American Journal of Microbiological Research, 2020, Vol. 8, No. 1, 24-33
Available online at http://pubs.sciepub.com/ajmr/8/1/4
Published by Science and Education Publishing
DOI:10.12691/ajmr-8-1-4

Application of Hazard Analysis Critical Control Point (HACCP) Concept Using Sodium Metabisulphite and Hypochlorite to Enhance Microbial Safety of Shrimps

Bernard Johnson Okpako Efiuvwevwere[1,*], Chimezie Jason Ogugbue[2], Godwin Emoghene[3], Augustine Kpeedee Ngbara-ue[4]

[1]Department of Microbiology, Faculty of Science,University of Port Harcourt, Port Harcourt, Nigeria
[2]Department of Microbiology, Faculty of Science,University of Port Harcourt, Port Harcourt, Nigeria
[3]Department of Microbiology, Faculty of Science, University of Port Harcourt, Port Harcourt, Nigeria
[4]Department of Microbiology, Faculty of Science, University of Port Harcourt, Port Harcourt, Nigeria
*Corresponding author: bjefiuvw@yahoo.com

Received December 27, 2019; Revised February 06, 2020; Accepted February 23, 2020

Abstract Shrimps are highly valued worldwide. However, they deteriorate rapidly after harvest except preserved or subjected to hazard analysis critical control point (HACCP). Therefore, this investigation was undertaken to determine the microbiological and physico-chemical [pH and trimethylamine (TMA)] characteristics of shrimps subjected to several critical control points (CCPs) including 100 ppm sodium metabisulphite ($Na_2S_2O_5$) alone or followed by 10 ppm calcium hypochlorite $Ca[OCL]_2$ before ambient (27-35°C) or refrigeration (4-6°C) storage. Also, shrimp types (whole, head or tail subjected to CCPs (iced or un-iced storage) wereanalysed for total viable counts (TVCs), coliforms, Staphylococcus spp.,Salmonella spp. and Vibrio spp. counts; pH and TMA contents. Significant ($p<0.05$) maximum TVCs of 1.8 x 108cfu/g occurred in tail samples subjected to $Na_2S_2O_5$ followed by $Ca[OCL]_2$ before 27-35°C storage. Significant different bacterial populations occurred with the un-iced whole shrimps showing maximum population (2.9 x 105cfu/g) of coliforms. Additionally, Staphylococcus spp. had the maximum count (8.2 x 103cfu/g) in un-iced whole samples while the lowest (7.8 x 101cfu/g) occurred in tail samples treated with $Na_2S_2O_5$ followed by $Ca[OCL]_2$ before 4-6°C storage. Variations in bacterial profiles were influenced by the CCPs resulting in diverse bacteria with iced head samples showing Bacillus spp.,E.coli, Pseudomonas spp., Salmonella spp. and Staphylococcus aureus while the others differed. Most bacterial pathogens occurred in tail samples subjected to CCPs before 27-35°C storage. Highest pH (7.85) and TMA (37.48mgN/100g) occurred in tail samples treated with Na2S2O5 followed by $Ca[OCL]_2$ before ambient storage. Significant positive correlation occurred between TVCs and coliforms (r = 0.9011) and others. However, pH and TMA showed negative or poor correlation against the different bacterial groups.Percentage frequency of bacterial occurrence differed. This study has demonstrated the importance of HACCP and the need to adopt its concept and application to enhance microbial safety of shrimps.

Keywords: *shrimps, HACCP, critical control measures, microbial safety*

Cite This Article: Bernard Johnson Okpako Efiuvwevwere, Chimezie Jason Ogugbue, Godwin Emoghene, and Augustine Kpeedee Ngbara-ue, "Application of Hazard Analysis Critical Control Point (HACCP) Concept Using Sodium Metabisulphite and Hypochlorite to Enhance Microbial Safety of Shrimps." *American Journal of Microbiological Research*, vol. 8, no. 1 (2020): 24-33. doi: 10.12691/ajmr-8-1-4.

1. Introduction

Shrimps are important seafoods globally and have high commercial value in export/international trade [1,2,3]. However, the quality attributes of shrimps deteriorate soon after harvest leading to substantial economic losses [4,5].

Shrimps harvested from developing countries are usually by artisanal fishermen who are not equipped with refrigeration facilities. This often results in high level of post-harvest losses due to substantial perishability [6,7].

A number of factors including contamination of shrimp ecosystems, poor handling practices, lack of adequate storage facilities and inadequate preservative measures contribute considerably to shrimp losses [5,8,9].

Application of sulphiting agents in seafoods is permitted in many countries at permissible levels [4,10,11] with the main purpose of minimizing or preventing melanosis (black spot formation). The most commonly used agent is sodium metabisulphite which has also shown inconsistent reduction in microbial populations [1,11].

Changes in the pH of shrimp under different storage conditions have been reported to be minimal [5] while increase in trimethylamine (TMA) content of shrimp

occurred during storage under refrigeration temperature [1,12].

HACCP application is highly recommended for sustenance of seafood quality and safety enhancement. However, HACCP is rarely adopted by seafood handlers and industries as well as regulatory agencies especially in developing countries including Nigeria [13,14]. More importantly, little or no work has focused attention on HACCP application for shrimp processing as a comprehensive approach to maintenance of shrimp quality after harvest [3,7].

The present work was therefore undertaken to determine the effects of HACCP concept on microbiological profiles and physico-chemical characteristics of shrimps based on the application of sodium metabisulphite ($Na_2S_2O_5$), calcium hypochlorite $Ca[OCl]_2$, headed versus tail portion and storage temperature as critical control points.

2. Materials and Methods

2.1. Sample Collection

Freshly harvested shrimps (*Penaeus notialis*) (approximately 3kg) from Andoni River, Rivers State, Nigeria were purchased from seafood harvesters following prior arrangements. The samples ranged from 6-10g (with average weight of about 7g).

2.2. Processing and Treatment of Shrimps

The shrimps were sorted into comparable sizes (approx. 7g each) and divided into two portions; one kept in ice-box (4-6°C) while the other in un-iced box. Figure 1 shows the critical control points as deemed appropriate in shrimp processing to improve microbial safety.

On arrival at the laboratory, the samples (iced and un-iced) were aseptically de-headed respectively into head and abdominal (tail) portions and analyzed for microbiological and physico-chemical characteristics.

The fresh abdominal (tail) portions (being more commercially valued and acceptable) were subjected to various critical control points (CCPs) or treatments as shown in Figure 1.

Following these CCPs/treatments, the samples were analyzed for microbiological and physico-chemical attributes.

2.3. Microbiological Analysis

Serial dilutions were prepared by blending 25g of pooled samples in 225ml 0.1% peptone water using a stomacher (model BA 6021, Seward Medical, London, UK).Further 10-fold dilutions were prepared and spread-plated (0.1ml aliquot) in triplicate on surface-dried plate count agar, MacConkey agar, Mannitol salt agar, Thiosulphite-citrate-bile-sucrose agar and Salmonella-Shigella agar and incubated at 37°C for18-24hr. The plates were then examined for colonial growth and enumeration astotal viable counts, coliforms, *Staphylococcus* spp., *Vibrio* spp and *Salmonella*- spp counts. All the culture media used were products of Titan Biotech. Ltd., India.

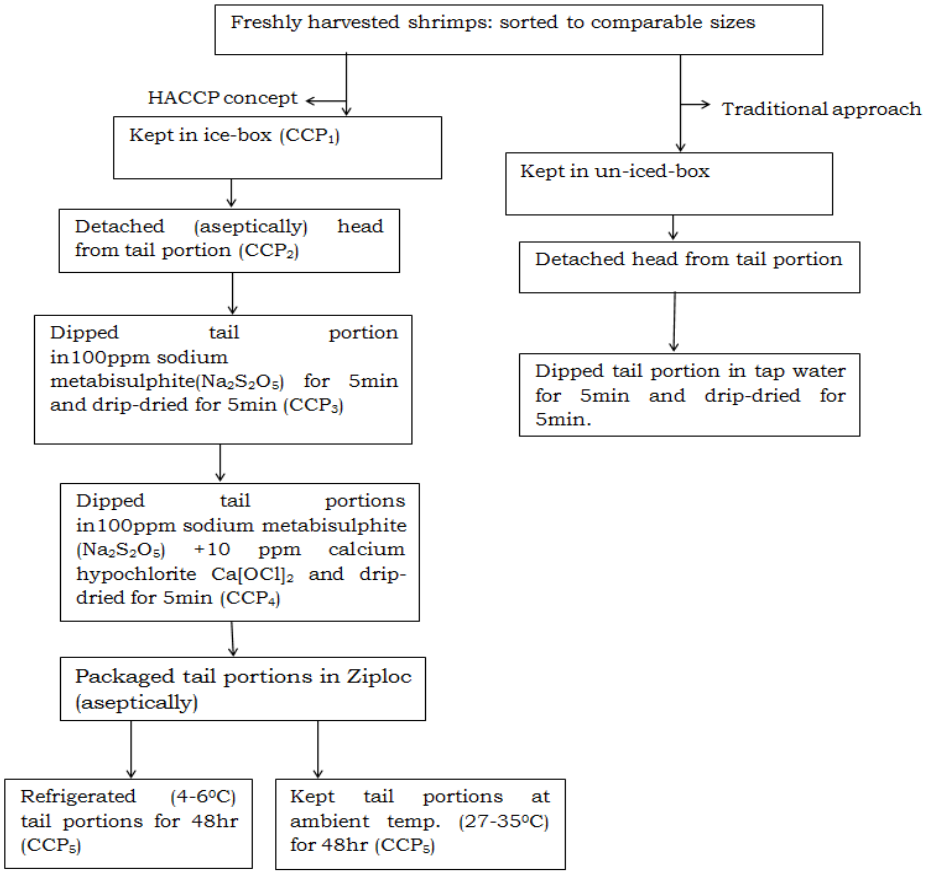

Figure 1. Flow diagram showing critical control points (CCPs) for shrimp processing

2.4. Identification of Microbial Isolates

Typical colonies were randomly picked from plates showing 30-300 colonies, purified and identified based on cultural, morphological and biochemical characteristics. The identification procedure included colonial and cellular morphological features coupled with biochemical characteristics (IMViC, triple sugar iron agar, catalase, oxidase, sugar fermentation:glucose, lactose, sucrose, mannitol [15,16,17].

2.5. Physico-Chemical Analysis

pH and Trimethylamine (TMA)

2.5.1. The pH of the pooled 10g samples of the respectively treated samples were determined after blending in 20ml distilled water (1:2 ratio) [18] using a calibrated pH meter (model Jenco 6177, USA).

2.5.2. The TMA contents of the respective triplicate samples were determined as described by Malle and Poumeyrol [19]. The determination involved use of Kjedahl distillation unit 2100 (Foss, Sweden).

2.6. Statistical Analysis

The obtained data were analyzed using Analysis of variance (ANOVA) software of SPSS version 15 and the significance of the mean differences determined at $p<0.05$.

3. Results

3.1. Microbiological Populations of Shrimps as Affected by Critical Control Points/Treatments

The microbiological qualities of the shrimps varied due to the critical control points/treatments (Table 1). The maximum total viable count (1.8 x 10^8cfu/g) occurred in tail samples stored at ambient temperature following 100 ppm $Na_2S_2O_5$ and 10ppm $Ca[OCl]_2$ treatment which was significantly different from the other samples (Table 1). Additionally, the other samples differed significantly from each other but those treated with $Na_2S_2O_5$ and $Ca[OCl]_2$ before refrigeration temperature showed the lowest total viable counts (Table 1). Coliform counts exhibited similar trends but un-iced samples on arrival in the laboratory had the highest significant population of 2.9 x 10^5 cfu/g (Table 1). Similarly, *Staphylococcus* spp counts showed significant maximum population of 8.2 x 10^3cfu/g in un-iced whole samples while the lowest count (7.8 x 10^1 cfu/g) was observed in tail samples treated with $Na_2S_2O_5$and $Ca[OCL]_2$prior to refrigeration storage (Table 1). Less significant variations in *Vibrio*spp counts occurred as affected by the critical control points but samples stored at ambient temperature had the highest population of 2.2x10^5cfu/g (Table 1). Similarly, maximum *Salmonella* spp. population (3.3 x 10^5cfu/g) was observed in tail sample subjected to $Na_2S_2O_5$ treatment followed by $Ca[OCl]_2$ treatment before storage at ambient temperature. However, un-iced whole shrimp sample, iced whole shrimp sample and tail sample dipped in tap water (control) and drip-dried showed no significant ($p<0.05$) difference among these three treatments (Table 1).

3.2. Microorganisms Isolated from Shrimps as Affected by Critical Control Points/Treatments

Table 2 shows the variety of microorganisms isolated from the shrimp samples as influenced by the critical control points/treatments. Maximum of five different bacterial genera were isolated from iced head sample, tail sample treated with 100ppm sodium metabisulphite alone, tail sample treated with 100ppm $Na_2S_2O_5$ and 10ppm $Ca[OCl]_2$ and tail sample subjected to 100ppm sodium metabisulphite and 10ppm calcium hypochlorite prior to refrigeration storage (Table 2). However, control sample dipped in tap water showed six bacterial genera (Table 2). But four different bacterial genera were isolated from the other samples.Of the bacterial isolates, most pathogens (*Bacillus*spp., *E. coli*, *Salmonella* spp., *Staphylococcus* spp.and *Vibrio* spp.) occurred in control samples, un-iced whole samples, iced head sample, tail samples treated with 100pm sodium metabisulphitealone, tail samples treated with 100ppm $Na_2S_2O_5$and 10ppm $Ca[OCl]_2$ and tail samples treated with 100 ppm sodium metabisulphite followed by calcium hypochlorite prior to ambient temperature storage (Table 2). In addition, *Pseudomonas* spp.were mostly isolated from iced or refrigerated samples irrespective of sample type (whole, head and tail) (Table 2).

Table 1. Total viable counts, Coliforms, Staphylococcus spp., *Vibrio* spp. and *Salmonella* spp. counts (cfu/g) of shrimps as influenced by critical control points CCPs/treatments and storage

Sample/CCPs/Treatments	Microbiological		Quality		
	TVCs	Coliforms	Staph	Vibrio	Salm
Whole fresh: un-iced	1.8 x 10^6 b	2.9x10^5 a	8.2 x 10^3 a	2.8 x 10^3 b	8.6x 10^2 b
Whole fresh: iced	8.6 x 10^4 d	3.1x10^3 e	2.3 x10^3 d	7.6 x 10^1 c	5.1 x 10^2 b
Head fresh: iced	4.7 x 10^4 e	3.2 x 10^3 e	3.0 x 10^3 c	7.2 x 10^1 c	2.9 x 10^2 c
Tail fresh: iced	8.5 x 10^4d	2.7 x 10^3 e	3.9 x 10^3b	2.1 x 10^3 b	3.2 x 10^2 c
Tail fresh: tap water (control)	1.6 x 10^5 c	6.5 x 10^3 d	4.3 x 10^3 b	3.0 x 10^3 b	6.3 x 10^2 b
Tail fresh: 100ppm $Na_2S_2O_5$	3.6 x 10^4f	2.1 x10^3 f	1.4 x 10^3 e	1.7 x 10^3 b	1.7 x 10^2 c
Tail fresh: 100ppm $Na_2S_2O_5$+ 10ppm $Ca[OCl]_2$	1.1 x 10^3g	7.0 x10^1 g	8.0 x 10^1 f	8.0 x 10^1 c	4.0 x 10^1 d
Tail Fresh: 100ppm $Na_2S_2O_5$+ 10ppm $Ca[OCl]_2$+ Ref 48hr	8.6x10^4 d	3.2x10^4 c	7.8 x 10^1 f	7.9 x 10^1 c	1.7 x 10^2 c
Tail fresh: 100ppm $Na_2S_2O_5$+ 10ppm$Ca[OCl]_2$+48hr Amb.	1.8x10^8a	1.8x 10^5 b	2.8 x 10^3 c	2.2 x 10^5 a	3.3 x 10^5 a

CCPs/Treatments: $Na_2S_2O_5$ = Sodium Metabisulphite; $Ca[OCl]_2$ = Calcium hypochlorite; Ref = Refrigerated Storage; Amb= Ambient Storage. TVCs = Total viable counts; Staph = *Staphylococcus* spp; Salm = *Salmonella spp.* Values (means of triplicate determinations) in columns under different microbial groups having different letters are significantly ($p<0.05$) different.

Table 2. Microorganisms isolated from shrimp samples as affected by various critical control points (CCPs)/treatments and storage

Samples/CCPs/Treatments	Microorganisms Isolated
Whole fresh: un iced	*Bacillus* spp; *E.coli Salmonella* spp, *Staphylococcus aureus, Vibrio cholerae, Vibrio parahaemolyticus*
Whole fresh: iced	*E.coli; Pseudomonas* spp, *Proteus* spp, *Staphylococcus aureus, Staphylococcus epidermidis*
Head fresh: iced	*Bacillus* spp; *E.coli, Pseudomonas* spp, *Salmonella* spp, *Staphylococcus aureus*
Tail fresh: iced	*E. coli, Pseudomonas* spp, *Staphylococcus aureus, Vibrio parahaemolyticus*
Tail fresh: 100ppm $Na_2S_2O_5$ alone	*Bacillus* spp, *E. coli, Proteus* spp, *Staphylococcus* spp, *Vibrio parahaemolyticus*
Tail fresh: 100ppm $Na_2S_2O_5$ + 10 ppm $Ca[OCl]_2$	*Bacillus* spp; *E. coli, Proteus* spp, *Salmonella* spp, *Staphylococcus* spp
Tail fresh: tap water (control)	*E. coli, Proteus* spp, *Salmonella* spp, *Staphylococcus* spp, *Vibrio cholerae, Vibrio parahaemolyticus*
Tail fresh: 100ppm $Na_2S_2O_5$ + 10ppm $Ca[OCl]_2$ + Ref. 48hr	*E.coli; Proteus* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Streptococcus* spp
Tail fresh: 100ppm $Na_2S_2O_5$+ 10ppm $Ca[OCl]_2$ + Amb. 48hr	*Bacillus* spp. *E. coli, Salmonella spp, Staphylococcus aureus, Vibrio cholerae*

$Na_2S_2O_5$ = Sodium metabisulphite; $Ca[OCl]_2$ = Calcium hypochlorite; Ref = Refrigeration storage, Amb= Ambient Storage.

3.3. Physico-Chemical Characteristics of Shrimps as Affected by Critical Control Points (Ccps)/Treatments

3.3.1. pH Values

The pH values and TMA contents of the shrimps as influenced by the critical control points/treatments are shown in Table 3. The tail portion subjected to 100ppm sodium metabisulphite followed by 10ppm calcium hypochlorite prior to ambient temperature storage and that dipped in tap water (control) showed the highest significant pH values (Table 3). Significantly (p<0.05) higher pH value occurred in head portion compared with tail portions but the lowest pH value was observed in tail portions treated with 100 ppm sodium metabisulphite and 10 ppm calcium hypochlorite (Table 3).

3.3.2. TMA Contents

The tail portion treated with 100ppm sodium metabisulphite and 10ppm calcium hypochlorite prior to ambient temperature storage showed the highest TMA content (37.48 mgN/100g) which was followed by the control sample (Table 3). But un-iced whole sample and iced head sample had comparable TMA contents while the lowest TMA content (5.49 mgN/100g) occurred in shrimps treated with 100ppm sodium metabisulphite alone (Table 3).

Table 3. pH and Trimethylamine (TMA) contents of Shrimps as affected by Critical Control Points (CCPs)/treatments and storage

Sample/CCPs/Treatments	pH	TMA (mgN/100g)
Whole fresh: uniced	7.25e	21.01c
Whole fresh: iced	7.21e	14.58f
Head fresh: iced	7.63b	18.95c
Tail fresh: iced	7.15f	16.94d
Tail fresh: tap water (control)	7.69a	22.61b
Tail fresh: 100ppm $Na_2S_2O_5$ alone	7.45c	5.49h
Tail fresh: 100ppm $Na_2S_2O_5$ + 10 ppm $Ca[OCl]_2$	7.06g	15.84e
Tail fresh: 100ppm $Na_2S_2O_5$ + 10 ppm $Ca[OCl]_2$+ Ref. 48hr	7.34d	8.57g
Tail fresh: 100ppm $Na_2S_2O_5$ + 10 ppm $Ca[OCl]_2$+ Amb. 48hr	7.85a	37.48a

$Na_2S_2O_5$ = Soldiummetabisulphite; $Ca[OCl]_2$ = Calcium hypochlorite; Ref = Refrigerated storage, Amb = Ambient storage.

Each value represents the mean of triplicate determinations. Mean values in columns under pH and TMA having different letters are significantly (p<0.05) different.

3.4. Correlation of Variables and the Coefficients

The correlation values of the different variables and the levels of significance are presented in Table 4. Highly significant positive correlation occurred between total viable counts and coliform counts (r=0.9011), between coliform counts and *Salmonella* spp. population (r = 0.9692), between *Vibrio* counts and *Salmonella* spp. population (r=0.9732) and total viable counts versus *Salmonella* spp. population (r = 0.7803) (Table 4). However, total viable counts versus *Vibrio* spp. counts showed weak positive correlation (r = 0.6516) while non-significant negative correlation occurred between pH and total viable counts (r=-0.3406), between pH and coliforms (r = -0.2867) and between TMA and total viable counts (r = - 0.3406). Similarly, a weak non-significant positive correlation (r = 0.3361) was observed between TMA and coliform counts (Table 4).

Table 4. Relationship between physico-chemical parameters (pH, TMA), microbial groups and among the microbial groupsin shrimps as affected by CCPs/Treatments

Variables correlated	Correlation values (r*; **)
pH versus TMA	0.1479
pH versus total viable counts (TVCs)	-0.3406
pH versus coliforms	-0.2867
pH versus *Staphylococcus*spp counts	0.1176
pH versus *Vibrio*spp counts	-0.1832
pH versus *Salmonella*spp counts	-0.1853
TMA versus TVCs	-0.3406
TMA versus *coliforms*	0.3361
TMA versus *Staphylococcus*spp counts	-0.1749
TMA versus *Vibrio*spp	-0.1832
TMA versus *Salmonella*spp	-0.1853
TVCs versus coliform counts	0.9011**
TVCs versus *Staphylococcus*spp counts	0.5572
TVCs versus *Vibrio*spp	0.6576*
TVCs versus *Salmonella*spp	0.7803**
Coliform counts versus *Vibrio*spp	0.2110
Coliform count versus *Salmonella*spp	0.9692**
Vibrio counts versus *Salmonella*spp	0.9732**

Correlation (r* and r** = 0.01 and 0.001 level of significance respectively). Correlation coefficients are based on overall means of 10 determinations of 3 replicates (n=30).

3.5. Percentage Frequency of Occurrence of Bacterial Isolates

The percentage frequency of occurrence of bacteria isolated from un-iced whole fresh shrimps and iced whole fresh samples is shown in Figure 2a and Figure 2b respectively.Whereas five bacterial genera were isolated from un-iced samples, four bacterial genera occurred in iced samples with *E. coli*being the most predominant (26%) in un-iced samples followed by *Salmonella* spp. (22%); *Staphylococcus* spp. was least prevalent (Figure 2a).

In contrast, *Pseudomonas* spp. was the most prevalent (30%) and *Proteus* spp.was least dominant (18%) in iced whole fresh shrimps (Figure 2b).

The iced head portion of the shrimps showed *Pseudomonas* spp. having the highest frequency of occurrence (28%) followed by *Bacillus*spp. (24%) while the least was *Salmonella* spp. (15%) (Figure 3a).

Conversely, iced tail portion had *Bacillus* spp. as the most dominant (30%) and *E.coli* as the least prevalent (11%). Tail portion of shrimps subjected to critical control point using 100ppm sodium metabisulphitealone showed *Bacillus* spp. havingmaximum frequency of occurrence (33%) while the lowest frequency of occurrence was 12% obtained for*Vibrio* spp. (12%) (Figure 3b).

On the other hand, tail portion treated with 100ppm sodium metabisulphite and followed by 10ppm calcium hypochlorite had much higher *Staphylococcus* spp. (28%) compared to that treated only with 100ppm sodium metabisulphite (Figure 4 and Figure 5).

In addition, no *Vibrio* spp. was observed in Figure 5. The tail portion subjected to critical control point involving tap water (control) had six bacterial isolates with *E.coli* showing the maximum frequency of occurence (24%) followed by *Bacillus* spp. (20%) and *Staphylococcus* spp. (10%) (Figure 6).

Figure 7a shows percentage frequency of occurrence of bacteria isolated from tail portion treated with 100ppm sodium metabisulphite and 10ppm calcium hypochlorite before refrigeration storage for 48hr. Much higher percentage of *Pseudomonas* spp. occurred (34%) followed by *E. coli* (22%) and the lowest,*Staphylococcus* spp. (11%) (Figure 7a).

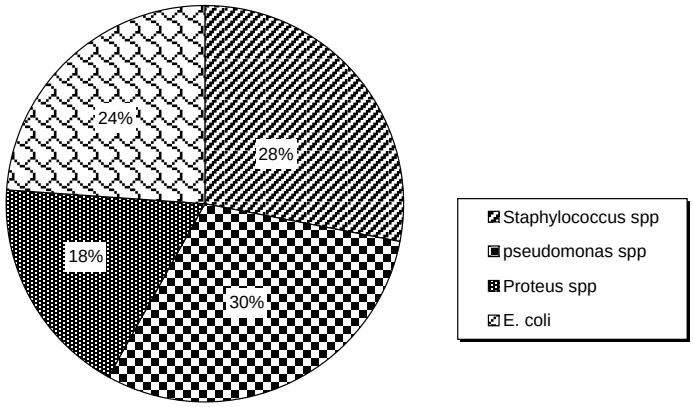

Figure 2a. Percentage frequency of occurrence of bacteria isolated from un-iced whole fresh shrimps

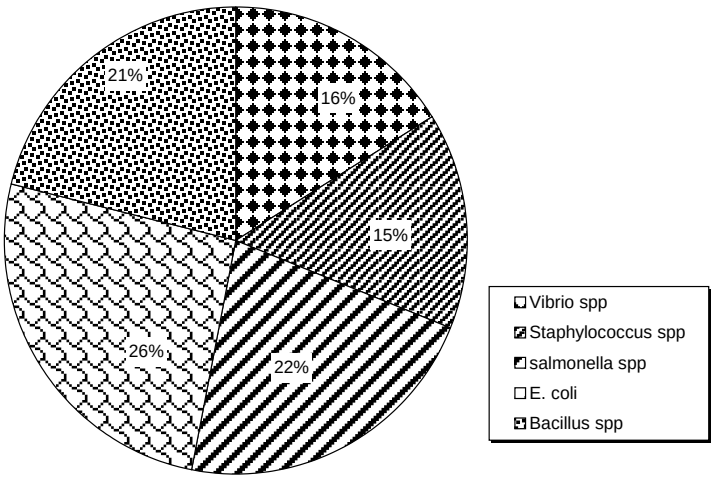

Figure 2b. Percentage frequency of occurrence of bacteria isolated from iced whole fresh shrimps

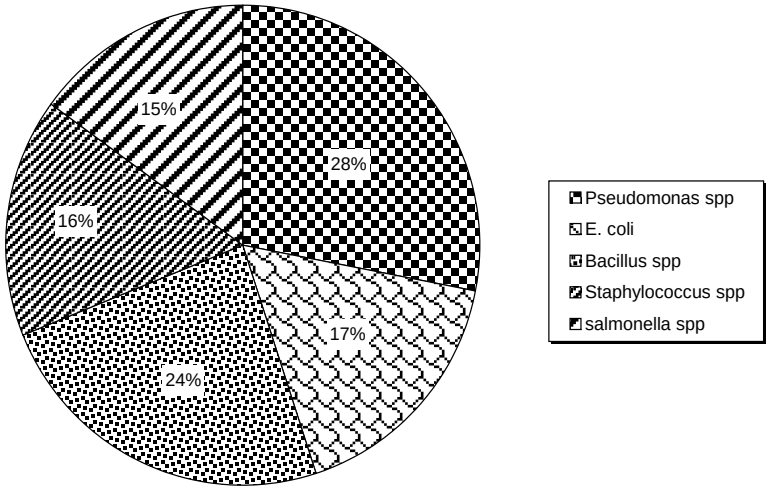

Figure 3a. Percentage frequency of occurrence of bacteria isolated from iced head portion of shrimps

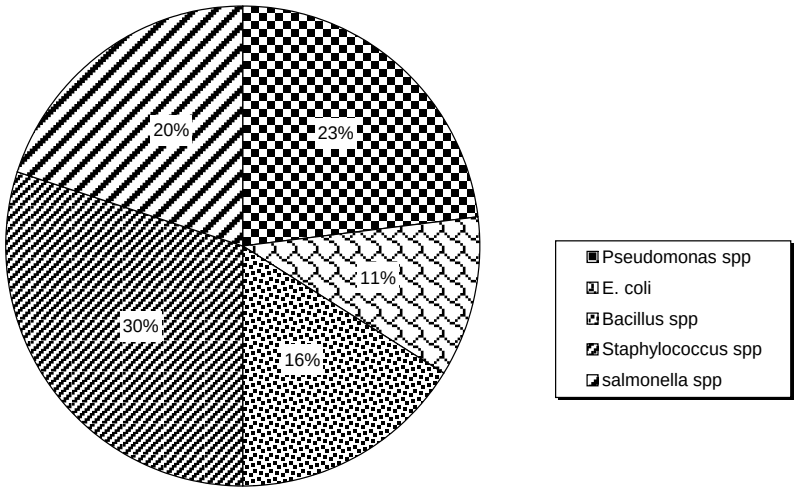

Figure 3b. Percentage frequency of occurrence of bacteria isolated from iced tail portion of shrimp.

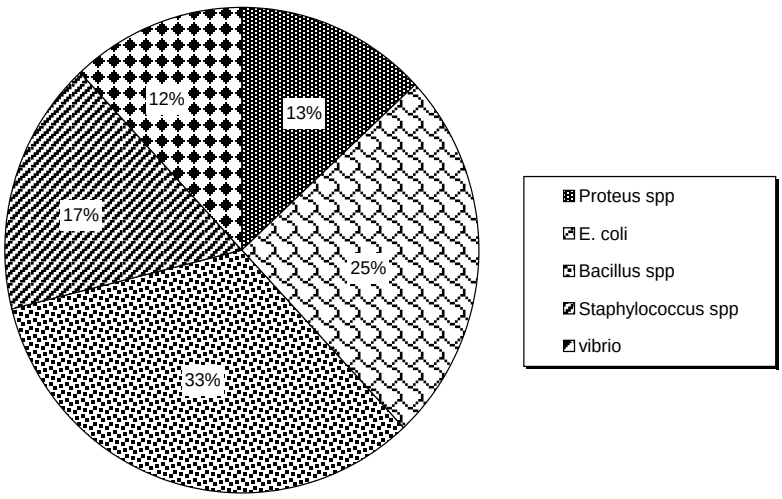

Figure 4. Percentage frequency of occurrence of bacteria isolated from tail portion of shrimp treated with 100 ppm sodium metabisulphite

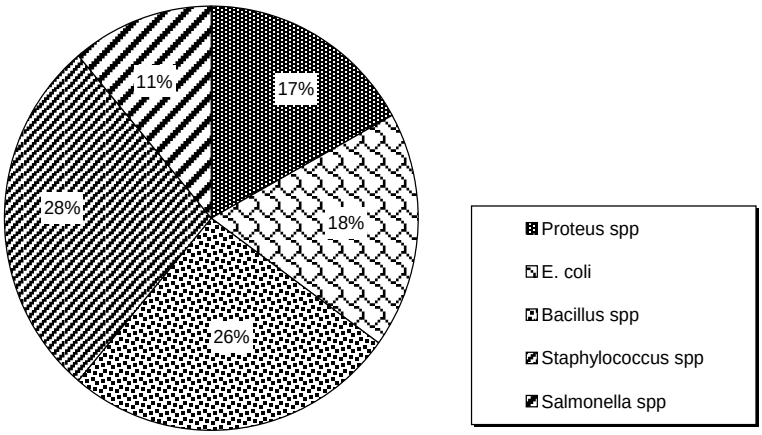

Figure 5. Percentage frequency of occurrence of bacteria isolated from tail portion of shrimp treated with 100 ppm sodium metabisulphite and 10ppm calcium hypochlorite

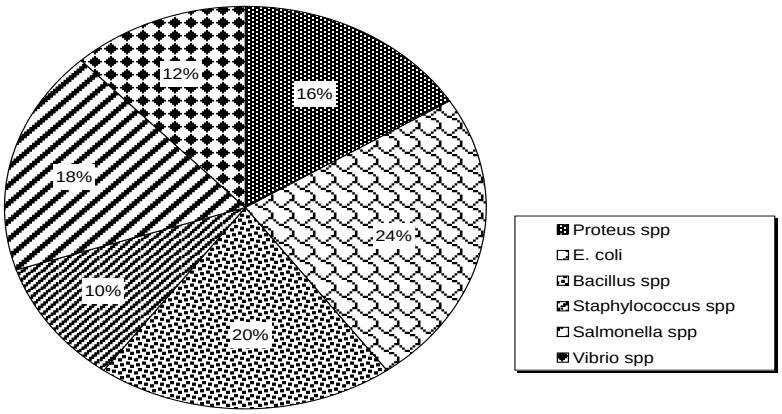

Figure 6. Percentage frequency of occurrence of bacteria isolated from tail portion of shrimp immersed in tap water and drip-dried (control; traditional practice)

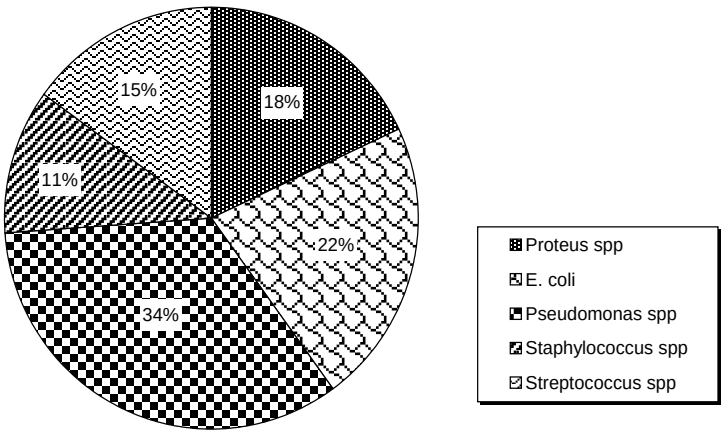

Figure 7a. Percentage frequency of occurrence of bacteria isolated from tail portion of shrimp treated with 100 ppm sodium metabisulphite and 10 ppm calcium hypochlorite prior to refrigerated storage for 48hr

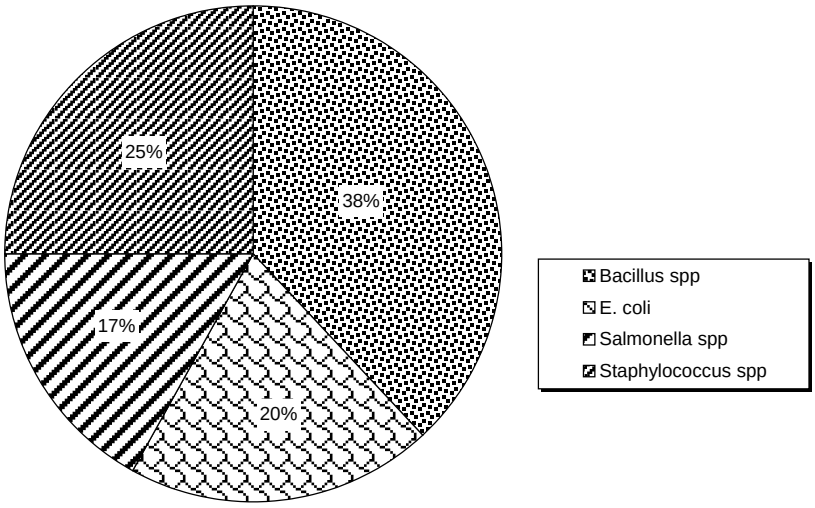

Figure 7b. Percentage frequency of occurrence of bacteria isolated from tail portion of shrimp treated with 100ppm sodium metabisulphite and 10ppm calcium hypochlorite prior to ambient temperature storage for 48hr

But fewer bacterial genera were observed in tail sample treated with 100ppm sodium metabisulphitefollowed by 10ppm calcium hypochlorite before ambient temperature storage (27-35°C) for 48hr (Figure 7b) which was dominated by *Bacillus* spp. (38%) and *Staphylococcus* spp. (25%)(Figure 7b).

4. Discussion

Critical control points (CCPs) or control measures applied in food production systems are often aimed at minimizing or preventing hazards in foods to enhance food safety [4,13,20]. Storage temperature of food and use of preservatives are among the critical variables associated with microbial growth, proliferation and safety in foods [8,18,21].

The present results (Table 1) have clearly shown the importance of storage temperature as a critical control point in shrimp production process where samples stored at ambient temperature exhibited maximum total viable counts (TVCs) in spite of treatment with $Na_2S_2O_5$ followed by $Ca[OC]_2$ (Table 1). Similarly, un-iced whole shrimps compared with iced whole shrimp had significantly much higher microbial population ($1.8x10^6$ cfu/g)than the latter (Table 1). The variations in the TVCs as influenced by the respective critical control points (Table 1) underscore the need for application of CCPs in shrimp processing. For example, the application of sodiummetabisulphite alone was beneficial but sequential application of calcium hypochlorite was significantly more effective in reduction of TVCs count of and other microbial groups (Table 1). This gives credence to the report by Pyle and Koburger [11] that the antimicrobial effects of sodium metabisulphite treatment were dependent on order of application such as the sequential treatment of shrimp with bisulphite followed by hypochlorite which led to greater microbial reduction over either treatment alone (Table 1). The occurrence of similar trends among the different bacterial groups (Coliforms, *Staphylococcus* spp.,*Vibrio* spp. and *Salmonella* spp.) suggest the effects of CCPs where gram negative bacteria predominated in samples stored at low temperature or subjected to iced-storage as earlier reported [1,5,8].

The high microbial populations observed in the un-iced and ambient temperature stored samples (Table 1) corroborate the findings by other investigators who showed highest pathogenic microbial loads during the summer [22]. Apparently, based on the TVCs safety limit of 10^5cfu/g of shellfish [6,23,24]. un-iced whole shrimp, tail sample subjected to tap water (control) and sample treated with 100pm $Na_2S_2O_5$ followed by 10ppm $Ca[OCL]_2$ prior to ambient temperature storage (Table 1) are therefore microbiologically unsafe having exceeded the limit. Thus, the application of CCPs involving low temperature and/or preservatives enhanced the microbial safety of the samples.

The isolation of fewer bacterial genera (five) from sodium metabisulphite and calcium hypochlorite treated samples is indicative of their inhibitory effects but such impacts were negligible in ambient temperature stored samples (Table 2). Thus, application of adequate CCPs should always be recommended as evidenced by the diversity of bacterial genera (six) in control sample (Table 2). In addition, whereas fewer bacterial isolates were observed in the ambient temperature stored samples (Table 2), virtually all of these microorganisms isolated from such samples were pathogens commonly associated with mesophilic growth temperature [4] (Table 2).

The pH of seafoods serves as an indicator of quality [1,5]. In the present work, the occurrence of highest pH values in control and 100ppm sodiummetabisulphiteand 10ppm calcium hypochlorite treated samples prior to ambient temperature storage (Table 3) clearly reflect the impacts of CCPs in which the maximum pH of such samples is indicative of spoilage (Table 3). These results corroborate some earlier findings which showed that pH of shrimps is a useful indicator of freshness or spoilage [1,12]. It is likely that the autocatalytic phenomenon involving bisulphite reaction with hypochlorite must have

led to formation of hydrogen ions which probably reduced the pH and enhanced the antimicrobial effects [11].

TMA is a known product of bacterial activity which is used as an index of seafood quality [25,26]. The significantly higher TMA contents observed in the samples subjected to 100ppm sodium metabisulphite followed by 10ppm calcium hypochlorite before ambient temperature storage or control sample (Table 3) clearly indicate the impacts of microbial activity as influenced by storage temperature or lack of preservative (ie control) (Table 3).It is therefore evident that the beneficial effects of sodium metabisulphite and calcium hypochlorite coupled with low temperature are reflected by the low TMA contents (Table 3). These results are in agreement with some other findings involving use of ice-storage of shrimps [1]. However, the significantly high TMA contents (Table 3) that occurred in control and un-iced whole and iced head samples are indicative of the importance of sample portion/type [6] and CCPs in relation to shrimp deterioration or shelf life extension [1,9,12].

Thus, based on the range of 10-15mgN TMA/100g for seafood spoilage-detection and unacceptability [4,12,18], it is therefore evident that virtually all the samples except three, namely; (i) those subjected to 100ppm sodium metabisulphitealone (ii) those subjected to 100ppm sodium metabisulphite followed by 10ppm calcium hypochlorite prior to refrigeration storage and (iii) whole shrimp subjected to iced storage were considered unacceptable having exceeded the limit of 15mg TMA/100g (Table 3).

Correlation analysis showed that total viable counts were significantly positively related to the coliforms and *Salmonella* spp.counts respectively. Such a relationship was also established between coliforms and *Salmonella* spp. counts as well as between *Vibrio* spp. and *Salmonella* spp. populations (Table 4). Thus, each of these microbial groups may be used as predictor of the other. In contrast, the physico-chemical parameters (pH and TMA) either correlated poorly or negatively against the microbial groups (Table 4) which suggest that they are poor indices of microbial predictability in these samples probably due to the influence of the various treatments.

The differential percentage frequency of bacterial occurrence between un-iced and iced whole shrimp samples clearly suggests the effects of storage temperature since more bacterial diversity was observed in the samples exposed to high ambient temperature [27-35°C] with particularly *Bacillus* spp. and *Staphylococcus* spp. being most dominant which probably may be attributed to their mesophilic characteristics [4,15]. On the other hand, the dominance of *Pseudomonas* spp. in iced whole samples (Figure 2b) confirms their proliferation under low temperature storage conditions as previously reported [4,18]. Whereas the bacterial distribution (five) is comparable between the two sample portions, the head portion was dominated by *Bacillus* spp. while *Staphylococcus* spp.were most prevalent in the tail portion (Figure 3a and b). This differential bacterial profile could be attributed to tissue anatomical structure and exposure as well as bacterial ability to survive under different conditions since *Bacillus* spp. are spore-formers and *Staphylococcus* spp. are more associated with aerial and food processing contamination [4,6]. Similarly, the predominance of *Bacillus* spp. and *Staphylococcus* spp. in samples treated with the preservatives suggeststheir tolerance as spore-formers and the sensitivity of gram-negativeasporogenous bacterial genera (Figure 4 and 5). Evidently, the occurrence of maximum bacterial diversity of six genera (mostly pathogens)in control samples indicates the potential hazards posed by such samples to consumers (Figure 6) hence, CCPs are highly recommended. Application of low storage temperature is highly advisable but often, not available in developing countries including Nigeria due to inadequate (erratic) power supply [18,27]. Nevertheless, samples stored under refrigeration exhibited substantial prevalence of *Pseudomonas* spp. which are known to play a major role in spoilage of such seafoods [3,4].

On the contrary, the occurrence of fewer bacterial genera in samples subjected tosodium metabisulphite and calcium hypochlorite before ambient temperature storage (Figure 7b) but dominated by *Bacillus* spp. (38%) and followed by *Staphylococcus* spp. clearly underscores the critical importance of storage temperature since the effects of high ambient temperature (27-35°C) outweighed the benefits of the preservatives (Figure 7b). These results clearly indicate the essence of hurdle technology and application of HACCPconcept in the seafood industry.

5. Conclusions

Application of critical control points (CCPs) in shrimp processing has shown that use of sodium metabisulphite followed by calcium hypochlorite significantly improved microbial safety of shrimps. Further enhancement of shrimp safety was achieved when such preserved samples were subjected to iced or refrigeration storage. However, ambient temperature storage of shrimps impacted negatively with respect to microbial and physico-chemical parameters in spite of use of sodium metabisulphite and calcium hypochlorite as CCPs. Additionally, the prevalence of pathogens in samples subjected to ambient temperature storage is a major concern. Whereas total viable counts correlated positively with other bacterial groups, poor or negative correlation was observed between the physico-chemical parameters (pH and TMA) and the microbial groups. Therefore, they are poor predictors of microbial quality/safety in these samples. This study has clearly demonstrated the benefits of application of sodium metabisulphite followed by calcium hypochlorite as critical control points in shrimp processing. But these effects waned when such samples were subjected to ambient temperature storage. It is therefore evident that application of HACCP concept with focus on various relevant control measures is necessary to achieve microbiologically safe food products. Thus, this study has provided information (not currently available) on the concept and application of HACCP to seafood harvesters, processors and regulatory agencies such that its adoption would enhance microbial safety of shrimps and other food products.

Acknowledgements

Tertiary Education Trust Fund (TetFund) is gratefully

acknowledged for the sponsorship of this research project (TETFUND/DESS/UNI/P-HARCOURT/IBR/2016/VOL.1) to Prof. B.J.O Efiuvwevwere, the Lead Researcher of the team.

Competing Interests

Authors have declared that no competing interests exist.

References

[1] Omar, M. I. V. Utilization of sodium metabisulphite for preservation of frozen thaivel shrimp *(Pandaleus borealis).* The United Nations University, Fisheries Training Programme, Iceland, Final Project 1998.

[2] Jimoh, A.A. and Lemomu, L.P. Shell fish resources in Nigeria. Proceedings of Fisheries, Society of Nigeria (FISON), ASCON, Badagry, 25th -29th Oct. 2010; 683-693.

[3] Ramesh B.K., Govindaw, R.V Krishna, N.M Geetha, S. and Kakara, R.R Assessment of bacteriological quality in selected commercially important shrimps of Visakhapatnawm, East coast of India.*International J.Microbiological Biotechnology* 2017; 2(2): 102-105

[4] Jay, J. M. *Modern Food Microbiology* 6th edition Aspen; Maryland USA. 2000.

[5] Condurso, C. Tripodi, G; Cincotta, F; Lanza, C.M Mazzagha, A. and Verzera, A. Quality Assessment of Mediterranean shrimps during storage. *Italian J. Food Science* 2016; 28: 497-509.

[6] Samia, S; Galib, H.T., Tanvir, A. S; Basudob, C.S; Walliullah, M.D; Tasniaw, A; Sakil, M.D; Afsana, F.N; Sadia, K.P Kamal, K.D; Mrityunjoy, A Nusrat, J.UT asminia, R. and Rashed, N. Microbiological quality analysis of shrimps collected from local market around Dhaka city. *International Food Research J.* 2014; 21(1): 33-38.

[7] Miget, R. *Shellfish handling practices-shrimps and molluscs*. Southern Regional Aquaculture center, (SRAC), Texas. SRAC Publication No. 4902. May 2010.

[8] Jonnalagadda, P.R. Sudershan, R.V., Raji, N.S. and Rao, D.R. Identification of critical control points in the two selected HACCP-certified prawn processing units. *J. Food Quality* 2009; 32:177-189.

[9] Ronholm, J., Lau, F. and Banerjee, S.K Emerging seafood preservation techniques to extend freshness and minimize *Vibrio* contamination: *Frontiers in Microbiology* 2016; 7:1-7.

[10] Martinez, Alvarez, O Gomez-Gullen, M.C and Montero, P. Role of sulfites and 4-hexylresorcinol in microbial growth and melanosis prevention of deepwater pink shrimp (*Parapenaculongirostris)* using a controlled atmosphere. *J. Food Protection*, 2005; 68 (1): 98-104.

[11] Pyle M.L. and Koburger J.A. Increased sensitization of shrimp microflora to hypochlorite following a sodium bisulphite dip. *J. Food Protection* 1984; 47 (5): 375-377.

[12] Khodanazary, A. Freshness assessment of shrimp *Metapenaeusaffnis* by quality index method and estimation of its shelf life. *International J. Food Properties* 2019; 22(1): 309-319.

[13] Ehiri, J.E Azubuike, M.C Ubbaonu, C.NAnyanwu, E. C Ibe, K. M. and Ogbonna, M.O. Critical control points of complementary food preparation and handling in Eastern Nigeria. *Bulletin World Health Organization*. 2001; 7(5): 423-435.

[14] Nahemiah, D; Bankole, O.S; Tswako, MNma-Usman, K.I Hassan, H. and Fati, K.I. Hazard analysis critical control points (HACCP) in the production of soy-Kununzaki: A traditional cereal-based fermented beverage of Nigeria. *American Food Science Technology*. 2014; 2(6): 196-202.

[15] Cheesbrough M. *District Laboratory Practice in Tropical countries Part 2*. Cambridge University Press, 2000; 400-434.

[16] APHA (American Public Health Association). In F.P. Downes and K. Ito (Eds).*Compendium of methods for the microbiological examination of foods*.(4th edition).Washington, DC; American Public Health Association; USA. 2001.

[17] Sneath, P. H.A; Mair, N.S. Sharpe, M.E and Halt, J.G. *Bergey's Manual of Systemic Bacteriology* vol. 2. Williams and Wilkins; Baltimore, 1986.

[18] Oruwari, B.O. and Efiuvwevwere, B.J.O. The effects of different storage temperatures on the microbial, physico-chemical and organoleptic quality changes in the shellfish "Ngolo" (Thais-califera) from Nigeria. *British Microbiology Research J.*2016; 16(2):1-11.

[19] Malle, P and Poumeyrol, M.A. New chemical criterion for determination of trimethylamine and total volatile base nitrogen.*J. Food Protection*1989; 52:419-423.

[20] FAO/WHO Guidance to governments on the application of HACCP in small and/or less-developed food businesses. FAO Food and Nutrition: Paper 86; 2014; FAO/WHO, Geneva.

[21] Sudheer, K.P; Sankalpa, K.B. and Saranya, S. Effect of preservatives and temperature on microbial and physico-chemical attributes of minimally processed pineapple. *International J. Current Microbiology. Applied Sciences* 2019; 8(2):541-553.

[22] Depaola, A Jones, J. L Woods, J. Burkhardt, W Cala, K.R Krantz, J.A, Bowers, J.C., Kasturi, K Byara, R.H. Jacobs, E. Williams-Hill, D. and Nabe, K. Bacterial and viral pathogens in live oysters: 2007 United States Market survey. *Applied and Environmental Microbiology.* 2010; 7b(9): 2754-2768.

[23] International Commission on Microbiological Specification for Foods (ICMSF). *Microorganisms in Foods. 2. Sampling for Microbiological Analysis. Principles and Specific Applications.*2nd edition.Blackwell Scientific Publications 1986.

[24] FAO Corporate Document on Repository Assessment and Management of Seafood Safety and Quality (EEC 93/493); 1993.

[25] Gram, L. and Huss, H.H. Microbiological spoilage of fish and fish products. *International J.Food Microbiology* 1996; 33:121-137.

[26] Efiuvwevwere, B.J.O. and Amadi, L.O. Effects of preservatives on the bacteriological, chemical and sensory qualities of mangrove oyster (*Crassostreagasar*). *British J. Applied Science Technology* 2015; 5(1): 76-84.

[27] Cahill, S. M. and Jouve, J.L. R. Microbiological risk assessment in developing countries. *J. Food Protection* 2004; 67(9): 2016-2023.