan have been reported by various investigators. No et al. 2002 reported that chitosan of 746 kDa appeared most effective against Gram-negative bacteria while chitosan of 470 kDa work best against Gram-positive bacteria [111]. The results were different from ours, due to our results having very close range in MW of chitosan samples processed and the molecular weight range used (750 kDa to 168 kDa).

Figures 5-9 show reactions of higher molecular weight chitosan (650 kDa to 487 kDa) against *E. coli* K-12. The samples were processed at 100 MPa homogenization pressure from 0 to 5 times. Inoculation of *E. coli* was ca, 10^5 CFU. All compounds showed bactericidal activity. Viable numbers were reduced by up to 3 log cycles within 2 h of

exposure.

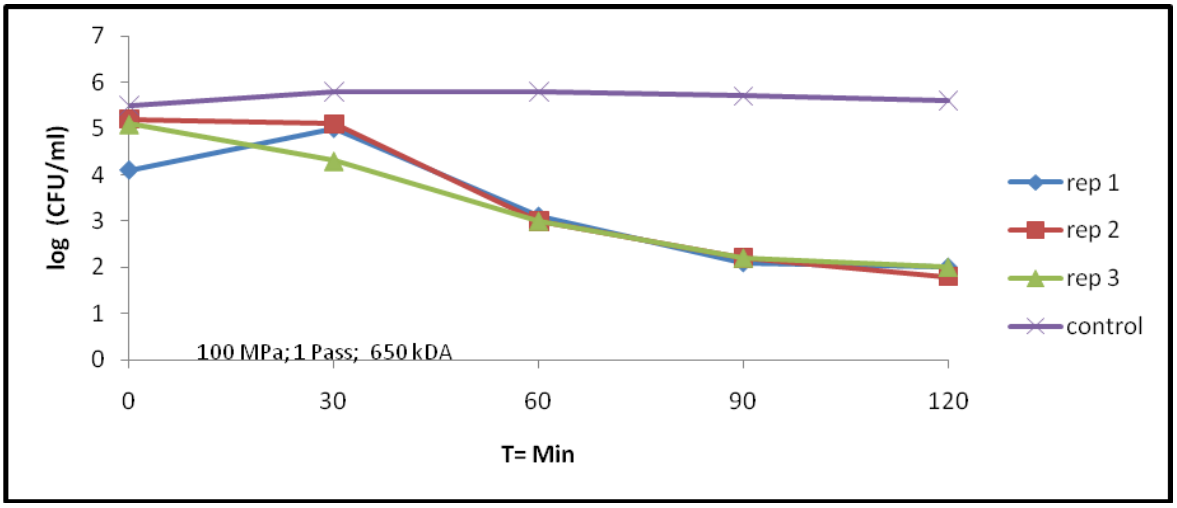

Figure 5. Inactivation of *E. coli* K-12 in chitosan (650 kDa) solution over 120 min after HPH processing at 100 MPa.

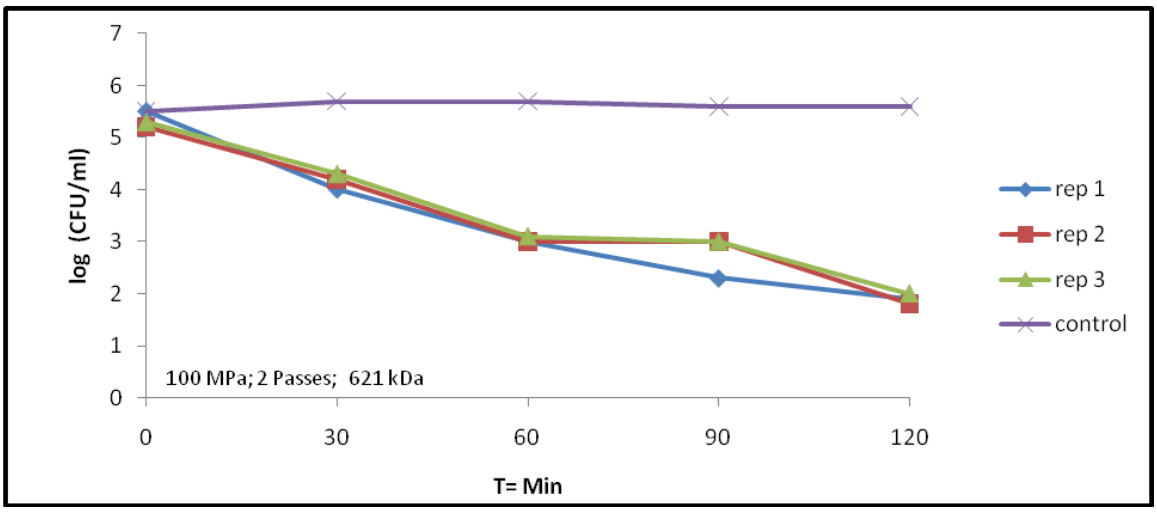

Figure 6. Inactivation of *E. coli* K-12 in chitosan (621kDa) solution over 120 min after HPH processing at 100 MPa.

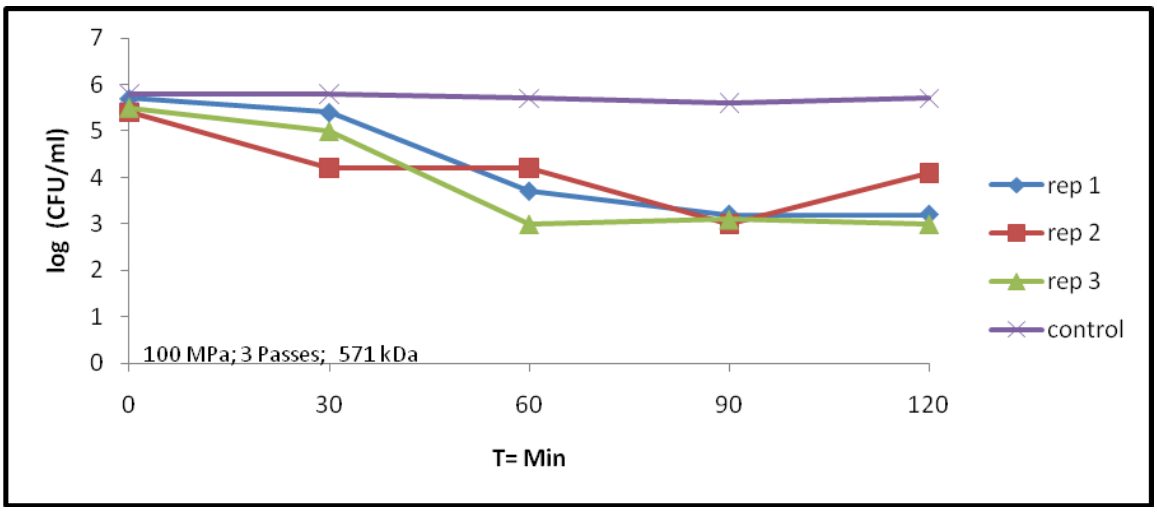

Figure 7. Inactivation of *E. coli* K-12 in chitosan (571 kDa) solution over 120 min HPH processing at 100 MPa.

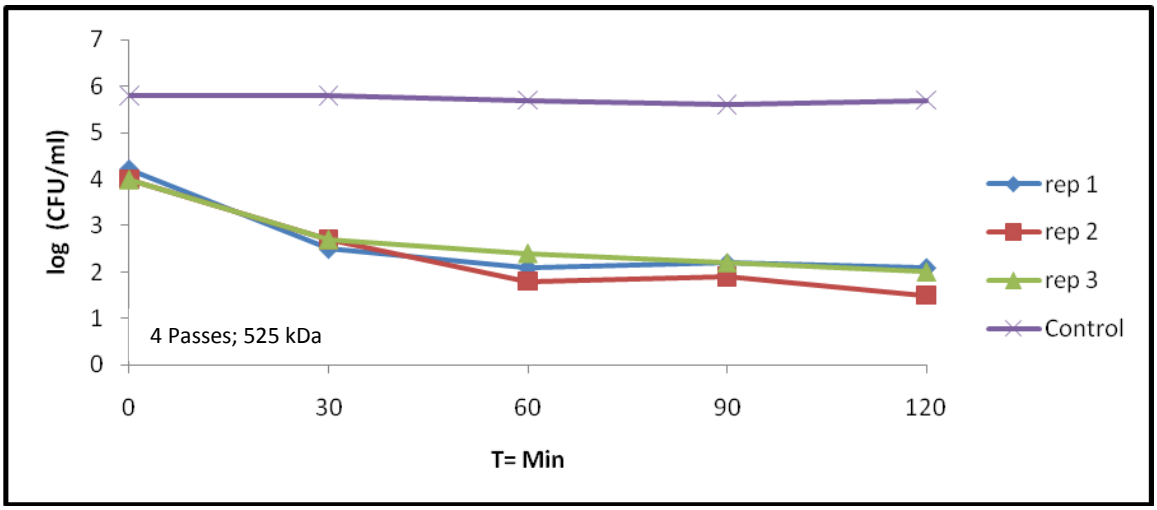

Figure 8. Inactivation of *E. coli* K-12 chitosan (525 kDa) solution over 120 min HPH processing at 100 MPa.

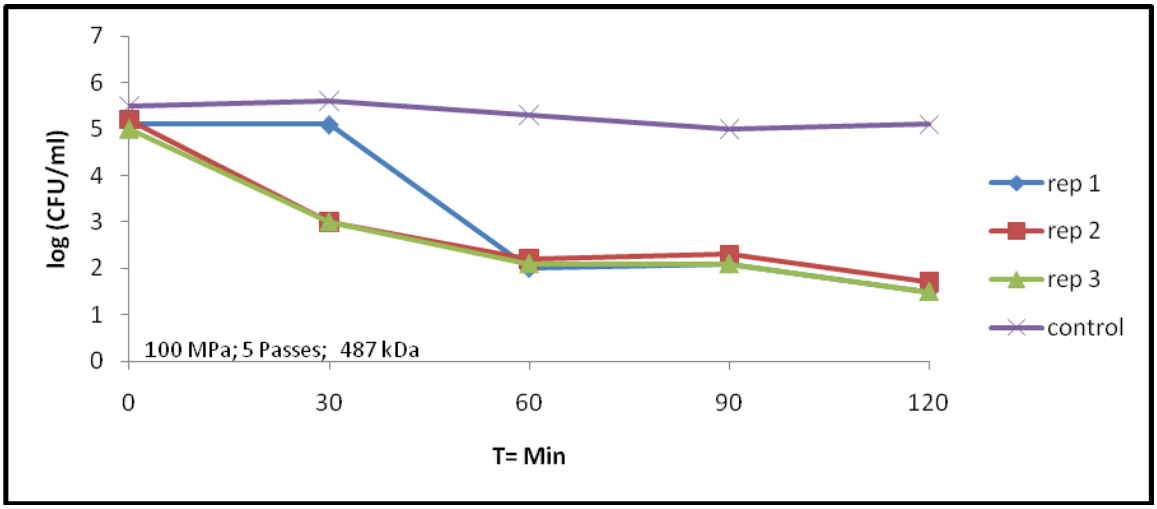

Figure 9. Inactivation of *E. coli* K-12 in chitosan (487 kDa) solution over 120 min. HPH processing at 100 MPa.

Figure 6 shows results of our medium molecular weight range of 410 kDa to 261 kDa, The pressure was increased to 200 MPa, passes 0-5. Figures 10-14 shows samples produced for Mw's 410, 340, and 261 kDa a 3 log reduction. However results for MW's 278 and 308 kDa showed the least reduction with a 1 log, for these results further research is required to ascertain possible mechanism's that may have influenced samples. In prior research there has been clear connection that chitosan's antimicrobial activity depends on specific molecular weight ranges. In this respect I have found no prior research to compare results to due to the fact that most chitosan samples are usually not so close together in MW.

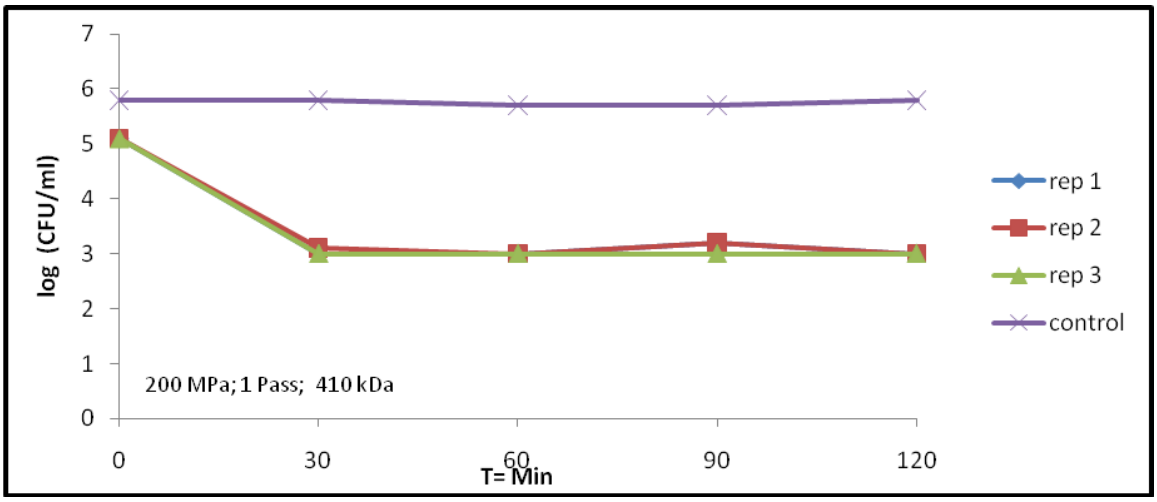

Figure 10. Inactivation of *E. coli* K-12 in chitosan (410 kDa) solution over 120 min after HPH processing at 200 MPa

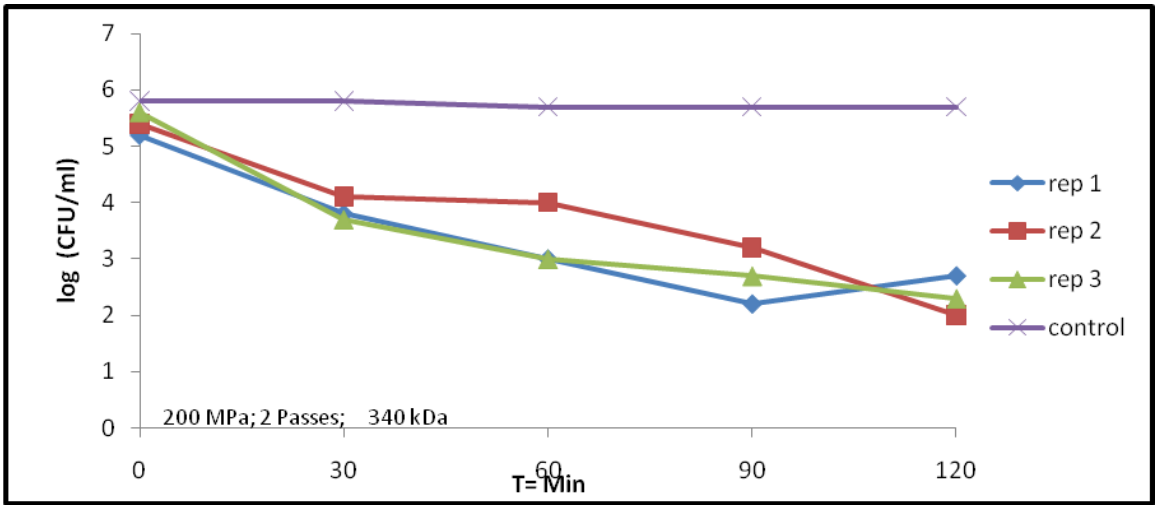

Figure 11. Inactivation of *E. coli* K-12 in chitosan (340 kDa) solution over 120 min after HPH processing at 200 MPa.

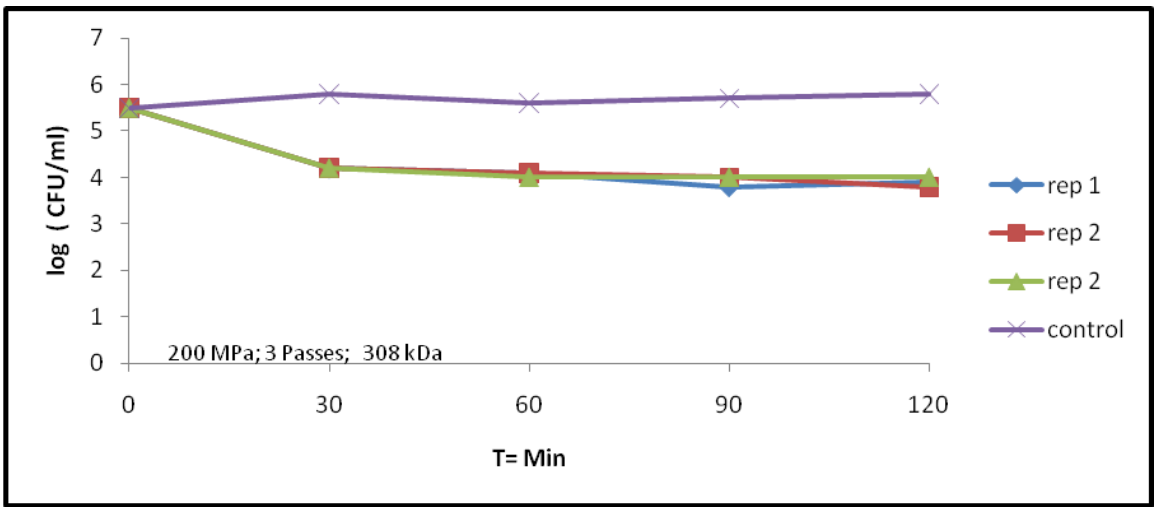

Figure 12. Inactivation of *E. coli* K-12 in chitosan (308 kDa) solution over 120 min after HPH processing at 200 MPa.

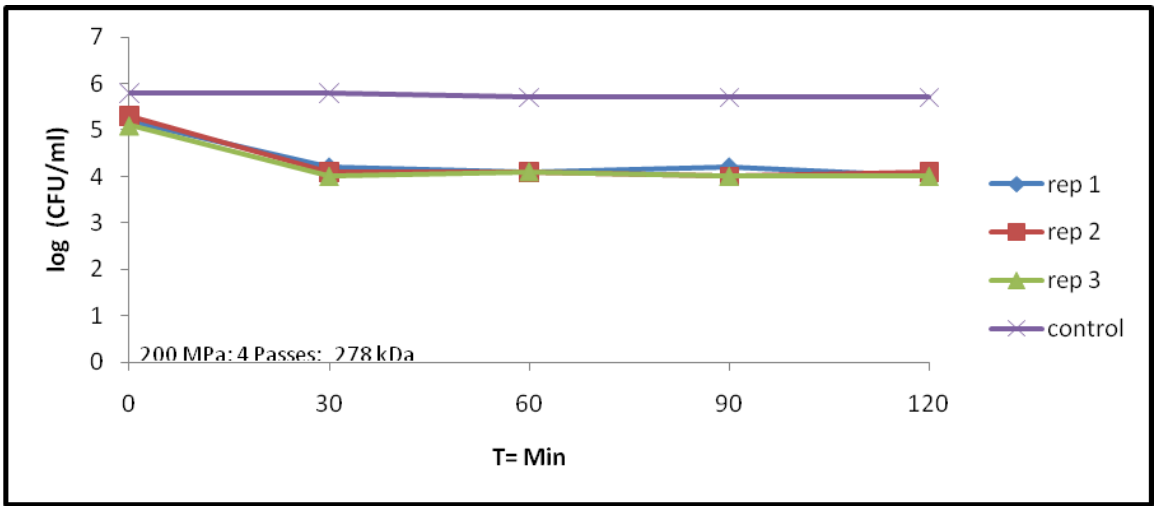

Figure 13. Inactivation of *E. coli* K-12 in chitosan (278 kDa) solution over 120 min after HPH processing at 200 MPa.

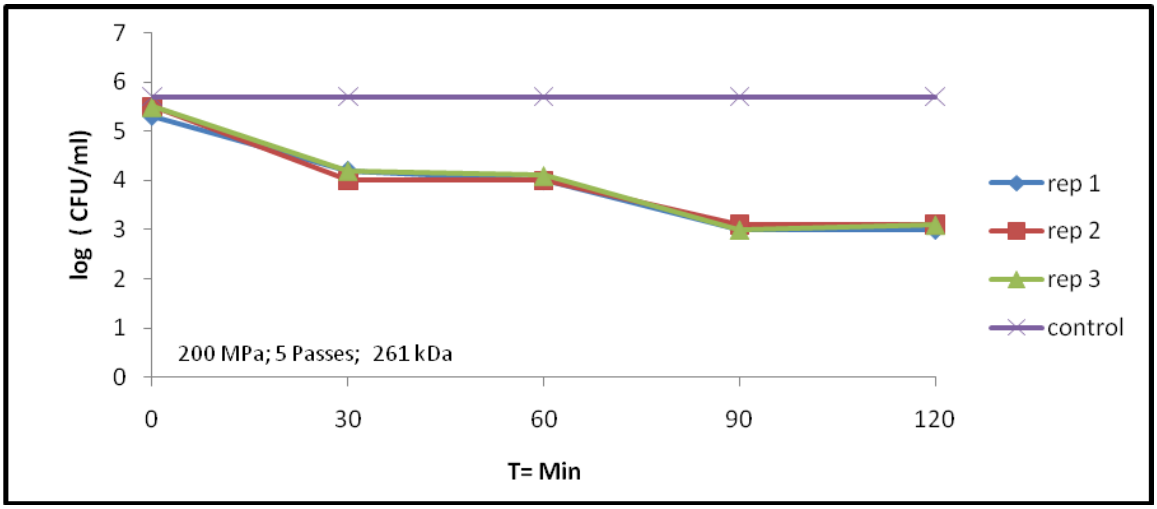

Figure 14. Inactivation of *E. coli* K-12 in chitosan (261 kDa) solution over 120 min after HPH processing at 200 MPa.

Figures 15-19 shows results of experiments using 300 MPa the molecular weight of chitosan is altered from 293 kDa to 168 kDa, as the number of passes increased from 0-5 passes. Average log reduction of 4 to 5 logs decrease in bacterial population this finding is consistent with N. Liu et al. 2006 the antibacterial activity of low MW chitosan is higher than that of higher MW samples.

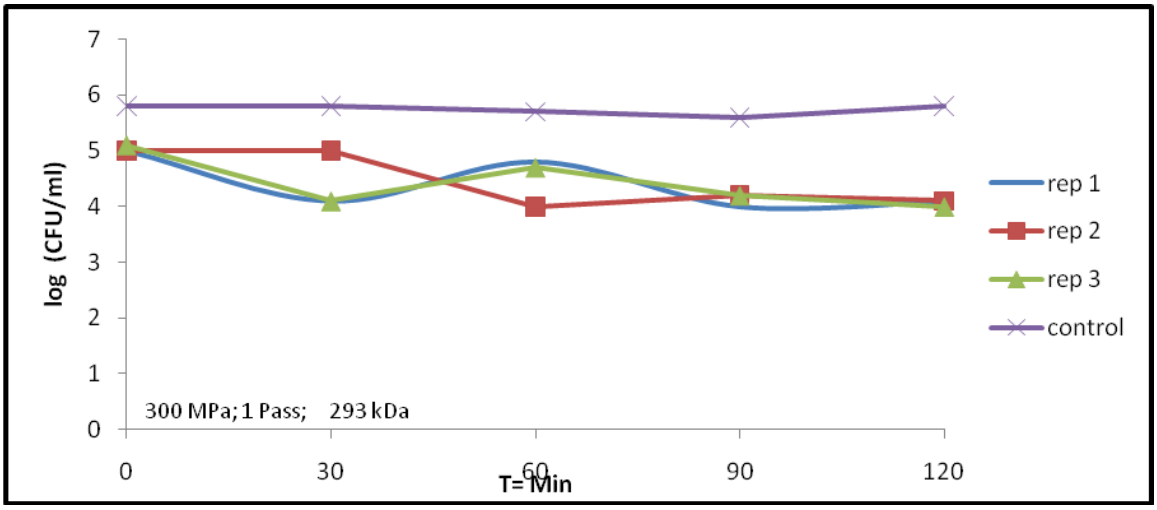

Figure 15. Inactivation of *E. coli* K-12 in chitosan (293 kDa) solution over 120 min after HPH processing at 300 MPa.

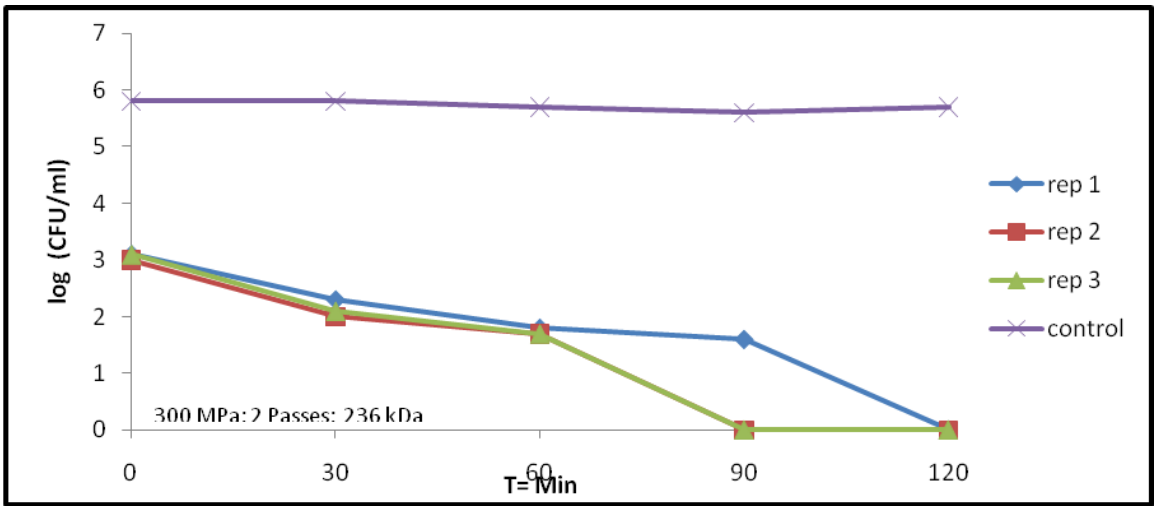

Figure 16. Inactivation of *E. coli* K-12 in chitosan (236 kDa) solution over 120 min after HPH processing at 300 MPa.

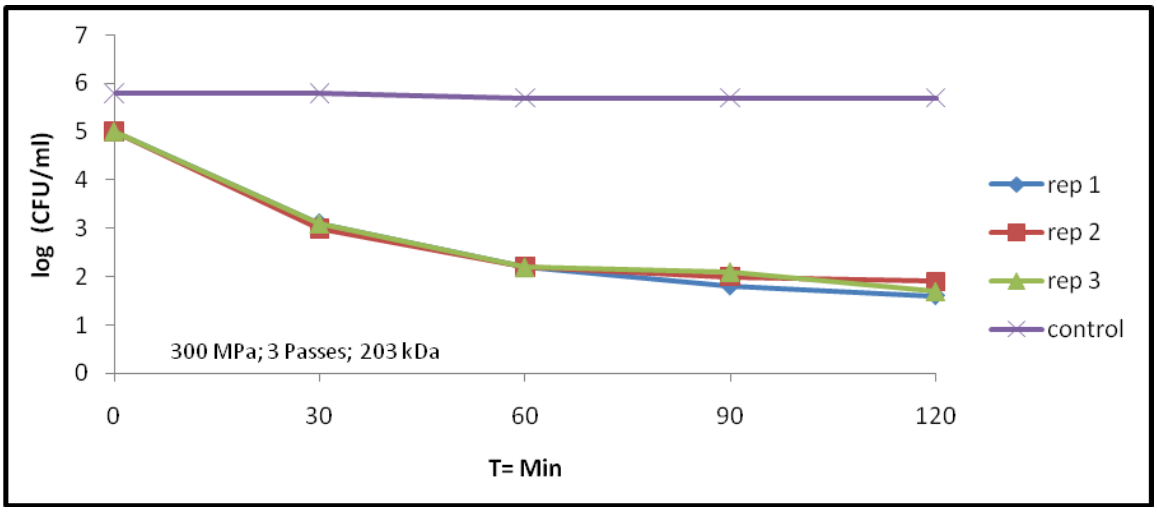

Figure 17. Inactivation of *E. coli* K-12 in chitosan (203 kDa) solution over 120 min after HPH processing at 300 MPa.

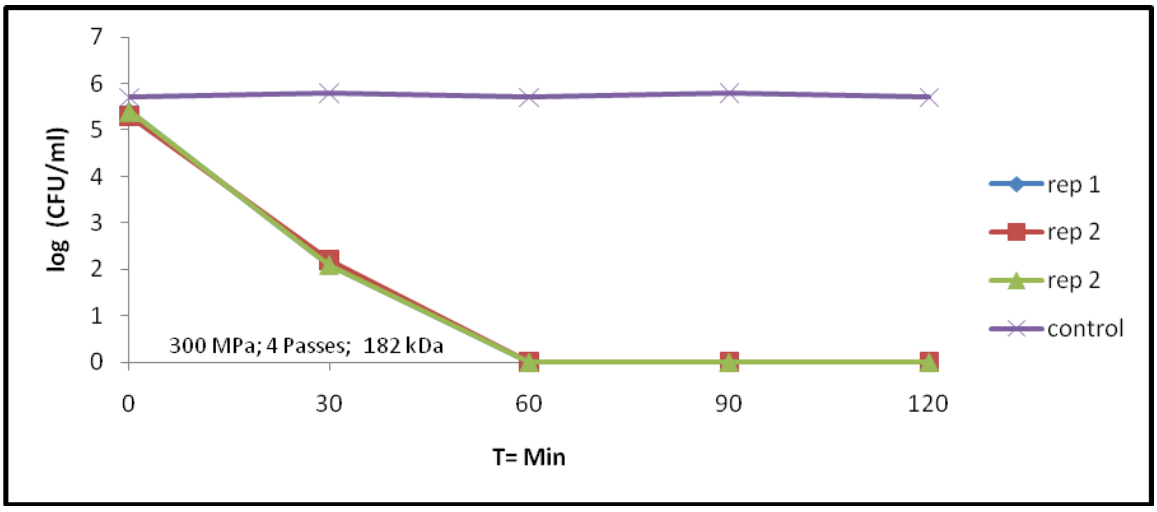

Figure 18. Inactivation of *E. coli* K-12 in chitosan (182 kDa) solution over 120 min after HPH processing at 300 MPa.

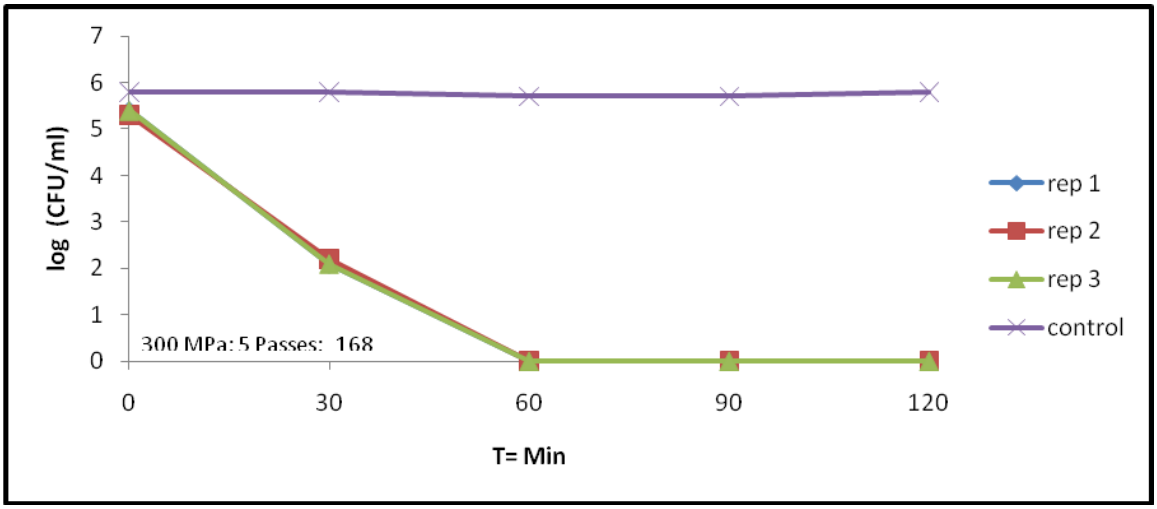

Figure 19. Inactivation of *E. coli* K-12 in chitosan (168 kDa) solution over 120 min after HPH processing at 300 MPa.

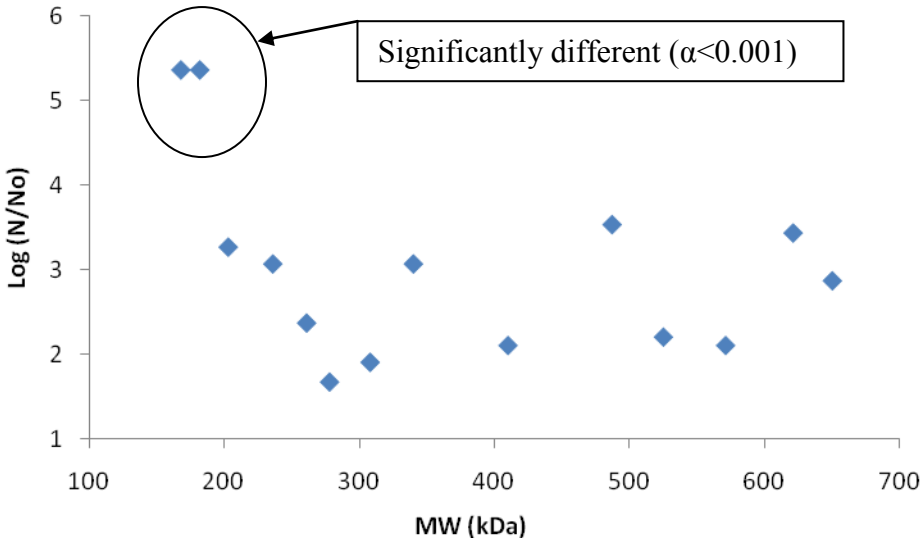

Figure 90. Statistical analysis of effect of molecular weight of chitosan on E. coli K-12 at 2 hours

The results indicate that a one percent (w/v) chitosan solution inhibits the Gram-negative bacteria *E. coli* K-12 to non-detectable levels at molecular weight below cs. 200 kDa after two hours of exposure. Results also suggest that with an extension of time perhaps complete inhibition could have been achieved for MW ranges 658 kDa to 487 kDa.

CONCLUSIONS AND RECOMMENDATIONS

High pressure homogenization has been shown to be an effective tool in decreasing viscosity of a chitosan while producing a specific range in polydispersity and with further research could be used as an instrument to achieve specific molecular weights. The antimicrobial effect of chitosan is strongly dependent on Mw of chitosan sample used. However results showed all chitosan samples to have an effect on *E. coli* in decreasing bacterial population.

Future research would consist of running solubilized chitosan samples through HPH several more times to compare prior results, while increasing parameters to include a deduction of chitosan concentration used in each sample, measurements of polydispersity and application against Gram- positive and Gram-negative bacteria.

REFERENCES

1. **GOULD, G.W., *PRESERVATION: PAST, PRESENT AND FUTURE.* BRITISH MEDICAL BULLETIN, 2000. 56(1): P. 84-96.**

2. **INTVELD, J., *MICROBIAL AND BIOCHEMICAL SPOILAGE OF FOODS: AN OVERVIEW.* INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 1996. 33(1): P. 1-18.**

3. **HUIS IN'T VELD, J.H.J., *MICROBIAL AND BIOCHEMICAL SPOILAGE OF FOODS: AN OVERVIEW.* INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 1996. 33(1): P. 1-18.**

4. **LACROIX, M. AND B. OUATTARA, *COMBINED INDUSTRIAL PROCESSES WITH IRRADIATION TO ASSURE INNOCUITY AND PRESERVATION OF FOOD PRODUCTS - A REVIEW.* FOOD RESEARCH INTERNATIONAL, 2000. 33(9): P. 719-724.**

5. **LACROIX, M. AND B. OUATTARA, *COMBINED INDUSTRIAL PROCESSES WITH IRRADIATION TO ASSURE INNOCUITY AND PRESERVATION OF FOOD PRODUCTS -- A REVIEW.* FOOD RESEARCH INTERNATIONAL, 2000. 33(9): P. 719-724.**

6. **MORRIS, C., A.L. BRODY, AND L. WICKER, *NON-THERMAL FOOD PROCESSING/PRESERVATION TECHNOTOGIES: A REVIEW WITH PACKAGING IMPLICATIONS.* PACKAGING TECHNOLOGY AND SCIENCE, 2007. 20(4): P. 275-286.**

7. **MORRIS, C., A.L. BRODY, AND L. WICKER, *NON-THERMAL FOOD PROCESSING/PRESERVATION TECHNOLOGIES: A REVIEW WITH PACKAGING IMPLICATIONS.* PACKAGING TECHNOLOGY AND SCIENCE, 2007. 20(4): P. 275-286.**

8. **YALDAGARD, M., S.A. MORTAZAVI, AND F. TABATABAIE, *THE PRINCIPLES OF ULTRA HIGH PRESSURE TECHNOLOGY AND ITS APPLICATION IN FOOD PROCESSING/PRESERVATION: A REVIEW OF MICROBIOLOGICAL AND QUALITY ASPECTS.* AFRICAN JOURNAL OF BIOTECHNOLOGY, 2008. 7(16): P. 2739-2767.**

9. **ONISHI, H. AND Y. MACHIDA, *BIODEGRADATION AND DISTRIBUTION OF WATER-SOLUBLE CHITOSAN IN MICE.* BIOMATERIALS, 1999. 20(2): P. 175-182.**

10. **CHIRKOV, S.N., *THE ANTIVIRAL ACTIVITY OF CHITOSAN (REVIEW).* APPLIED BIOCHEMISTRY AND MICROBIOLOGY, 2002. 38(1): P. 1-8.**

11. **NO, H.K., ET AL., *ANTIBACTERIAL ACTIVITIES OF CHITOSANS AND CHITOSAN OLIGOMERS WITH DIFFERENT MOLECULAR WEIGHTS ON SPOILAGE BACTERIA ISOLATED FROM TOFU.* JOURNAL OF FOOD SCIENCE, 2002. 67(4): P. 1511-1514.**

12. **RABEA, E.I., ET AL., *CHITOSAN AS ANTIMICROBIAL AGENT: APPLICATIONS AND MODE OF ACTION.* BIOMACROMOLECULES, 2003. 4(6): P. 1457-1465.**

13. **QI, L.F., ET AL., *PREPARATION AND ANTIBACTERIAL ACTIVITY OF CHITOSAN NANOPARTICLES.* CARBOHYDRATE RESEARCH, 2004. 339(16): P. 2693-2700.**

14. **SCALLAN, E., ET AL., *FOODBORNE ILLNESS ACQUIRED IN THE UNITED STATES - UNSPECIFIED AGENTS.* EMERGING INFECTIOUS DISEASES. 17(1): P. 17 (1).**

15. **COGHLAN, A., *IS ANYTHING SAFE TO EAT?* NEW SCIENTIST, 1998. 157(2115): P. 4-4.**

16. **MAURICE, J., *THE RISE AND RISE OF FOOD POISONING.* NEW SCIENTIST, 1994. 144(1956): P. 28-33.**

17. **DOYLE, M.P., L. R. BEUCHAT, T.J. MONTVILLE, T. ZHAO, J. MENG, AND S. ZHAO, ED. , *FOOD MICROBIOLOGY: FUNDAMENTALS AND FRONTIERS. THIRD EDITION.* FOOD MICROBIOLOGY: FUNDAMENTALS AND FRONTIERS. THIRD EDITION., 2007: P. ED. 3, XVIII + 1038PP.**

18. **NYACHUBA, D.G., *FOODBORNE ILLNESS: IS IT ON THE RISE?* NUTRITION REVIEWS, 2010. 68(5): P. 257-269.**

19. **FRANKLIN, T.J.A.G.A.S., ED. *BIOCHEMISTRY OF ANTIMICROBIAL ACTION*. ED. C.A. HALL. 1981: LONDON.**

20. **TAKEMONO, K., J.SUNAMOTO, AND A. MITA ED. *POLYMERS AND MEDICAL CARE*. 1989; : TOKYO.**

21. **KONG, M., ET AL., *ANTIMICROBIAL PROPERTIES OF CHITOSAN AND MODE OF ACTION: A STATE OF THE ART REVIEW.* INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 2010. 144(1): P. 51-63.**

22. **LI, Y., ET AL., *PHYSICOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL PROPERTY OF CHITOSAN ACETATES.* CARBOHYDRATE POLYMERS, 2007. 67(2): P. 227-232.**

23. **JUNG, E.J., D.K. YOUN, S.H. LEE, H.K. NO, J.G. HA, AND W. PRINYAWIWATKUL, *ANTIBACTERIAL ACTIVITY OF CHITOSANS WITH DIFFERENT DEGREES OF DEACETYLATION AND VISCOSITIES.* INTERNATIONAL JOURNAL OF FOOD SCIENCE AND TECHNOLOGY 2010. 45: P. 676-682.**

24. **JUNG, E.J., ET AL., *ANTIBACTERIAL ACTIVITY OF CHITOSANS WITH DIFFERENT DEGREES OF DEACETYLATION AND VISCOSITIES.* INTERNATIONAL JOURNAL OF FOOD SCIENCE AND TECHNOLOGY, 2010. 45(4): P. 676-682.**

25. **TSAI, G.J., S.L. ZHANG, AND P.L. SHIEH, *ANTIMICROBIAL ACTIVITY OF A LOW-MOLECULAR-WEIGHT CHITOSAN OBTAINED FROM CELLULASE DIGESTION OF CHITOSAN.* JOURNAL OF FOOD PROTECTION 2004. 67: P. 396-398.**

26. **TSAI, G.J., S.L. ZHANG, AND P.L. SHIEH, *ANTIMICROBIAL ACTIVITY OF A LOW-MOLECULAR-WEIGHT CHITOSAN OBTAINED FROM CELLULASE DIGESTION OF CHITOSAN.* JOURNAL OF FOOD PROTECTION, 2004. 67(2): P. 396-398.**

27. **SAGOO, S., R. BOARD, AND S. ROLLER, *CHITOSAN INHIBITS GROWTH OF SPOILAGE MICRO-ORGANISMS IN CHILLED PORK PRODUCTS.* FOOD MICROBIOLOGY, 2002. 19(2-3): P. 175-182.**

28. **DARMADJI, P. AND M. IZUMIMOTO, *EFFECT OF CHITOSAN IN MEAT PRESERVATION.* MEAT SCIENCE, 1994. 38(2): P. 243-254.**

29. **KIM, S.H., H.K. NO, AND W. PRINYAWIWATKUL, *EFFECT OF MOLECULAR WEIGHT, TYPE OF CHITOSAN, AND CHITOSAN SOLUTION PH ON THE SHELF-LIFE AND QUALITY OF COATED EGGS.* JOURNAL OF FOOD SCIENCE 2007. 72: P. S44-S48.**

30. **KIM, S.H., H.K. NO, AND W. PRINYAWIWATKUL, *EFFECT OF MOLECULAR WEIGHT, TYPE OF CHITOSAN, AND CHITOSAN SOLUTION PH ON THE SHELF-LIFE AND QUALITY OF COATED EGGS.* JOURNAL OF FOOD SCIENCE, 2007. 72(1): P. S44-S48.**

31. **IRITI, M., ET AL., *CELL DEATH-MEDIATED ANTIVIRAL EFFECT OF CHITOSAN IN TOBACCO.* PLANT PHYSIOLOGY AND BIOCHEMISTRY, 2006. 44(11-12): P. 893-900.**

32. **KULIKOV, S.N., S.N. CHIRKOV, A.V. IL'INA,S.A. LOPATIN, AND V.P. VARLAMOV *EFFECT OF THE MOLECULAR WEIGHT OF CHITOSAN ON ITS ANTIVIRAL ACTIVITY IN PLANTS.* APPLIED BIOCHEMISTRY AND MICROBIOLOGY 2006. 42: P. 200-203.**

33. **KULIKOV, S.N., ET AL., *EFFECT OF THE MOLECULAR WEIGHT OF CHITOSAN ON ITS ANTIVIRAL ACTIVITY IN PLANTS.* APPLIED BIOCHEMISTRY AND MICROBIOLOGY, 2006. 42(2): P. 200-203.**

34. **EL HADRAMI, A., ET AL., *CHITOSAN IN PLANT PROTECTION.* MARINE DRUGS, 2010. 8(4): P. 968-987.**

35. **FALCON, A.B., ET AL., *THE EFFECT OF SIZE AND ACETYLATION DEGREE OF CHITOSAN DERIVATIVES ON TOBACCO PLANT PROTECTION AGAINST PHYTOPHTHORA PARASITICA NICOTIANAE.* WORLD JOURNAL OF MICROBIOLOGY & BIOTECHNOLOGY, 2008. 24(1): P. 103-112.**

36. **CHIRKOV, S.N., A. V. IL'INA, N. A. SURGUCHEVA, E. V. LETUNOVA, YU. A. VARITSEV, N. YU. TATARINOVA, AND V. P. VARLAMOV, *EFFECT OF CHITOSAN ON SYSTEMIC VIRAL INFECTION AND SOME DEFENSE RESPONSES IN POTATO PLANTS.* FIZIOL. RASTENII 2001. 48(6): P. 890–896.**

37. **UMEMURA, M., ET AL., *DESIGN OF A SIALYLGLYCOPOLYMER WITH A CHITOSAN BACKBONE HAVING EFFICIENT INHIBITORY ACTIVITY AGAINST INFLUENZA VIRUS INFECTION.* JOURNAL OF MEDICINAL CHEMISTRY, 2008. 51(15): P. 4496-4503.**

38. **SU, X.W., S. ZIVANOVIC, AND D.H. D'SOUZA,, *EFFECT OF CHITOSAN ON THE INFECTIVITY OF MURINE NOROVIRUS, FELINE CALICIVIRUS, AND BACTERIOPHAGE MS2.* JOURNAL OF FOOD PROTECTION, 2009. 72(12): P. 2623-2628.**

39. **MENG, X.H., ET AL., *EFFECTS OF CHITOSAN AND OLIGOCHITOSAN ON GROWTH OF TWO FUNGAL PATHOGENS AND PHYSIOLOGICAL PROPERTIES IN PEAR FRUIT.* CARBOHYDRATE POLYMERS, 2010. 81(1): P. 70-75.**

40. **MENG, X.H., L.Y. YANG, J.F. KENNEDY, AND S.P. TIAN, *EFFECTS OF CHITOSAN AND OLIGOCHITOSAN ON GROWTH OF TWO FUNGAL PATHOGENS AND PHYSIOLOGICAL PROPERTIES IN PEAR FRUIT.* CARBOHYDRATE POLYMERS, 2010. 81: P. 70-75.**

41. **CHIEN, P.J. AND C.C. CHOU, *ANTIFUNGAL ACTIVITY OF CHITOSAN AND ITS APPLICATION TO CONTROL POST-HARVEST QUALITY AND FUNGAL ROTTING OF TANKAN CITRUS FRUIT (CITRUS TANKAN HAYATA).* JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE, 2006. 86(12): P. 1964-1969.**

42. **ELGHAOUTH, A., ET AL., *CHITOSAN COATING EFFECT ON STORABILITY AND QUALITY OF FRESH STRAWBERRIES.* JOURNAL OF FOOD SCIENCE, 1991. 56(6): P. 1618-&.**

43. **ROLLER, S. AND N. COVILL, *THE ANTIFUNGAL PROPERTIES OF CHITOSAN IN LABORATORY MEDIA AND APPLE JUICE.* INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 1999. 47(1-2): P. 67-77.**

44. **FILTEN, O., J. C. FRISHVAD AND U. THRANE, *MOULDS IN FOOD SPOILAGE.* INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 1996. 33: P. 85-102.**

45. **VELD, J.H.J.H.I.T., *MICROBIAL AND BIOCHEMICAL SPOILAGE OF FOODS: AN OVERVIEW* INT J. OF FOOD MICROBIOLOGY, 1996. 33: P. 1-18.**

46. **SUTHERLAND, L., ED. *MODELING FOOD SPOILAGE*. IN FOOD PRESERVATION TECHNIQUES. 2003, CRC PRESS: BOCA RATON, FL.**

47. **GRAM, L. AND H.H. HUSS, *MICROBIOLOGICAL SPOILAGE OF FISH AND FISH PRODUCTS.* INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 1996. 33(1): P. 121-137.**

48. **THARANATHAN, R.N., AND KITTUR, F.S. , *CHITIN—THE UNDISPUTED BIOMOLECULE OF GREAT POTENTIAL.* CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION, 2003. 43: P. 61-87.**

49. **KURITA, K., *CHITIN AND CHITOSAN: FUNCTIONAL BIOPOLYMERS FROM MARINE CRUSTACEANS.* MARINE BIOTECHNOLOGY. , 2006. 8: P. 203-226.**

50. **ZHANG, M., HAGA, A., SEKIGUCHI, H. AND HIRANO, S. , INT. J. BIOLOGICAL MACROMOLECULES, 2000. 27 P. 99–105**

51. **XU, Y., C. GALLERT, J. WINTER, *CHITIN PURIFICATION FROM SHRIMP WASTES BY MICROBIAL DEPROTEINATION AND DECALCIFICATION.* ENVIROMENTAL BIOTECHNOLOGY, 2008. 79: P. 687-697.**

52. **TOLAIMATE, A., J. DESBRIERES, M. RHAZI, A. ALAQUI, M. VINCENDON, P. VOTTERO, *ON THE INFLUENCE OF DEACETYLATION PROCESS ON THE PHYSICOCHEMICAL CHARACTERISTICS OF CHITOSAN FROM SQUID CHITIN.* ELSEVIER SCIENCE LTD, 2000. 7: P. 2463-2469.**

53. **PARK, P.J., ET AL., *ANTIMICROBIAL ACTIVITY OF HETERO-CHITOSANS AND THEIR OLIGOSACCHARIDES WITH DIFFERENT MOLECULAR WEIGHTS.* JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, 2004. 14(2): P. 317-323.**

54. **AGULLO, E., M. S. RODRIQUEZ,V. RAMOS, AND L. ALBERTENGO *PRESENT AND FUTURE ROLE OF CHITIN AND CHITOSAN IN FOOD.* MACROMOLECULAR BIOSCIENCE, 2003. 3: P. 521- 530.**

55. **LI, X.F., X. Q. FENG, S. YANG, G.Q. FU, T. P. WANG, AND Z. X. SU *CHITOSAN KILLS ESCHERICHIA COLI THROUGH DAMAGE TO BE OF CELL MEMBRANE MECHANISM.* CARBOHYDRATE POLYMERS, 2010. 79: P. 493-499.**

56. **JAE- YOUNG, J., AND K. SE- KWON *CHITOSAN DERIVATIVES KILLED BACTERIA BY DISRUPTING THE OUTER AND INNER MEMBRANE.* J. AGRIC. FOOD CHEM, 2006. 54: P. 6629-6633.**

57. **RAVI- KUMAR, M.N.V., R. A. A. MUZZARELLI, C. MUZZARELLI, H. SASHIWA, AND A. J. DOMB II. , *CHITOSAN CHEMISTRY AND PHARMACEUTICAL PERSPECTIVE.* CHEM. REV, 2004. 104: P. 6017-6084**

58. **POSPIESZNY, H., H. STRUSZCZYK, S.N. CHIRKOV AND J.G. ATABEKOV, ED. *NEW APPLICATIONS OF CHITOSAN IN AGRICULTURE*. 1994: WIRTSCHAFTSVERLAG, BREMERHAVEN. 246-254.**

59. **LI, Y., X.G. CHEN, N. LIU, C.S. LIU, C.G. LIU, X.H. MENG, L.J. YU, AND J.F. KENENDY, *PHYSICOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL PROPERTY OF CHITOSAN ACETATES.* CARBOHYDRATE POLYMERS 2007. 67: P. 227-232.**

60. **GOOSEN, M.F.A., ED. *APPLICATIONS OF CHITIN AND CHITOSAN* 1996, CRC PRESS LLC: BOCA RATON, FLORIDA. 336.**

61. **SHAHIDI, F., J. K.ARACHCHI,Y. J. JEON *FOOD APPLICATIONS OF CHITIN AND CHITOSANS.* TRENDS IN FOOD SCIENCE & TECHNOLOGY, 1999. 10: P. 37-51.**

62. **RINAUDO, M., *CHITIN AND CHITOSAN:NEXT TERM PROPERTIES AND APPLICATIONS.* PROGRESS IN POLYMER SCIENCE, 2006. 31(7): P. 603-632**

63. **KANKE, M., H. KATAYAMA, S. TSUZUKI AND H. KURAMOTO,, *APPLICATION OF CHITIN AND CHITOSAN TO PHARMACEUTICAL PREPARATIONS.* CHEM PHARM BULL 1989. 37: P. 523–525.**

64. **YOUNG- WOO, C., J. JANG, C. R. PARK, AND S. W. KO, *PREPARATION AND SOLUABILITY IN ACID AND WATER OF PARTIALLY DEACETYLATED CHITINS.* BIOMACROMOLECULES, 2000. 1: P. 609-614.**

65. **NO, H.K., S.P. MEYERS, W. PRINYAWIWATKUL, AND XU, Z. , *APPLICATIONS OF CHITOSAN FOR IMPROVEMENTS OF QUALITY AND SHELF LIFE OF FOODS: A REVIEW.* J. FOOD SCIENCE, 2007. 72: P. 87-100.**

66. **JOAS, J., Y. CARO, M. N. DUNCAMP, M. REYNES, *POSTHARVEST CONTROL OF PERICARP BROWNING OF LITCHI FRUIT (LITCHI CHINENSIS SONN CV KWAI MI) BY TREATMENT WITH CHITOSAN AND ORGANIC ACIDS I. EFFECT OF PH AND PERICARP DEHYDRATION.* POSTHARVEST BIOLOGY TECHNOLOGY, 2005. 38: P. 128-136.**

67. **JIANG, Y., Y. LI, *EFFECTS OF CHITOSAN COATING ON POSTHARVEST LIFE AND QUALITY OF LOGAN FRUIT.* FOOD CHEMISTRY, 2001. 73: P. 139-143.**

68. **ZHANG, D., AND P.C. QUANTICK *EFFECT OF CHITOSAN ON MEMBRANE PERMEABILITY OF SUSPENSION CULTURED GLYCINE MAX AND PHASEOLUS VULGARIS CELLS.* PLANT PHYSIOLOGY, 1997. 70(1): P. 449-454.**

69. **EL GHAOUTH, A., J. ARUL, R. PONNAMPALAM, M. BOULET *CHITOSAN COATING EFFECT ON STORABILITY AND QUALITY OF FRESH STRAWBERRIES.* J. FOOD SCIENCE 1991. 56: P. 1618–1620.**

70. **PARK, S., S.D. STAN, M.A. DAESCHEL, Y. ZHAO *ANTIFUNGAL COATINGS ON FRESH STRAWBERRIES (FRAGARIA × ANANASSA) TO CONTROL MOLD GROWTH DURING COLD STORAGE* J. FOOD SCIENCE, 2005. 70: P. M202–M207.**

71. **PARK S. I., S.S.D., DAESCHEL M. A. AND ZHAO Y. , *ANTIFUNGAL COATINGS ON FRESH STRAWBERRIEW (FRAGARIA X ANANASSA) TO***

CONTROL MOLD GROWTH DURING COLD STORAGE. **J. FOOD SCIENCE, 2005. 70(4): P. 202-207.**

72. **REDDY B.M.V., K.B., R. CORCUFF , F. CASTAIGNER ,J. ARUL *EFFECT OF PRE-HARVEST CHITOSAN SPRAYS ON POST-HARVEST INFECTION BY BOTRYTIS CINERA AND QUALITY OF STRAWBERRY FRUIT.* POSTHARVEST BIOL TECHNOL, 2000. 20: P. 39-51.**

73. **HAN C., Y.Z., S. W. LEONARD, AND M. G TRABER *EDIABLE COATINGS TO IMPROVE STORAGEABILITY AND ENHANCE NUTRTIONAL VAULE OF FRESH AND FROZEN STRAWBERRIES (FRAGARIA X ANANASSA) AND RASPBERRIES (RUBUIS IDEAUS).* POSTHARVEST BIOL TECHNOL, 2004. 33: P. 67-78.**

74. **HERNANDEZ- MUNOZ P., E.A., M. J. OCIO , R. GAVARA *EFFECT OF CALCIUM DIPS AND CHITOSAN COATINGS ON POSTHARVEST LIFE OF STRAWBERRIES (FRAGARIAX ANANASSA).* POSTHARVEST BIOL TECHNOL, 2006. 39: P. 247-253.**

75. **VARGAS M, A.A., A. CHIRALT, AND C. GONZALEZ- MARTINEZ *QUALITY OF COLD-STORED STRAWBERRIES AS AFFECTED BY CHITOSAN-OLEIC ACID EDIABLE COATINGS.* POSTHARVEST BIOL TECHNOL, 2006. 41: P. 164-171.**

76. **OUATTARA B. , R.E.S., G. PIETTE, A. BÉGIN,AND R.A. HOLLEY AND *DIFFUSION OF ACETIC AND PROPIONIC ACIDS FROM CHITOSAN-BASED ANTIMICROBIAL PACKAGING FILMS.* J. OF FOOD SCIENCE, 2006. 65(5): P. 768-773.**

77. **HUI L., Y.D., X. WANG, AND L. SUN, *CHITOSAN KILLS BACTERIA THROUGH CELL MEMBRANE DAMAGE.* INT J. FOOD MICROBIAL, 2004. 95: P. 147-155.**

78. **LIU, H., Y. DU, X. WANG, AND L. SUN, *CHITOSAN KILLS BACTERIAL THROUGH CELL MEMBRANE DAMAGE.* INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 2004. 95: P. 147-155.**

79. **YOUNG, D.H., H. KOHLE, AND H. KAUSS, *EFFECT OF CHITOSAN ON MEMBRANE PERMEABILITY OF SUSPENSION-CULTURED GLYCINE MAZ AND PHASEOLUS VULGARIS CELLS.* PLANT PHYSIOLOGY, 1982. 70: P. 1449-1454.**

80. **ZHENG, L.Y., AND J. F. ZHU. , *STUDY ON ANTIMICROBIAL ACTIVITY OF CHITOSAN WITH DIFFERENT MOLECULAR WEIGHTS.* CARBOHYDRATE POLYMERS, 2003. 54: P. 527-530**

81. **CHUNG, Y.C., Y. P. SU, C. C. CHEN, G. JIA, H. L. WANG, AND J. C. WU. , *RELATIONSHIP BETWEEN ANTIBACTERIAL ACTIVITY OF CHITOSAN AND SURFACE CHARACTERISTIC OF CELL WALL.* ACTA PHARMACOLOGICA SINICA, 2004. 27: P. 932-936.**

82. **HELANDER, I.M., E. L. NURMIAHO-LASSILA, R. AHVENAINEN, J. RHOADES, AND S. ROOLER, *CHITOSAN DISRUPTS THE BARRIER PROPERTIES OF THE OUTER MEMBRANE OF GRAM-NEGATIVE BACTERIA.* INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 2001. 71: P. 234-244.**

83. **TOKURA, S., K. UENO, S. MIYAZAKI, AND N. NISHI, *MOLECULAR WEIGHT DEPENDENT ANTIMICROBIAL ACTIVITY OF CHITOSAN.* MACROMOLECULAR SYMPOSIUMS, 1997. 120: P. 1-9.**

84. **PAPINEAU, A.M., D. G. HOOVER, D. KNORR, AND D. F. FARKAS, *ANTIMICROBIAL EFFECT OF WATER- SOLUBLE CHITOSAN WITH HIGH HYDROSTATIC PRESSURE.* FOOD BIOTECHNOLOGY, 1991. 5: P. 45-57.**

85. **SUDARSHAN, N.R., D. G. HOOVER, AND D. KNOOR, *ANTIBACTERIAL ACTION OF CHITOSAN.* FOOD BIOTECHNOLOGY, 1992. 6: P. 257-272.**

86. **FERNANDES, J.C., F. K. TAVARIA, J. C. SOARES, O. S. RAMOS, M. J. MONTERIO, M. E. PINTADO, AND F. X. MALCATA. , *ANTIMICROBIAL EFFECTS OF CHITOSANS AND CHITOOLIGOSACCHARIDES, UPON STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI, IN FOOD MODEL SYSTEMS.* FOOD MICROBIOLOGY, 2008. 25: P. 922-928.**

87. **ROLLER, S.A.N.C., *THE ANTIFUNGAL PROPERTIES OF CHITOSAN IN LABORATORY MEDIA AND APPLE JUICE.* INT J. FOOD MICROBIAL, 1999(47): P. 66-77.**

88. **OKUYAMA, K., K. NOGUCHI, M. KANENARI, T. EGAWA, K. OSAWA AND K. OGAWA, *STRUCTURE DIVERSITY OF CHITOSAN AND ITS COMPLEXES.* CARBOHYDR. POLYM, 2000. 41 P. 237-247**

89. **SEO, S., J.M. KING AND W. PRINYAWIWATKUL, *SIMULTANEOUS DEPOLYMERIZATION AND DECOLORIZATION OF CHITOSAN BY OZONE TREATMENT.* J. FOOD SCI. , 2007. 72: P. 522-526.**

90. **JEON, Y.J.A.S.K.K., *ANTIMICROBIAL EFFECT OF CHITOOLIGOSACCHARIDES PRODUCED BY BIOREACTOR.* CARBOHYDATE POLYMERS, 2001. 44: P. 71-76**

91. **NO H.K., S.P.M., W. PRINYAWIWATKUL,AND Z. XU, *APPLICATIONS OF CHITOSAN FOR IMPROVEMENT OF QUALITY AND SHELF LIFE OF FOODS: A REVIEW.* JOURNAL OF FOOD SCIENCE, 2007. 72(5): P. R87-R100.**

92. **XIA W.S., A.Y.N.W., *FUNCTIONAL PROPERTIES OF CHITOOLIGOSACCHARIDES* J. WUXI UNIVERSITY LIGHT INDUSTRY, 1996. 15: P. 297-302.**

93. **NO, H.K., N.Y. PARK, S.H. LEE AND S.P. MEYERS, *ANTIBACTERIAL ACTIVITY OF CHITOSANS AND CHITOSAN OLIGOMERS WITH DIFFERENT MOLECULAR WEIGHTS.* INT. J. FOOD MICROBIOLOGY, 2002. 74: P. 65-72.**

94. **UCHIDA, Y., M. IZUME AND A. OHTAKARA, ED. *PREPARATION OF CHITOSAN OLIGOMERS WITH PURIFIED CHITOSANASE AND ITS APPLICATION*. CHITIN AND CHITOSAN: IN SOURCES, CHEMISTRY, BIOCHEMISTRY, PHYSICAL PROPERTIES AND APPLICATIONS. 1989.**

95. **KITTUR, F.S., A. B. VISHU KUMAR, L. R. GOWDA AND R. N. THARANATHAN *CHITOSANO LYSIS BY AN ISOZYME OF ASPERIGILLUS NIGER- A NON- SPECIFIC ACTIVITY.* CARBOHYDR POLYM, 2003. 55: P. 191-196.**

96. **PANTALEONE, D., M. YALPANI AND M. SCOLLER, *UNUSUAL SUSCEPTIBILITY OF CHITOSAN TO ENZYMIC HYDROLYSIS.* CARBOHYDR. RESC. , 1992. 237: P. 325-332.**

97. **ANTHONSEN, M.W., K. M. VARUM AND O. SMIDSROD, *SOLUTIONS PROPERTIES OF CHITOSANS- CONFORMATION AND CHAIN STIFFNESS OF CHITOSANS WITH DIFFERENT DEGREE OF N-ACETYLATION.* CARBOHYDR. POLYM, 1993.**

98. **OTTOY, M.K., K. M. VARUM AND O. SMIDSROD, *COMPOSITIONAL HETEROGENEITY OF HETEROGENEOUSLY DEACETYLATED CHITOSANS.* CARBOHYDR POLYM. , 1996. 29: P. 17-24.**

99. **WANG, W., S. BO, S. LI AND W. QIN, *" DETERMINATION OF THE MARK-HOUWINK EQUATION FOR CHITOSAN WITH DIFFERENT DEGREE OF DEACETYLATION"*. INT J. BIOL MACROMOL, 1991. 13: P. 281-285.**

100. **DRAGET, K.I., VARUM, K. M., MOEN, E., GYNNILD, H., AND SMIDSRΦD, O., BIOMATERIALS, 1992. 13: P. 635-638.**

101. VÅRUM, K.M., M. H. OTTOY, AND O. SMIDSRØD, *ACID HYDROLYSIS OF CHITOSANS.* CARBOHYDATE POLYMERS, 2001. 46: P. 89-98.

102. HARTE, F., AND R. VENEGAS, *A MODEL FOR VISCOSITY REDUCTION IN POLYSACCHARIDES SUBJECTED TO HIGH-PRESSURE HOMOGENIZATION.* JOURNAL OF TEXTURE STUDIES, 2009. 41: P. 49-61.

103. SINGH, R.P.A.D.R.H., ED. *INTRODUCTION TO FOOD ENGINEETING 4TH EDITION*. FOURTH ED. FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL SERIES, ED. S.L. TAYLOR. 2009, ELSEVIER SCIENCE LTD.: BURLINGTON, MA. 155- 159.

104. ARVANITOYANNIS, I.S., *TOTALLY AND PARTIALLY BIODEGRADABLE POLYMER BLENDS ON NATURAL AND SYNTHETIC MACROMOLECULES: PREPARATION, PHYSICAL PROPERTIES, AND POTENTIAL AS FOOD PACKAGING MATERIALS.* JOURNAL OF MACROMOLECULAR SCIENCE, 1999. 39: P. 207-271.

105. ARVANITOYANNIS, I.S., A. NAKAYAMA, S. AIBA, *CHITOSAN AND GELATIN BASED EDIABLE FILMS : STATE DIAGRAMS, MECHANICAL AND PERMEATION PROPERTIES.* CARBOHYDRATE POLYMERS, 1998. 37: P. 371-382.

106. HAGUE, T., H. CHEN, W. OUYANG, C. MARTONI, B. LAWUYI, A. M. URBANSKA., *SUPERIOR CELL DELIVERY FEATURES OF POLY(ETHYLENE GLYCOL) INCORPORATED ALGINATE, CHITOSAN AND POLY-L-LYSINE MICROCAPSULES.* MOLECULAR PHARMACEUTICALS, 2005. 2: P. 29-36.

107. YAMADA, K., Y. AKIBA, T. SHIBUYA, A. KISHIWADA, K. MATSUDA, AND M. HIRATA, *WATER PURIFICATION THROUGH BIOCONVERSION OF PHENOL COMPOUNDS BY TYROSINASE AND CHEMICAL ADSORPTION BY CHITOSAN BEADS.* BIOTECHNOLOGY PROGRESS, 2005. 21: P. 823- 829.

108. JEON, Y.J., P.J. PARK AND S.K. KIM, *ANTIMICROBIAL EFFECT OF CHITOOLIGOSACCHARIDES PRODUCED BY BIOREACTOR* CARBOHYDATE POLYMERS, 2001. 44(1): P. 71-76.

109. MUZZARELLI, R.A.A., M. TOMASETTI, AND P. ILARI, *DEPOLYMERIZATION OF CHITOSAN WITH THE AID OF PAPAIN.* ENZYME AND MICROBIAL TECHNOLOGY, 1994. 16(2): P. 110-114.

110. CHO, Y.-W., ET AL., *PREPARATION AND SOLUBILITY IN ACID AND WATER OF PARTIALLY DEACETYLATED CHITINS.* BIOMACROMOLECULES, 2000. 1(4): P. 609-614.

111. NO, H.K., ET AL., *ANTIBACTERIAL ACTIVITY OF CHITOSANS AND CHITOSAN OLIGOMERS WITH DIFFERENT MOLECULAR WEIGHTS.* INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 2002. 74(1-2): P. 65-72.

VITA

Deidra Shannon Lyons was born in Nashville Tennessee, on April 27th. She grew up in Nashville and attended Middle Tennessee State University where she received a B.S. in Animal Science in May 2007. She got her M.S. in Food Science and Technology from the University of Tennessee Knoxville in May 2011, where she worked with Dr. Federico Harte on novel approaches using high pressure homogenizer to process chitosan. Deidra is planning to pursue a career in product development within the food industry and is looking forward to future challenges.