PRINT ISSN 1119-8362
ELECTRONIC ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 23 (3) 411-415 March 2019

Changes in pH affects Bioactivity of Chitosans from *Callinectes sapidus*

***OMOGBAI, BA; IKENEBOMEH, MJ; OBAZENU, EI; IMONI, AA**

Department of Microbiology, Food and Industrial Division, Faculty of Life Sciences, University of Benin, Benin City, Nigeria
**Corresponding Authors Email: barry.omogbai@uniben.edu, Tel: +2348023579328*

ABSTRACT: The effect of pH on the antimicrobial activity of chitosans was evaluated with five different molecular weight chitosans (DMPAC: M_W 152; DMPCA: M_W 338; $DPMCA_{(2)}$: M_W 418; DMCPA: M_W550 and DCMPA,M_W 558) at 500µg/ml concentration on different food-borne bacteria and fungi. Studies on pH was carried out at pH 3.0, 3.5, 4.0, 5.0 and 5.8 with 500µg/ml chitosan using an overnight broth of bacteria (0.05ml) sub-cultured in nutrient broth or MRS broth (for lactic acid bacteria). Fungi were incubated at 28± 2^0C for 72h and enumerated on sabouraud dextrose agar. The viable cell count of *Staphylococcus aureus* at pH 3.0 for all chitosans ranged between 1.23-1.76Log_{10}CFU/ml while at pH 5.8 viable cell numbers was 2.38-5.26log_{10}CFU/ml compared to the initial inoculum number of 7.06Log_{10}CFU/ml. The growth of *Listeria monocytogenes* was totally suppressed by 500µg/ml chitosan at or below pH 5.0. *Bacillus subtilis* was susceptible to inhibition at low pH and had no detectable viable cell counts at pH 3.0-4.0. The viable cell numbers of *Escherichia* coli 0157:pH7 were reduced by approximately 2 log_{10} cycles at pH 5.8 and by more than 5 logs at pH3.0 with DMPAC chitosan. *Rhizopus Stolonifer* was reduced to non-detectable levels by DMPAC chitosan at pH 3-3.5.This mould was more sensitive to chitosan (500µg/ml) at all pH compared to *Penicillium expansum* and *Aspergillu sniger. Saccharomyces cerevisiae* and *Pichia fermentans* were similarly affected by low pH. The results of the present study show that application of chitosan to acidic foods such as fruit juices will enhance its effectiveness as a natural preservative.

DOI: https://dx.doi.org/10.4314/jasem.v23i3.7

Dates: Received: 07 February 2019; Revised: 21 March 2019; Accepted 27 March 2019

Keywords: Antimicrobial, chitosans, Crab, Acidity, Microorganisms

Chitosan is a natural polysaccharide comprising copolymer of glucosamine and N-acetylglucosamine. It can be obtained by the deacetylation of chitin from crustacean shells. (No and Meyers, 1989). Chitin and chitosan have very similar chemical structures. Chitin exhibits structural similarity to cellulose and differs from it with the replacement of C-2 hydroxyl residues by acetamide groups (Kurita, 1998). Chitin can be transformed into chitosan that has free amino groups by removing acetyl groups (CH_3-CO) from chitin molecules. Thus chitosan is the deacetylated form of chitin, meaning that the acetamide groups (CH_3CO-NH) in chitin are substituted into amino groups (-NH_2) in chitosan (Sakthivel *et al*., 2015). Hsu *et al*. (2002) reported that chitosan is insoluble in water, alkali and organic solvents, but soluble in most diluted acids with pH less than 6. When chitosan is dissolved in an acid solution, it becomes a cationic polymer due to the protonation of free amino groups on the C-2 position of the pyranose ring. The cationic properties of chitosan in acidic solutions give it the ability to interact readily with negatively charged molecules such as lipids and cholesterol. In this respect, chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility, biodegradability, absorption, ability to form films and to chelate metal ions (Li *et al*., 1992).Chitosan have been reported to have antimicrobial activity (Omogbai and Ikenebomeh,2013; Sakthivel *et al*., 2015).In the light of the above, this paper examines the effect of changes in acidic pH on the antimicrobial properties of chitosan.

MATERIALS AND METHODS

Source of microorganisms: The microorganisms used in the study were bacteria including *Salmonella typhimurium, Escherichia coli* 0157:H7, *Pseudomonas fluorescens, Vibrio parahaemolyticus* and *Listeria monocytogenes* which were stool isolates obtained from Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria. *Staphylococcus aureus (SAUBT$_1$),* was a clinical wound isolate obtained from the Department of Medical Microbiology, University of Benin Teaching Hospital, Benin City, Nigeria. *Bacillus subtilis, Lactobacillus casei and Lactobacillus plantarum* were obtained from fruit wastes. Microorganisms were characterized based on shape, size and colour of colony and inspected by light microscopy. The bacteria were Gram-stained (Roberts *et al*., 1995). Phenotypic profiling of both Gram-positive and Gram-negative bacteria was undertaken using API 50CHB and API 20E strips (BioMerieux, Marsielle, France) respectively. Fungi (*Saccharomyce scerevisiae, Pichia fermentans, Penicillium expansum, Aspergillus niger* and *Rhizopus stolonifer*) employed in the studies were

isolated from tropical fruits wastes of pineapple and watermelon.

Source of chitosan: This was obtained from *Callinectes sapidus* by unconventional methods outlined by Omogbai and Ikenebomeh (2016).

Effect of pH on the antimicrobial activity of chitosan: modified method of Youssuf and Munir (2007) was employed. The effect of pH on the antimicrobial activity of chitosans was evaluated with five different molecular weight chitosans (DMPAC,M_W 152; DMPCA, M_W 338;DPMCA$_{(2)}$,M_W 418; DMCPA M_W, 550;DCMPA,M_W 558) at a 0.05% (500µg/ml) concentration on different food-borne bacteria and fungi. The upper pH value studied was limited to 5.8 because chitosan is soluble in most organic acid solutions with less than pH 6 (Muzzarelli, 1973). Studies on pH were carried out at pH 3.0, 3.5, 4.0, 5.0 and 5.8. The solutions were adjusted to these pH values with 1N HCL and 1N NaOH. Then 0.05ml of overnight broth of each microorganism subcultured in nutrient broth or MRS broth (for lactic acid bacteria) were inoculated into 10ml of nutrient broth or MRS broth containing 0.05% chitosan and incubated at 37^0C for 24h. Viable cells were enumerated on nutrient agar or MRS agar by pour plating 1ml after serial dilutions of the chitosan solutions followed by incubation at 37^0C for 24h.Fungi were enumerated on sabouraud dextrose agar and incubated at 28 ± 2^0C for 72h.

RESULTS AND DISCUSSION

The effects of pH 3.0-5.8 on antimicrobial activity of crab chitosans are shown in Tables 1-3. The effect of pH on the antibacterial activity of chitosans was evaluated with five different molecular weight chitosans at 0.05% (500µg/ml) concentration on five Gram-positive bacteria (Table 1).As shown in Table 1, the antibacterial activity of chitosan was affected by pH, with greater activity at lower pH values. The viable cell count of *Staphylococcus aureus*at pH 3.0 for all chitosans ranged between 1.23-1.76Log_{10}CFU/ml while at pH 5.8 viable cell numbers was 2.38-5.26log_{10}CFU/ml compared to the initial inoculum number of 7.06Log_{10}CFU/ml. Among the chitosans, DMPAC had the lowest viable cell count of 1.23Log_{10}CFU/ml at pH3.0. Chitosan DCMPA had the highest viable cell count (5.26Log_{10}CFU/ml) for this organism at pH 5.8. The growth of *Listeria monocytogenes* was totally suppressed by 500µg/ml chitosan at or below pH 5.0. *Bacillus subtilis* was susceptible to inhibition at low pH and had no detectable viable cell counts at pH 3.0-4.0 using 500µg/ml with chitosans DMPAC, DMPCA, DPMCA $_{(2)}$ and DMCPA. The chitosan DCMPA although suppressed the growth of this organism had viable cell numbers of 1.18Log_{10}CFU/ml at pH 3.0 and 4.06Log_{10}CFU/ml at pH 5.8.

Lactobacillus casei and *Lactobacillus plantarum* also had their cell numbers reduced at low pH than at higher pH.At pH 3.0 the viable cell numbers of *Lactobacillus casei* ranged between 1.08-2.03Log_{10}CFU/ml, 1.21 -2.16Log_{10}CFU/ml (at pH 4.0) and 2.60-4.03Log_{10}CFU/ml (at pH 5.8). The antibacterial activity of all chitosans was stronger with decrease in pH against *Lactobacillus plantarum*. At pH 3.0 and 3.5 no viable cells were detected for DMPAC chitosan, 2.05 Log_{10}CFU/ml at pH 4.0, and 2.52Log_{10}CFU/ml at pH 5.8. Thus for all gram-positive bacteria tested, the lower the pH, the greater the antimicrobial activity of all chitosans used.

The effect of pH on the antibacterial activity of chitosans on Gram-negative bacteria is illustrated in Table 2. The most antimicrobial activity was found at low pH values. The viable cell numbers of *Escherichia* coli 0157:H7 were reduced by approximately 2 log_{10} cycles pH 5.8 and by more than 5 logs at pH3.0 with DMPAC chitosan. The range of viable cell reduction by chitosans at pH 3.0 for *Escherichia coli* 0157:H7 was 2.05-2.42Log_{10}CFU/ml, 3.30-3.62Log_{10}CFU/ml (pH 3.5), 4.65-4.92Log_{10}CFU/ml (pH 4.0), 5.32-5.83Log_{10}CFU/ml (pH5.0) and 5.68-5.95Log_{10}CFU/ml (pH 5.8) respectively (Table 2). The viable cell numbers of *Salmonella typhimurium*in a control experiment at pH 3.0 increased from 7.57 -9.23Log_{10}CFU/ml. The addition of chitosans to the medium caused a reduction in the viable cell count in the range 2.08-2.61Log_{10}CFU/ml. At pH 3.5, the viable cell count also reduced to 3.41 - 4.82Log_{10}CFU/ml. With DMPAC chitosan, *Salmonella typhimurium* was reduced by approximately 5.5 log cycle at pH 3.0 compared to 2 log cycle at pH 5.8.The growth of *Pseudomonas fluorescens* and *Vibrio parahaemolyticus* were similarly affected by low acidic pH values. With DMPCA chitosan addition at pH 3.0 the viable cell count of these bacteria was reduced to non-detectable levels but at pH 5.8 the viable cell counts were reduced to 1.94 and 3.22Log_{10}CFU/ml respectively (Table 2).

The effect of pH on the antifungal activity of chitosan on yeast and moulds is shown in Table 3. Although yeasts and moulds can survive in acidic pH, their numbers were decimated considerably with the addition of 500µg/ml of chitosan to the growth medium. *Saccharomyces cerevisiae* for example at pH 3.0 was reduced to 1.35 -1.59Log_{10}CFU/ml compared to 4.25-4.46Log_{10}CFU/ml at pH 5.8. *Saccharomyces cerevisiae* at pH 3.5 was reduced by 3log cycles by DCMPA (Mw, 558 KDA) compared to less than 1 log cycle reduction at pH 5.8.

Table 1: Effect of pH on the Antibacterial Activity of Chitosans based on Colony Counts ($LOG_{10}CFU/ml$)[1] on Gram-positive Bacteria

Microorganisms(Bacteria)	**pH**				
	3.0	**3.5**	**4.0**	**5.0**	**5.8**
Staphylococcus aureus					
Initial	7.06	7.06	7.06	7.06	7.06
Control	9.21	9.24	9.26	9.28	9.31
DMPAC (152)	1.23e	1.56d	2.08c	2.25b	3.02a
DMPCA(338)	1.58d	1.76c	2.30b	3.36a	2.38b
$DPMCA_{(2)}$(418)	1.49d	1.65c	2.47b	3.31b	4.23a
DMCPA(550)	1.54d	1.72c	2.65b	2.68b	5.04a
DCMPA(558)	1.76c	1.81c	2.82b	2.94b	5.26a
Listeria monocytogenes					
Initial	6.45	6.45	6.45	6.45	6.45
Control	8.60	8.76	8.81	8.85	8.89
DMPAC (152)	ND[2b]	NDb	NDb	NDb	2.26b
DMPCA (338)	NDb	NDb	NDb	NDb	2.48a
$DPMCA_{(2)}$ (418)	NDb	NDb	NDb	NDb	2.53a
DMCPA (550)	NDb	NDb	NDb	NDb	2.60a
DCMPA(558)	NDb	NDb	NDb	NDb	2.73a
Bacillus subtilis					
Initial	6.30	6.30	6.30	6.30	6.30
Control	7.85	7.90	9.94	8.01	8.12
DMPAC (152)	NDc	NDc	NDc	1.13b	2.15a
DMPCA (338)	NDc	NDc	NDc	1.24b	2.20a
$DPMCA_{(2)}$ (418)	NDb	NDb	NDb	NDb	324a
DMCPA (550)	NDb	NDb	NDb	NDb	3.58a
DCMPA (558)	1.18c	1.21c	NDd	2.05b	4.06a
Lactobacillus casei					
Initial	6.75	6.75	6.75	6.75	6.75
Control	8.23	8.67	8.89	8.99	9.24
DMPAC (152)	1.08e	1.15d	1.21c	1.56b	2.60a
DMPCA (338)	1.43d	1.49c	1.48c	1.78b	2.87a
$DPMCA_{(2)}$ (418)	1.62d	1.73c	1.85b	1.91b	3.52a
DMCPA (550)	1.65d	1.72c	1.96b	1.98b	3.49a
DCMPA (558)	2.03d	2.10c	2.16b	2.20b	4.03a
Lactobacillus plantarum					
Initial	6.71	6.71	6.71	6.71	6.71
Control	8.76	8.85	8.91	8.96	9.09
DMPAC (152)	NDd	NDd	2.05c	2.17b	2.52a
DMPCA (338)	NDd	1.24d	1.56c	2.89b	3.17a
$DPMCA_{(2)}$ (418)	1.58d	1.72c	1.84b	2.92b	3.86a
DMCPA (550)	2.57e	2.68d	3.04c	3.15b	4.10a
DCMPA (588)	3.06cd	3.13c	3.19c	3.40b	4.33a

NOTE: *a - e Means with different letters within a row indicate significant difference ($p < 0.05$); 1. Viable cells after incubation without (control) and with 0.05% chitosan for 24h at 37°C; 2. ND = Not detected. Figures in parathenses are Molecular weight*

The yeast *Pichia fermentans* was similarly affected by pH. At pH3.0 the log reduction was in the range 1.48-2.20Log_{10}CFU/ml, 2.31-2.50Log_{10}CFU/ml at pH 3.5, 2.62-2.72Log_{10}CFU/ml at pH 4.0, 2.70-2.85Log_{10}CFU/ml at pH 5.0 and 3.17-3.41Log_{10}CFU/ml, at pH 5.8. The log reduction in counts of this organism decreased with increasing pH (Table 3).

The growth of the moulds *Penicillium expansum, Aspergillus niger* and *Rhizopus stolonifer* were affected by pH on the addition of 500 µg/ml of chitosans to the medium. In the control experiment with no chitosan, *Penicillium expansum* grew from 5.20-8.56Log10CFU/ml at pH 3.0 and 5.20-9.02 log cfu/ml at pH 5.8. With chitosan addition *Penicillium expansum* showed 1.11-1.87 viable cell log_{10} number at pH 3.0, 1.51-2.17 (pH 3.5), 3.02-3.31 (pH 4.0), 3.35-3.63 (pH 5.0) and 4.02-4.17 (pH 5.8) in that order. The log reduction of *Aspergillus niger* was greater at low pH values. With DMPAC chitosan, a 3.23log_{10} reduction occurred at pH 3.0 compared to 1.30log_{10} reduction at pH 5.8. *Rhizopus Stolonifer* was reduced to non-detectable levels by DMPAC chitosan at pH 3-3.5.This mould was more sensitive to chitosan (500µg/ml) at all pH compared to *Penicillium expansum* and *Aspergillus niger.* At pH 5.8 the range of log reduction was 4.02-4.17, 4.0-4.50 and 2.30-2.86 for *Penicillium expansum, Aspergillus niger* and *Rhizopus stolonifer* respectively (Table 3).

The antimicrobial activity of chitosan was found to increase with decreasing pH. Decrease in pH increased solubility by forming polycationic polymer. This is due to the fact that amino groups of chitosan become ionized at pH below 6 and carry a positive charge. (Muzzarelli, 1973).

The greater the positive charge the more active this polymer becomes. It is worthwhile to note that growth of *Listeria monocytogenes* and *Bacillus subtilis* were completely deactivated or suppressed by 500µg/ml chitosan at or below pH 5.5. The presence of *Listeria monocytogenes* in foods has become a concern in recent years.

Table 2: Effect of pH on Antibacterial Activity of Chitosans based on Colony Counts ($LOG_{10}CFU/ml$)[1]on Gram-negative Bacteria

Microorganisms (Bacteria)	pH				
	3.0	3.5	4.0	5.0	5.8
Escherichia coli 0157:H7					
Initial	7.67	7.67	7.67	7.67	7.67
Control	8.88	8.94	9.10	9.45	9.62
DMPAC (152)	2.05e	3.30d	4.65c	5.32b	5.68a
DMPCA (338)	2.13d	3.43c	4.78c	5.68b	5.85a
DPMCA$_{(2)}$ (418)	2.28e	3.51d	4.81c	5.76b	5.91a
DMCPA (550)	2.36e	3.58d	4.86c	5.79ab	5.95a
DCMPA (558)	2.42d	3.62c	4.92b	5.83a	5.90a
Salmonella typhimurium					
Initial	7.57	7.57	7.57	7.57	7.57
Control	9.23	9.35	9.48	9.49	9.54
DMPAC (152)	2.08e	3.41d	4.73c	5.44b	5.74a
DMPCA (338)	2.22e	3.46d	4.85c	5.57b	5.78a
DPMCA$_{(2)}$ (418)	2.31d	3.62c	4.90b	5.69b	6.01a
DMCPA (550)	2.56e	3.75d	4.96c	5.78b	6.21a
DCMPA (558)	2.61d	4.82c	4.98c	5.92b	6.32a
Pseudomonas fluorescens					
Initial	7.82	7.82	7.82	7.82	7.82
Control	9.25	9.33	9.38	9.54	9.56
DMPAC (152)	NDc	NDc	1.15b	1.18b	2.18a
DMPCA (338)	NDcd	1.14c	NDcd	1.83a	1.94a
DPMCA$_{(2)}$ (418)	1.34e	1.45d	1.52c	1.98b	3.10a
DMCPA (550)	1.42cd	1.51c	NDe	2.19b	3.42a
DCMPA (558)	1.48	1.58	1.64	2.33	3.58
Vibrio parahaemolyticus					
Initial	6.70	6.70	6.70	6.70	6.70
Control	8.95	8.99	9.21	9.36	9.48
DMPAC (152)	NDb	NDb	NDb	NDb	3.06a
DMPCA (338)	NDe	1.34d	1.44cb	1.58b	3.22a
DPMCA$_{(2)}$ (418)	NDe	1.42d	1.62cb	1.74b	3.41a
DMCPA (550)	NDe	1.53d	1.65c	1.78b	3.57a
DCMPA (558)	NDe	1.61d	1.71c	1.83b	4.14a

NOTE: *a - e Means with different letters within a row indicate significant difference ($p < 0.05$). 1. Viable cells after incubation without (control) and with 0.05% chitosan for 24h at 37°C 2. ND = Not detected, Figures in parathenses are Molecular weight*

Table 3: Effect of pH on Antifungal Activity of Chitosans Based on Colony Counts ($Log_{10}CFU/ml$) [1] on Yeast and Moulds

Microorganisms (Fungi)	pH				
	3.0	3.5	4.0	5.0	5.8
Saccharomyces cerevisiae					
Initial	5.30	5.30	5.30	5.30	5.30
Control	7.87	7.89	8.03	7.96	8.09
DMPAC (152)	1.35e	2.09d	3.16c	3.41b	4.25a
DMPCA (338)	1.46de	2.15d	3.20c	3.45b	4.30a
DPMCA$_{(2)}$ (418)	1.51e	2.18d	3.26c	3.48b	4.37a
DMCPA (550)	1.56e	2.25d	3.30c	3.53b	4.40a
DCMPA (558)	1.59e	2.30d	3.38c	3.59b	4.46a
Pichiafermentans					
Initial	5.54	5.54	5.54	5.54	5.54
Control	8.66	8.71	8.75	8.80	8.89
DMPAC (152)	1.48d	2.31c	2.62b	2.70b	3.17a
DMPCA (338)	1.52e	2.35d	2.64c	2.74b	3.20a
DPMCA$_{(2)}$ (418)	2.05d	2.38c	2.64b	2.78b	3.25a
DMCPA (550)	2.11e	2.42d	2.67bc	2.81b	3.30a
DCMPA (558)	2.20e	2.50d	2.72c	2.85b	3.41a
Penicilluma purpurogenum					
Initial	5.20	5.20	5.20	5.20	5.20
Control	8.56	8.68	8.78	8.86	9.02
DMPAC (152)	1.11e	1.51d	3.02c	3.35b	4.02a
DMPCA (338)	1.25e	1.58d	3.14c	3.42b	4.05a
DPMCA$_{(2)}$ (418)	1.78de	1.85d	3.18c	3.50b	4.10a
DMCPA (550)	1.83e	2.03d	3.25c	3.56b	4.11a
DCMPA (558)	1.87de	2.17d	3.31c	3.63ab	4.17a
Aspergillus niger					
Initial	5.30	5.30	5.30	5.30	5.30
Control	8.75	8.89	9.26	9.31	9.45
DMPAC (152)	2.07e	2.63d	3.20c	3.61ab	4.00a
DMPCA (338)	2.18e	2.66d	3.26c	3.65b	4.19a
DPMCA$_{(2)}$ (418)	2.25e	2.75d	3.31c	3.69b	4.26a
DMCPA (550)	2.37e	2.87d	3.45c	3.74b	4.34a
DCMPA (558)	2.48e	2.94d	3.58c	3.86b	4.50a
Rhizopus stolonifer					
Initial	5.60	5.60	5.60	5.60	5.60
Control	9.34	9.39	9.46	9.35	9.47
DMPAC (152)	NDd	NDd	1.16bc	1.41b	2.75a
DMPCA (338)	NDdc	1.12c	1.18c	1.53b	2.78a
DPMCA$_{(2)}$ (418)	1.23d	1.36c	1.44c	1.57b	2.82a
DMCPA (550)	1.28d	1.42c	1.30d	1.63b	2.86a
DCMPA (558	1.35c	1.15d	1.35c	1.71b	2.30a

NOTE: *a - e Means with different letters within a row indicate significant difference ($p < 0.05$). 1. Viable cells after incubation without (control) and with 0.05% chitosan for 24h at 37°C. 2. ND = Not detected, Figures in parathenses are Molecular weight.*

Confirmed outbreaks of human Listeriosis have been associated with consumption of contaminated foods from plant and animal sources. The ability of *Listeria monocytogenes* to proliferate at refrigeration temperatures and cause serious illness have been reported (Ahamad and Marth, 1989), Thus, a significant health hazard could result by consumption of foods contaminated with this organism but possibly could be reduced or prevented by proper chitosan treatment. The pH values of tropical fruit juices are usually acidic and range between 3.54 and 5.56. At low pH, both *Escherichia coli* 0157:H7 and *Salmonella typhimurium* survive for several days, especially when stored at refrigeration temperatures as reported by McClure and Hall (2000); Youssuf and Munir (2007). Thus the acidic nature of unpasteurized fruit juices does not ensure its safety as some pathogens may survive for extended periods of time and cause disease (CDC, 1997). While some pathogens gradually died off with chitosan addition at low pH, others were killed rapidly showing the potential efficacy for use of chitosan for fruit juice preservation.

Conclusion: The bioactivity of chitosan was affected by acidic pH showing the inhibition and total killing of food-borne bacteria and fungi which are either pathogenic or spoilage organisms. Thus the results of the present study clearly show that application of chitosan to acidic foods such as fruit juices will enhance its effectiveness as a natural preservative.

REFERENCES

Ahamad, N; Marth, EH (1989). Behaviour of *Listeria monocytogenes* at 7, 13, 21 and 35^{0}C in tryptose broth acidified with acetic, citric, or lactic acid. *J. Food Protect*. 52**:** 688 - 695.

CDC (1997). Outbreak of *Escherichia coli* 0157:H7 infection and criptosporidiosis associated with drinking unpasteurized apple cider-Connecticut and New York. *J. American Med. Assoc.*277**:** 781-787.

Hsu, SC; Don, TM; Chiu, WY (2002). Free radical degradation of chitosan with potassium persulfate. *Polymer Degrad. Stab.*755: 73 – 83.

Kurita, K (1998). Chemistry and application of chitin and chitosan. *Polymer Degrad. Stab.*59: 117 – 120.

Li, Q; Dunn, EJ; Grandmaison, EW; Goosen, MF (1992). Applications and properties of chitosan. *J. Bioactive Comp. Polymers*. **7**: 370 – 397.

Mcclure, PJ; Hall, S (2000). Survival of *Escherichia coli* in foods. *J. Appl. Microbiol.supplement* .88**:** 61s-70s.

Muzzarelli, RAA (1973). *Natural Chelating Polymers* Pergamon Press, Oxford, U.K.

No, HK; Meyers, SP (1989). Crawfish chitosan as a coagulant in recovery of organic compounds from seafood processing streams. *J. Agric. Food Chem.*37**:** 580 – 583.

Omogbai, BA; Ikenebomeh, MJ (2013). Antimicrobial and toxicological evaluation of food grade chitosan from crab (*Callinectes sapidus*). *NISEB J.* 13 (1&2): 23-28.

Omogbai, BA**;** Ikenebomeh, MJ (2016).Sub-chronic toxicity study of a characterized food grade chitosan from crab (*Callinectes sapidus*).*NISEB J.* 16 (1): 34-44.

Sakthivel, D; Vijayakumar, N; Anandan, V (2015). Extraction of chitin and chitosan from Mangrove crab (*Sesarmaplicatum*) from Thengaithittu estuary Pondicherry Southeast coast of India. *Intl. J. Pharm. Pharmceu. Res.* 4(1); 12-24.

Yossuf, AA; Munir, MA (2007). Experimental studies on the potential for acid tolerance, growth and survival of *Salmonella enteric* Serovar *Typhimurium* and *Escherichia coli* 0157: H7 in orange juices. *Adv. Biol. Res.*1 (3-4): 99-107.