

EFFECT OF CHITOSAN COATING ON SHELF LIFE OF BLACK TIGER SHRIMP (*PENAEUS MONODONS*)

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ABSTRACT: Chitosan coating serve as an antioxidant and micro-diffusion barrier and prevents the loss of water, texture, odour, color or overall accessibility in seafood. The preservation of shrimps using chitosan dips seems promising and effective, as demonstrated in this study. The antimicrobial property of chitosan is infhenod by slightly acidic pH. This work also showed that the shelf life of *Penaeus monodon* coated with chitosan dips extends the shelf life of shrimp.

Key words: Antimicrobial, Antioxidant, Chitosan coating, Micro-diffusion, Preservation, Shelf life, *Penaeus monodon*.

INTRODUCTION

The shrimp waste contains biopolymers chitin, chitosan, protein with high economical values. Chitosan [β -(1 – 4)-linked N-acetyl-D-glucosamine] is the major structural component of the exoskeleton of crustacean. It is a non-toxic, biodegradable polymer of high molecular weight, and is very much similar to cellulose.

In South-east Asia, the total waste produced in fish industry is over 2 million metric tonnes per year. (Hossain 2003). Production cost for 1 kg of chitosan is about US\$ 15-20/kg 12 kg better quality chitosan is obtained from 200 kg shrimp bio waste (Stevens *et al.* 1998). Chitosan is a new promising technology developed to kill/ inactivate undesirable micro organism in more environmental friendly way

with out affecting food quality. Shellfish chitosan from crab and shrimp comprises of 17-32% of the dry weight of the shell. (No and Meyers 1992) Chitosan emerged from chitin, which is available commercially and used by a lot of people across the globe. Many studies have shown that chitosan is biodegradable polymer (Davies *et al.* 1989). Chitosan is a biomaterial with antiseptic, bioactive, and biocompatible properties (Shigemasa *et al.* 1994). Chitosan has been approved as functional food in some south-east Asian countries during the last decade. The inclusion of chitin and chitosan was considered in 2003 by the Codex Alimentarius Commission. Although several studies have shown that this compound is non-toxic, no long term effects

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on human have been reported.

Sea food industry suffers from different problems like post-processing contamination by bacteria like *Escherichia coli* and *Staphylococcus aureus* during retail display and handling, Lipid oxidation due to contact with oxygen. The above problems shows the way for future research on the use of chitosan as coating film to improve shelf life of products like PUD shrimps, fillets etc. that are kept on retail display.

Chitosan coating helps to extend shelf life of *Penaeus monodon* by reducing microbial load and respiration rate, act as antioxidant and oxygen barrier. The performance of the films will be influenced by the acetic acid (used to soluble chitosan) also. Suitability of the film will also depend on the conditions of storage, namely the temperature and humidity.

Keeping this in view, the major objectives of the present study were to determine the proximate composition of shrimp waste, to extract chitosan from shrimp waste for determining its antimicrobial activity on *S. aureus* and *E. coli*. To assess the storage life of *P. monodon* coated with chitosan film.

MATERIALS AND METHODS

Preparation of samples

P. monodon with an average weight of 35 g were purchased and were transported packed in ice to laboratory in insulated box. The treatment process involved washing with tap water and beheading and peeling is done prior to dipping in the chitosan solution, followed by packing in low density polyethylene (LDPE) bags of 200 gauges and then storage at 4°C on ice.

Preparation of chitosan from shrimp shell

Chitin and chitosan was produced from

shrimp shell waste through steps of process control. Shrimp shell was collected from local south Kolkata processing factory during December, 2009. Chitosan was prepared from shrimp shell by involving size reduction, deproteinization, demineralization, and deacetylation as per Djaeni *et al.*(2002).

The shrimp shell powder was then added by 3.5% NaOH (w/v) for 2 hr at 65°C, in the ratio of (1:10w/v) to obtain chitin. After that, the mixture was then cooled and filtered. The deproteinized shrimp shell was washed by distilled water and dried until 10% water content (Djaeni *et al.* 2002).

The deproteinized shrimp shell was reacted with 1N HCl to remove CaCO₃ content. The process is done in stirred mixer for 1 h at room temperature. CaCO₃ was converted to CaCl₂ that was soluble in water. The mixture was then filtered by vacuum filter to separate solid and liquid phase. The solid phase was washed by pure water and then dried in hot air oven 90 - 100°C for 2 h to yield dry chitin. Then decoloration (bleaching) was done with washing with 0.315% NaOCl (w/v) for 5 min at room temperature [solid: solvent (1:10 w/v)].

The dry chitin is a raw material to produce chitosan through deacetylation process. The chitin powder was mixed with high concentration of NaOH to remove acetyl groups bounded in amine groups of chitin. Here, the acetyl was reacted with 50% NaOH for 2 h at 115 psi or 121°C, solid: solvent (1:10, w/v) to form sodium acetate (Mekawati *et al.* 2000).

Sodium acetate dissolved in the solution, while deacetylated chitin namely chitosan was obtained as solid product. The mixture was separated by vacuum filter to obtain chitosan as solid phase (cake). The cake was washed by

distilled water and then dried in electric oven for 2-4 h at 105°C. The dried chitosan is weighed using electrical balance. The chitosan product is analyzed for water content and ash content to determine the purity of chitosan.

Preparation of the film forming solutions

Chitosan (1% w/v) was dispersed in an aqueous solution of glacial acetic acid (1% v/v), at 40°C. Tween 80 at 0.1% (v/v) was added to improve wettability. After 8 h of stirring, chitosan solution reached final concentration of 1% and 2 % (v/v) respectively. An aqueous solution of 1% acetic acid was produced for estimating the antimicrobial effect of acetic acid. Distilled water was used as control in the experiment. pH was adjusted to 5 with 1N NaOH according to Jumaa *et al.* 2002.

Coating

Selected shrimps were dipped in the film-forming solutions for 30 seconds. Then these were held in for 2 min followed by further dipping for proper coating. Samples were dried by natural convection for 1 h at 40°C and were packed in low density polyethylene (LDPE) bags of 200 gauges and then were stored at 4±1°C.

Study on raw material :

Sensory characteristics

The raw material was subjected for sensory evaluation parameters like general appearance, odor, texture and flavor.

Physical characteristics

Average length and weight were measured for the shrimps were accomplished.

Chemical characteristics

The chemical characteristics like proximate

composition, PV, FFA, TVB-N, TMA of raw material were analyzed.

Microbiological characteristics

The raw material was tested for plate counts (TPC) for bacterial count and agar diffusion method of *Staphylococcus aureus* and *Escherichia coli* were done.

Sampling technique for storage study

Products stored at 4±1°C were opened at an interval of every 2 days for physical, chemical and microbiological analyses. The chemical parameters include proximate composition, PV, FFA, TMA and TVBN. The microbiological analyses included TPC and agar diffusion method. The organoleptic parameters included were color, flavor, taste, texture and overall acceptability.

Proximate composition and quality characteristics :

Determination of moisture content

The moisture content was determined by the standard hot air oven method (A.O.A.C. 1984). About 5 g of finely chopped meat of dry samples were taken in moisture bottles and dried in a hot air oven, maintained at 105±2°C till a constant weight was obtained. The weight loss was expressed as percent moisture content of the sample.

$$\% \text{SOLIDS} = \frac{\text{Weight of solids (after drying)}}{\text{Weight of sample (before drying)}}$$

$$\% \text{ moisture} = 100 - \% \text{ solids}$$

Protein estimation

The method of protein estimation was based on the conversion of organic nitrogen to

inorganic nitrogen following Kjeldahl's method (A.O.A.C. 1995).

About 1 g of sample was transferred to 250 ml of digestion tube and 10-12 ml sulphuric acid was added with 0.2 g of digestion mixture and digested in a digestion chamber till a clear digest was obtained. After cooling the volume was made up to 100 ml with distilled water. Two ml of the solution was taken for distillation in Kjeldahl's distillation unit. The liberated ammonia was absorbed in 2% boric acid solution containing mixed indicator (2% methyl red and 2% methylene blue in 1:1 ratio dissolved in ethyl alcohol), the pink color of boric acid turns green. Then the boric acid was titrated against N/140 standard hydrochloric acid until a pink color end point was obtained.

Total Nitrogen % =

$$\frac{14}{100 \times \frac{1}{2} \times 100} \left\{ \frac{\text{Normality of HCl (Volume random of 1/140 N X HCl - Blank)} \times 1000}{\text{Sample weight}} \right.$$

Determination of fat content

The Soxhlet method (A.O.A.C. 1975) was used to determine the fat content of samples. 2g each of moisture-free samples were extracted with suitable solvent of 55°C to 65°C. On evaporation of the solvent, the fat was left behind in the flask. The difference of weight of the empty flask and the flask with fat gives the fat content of samples.

Determination of ash content

Ash content of samples was estimated as per A.O.A.C. (1984). The inorganic residue as

oxides, sulfates, silicates and chlorides left behind sample heated to temperatures of 500-600°C in muffle furnace for 4-5 h and values were expressed on wet weight basis as percentages.

Quality characteristics :

Total volatile base-nitrogen (TVB-N)

The TVB-N was determined as per Executive Indian Council (1995) recommended method. In this method ammonia and other aliphatic amines in the meat and products were estimated. For this, 100 g of sample was blended with 300 ml of 5% trichloroacetic acid (TCA) solution and filtered to obtain clear extract. 5 ml extract was heated with 5 ml of 2N NaOH. The distillate was collected in 15

ml of 0.01N HCl containing 0.1 ml of resolic acid indicator. After distillation, excess acid was titrated by using 0.01N NaOH to a pale pink end point. One blank was also determined. The result was expressed as mg/100 g of sample.

Trimethylamine (TMA)

The TMA content of the sample was determined by the Conway micro-diffusion method described by Beatty and Fougere (1957). The sample treated with 20% TCA a pre-determined (1 ml) quantity was taken in the inner chamber of Conway micro diffusion

unit. On the outer chamber formalin and concentrated potassium carbonate was laid. After keeping inside a dark place for 3-6 h, it was titrated with 0.002 N sulfuric acid and calculated for TMA content of the sample.

pH

The pH of the sample was determined by the method described by Suzuki (1981). 10 g sample was blended with 20 ml distilled water and pH of the homogenate was measured using a pH meter by immersing the electrodes well inside the blend. The instrument was set using a standard pH buffer.

Peroxide value (PV)

The PV of the lipid was determined from the lipid extract using iodometric method as described by Jacobs (1958). 10 g sample was taken and ground well with 20 g anhydrous Na_2SO_4 . The blend was shaken thoroughly in distilled chloroform for 5-10 min and filtered. Fat content aliquot was determined in 10ml by evaporating it. In another 10ml of aliquot, 20ml of glacial acetic acid was poured and a pinch of potassium iodide was added. The flask was closed and sealed with solution of KI. It was shaken well and kept in the dark place for 30 min. The flask was taken out and sides were washed with distilled water. A few drops of starch indicator were added and titrated immediately against $\text{N}/500 \text{ Na}_2\text{S}_2\text{O}_3$. The end was denoted by the disappearance of the blue color. It is expressed as milliequivalent of O_2 per kg of fat.

Microbiological characteristics

Microbiological characteristics were carried out as per the Standard methods (APHA 1984). Appropriate dilution of the homogenate was

made in a physiological saline (0.85%), plated in duplicate on nutrient agar by spread plate method. The plates were incubated at 37°C for 48 h and TPC was calculated accordingly. Like wise mould count was enumerated on potato dextrose agar at 30°C for 5 days.

Assessment of organoleptic quality

Organoleptic evaluation was carried out by highly experienced judges on a 9-point hedonic scale. The 9-point hedonic scale and score card was adopted.

Statistical analyses

Statistical analyses were performed as per Snedecor and Cochran (1968). Correlation coefficient (r) was calculated for the chemical quality parameters for the raw material to observe their acceptance level. One way analysis of variance (ANOVA) and three way ANOVA followed by least significant test in the form of critical difference was performed to test the significant difference between samples and storage days in the case of dried fish product.

Testing antimicrobial effectiveness of antimicrobial chitosan film :

Agar diffusion method (zone inhibition assay)

Antimicrobial activity test was carried out using agar diffusion method. Indicator cultures were *Staphylococcus sp.* and *Escherichia coli*, representing Gram-positive and Gram-negative bacteria. 100 μl inoculum was added to 5ml of the appropriate soft agar, which was overlaid onto hard agar plates.

Each film was cut into squares (1cm x 1cm) and was placed on the bacterial lawns. Duplicate agar plates were prepared for each

type of film and control film. The plates were incubated for 48 h at 37°C in the appropriate incubation chamber (aerobic chamber for *E.coli*). The plates were visually examined for inhibition zones around the film disc and the size of the zone diameter was measured at two cross sectional points and the average was taken as the inhibition zone. This method was slightly modified from Padgett *et al.* (2000).

RESULTS AND DISCUSSION

The sensory characteristics of whole *P. monodon* are presented in Table 2. The results show that *P. monodon* was having bright and shining appearance with no discoloration. There was no evidence of black spot on the shell and meat and texture was firm and elasticity of muscle remain is perfect. No objectionable foreign material is present in any part of shell or meat. Considering these sensory characteristics, the quality of the raw material was considered as very good.

Chemical parameters like pH, PV, FFA, TMA, TVB-N, and TPC are presented in Tables 1 and 3. In the present study, all the parameters were within the acceptable limit in meat and waste of *P. monodon*.

The recommended microbiological limits for fresh and frozen shrimp are a total plate count of 5×10^5 /g. In this study, the total plate count of the *P. monodon* meat and shrimp waste were 3.4×10^4 /g and 4.3×10^5 /g respectively which is also within the acceptable limit.

Several researchers have reported that the fat content of shrimp waste has a greater value than fresh *P. monodon* meat. In the present study, similar results are encountered.

Storage study of chitosan coated or non-coated *P. monodon*.

Chemical characteristics

PV

The effect of chitosan coating on the changes of PV of *P. monodon* lipids is depicted in Table 1. The initial PV in the samples analyzed was 8.55 miliequivalent of oxygen/kg of fat. The PV values of the control and coated samples increased significantly ($p < 0.05$) with storage time.

The PV up to 30 miliequivalent of oxygen/kg of fat is considered acceptable without any objectionable off taste and odour. It was evident that the control sample crossed the limit of acceptability on 10th day, however the treated samples were well within the acceptable limit.

FFA

It is recommended that a level of FFA in sea foods is 10-12 mg% as a limit of acceptability. The effect of chitosan coating on the FFA in lipids is depicted in Table 1. The initial FFA value in the samples analyzed was 2.23 mg%. The FFA values of the control and coated samples increased significantly ($p < 0.05$) with storage time.

pH

During 10 days of storage study, the pH of uncoated samples increased significantly from 7.26 to 8.67 ($p < 0.05$). It was observed that the pH increased with storage time. But in the case of coated samples, the coating treatments reduced the pH up to 2nd day of storage. The reduction of pH was probably caused by acidic coatings formed on the surface of the samples. The increase in pH indicated the growth of bacteria in the seafood. Generation of a higher concentration of ammonium compounds and other alkaline molecules resulted in higher

microbiological growth and enzymatic activity that was registered during refrigerated storage is the probable reasons for pH rise. It is well known that the pH value of fish tissue gives valuable information about its hygienic condition. As a consequence, this parameter is governed by food legislation in some countries. The pH value must be lower than 7.0-7.5 for the product to be acceptable.

TMA

Trimethylamine (TMA) is a most known compound to indicate freshness quality and degree of spoilage in seafood. TMA is associated with fishy odor of spoilage and formed from TMAO indicating different enzymes. After death, the spoilage bacterial enzymes activate the TMAO and generate TMA.

A level of TMA in sea foods is 5-10 mg N/100 g indicates very good and fair condition of flesh of prawns, but TMA value of 10-12 mg N/100 g as a limit of acceptability. In the present study, the initial TMA value of *P. monodon* was 0.32 mg/100 g. In the case of 1% and 2% chitosan treated samples after 10th day, the samples were not beyond the acceptable limit. But in coating variations also, 1% chitosan coating showed lower TMA values, as 1% chitosan sample had greater antimicrobial effect than other treated or non-treated samples and considering that TMA production was mainly due to specific microbial activity, it may be inferred that the microorganisms load involved in the spoilage in the shrimp samples were probably different, since we would have expected the TMA production from the samples to be correlated with the microbial counts.

Total volatile base nitrogen (TVB-N)

Total volatile base nitrogen (TVB-N), which is mainly composed of ammonia and primary, secondary and tertiary amines, is widely used as an indicator of meat deterioration. Its increase is related to the activity of spoilage bacteria and endogenous enzymes.

A TVB-N value of 20 mg/100 g of muscle indicates that the flesh is in a very good condition, between 20-30 mg/100 g rated as good to fair and a level of 35-40 mg TVB-N / 100 g is regarded as spoiled. There were some significant differences in TVB-N concentrations in *P. monodon* during the study.

Microbiological characteristics

TPC

Seafood provides a good niche for growth of different microorganisms. The initial total plate count of fresh *P. monodon* samples were 3.02 log CFU/g. Chitosan coatings resulted in upto 2-3 log reductions in total plate count between coated samples and control after 12 days of storage of herring and cod in refrigerated storage condition. Chitosan coating significantly lowered the TPC in fish samples ($p < 0.05$) with 0.60-1.19 log CFU/g reduction being obtained in coated samples and the TPC of coated samples were below 10^7 CFU/g during first 2 weeks of cold storage. In the present study chitosan coatings resulted in 3-4.5 log CFU/g reduction in total plate count at $4 \pm 1^\circ\text{C}$ storage temperature as compared to uncoated one.

Chitosan coating consisting of a blend of acetic acid and 1% chitosan exerts an inhibitory effect on the gram- negative flora of fish patties. Various factors affect the antimicrobial action of chitosan and its mechanism of action appears

to be related to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membrane and act as a barrier against oxygen transfer. From the results of this study, it may be also concluded that chitosan coating has a significant ($p < 0.05$) advantage in retarding the growth of bacteria. From the result of the study indicated that 1% chitosan solution coating was effective as 2% chitosan coated samples for extending the shelf life at $4^{\circ} \pm 1^{\circ} \text{C}$ storage temperature, attributing to the better inhibitory effect of 1% chitosan on spoilage bacteria.

Sensory assessment

The results of the sensory evaluation of shrimp samples showed significant decline in all samples. The control and 1% acetic acid treated samples became unacceptable to the testing panels after 6th and 8th day of storage respectively. It is well known that the texture of shrimp is a very good indicator of freshness from an initial firm and slightly chewy to gradual breakdown in structure until soft and mushy at rejection. In the present study, chitosan treated samples maintained all the attributes of colour, odour and taste with acceptable limit till the 10th day of storage. The final acceptability score reaching 6.42 which was significantly higher than the other treatments.

Effect of Chitosan on Microbial Activity Inhibition of *Escherichia coli* on Agar Plate Test

Low molecular weight chitosan seemed to be a more effective inhibitor of microbial growth for some organisms such as *E. coli*. Although chitosan generally show stronger bactericidal effect on gram positive bacteria,

in the present study it is found to be effective in inhibiting the gram negative bacteria *E. coli* at a concentration of both 1% and 2% level. The effect of chitosan may be due to the change in the outer membrane of *E. coli* cell, thereby affecting the barrier properties of bacterial cell. Leakage of intracellular material was one of the mechanisms of chitosan actions which mainly signify the present study.

Inhibition of *Staphylococcus aureus* on Agar Plate Test

The use of chitosan is effective against Gram-positive bacteria like *Staphylococcus aureus*. Effect of chitosan and its derivatives is shown in inhibiting *Staphylococcus aureus* and other Gram-positive bacteria. The antibacterial mechanism of chitosan may be attributed to the interaction of charged amino group of chitosan with negatively charged microbial cell membrane leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms.

Characteristics of raw materials

In the present study, the chemical parameters like pH, PV, FFA, TMA, TVB-N, and TPC were within the acceptable limit in meat and waste of *P. monodon*. This report is compatible with previous report of Giri *et al.* 2004.

Chitosan possesses the functional antioxidant, antimicrobial and oxygen barrier properties (Fan *et al.* 2008). No *et al.* (2002) reported that 1% chitosan concentration can retard gram negative bacteria like *E. coli*, *Vibrio*, *Salmonella* etc. The effect of chitosan may be due to the change in the outer membrane of *E. coli* cell, thereby affecting the barrier properties of bacterial cell (Helander *et al.* 2001). Sudarshan *et al.* (1992) reported

Table 1. Chemical parameters of *P. monodon* meat.

pH	7.28
PV	8.55 (milliequivalent of O ₂ /kg of fat)
FFA	2.23(% of oleic acid)
TMA	0.32 mg/100 g
TVBN	17.74(mg %)
TPC	3.4×10 ⁴ /g

Table 2. Sensory characteristics of *P. monodon* meat.

Appearance	Bright and shining
Color	Body has even grey color, edge of meat stripes well defined
Texture	Firm, body has clearly defined shape
Flavours	Very acceptable
Black spot on meat	None
Discoloration of meat	None
Objecti onable foreign material	None
Overall Quality	Mostly acceptable

Table 3. Chemical parameters of shrimp waste.

pH	7.23
PV	8.42 (milli equivalent of O ₂ /kg of fat)
FFA	2.97(% of olei c acid)
TMA	0.39 mg/100 g
TVBN	15.9 (mg %)
TPC	4.3×10 ³ /g

that leakage of intracellular material was one of the mechanisms of chitosan actions which mainly signify the present study. No *et al.* (2002) reported the use of chitosan is effective against Gram- positive bacteria like *Staphylococcus aureus*. Muzzarelli *et al.* (1990) reported the effect of chitosan and its derivatives in inhibiting *Staphylococcus aureus* and other gram-positive bacteria. The antibacterial mechanism of chitosan may be attributed to the interaction of charged amino group of chitosan with negatively charged microbial cell membrane leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms. (Shahidi *et al.* 1999).

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REFERENCES

A.O.A.C.(1975). Official methods of analysis. 12th edn. Washington DC.

A.O.A.C.(1984). Official methods of analysis. 14th edn. Washington DC.

A.O.A.C.(1995). Official methods of analysis. 16th edn. Washington DC.

APHA.(1984). In: Compendium of methods for the Microbiological Examination of Foods. ed. Speck, M. L., 2nd edn. American Public

Health Association. Washington. DC.

Beatty SA and Fougere H.(1957). The processing of Dried salted fish. Bulletin No.112. Fisheries Research Board of Canada. Ottawa.

Davies DH, Elson CM and Hayes ER.(1989). N, O-carboxymethyl chitosan, a new water soluble chitin derivative. In: Skjak-Braek G., Anthonsen T. and Sandford P. (eds.) Chitin and chitosan: sources, chemistry, biochemistry, physical properties and applications. Elsevier Applied Science. England. p.467-472.

Djaeni M, Nur R, Hargono and Aji P.(2002). Prospect of Chitosan from Shrimp Shell as Expoted Commodity. Community Service Report. Diponegoro University.

Fan W-J, Chi YL and Zhang S.(2008). The use of a tea polyphenol dips to extend the shelf life of silver carp (*Hypophthalmichthys molitrix*) during storage in ice. *Food Chem.* 108: 148-153.

Giri A and Dora KC.(2004). Shrimp waste utilization with special emphasis on flavour extraction and value addition. M.F.Sc. thesis submitted to West Bengal University of Animal and Fishery Sciences. Kolkata. India.

Helander IM, Lassila EL, Ahvenainen R, Rhoades J and Roller S.(2001). Chitosan disrupts the outer membrane of gram-negative bacteria. *Internat. J. Food Microbiol.* 71: 235-244.

Hossain MI.(2003). Study on conservation of shrimp biowaste to environmental friendly products. B.Sc. Thesis. BGE Discipline. Khulna University. Khulna. Bangladesh.

Jacobs MB.(1958). The chemical analysis of foods and food products. New York Krieger Publishing Co. Inc. p.393-394.

Jumaa M, Furkert FH and Muller BW.(2002). A new lipid emulsion formulation with high antimicrobial efficacy using chitosan. *European J. Pharm. Biopharm.* 53: 115-123.

Mekawati, Fachriyah E and Sumarjo D.(2000). Application of chitosan from shrimp shell to adsorb Plumbum ion. *J. Sains. Matematika.* 8(2): 51-54.

Muzzarelli R, Tarsi R, Filippini O, Giovanetti E, Biagini G and Varaldo PE.(1990). Antimicrobial properties of N-carboxybutyl chitosan. *Antimicrob. Agents Chemother.* 34(10): 2019-2023.

No HK and Meyers SP.(1992). Utilization of crawfish processing wastes as carotenoids, chitin, and chitosan sources. *J. Korean Soc. Food Nutri.* 21(3): 319-26.

No HK, Park NY, Lee SH and Meyers SP.(2002). Antimicrobial activity of chitosan and chitosan oligomers with different molecular weights. *J. Food Microbiol.* 74: 65-72.

Padgett T, Han IY and Dawson PL.(2000). Effect of Loric acid addition on the

antimicrobial efficacy and water permeability of corned zein films containing nisin. *J. Food Pro. Pres.* 24:423-432

Shahidi F, Arachchi JKV and Jeon YJ.(1999). Food applications of chitin and chitosans. *Trends Food Sci. Tech.* 10(2): 37-51.

Shigemasa Y, Saito K, Sashiwa H and Saimoto H.(1994). Enzymatic degradation of chitins and partially deacetylated chitins. *Internat. J. Biol. Macromol.* 16(1): 43-9.

Snedecor GW and Cochran WG.(1968) Statistical Methods. Oxford and IBH Pub. Co. SOFT (India) Publication Calcutta. p.221-231.

Stevens WF, Cheypratub P, Lertsutthiwong P, Hein S, Ng CH and S Chandkrachang.(1998). Alternatives in Shrimp Biowaste Processing. In: Advances in Shrimp Biotechnology. p.19-25.

Sudarshan NR, Hoover DG and Knorr D.(1992). Antibacterial action of chitosan. *Food Biotech.* 6(3): 257-272.

Suzuki T.(1981). Fish and Krill protein processing technology. Applied Science Publication Ltd. London. p.62-112.