Science World Journal Vol 14(No 1) 2019
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

Full Length Research Article

SURVIVAL OF SOME FOOD-BORNE PATHOGENIC MICROORGANISMS IN OZONATED TROPICAL FRUITS JUICES TREATED WITH CHITOSAN

Barry Aigbodion Omogbai[1*] and Marcel James Ikenebomeh

[1]Department of Microbiology, Food and Industrial division, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

*Corresponding Author Email Address barry.omogbai@uniben.edu.ng

ABSTRACT
Enteric pathogens in non-pasteurized fruit juices can cause gastroenteritis. The ability of chitosan to inactivate food-borne pathogens in tropical fruit juices was investigated. Challenge studies was conducted with Salmonella *typhimurium, Escherichia coli* 0157:H7*, Listeria monocytogenes and Staphylococcus aureus.* Tropical fruit juices employed were orange, pineapple, watermelon and a mix of the three. Fruit juices samples (100ml) were dispensed into 150ml conical flasks. Chitosan powder was added directly to the juice to give a final concentration of 1000µg/ml, 1500µg/ml and 2000µg/ml respectively. For challenge testing, 0.1ml of 24h old broth cultures of the microorganisms were inoculated into the flasks at a level of ≤ 6.69Log$_{10}$CFU/ml. The juices were then stored at 28^{o}C and 4^{o}C for 7-14 days and 20days respectively. Chitosan concentration of 2000µg/ml was effective in killing *Escherichia coli* 0157:H7 in all fruit juices in 36 h irrespective of temperature of storage. *Salmonella* only survived up to 24h in pineapple and mixed fruit juices treated with ≥1500µg/ml chitosan. A greater killing occurred for Listeria *monocytogenes* with the application of concentration of 1500-2000µg/ml chitosan. At these concentrations *Listeria monocytogenes* counts were reduced from an initial challenge inoculum of 5.82log$_{10}$CFU/ml to zero count in 12h to 24h in all fruit juices. The addition of 1500µg/ml or 2000µg/ml chitosan to the juices reduced *Staphylococcus* counts to zero in 18h in watermelon and orange juices. The ≥5log reduction of these pathogens by chitosan demonstrates its potential preservative power for unpasteurised fruit juices.

Keywords: Chitosan, juices, foodborne pathogens, inactivation, preservative.

INTRODUCTION

Juices may be squeezed directly from fruits or vegetables. They can also be produced from macerated or crushed material so as to include a considerable amount of pulp, or may be extracted by water as in prune juice. Juices may be used in their natural concentrations or may be concentrated by evaporation or freezing. They may be preserved by canning, freezing, or drying. Juices extracted from fruits are more or less acidic depending on the product. The pH of juices range from about 2.4 for lemon or cranberry juice to 4.2 for tomato juice. They all contain sugars, the amount varying from about 2% in lemon juice up to 17% in some samples of grape juice (Stratford *et al*., 2000).

An important current trend in food preservation is towards the use of procedures that deliver food products that are less "heavily" preserved, higher in quality, perceived as being more "natural", contain less additives, and are nutritionally healthier. Some new and "emerging" techniques aim to meet some of these objectives. Most of these techniques act by inactivating microbes. Many naturally occurring antimicrobials have been explored for use as food preservatives, but few have yet been widely exploited (Lee and Kang, 2004).

It is of great importance for manufacturers to assess the microbiological safety and stability of any new or reformulated product. Three ways of achieving this objective are: predictive microbiology, storage trials and challenge testing. Predictive microbiology is an excellent tool for examining the consequences of formulation changes in products. Storage trials involve assessing the product over its storage period to establish shelf-life of the product including assessing sensory and chemical changes as well as microbiological stability. Challenge testing encompass the use of inoculated packs studies to establish the safety of products. This being especially important for chilled foods (Smith, 2004).

With the increased pressure on food manufacturers to remove or reduce the traditional chemical preservatives in their foods, and to adopt more natural alternatives for the maintenance or extension of shelf-life, there is considerable interest in the use of natural ingredients as microbial growth inhibitors. Numerous naturally occurring antimicrobial agents are present in animal and plant tissues, where they probably evolved as part of the defense mechanisms of the host against microbial invasion. Chitosan from crab holds great promise in this direction.

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). 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). This investigation attempts to provide a base of scientific knowledge to support innovation and development of new functional tropical fruit juices with an assurance of consumer protection.

MATERIALS AND METHODS

Sources of microorganisms

The microorganisms used in the study were bacteria including *Salmonella typhimurium, Escherichia coli* 0157:H7 and *Listeria monocytogenes* which were stool isolates obtained from Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria. *Staphylococcus aureus (SAUBT1),* was a clinical wound isolate obtained from the Department of Medical Microbiology, University of Benin Teaching Hospital, Benin City, Nigeria. 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.

Source of chitosan

This was obtained according to the methods outlined by Omogbai and Ikenebomeh (2016).

Preparation of fruit juices

Freshly harvested fruits of pineapple, orange and watermelon were sorted, washed, peeled and diced into cubes using a sharp sterile stainless steel knife. The diced or chopped pieces were fed into a sterilized Binatone juice extractor (Model JE-500, Binatone, England). The device was fitted with a special filter which enabled the fruit juice to be obtained directly without further filtration and homogenization. The juice obtained was poured into sterile bottles and corked after filling using manually operated bottle corking machine. Fresh juice samples were either refrigerated at 4^{o}C or kept at 28$\pm$2^{o}C until needed for use. Packaging materials used for storage include PET plastic bottles and polythene sachets (Omogbai, 2012).

Microbiological analysis of tropical fruit juices

Four fruit juices: pineapple juice, orange juice and water melon juice were analysed microbiologically for bacteria using standard methods.

Isolation and enumeration of microflora of juice samples

Fruit juice (100ml) was dispensed into 200ml containers (in duplicate for each treatment) and stored at 4^{o}C and 28$\pm$2^{o}C. Duplicate samples (10ml) were taken periodically from each container for microbiological analysis. The pour plate method was used for the isolation and enumeration of bacteria in the fruit juice samples. Ten-fold serial (1:10) dilutions of homogenized suspension were prepared in sterile peptone water –a maximal recovery diluent. Viable numbers were determined by pour-plating 1.0ml on nutrient agar for total counts. Violet red bile glucose agar was used for the enumeration of *Enterobacteriaceae* after incubation at 37^{o}C for 24h. Microbial counts were taken after incubation, calculated and expressed as colony forming units per milliliter (CFU/ml) of the juice samples tested (Omogbai and Ikenebomeh, 2016).

Ozonation of tropical fruit juices.

The modified method of Hunt and Marinas (1997) was employed in ozonation of fruit juice. Ozone gas was generated using an ozone generator (Model: TS-III, Tianjin Bioengineering Co., Ltd China) in a 500ml Erlenmeyer flask. An ozone concentration of 3.33mg/min was applied. Ozone concentration was recorded using an ozone analyzer (built-in ozone module in the device). Excess foaming was prevented with 10-20µl sterile antifoaming agent which was added before each ozone treatment. The juice samples were treated for 0-20min with sampling at 2min intervals. All experiments were carried out in duplicate and replicated at least twice. The efficacy of treatment was determined in terms of reduction or total killing of viable counts over time (Kim and Yousef, 2000).

Treatment of fruit juices with chitosan.

Fruit juices samples (100ml) were dispensed into 150ml conical flasks. Chitosan powder was added directly to the juice to give a final concentration of 1000µg/ml, 1500µg/ml and 2000µg/ml respectively. For challenge testing, 0.1ml of 24h old broth cultures of the microorganisms *E. coli* 0157:H7, *Salmonella typhimurium, Listeria monocytogenes* and *Staphylococcus aureus* were inoculated into the flasks at a level of ≤ 6.69Log$_{10}$CFU/ml. All treatments were prepared in duplicate. The juices were then stored at 28^{o}C and 4^{o}C for 7-14 days and 20days respectively. Juices containing no added chitosan were also inoculated with the selected bacteria or yeast as controls (C). Absolute controls (AC) containing no added inoculum and chitosan were also prepared. Single samples were removed from each duplicate batch of juice periodically during incubation for microbiological analysis (viable counting). Plating out was done in triplicate, therefore, mean counts for each time point were calculated from six replicate determinations (Omogbai, 2012).

STATISTICAL ANALYSIS

This was achieved according to Ogbeibu (2005).

RESULTS

Fruits juices challenge studies with bacterial pathogens

The survival of four foodborne pathogenic organisms in ozonated tropical fruits juices treated with chitosan is shown in **Tables 1-4**. When inoculated into ozonated tropical fruits juices, *Escherichia* coli 0157:H7 survived in all the untreated juices (control) for upwards of 72h at both 4±1^{o}C and 28±2^{o}C. In the presence of 1000µg/ml chitosan, log reductions of ≥ 2.87 log$_{10}$CFU/ml of *E. coli* 0157:H7 numbers occurred in all the juices irrespective of storage temperature over a period of 72h (**Table 1**).

With chitosan concentration of 1500µg/ml total killing of E. coli 0157: H7 occurred in watermelon juice at both 4±1^{o}C and 28±2^{o}C. In orange juice, E. coli 0157: H7 was not detected beyond 12h with 1500µg/ml chitosan treatment. Marked reduction of log number was seen in pineapple (≥4.65 logCFU/ml) and in mixed fruits juices (≥4.67 log CFU/ml) at both 4±1oC and 28±2^{o}C. Chitosan concentration of 2000µg/ml was effective in killing Escherichia coli 0157:H7 in all fruit juices in 36h irrespective of temperature of storage (Table 1).

The survival of *Salmonella typhimurium* in ozonated tropical fruits juices treated with chitosan is illustrated in **Table 2**. In untreated controls *Salmonella* populations increased at 28±2^{o}C but had little or no growth at 4±1^{o}C during 72h incubation in all fruit juice samples. At 1000µg/ml chitosan concentration, counts of *Salmonella* decreased steadily from a challenge concentration of 6.68 log$_{10}$ CFU/ml to 0.0 and 2.24 log$_{10}$CFU/ml at 28±2^{o}C and

4±1°C respectively in orange juice over 72h. In watermelon juice this *Salmonella* was reduced to zero count in 60h at 28±2°C. A concentration of 1500 and 2000µg/ml reduced the initial challenge cell concentration of 6.68log_{10}CFU/ml to zero by 36h in all juices irrespective of storage temperature. Thus *Salmonella* only survived up to 1day or 24h in pineapple and mixed fruit juices treated with ≥1500µg/ml chitosan (**Table 2**).

In **Table 3** the Gram-positive bacteria *Listeria monocytogenes* increased slightly in cell population in the control experiment (fruit juices without chitosan) stored at 4±1°C by 1.01-1.9 log_{10}CFU/ml. In juices stored at 28±2°C there was 0.5-1.l4 log_{10}CFU/ml increase in growth of *Listeria monocytogenes.* The introduction of chitosan into the juices caused a reduction in the number of this bacterium. *Listeria* counts fell from an initial of 5.82log_{10}CFU/ml by ≥3.21 log_{10}CFU/ml in juices stored at 28±2°C and ≥3.37 log_{10}CFU/ml for 4±1°C over a period of 42h. However greater killing effect was seen with the application of concentration of 1500-2000µg/ml. At these concentrations *Listeria monocytogenes* counts were reduced from an initial challenge inoculum of 5.82log_{10}CFU/ml to zero count in 12h (in watermelon juice), 18h (in orange juice) and 24h (in pineapple and mixed fruit juice) respectively (**Table 3**).
The effect of chitosan concentration and storage time on the growth and survival of *Staphylococcus aureus* in tropical fruit juices stored at both and 4±1°C and 28±2°C showed reductions in the bacterial populations (**Table 4**).Chitosan concentration of 1000µg/ml achieved reduction of initial challenge inoculum of 5.75log_{10}CFU/ml to zero count in orange, pineapple and watermelon juices in 36h. In mixed fruit juice, this concentration achieved 3.84 and 3.89 log_{10}CFU/ml reduction in *Staphylococcus aureus* population at 4±1°C and 28±2°C. The addition of 1500µg/ml or 2000µg/ml chitosan to the juices reduced *Staphylococcus* counts to zero in 18h in watermelon and orange juices. Pineapple and mixed fruit juices however required 24h and 36h respectively for reduction of *Staphylococcus aureus* counts to zero at both 28±2°C and refrigerated 4±1°C storage.

Table 1: Effect of Chitosan (DMPAC) Concentration and Storage Time on the Growth and Survival of *Escherichia coli* 0157:H7 in Ozonated Tropical Fruit Juice Stored at 4±1°C and 28±2°C.

STORAGE TIME (H)	CHITOSAN CONCENTRATION (µg/ml)	SURVIVAL POPULATION (Log_{10}CFU/ml) IN TROPICAL FRUIT JUICES							
		ORANGE		PINEAPPLE		WATERMELON		MIXED FRUIT JUICE	
		28°C	4°C	28°C	4°C	28°C	4°C	28°C	4°C
0	0	6.40±0.03	6.40±0.03	6.40±0.03	6.40±0.03	6.40±0.03	6.40±0.03	6.40±0.03	6.40±0.03
	1000	5.62±0.01	6.10±0.01	5.84±0.02	6.18±0.01	5.43±0.02	5.67±0.01	5.87±0.00	5.91±0.02
	1500	3.53±0.01	4.15±0.02	4.26±0.01	4.77±0.01	3.26 ±0.01	3.85±0.00	4.45±0.01	4.63±0.01
	2000	2.81±0.04	3.09±0.00	3.75±0.02	3.94±0.00	2.48±0.01	2.37±0.01	3.93±0.02	4.15±0.02
12	0	6.33±0.02	6.40±0.03	6.48±0.01	6.40±0.03	6.36±0.00	6.40±0.03	6.34±0.00	6.40±0.03
	1000	5.48±0.01	5.82±0.00	5.57±0.02	5.73±0.01	5.03±0.02	5.25±0.01	5.63±0.02	5.72±0.00
	1500	1.38±0.02	2.46±0.01	2.95±0.01	3.06±0.01	1.30±0.01	1.46±0.00	3.01±0.01	3.18±0.01
	2000	1.23±0.00	1.45±0.01	2.08±0.01	2.38±0.00	1.16±0.00	1.21±0.02	2.22±0.01	2.31±0.00
24	0	6.25±0.01	6.35±0.00	6.55±0.00	6.31±0.00	6.36±0.00	6.34±0.00	6.25±0.01	6.31±0.01
	1000	4.27±0.01	5.04±0.02	4.43±0.01	5.25±0.02	4.56±0.01	4.86±0.01	4.95±0.02	5.02±0.00
	1500	0.00±0.00	1.53±0.02	1.38±0.01	1.75±0.00	0.00±0.00	0.00±0.00	1.54±0.01	1.73±0.01
	2000	0.00±0.00	0.00±0.00	1.25±0.00	1.41±0.01	0.00±0.00	0.00±0.00	1.38±0.00	1.41±0.00
36	0	6.38±0.00	6.28±0.00	6.43±0.02	6.31±0.00	6.30±0.01	6.25±0.00	6.37±0.02	6.26±0.01
	1000	3.85±0.02	4.16±0.02	3.94±0.02	4.66±0.01	4.18±0.01	4.30±0.00	4.10±0.00	4.23±0.03
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
48	0	6.26±0.00	6.28±0.00	6.33±0.01	6.38±0.01	6.15±0.02	6.25±0.00	6.43±0.01	6.20±0.01
	1000	3.53±0.02	3.76±0.02	3.65±0.01	3.87±0.02	3.79±0.00	3.87±0.02	3.83±0.02	3.95±0.02
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
60	0	6.20±0.01	6.25±0.01	6.28±0.00	6.30±0.00	6.27±0.01	6.25±0.00	6.19±0.02	6.20±0.00
	1000	3.21±0.03	3.78±0.01	3.44±0.01	3.56±0.01	3.36±0.01	3.52±0.01	3.56±0.01	3.67±0.00
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
72	0	6.20±0.01	6.25±0.01	6.28±0.00	6.30±0.00	6.20±0.00	6.25±0.00	6.15±0.01	6.29±0.01
	1000	3.21±0.03	3.52±0.02	3.32±0.00	3.41±0.00	3.01±0.00	3.33±0.00	3.44±0.02	3.53±0.02
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 2: Effect of Chitosan (DMPAC) Concentration and Storage Time on the Growth and Survival of *Salmonella typhimurium* in Ozonated Tropical Fruit Juices stored at 4±1°C and 28±2°C.

STORAGE TIME (H)	CHITOSAN CONCENTRATION (µg/ml)	SURVIVAL POPULATION (Log_{10}CFU/ml) IN TROPICAL FRUIT JUICES							
		ORANGE		PINEAPPLE		WATERMELON		MIXED FRUIT JUICE	
		28°C	4°C	28°C	4°C	28°C	4°C	28°C	4°C
0	0	6.69±0.01	6.68±0.01	6.68±0.01	6.68±0.01	6.68±0.01	6.68±0.01	6.68±0.01	6.68±0.01
	1000	5.05±0.00	6.10±0.02	5.76±0.02	5.88±0.00	4.73±0.03	4.85±0.01	5.81±0.00	5.87±0.02
	1500	4.26±0.02	4.52±0.01	4.41±0.00	4.52±0.02	3.24 ±0.04	3.61±0.04	4.52±0.03	4.73±0.01
	2000	3.18±0.01	3.36±0.02	3.52±0.02	3.87±0.01	2.31±0.02	2.62±0.00	4.02±0.01	4.24±0.01
12	0	6.75±0.02	6.68±0.01	6.89±0.00	6.56±0.00	6.71±0.04	6.68±0.01	6.83±0.02	6.54±0.02
	1000	4.67±0.01	4.83±0.03	5.10±0.01	5.47±0.01	4.51±0.02	4.72±0.03	5.44±0.02	5.56±0.02
	1500	1.65±0.02	2.29±0.00	2.35±0.00	3.63±0.02	1.38±0.00	1.95±0.01	2.64±0.01	2.78±0.01
	2000	1.19±0.02	2.10±0.01	1.36±0.03	1.58±0.00	1.05±0.00	1.27±0.01	2.25±0.02	2.43±0.00
24	0	7.25±0.01	6.54±0.01	7.18±0.01	6.50±0.02	6.96±0.01	6.75±0.02	6.97±0.02	6.36±0.01
	1000	4.26±0.01	4.67±0.02	4.48±0.02	4.23±0.01	2.86±0.02	3.07±0.01	5.01±0.00	5.18±0.00
	1500	0.00±0.00	0.00±0.00	1.21±0.00	1.86±0.02	0.00±0.00	0.00±0.00	1.45±0.02	1.56±0.02
	2000	0.00±0.00	0.00±0.00	1.09±0.00	1.19±0.00	0.00±0.00	0.00±0.00	1.37±0.01	1.41±0.01
36	0	7.85±0.01	6.48±0.01	7.27±0.02	6.57±0.01	7.58±0.02	6.88±0.01	7.38±0.00	6.48±0.02
	1000	2.94±0.02	4.58±0.00	3.63±0.01	3.18±0.02	2.29±0.00	2.55±0.04	4.48±0.01	4.60±0.01
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
48	0	8.30±0.01	6.56±0.00	7.39±0.00	6.46±0.00	7.74±0.01	6.71±0.00	7.48±0.02	6.57±0.02
	1000	2.23±0.01	3.06±0.01	3.21±0.02	3.56±0.03	1.24±0.03	2.18±0.01	3.86±0.02	4.12±0.03
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
60	0	7.64±0.02	6.63±0.01	7.46±0.02	6.70±0.00	7.96±0.02	6.83±0.02	7.55±0.00	6.63±0.02
	1000	2.25±0.00	2.63±0.00	2.74±0.01	3.12±0.03	0.00±0.00	1.37±0.02	3.17±0.02	3.67±0.00
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
72	0	7.35±0.01	6.65±0.00	7.41±0.02	6.61±0.02	8.10±0.02	7.15±0.03	7.62±0.01	6.74±0.02
	1000	0.00±0.00	2.24±0.01	1.16±0.01	2.75±0.01	0.00±0.00	0.00±0.00	2.73±0.02	3.04±0.02
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 3: Effect of Chitosan (DMPAC) Concentration and Storage Time on the Growth and Survival of *Listeria monocytogenes* in Ozonated Tropical Fruit Juices Stored at 4±1oC and 28±2°C.

STORAGE TIME (H)	CHITOSAN CONCENTRATION (µg/ml)	SURVIVAL POPULATION (Log_{10}CFU/ml) IN TROPICAL FRUIT JUICES							
		ORANGE		PINEAPPLE		WATERMELON		MIXED FRUIT JUICE	
		28°C	4°C	28°C	4°C	28°C	4°C	28°C	4°C
0	0	5.82±0.05	5.82±0.05	5.82±0.05	5.82±0.05	5.82±0.05	5.82±0.05	5.82±0.05	5.82±0.05
	1000	4.53±0.00	4.07±0.00	4.75±0.03	4.60±0.00	4.44±0.01	3.52±0.02	4.87±0.00	4.61±0.02
	1500	3.74±0.01	3.51±0.02	3.91±0.02	3.62±0.03	2.51 ±0.04	2.38±0.02	3.83±0.04	3.75±0.00
	2000	3.48±0.00	3.28±0.01	3.64±0.01	3.35±0.01	2.27±0.01	2.30±0.02	3.57±0.02	3.41±0.02
6	0	5.96±0.02	5.89±0.01	5.78±0.00	5.91±0.01	5.85±0.00	5.93±0.00	5.87±0.00	6.92±0.00
	1000	4.10±0.01	3.85±0.03	4.36±0.03	4.07±0.03	3.86±0.02	3.55±0.01	4.48±0.02	4.00±0.00
	1500	2.60±0.02	2.42±0.00	3.48±0.04	3.35±0.01	1.28±0.04	1.21±0.00	3.52±0.01	3.40±0.01
	2000	2.21±0.03	2.06±0.01	3.30±0.02	2.27±0.02	1.26±0.00	1.18±0.01	3.41±0.02	3.20±0.02
12	0	6.35±0.01	6.43±0.02	5.67±0.01	6.18±0.03	5.97±0.01	5.99±0.00	5.76±0.01	6.18±0.02
	1000	3.78±0.03	3.51±0.04	4.07±0.01	3.58±0.03	3.64±0.02	3.46±0.01	4.20±0.02	3.69±0.00
	1500	1.42±0.01	1.32±0.01	2.61±0.04	2.43±0.00	0.00±0.00	0.00±0.00	2.72±0.01	2.61±0.00
	2000	1.18±0.00	1.07±0.02	2.48±0.01	2.15±0.02	0.00±0.00	0.00±0.00	2.54±0.02	2.34±0.02
18	0	6.58±0.02	6.90±0.01	5.94±0.00	6.58±0.01	6.26±0.03	6.35±0.01	5.80±0.04	6.37±0.04
	1000	3.44±0.04	3.22±0.02	3.55±0.02	3.45±0.01	3.18±0.02	3.05±0.00	3.61±0.02	3.54±0.03
	1500	0.00±0.00	0.00±0.00	1.27±0.01	1.17±0.02	0.00±0.00	0.00±0.00	1.56±0.01	1.74±0.03
	2000	0.00±0.00	0.00±0.00	1.09±0.00	1.05±0.00	0.00±0.00	0.00±0.00	1.31±0.02	1.29±0.00
24	0	6.87±0.01	6.98±0.00	6.47±0.02	6.58±0.01	6.48±0.01	6.52±0.03	5.89±0.01	6.48±0.00
	1000	3.05±0.02	2.87±0.01	3.21±0.05	3.09±0.01	2.96±0.03	2.78±0.00	3.52±0.00	3.18±0.02
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
30	0	5.74±0.02	7.26±0.01	5.52±0.01	6.75±0.02	6.63±0.02	6.68±0.01	5.95±0.00	6.67±0.01
	1000	2.76±0.02	2.41±0.03	2.71±0.03	2.60±0.01	2.67±0.01	2.58±0.03	2.84±0.02	2.71±0.03
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
36	0	5.78±0.01	7.64±0.03	6.57±0.02	6.82±0.00	6.87±0.02	6.76±0.02	6.16±0.02	6.78±0.02
	1000	2.53±0.01	2.28±0.00	2.62±0.04	2.55±0.01	2.61±0.00	2.43±0.01	2.75±0.00	2.60±0.01
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
42	0	6.96±0.03	7.72±0.02	6.78±0.01	6.86±0.02	6.96±0.00	7.02±0.02	6.35±0.02	6.83±0.01
	1000	2.30±0.00	2.16±0.01	2.57±0.00	2.43±0.00	2.24±0.01	2.13±0.03	2.61±0.02	2.50±0.00
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 4: Effect of Chitosan (DMPAC) Concentration and Storage Time on the Growth and Survival of *Staphylococcus aureus* in Ozonated Tropical Fruit Juices Stored at 4±1°C and 28±2°C.

	CHITOSAN CONCENTRATION (µg/ml)	SURVIVAL POPULATION (Log_{10}CFU/ml) IN TROPICAL FRUIT JUICES							
		ORANGE		PINEAPPLE		WATERMELON		MIXED FRUIT JUICE	
		28°C	4°C	28°C	4°C	28°C	4°C	28°C	4°C
0	0	5.75±0.02	5.75±0.02	5.75±0.02	5.75±0.02	5.75±0.02	5.75±0.02	5.75±0.02	5.75±0.02
	1000	4.70±0.01	4.70±0.00	4.93±0.01	4.79±0.00	4.51±0.01	4.24±0.01	4.95±0.00	4.89±0.00
	1500	3.74±0.00	3.66±0.01	3.87±0.02	3.78±0.00	3.50±0.02	3.16±0.00	3.09±0.01	3.76±0.02
	2000	2.60±0.02	3.32±0.02	3.75±0.03	2.96±0.02	3.18±0.01	3.05±0.01	3.18±0.02	3.16±0.03
6	0	5.86±0.01	5.03±0.01	5.80±0.02	5.68±0.00	5.83±0.01	5.25±0.02	4.93±0.02	5.60±0.02
	1000	3.67±0.02	3.51±0.02	4.85±0.02	4.57±0.01	3.57±0.02	3.40±0.00	4.88±0.01	4.56±0.02
	1500	2.48±0.04	2.47±0.01	2.66±0.01	2.50±0.00	2.36±0.03	2.08±0.01	2.75±0.02	2.54±0.01
	2000	2.26±0.01	1.97±0.02	2.42±0.02	2.31±0.03	1.39±0.00	1.26±0.00	2.68±0.01	2.37±0.02
12	0	5.98±0.02	4.71±0.02	5.89±0.01	5.01±0.02	6.05±0.02	4.62±0.03	4.81±0.02	5.13±0.02
	1000	3.14±0.00	3.95±0.01	4.24±0.02	4.00±0.02	2.87±0.02	2.65±0.01	4.35±0.01	4.47±0.01
	1500	1.35±0.02	1.16±0.02	2.05±0.01	2.15±0.02	1.22±0.02	1.10±0.02	2.44±0.02	2.28±0.02
	2000	1.18±0.00	1.08±0.00	1.88±0.02	1.28±0.00	0.00±0.00	0.00±0.00	2.23±0.03	2.10±0.01
18	0	6.14±0.03	4.71±0.02	6.10±0.02	4.85±0.02	6.27±0.01	4.55±0.01	5.60±0.03	4.88±0.03
	1000	2.55±0.03	2.41±0.03	3.68±0.01	3.51±0.02	2.43±0.01	2.28±0.02	3.76±0.02	3.91±0.02
	1500	0.00±0.00	0.00±0.00	1.59±0.02	1.42±0.02	0.00±0.00	0.00±0.00	2.01±0.00	1.63±0.02
	2000	0.00±0.00	0.00±0.00	1.36±0.01	0.00±0.00	0.00±0.00	0.00±0.00	1.82±0.01	1.41±0.01
24	0	5.68±0.01	4.62±0.01	6.21±0.00	4.56±0.01	5.43±0.02	4.36±0.00	5.82±0.00	4.63±0.01
	1000	1.98±0.02	1.40±0.00	2.75±0.02	2.46±0.02	1.38±0.01	1.24±0.02	2.95±0.00	3.08±0.02
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.86±0.04	1.45±0.01
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.15±0.02	1.10±0.02
30	0	5.05±0.02	4.50±0.00	6.21±0.00	4.23±0.02	5.10±0.02	4.36±0.00	5.82±0.00	4.35±0.04
	1000	1.56±0.01	0.00±0.00	1.84±0.03	1.72±0.00	0.00±0.00	0.00±0.00	2.34±0.01	2.12±0.00
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.53±0.02	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
36	0	4.63±0.03	4.56±0.02	6.28±0.01	3.87±0.02	4.76±0.03	4.22±0.01	5.68±0.03	4.30±0.00
	1000	0.00±0.00	0.00±0.00	0.00±0.00	1.72±0.00	0.00±0.00	0.00±0.00	1.86±0.02	1.91±0.02
	1500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.41±0.04	0.00±0.00
	2000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Pathogen detection in tropical fruit juices by enrichment

In order to check the efficacy of chitosan treatments using concentrations of 1000, 1500 and 2000µg/ml in fruit juices, various enrichment treatments were investigated. Thus **Tables 5** illustrate pathogen detection in freshly prepared chitosan treated tropical fruit juices stored at both 4±1°C and 28±2°C. With the application of 1500 and 2000µg/ml chitosan the four pathogens tested (*Escherichia coli* 0157:H7*, Salmonella typhimurium, Listeria monocytogenes and Staphylococcus aureus*) did not survive more than one day at both 4±1°C and 28±2°C temperature in the juices. None was detected by enrichment except *Staphylococcus aureus* and *Listeria* which were detected on day 2 at 4±1°C storage in pineapple juices.

With chitosan concentration of 1000µg/ml the pathogens *Salmonella typhimurium* and *E. coli* 0157:H7 survived for up to 9 and 15 days respectively in orange juice at 28±2°C and 20 days at 4±1°C. *Listeria monocytogenes* and *Staphylococcus aureus* were recovered in orange juices for up to 3 and 13 days respectively at 28±2°C storage of orange juice (**Table 5**). In general the pathogens survived and were recovered in juice treated with a chitosan concentration of 1000µg/ml for up to 3days or the entire 20 day study period. Cell recovery by enrichment indicated that injured organisms were present in chitosan- treated (1000µg/ml) juices at either 4±1°C or 28±2°C (**Table 5-8**).

Table 5: Pathogen Detection in Freshly squeezed Chitosan Treated Orange Juice Stored at 4±1°C and 28±2°C.

Pathogen/Chitosan Concentration	STORAGE TEMPERATURE 28±2°C SAMPLING DAYS								4±1°C SAMPLING DAYS									
	0	1	3	5	9	13	15	19	0	2	6	8	10	12	14	16	18	20
Escherichia coli 0157:H7																		
Control+*E. coli* 0157:H7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Chitosan, 1500µg/ml	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Salmonella typhimurium																		
Control+ Salmonella typhimurium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
Chitosan, 1500µg/ml	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Listeria monocytogenes																		
Control + *Listeria monocytogenes*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	-
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Staphylococcus aureus																		
Control+ *Staphylococcus aureus*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-

+= Pathogen detected after enrichment - = Pathogen not detected after enrichment

Table 6: Pathogen Detection in Freshly Squeezed Chitosan Treated Pineapple Juice Stored at 4±1°C and 28±2°C.

Pathogen/Chitosan Concentration	STORAGE TEMPERATURE 28±2°C SAMPLING DAYS								4°C SAMPLING DAYS									
	0	1	3	5	9	13	15	19	0	2	6	8	10	12	14	16	18	20
Escherichia coli 0157:H7																		
Control+*E coli* 0157:H7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Salmonella typhimurium																		
Control+ *Salmonella typhimurum*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Listeria monocytogenes																		
Control + *Listeria monocytogenes*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Staphylococcus aureus																		
Controls+ Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-

+= Pathogen detected after enrichment -= Pathogen not detected after enrichment

Table 7: Pathogen Detection in Freshly Squeezed Chitosan Treated Watermelon Juice stored at 4±1°C and 28±2°C.

Pathogen/Chitosan Concentration	STORAGE TEMPERATURE 28±2°C SAMPLING DAYS								4±1°C SAMPLING DAYS									
	0	1	3	5	9	13	15	19	0	2	6	8	10	12	14	16	18	20
Escherichia coli 0157:H7																		
Control+*E. coli* 0157:H7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Salmonella typhimurium																		
Control+ *Salmonella typhimurium*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-	-
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Listeria monocytogenes																		
Control + *Listeria monocytogenes*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Staphylococcus aureus																		
Control+ *Staphylococcus aureu*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-

+= Pathogen detected after enrichmen -= Pathogen not detected after enrichment

Table 8: Pathogen Detection in Freshly Squeezed Chitosan Treated Mixed Fruit Juice Stored at 4±1°C and 28±2°C.

Pathogen\chitosan Concentration	STORAGE TEMPERATURE 28±2°C SAMPLING DAYS								4±1°C SAMPLING DAYS									
	0	1	3	5	9	13	15	19	0	2	6	8	10	12	14	16	18	20
Escherichia coli 0157:H7																		
Control+*E. coli* 0157:H7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Salmonella typhimurium																		
Control+ *Salmonella typhimurium*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Listeria monocytogenes																		
Control + *Listeria monocytogenes*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Staphylococcus aureus																		
Control+ *Staphylococcus aureus*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chitosan, 1000µg/ml	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
Chitosan, 1500µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Chitosan, 2000µg/ml	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-

+= Pathogen detected after enrichment - = Pathogen not detected after enrichment

DISCUSSION

The biocidal action of chitosan tested against *Escherichia coli* 0157:H7, *Salmonella typhimurium, Listeria monocytogenes* and *Staphylococcus aureus* were found to be concentration and temperature dependentthus corroborating Baskaran *et al*. (2010). At 1500 and 2000µg/ml, chitosan completely inactivated (negative by enrichment) *E coli* 0157:H7 after 24h in orange and watermelon fruit juices. However a concentration of 1000 µg/ml could not eliminate the pathogen from the fruit juices rather a gradual reduction occurred. Similarly *Staphylococcus aureus* and *Listeria monocytogenes* did not survive up to 1day when treated with ≥1500 µg/ml of chitosan. Both organisms rapidly died off from 5.57$\log_{10}$CFU/ml and 5.82$\log_{10}$CFU/ml to non-detection levels. Irrespective of the fruit juice type, 36h was required to completely eliminate *E coli* 0157:H7 (from 6.4$\log_{10}$CFU/ml) and *Salmonella* (from 6.68 Log_{10}CFU/ml) to levels that were undetectable using conventional microbiological techniques. Furthermore once inactivated, *E coli* 0157:H7 and *Salmonella* remained undetectable for the duration of the 20 days challenge test study. The non-detection of these pathogens with enrichment method presupposes that the cellular injury caused by chitosan was irreversible (Lee and Kang, 2004). The substantial reduction in the number of days (from 20 to 1) that *E. coli* 0157:H7 and *Salmonella typhimurium* were able to survive in all tropical fruit juices when treated with ≥1500 µg/ml and stored at 4±1^{o}C present an opportunity to improve the safety of unpasteurized fruit juices (Synder and Worobo, 2018).

Temperature was another factor that exerted a profound effect on the antibacterial activity of chitosan on the four challenge pathogens in this study, for example, 1000µg/ml chitosan decreased *E. coli* 0157: H7 counts from 6.4 $\log_{10}$CFU/ml to 3.21$\log_{10}$CFU/ml in 72 h at 28±2^{o}C. However the same concentration of chitosan reduced bacterial counts to 3.52$\log_{10}$CFU/ml over the same period at 4±1^{o}C. Similar differences in pathogen inactivation at these temperatures were also observed with higher chitosan concentrations, although not as prominent as that observed with 1000µg/ml. This finding agrees with that of Yuste and Fung (2004), who also reported a higher rate of inactivation of *E. coli* 0157:H7 and *Salmonella typhimurium* in apple juice by a combination of nisin and cinnamon at 20^{o}C compared to 5^{o}C. The enhanced antibacterial effect of chitosan at 28±2^{o}C could be attributed to the higher metabolic growth and death rates of *E.coli* 0157:H7 and *Salmonella typhimurium* at 28±2^{o}C compared to those at refrigeration (4±1^{o}C) temperature (Yuste and Fung, 2004). Similarly, *E. coli* 0157:H7 survived at a greater rate at 4±1^{0}C compared to 28±2^{o}C in the control samples, which could also be attributed to the lower metabolic, growth and death rate of the bacterium at this temperature. Zhao *et al.* (1993) reported that the survival of *E. coli* 0157: H7 was enhanced at 8^{o}C compared to that at 25^{o}C in apple cider with and without preservatives. The low pH of the juices could also have played a role in the antimicrobial activity of chitosan. The acidity of the fruit juices might have resulted in sublethal injury to the pathogens, which could enhance their susceptibility to cell membrane or cell wall damage activity of chitosan since this polymer is positively charged combined with its chelating ability (Li *et al.,* 1992).

Thus the results of this study indicate that chitosan at low concentrations (≥1500µg/ml) could potentially be used as an effective antimicrobial to inactivate *E. coli* 0157:H7, *Salmonella typhimurium, Listeria monocytogenes* and *Staphylococcus aureus* which may contaminate tropical fruit juices by chance during processing. The ≥5log reduction of these pathogens obtained in the present study would be seen to have met the demand of the National Advisory Committee on Microbiological criteria for foods, of the United States Department of Agriculture (USDA) and possibly National agency for Food, Drug administration and Control, Nigeria (NAFDAC) which recommends that manufacture of fruit juices should include effective treatments that can result in a cumulative 5-log reduction in *E. coli* 0157:H7 or pathogens of concern in the fruit juice being processed (USFDA, 2001).

Conclusion

This paper has shown the possible use of chitosan as an antimicrobial agent with a preservative potential for shelf life extension of tropical fruit juices.

REFERENCES

Baskaran, S. A., Amalaradjou, M.A.R., Hoagland, T and Kumar, V. (2010). Inactivation of *Escherichia coli* O157:H7 in apple juice and cider by trans-cinnamaldehyde. *International Journal of Food Microbiology*. 141:126-129.

Hsu, S.C., Don, T.M. and Chiu, W.Y. (2002). Free radical degradation of chitosan with potassium persulfate. *Polymer Degradation and Stability* **755**: 73 – 83.

Hunt, N.K. and Marinas, B.J. 1997. Kinectics of *E. coli* inactivation with ozone. *Water Research,* 31: 1355-1362.

Kim, J.G., Yousef, A.E. 2000. Inactivation kinetics of food-borne spoilage and pathogenic bacteria by ozone. *Journal of Food Science.* 65 : 521-528

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

Lee, S.Y. and Kang, D.H. (2004). Microbial safety of pickled fruits and vegetables and hurdle technology. *Internet Journal of Food Safety*. **4**: 21 – 32.

Li, Q., Dunn, E.J., Grandmaison, E.W and Goosen, M.F. (1992). Applications and properties of chitosan. *Journal of Bioactive and Compatible Polymers*. **7**: 370 – 397.

No, H.K and Meyers, S.P. (1989). Crawfish chitosan as a coagulant in recovery of organic compounds from seafood processing streams. *Journal of Agricultural and Food Chemistry*. **37:** 580 – 583.

Ogbeibu, A. E. (2005). *Biostatistics: A Practical Approach in Research and Data Handling.* Mindex Publishing Company Ltd., Benin City.265pp.

Omogbai, B.A. 2012. *Shelf life extension studies of tropical fruit juices with crab (Callinectes sapidus) chitosan.* Ph.D Thesis , University of Benin, Benin City. 388P.

Omogbai, B.A and Ikenebomeh, M.J. 2016. Sub-chronic toxicity study of a characterized food grade chitosan from crab (*Callinectes sapidus*) *NISEB Journal* 16(1): 34-44.

Roberts, D., Hopper, W and Greenwood, M. (1995). *Practical Food Microbiology*. Forum, Somerset, UK: Public Health Laboratory Service (PH LS). 205P.

Smith, J, (2004). *Technology of Reduced Additive Foods*. 2nd ed. Blackwell Science Publishing Company, Oxford, U.K. **221 pp.**

Stratford, M. Hofman, P.D. and Cole, M.B. (2000). Fruit Juices, Fruit drinks and Soft drinks. In: Lund, B.M., Baird-Parker,T.C. and Gould G.W. (Eds.). *The Microbiological*

Safety and Quality of Food. . Gaithersburg: Aspen Publishers Inc. **pp 836-869**

Synder, A. B and Worobo, R.W. (2018). Incidence and impact of Microbial spoilage in the production of fruit and vegetable juices as reported by manufacturers. *Food Control.* 85: 144-150.

United states food and Drug Administration (USFDA).(2001). Hazard Analysis and critical control point (HACCP). Procedures for the safe and sanitary processing and importing of juice: Final rule. *Federal Register* **66:** 6137-6202.

Yuste, J. and Fung, D.Y.C. (2004). Inactivation of *Salmonella typhimurium* and *Escherichia coli* O157:H7 in apple juice by a combination of nisin and cinnamon. *Journal of Food Protection.* 67: 371-377.

Zhao, T., Doyle, M.P., Besser, R.E. (1993). Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives. Applied *and Environmental Microbiology*. 59*: 2526-2530*