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Original Research Article

Critical Control Points Involving Calcium Hypochlorite and Storage Temperatures for Microbial Safety and Physico-Chemical Attributes of Oysters (*Crassostrea gasar*)

Bernard J.O. Efiuvwevwere[*], Chimezie J. Ogugbue, Godwin Emoghene and Augustine K. Ngbara-ue

Department of Microbiology, Faculty of Science, University of Port Harcourt, Port Harcourt, Nigeria

*Corresponding author

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Oysters (*Crassostrea gasar*) are globally important but highly susceptible to microbial hazards. Critical control points (CCPs) for oyster safety involving 10 ppm calcium hypochlorite $Ca(OCl)_2$, storage temperatures and traditional postharvest practices were employed. Microbiological and physico-chemical characteristics were analyzed. Un-iced storage (27-35°C) resulted in significant total viable counts (TVCs) exceeding recommended limits (approx. 5 $\log_{10}$ cfu/g). Diverse microbial profiles and hazardous levels occurred in traditionally handled samples compared to $Ca(OCl)_2$ treated samples. Regardless of $Ca(OCl)_2$ application, significant microbial populations (5.28 $\log_{10}$ cfu/g and 6.43 $\log_{10}$ cfu/g) and undesirable pH (<6.30) and trimethylamine contents of 37.65 and 45.84 mgN/100g were occasioned by 27-35°C storage. The preponderance of pathogenic Gram positive organisms (*Bacillus* and *Staphylococcus* spp.) occurred in 27-35^{0}C stored samples irrespective of $Ca(OCl)_2$ application. A remarkable increase in Gram positive flora to Gram negative profile ratio of approximately 5-fold occurred in 4-6^{0}C stored samples versus 27-35^{0}C stored samples; underscoring the impacts of storage temperature. These clearly demonstrate the critical role of storage temperature in spite of other CCPs employed. Overall, the need for adoption of CCPs has been demonstrated indicating that cold-chain practice is necessary to enhance microbial safety of oyster and maximize its local and international trade.

Introduction

Oysters (*Crassostrea gasar)* are of considerable economic importance and nutritional value globally (Galaviz- Villa *et al*., 2015; Jay, 2000). However, they are often exposed to a wide range of microorganisms in the aquatic ecosystem thereby accumulating several different microbiota since they are filter-feeders (Galaviz-Villa *et al.,* 2015; Depaola *et al*., 2010; APHA, 2001). Consequently, their levels of microbial contamination are usually very high and sometimes constitute public health hazards to consumers (Ozbay *et al.,* 2018; Jay,2000). Whereas there is abundance of oysters in the

Niger Delta region of Nigeria, the high levels of microbial hazards associated with them constitute a major concern. In addition, the potential of oysters and other seafoods to harbour microbial pathogens and eventually cause food-borne diseases is well documented for both developed and developing countries (Jonnalagadda *et al.*, 2009; Yonnes and Bartram, 2001). However, oysters remain acceptable if unshucked but they lose quality rapidly once shucked except preserved (Chen *et al.,* 2016; Efiuvwevwere and Amadi, 2015).

Hazard analysis critical control point (HACCP) has become a global systematic approach to ensuring food safety and wholesomeness as well as enhancement of international food trade (Galaviz-Villa *et al.*, 2015; WHO, 2007). Additionally, the benefits of HACCP to the seafood industry have been underscored by several workers elsewhere (National Seafood HACCP Alliance, 2017; Jonnalagadda *etal*; 2009; Rahman, 2007).

Therefore, the production of safe and high quality oysters in Nigeria and other countries for both domestic and export trade using HACCP concept is of critical economic importance and public health significance (Feltes *et al.*, 2017; Montanhini and Neto, 2015). Unfortunately, in spite of such benefits associated with HACCP application globally, very little or no research work on oysters to establish any critical control points or measures concerning microbial safety to consumers is available in Nigeria.

However, HACCP-related work on children's foods was carried out by Ehiri *et al.,* (2001).Thus, the present investigation was undertaken to focus on the application of HACCP using various parameters (such as calcium hypochlorite) to serve as critical control points or measures during processing in relation to microbial profiles and safety of oysters.

Materials and Methods

Collection of oyster samples

Freshly harvested oysters (*Crassostrea gasar)* from Andoni River, Rivers State, Nigeria were purchased from seafood harvesters based on prior arrangements. They were then transported to the laboratory in two polystyrene boxes (one containing ice packs and the other, no ice) within 4hr of harvest for analyses.

Processing and treatment of oysters

The oyster samples (approx. 5kg) were sorted into comparable sizes (approx.10g each) and divided into two portions. One portion was kept in a polystyrene box layered with polythene bag and packed with ice blocks (4-6^0C) while the other portion was kept in a polystyrene box without ice blocks. Both boxes were transported to the laboratory and on arrival, the samples were individually cleaned/washed thoroughly and shucked manually aseptically or as traditionally practised.

Following the CCPs/treatments, microbiological and physico-chemical characteristics were analyzed.

Microbiological analysis

A composite (25g) of shucked oyster samples was blended in 225 ml 0.1% (w/v) peptone water using a stomacher (model BA 6021, Seward Medical, London, UK) to obtain 10^{-1} dilution. 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 37^0C for 18-24hr.

The plates were then examined for growth of colonies and enumeration of total viable counts, coliforms, *Staphylococcus* spp., *Vibrio* spp. and *Salmonella* spp. counts was carried out. All the culture media used were products of Titan Biotech. Ltd., India.

Identification of bacterial isolates

Typical representative colonies were randomly picked from plates showing 25-250 colonies, purified, characterized using motility, Gram reaction, spore stain, catalase, coagulase, urease, citrate utilization, indole production, Methyl-Red (MR), Voges-Proskauer and sugar fermentation tests (triple sugar iron agar, glucose, sucrose, lactose and mannitol) and subsequently identified based on colonial, cellular and biochemical characteristics (APHA, 2001; Cheesbrough, 2000; Sneath *et al.*, 1986).

Chemical analysis: pH and trimethylamine (TMA)

The pH of composite (10g) oyster samples of the respectively treated samples were determined after blending in 20ml distilled water (1:2 ratio) (Efiuvwevwere and Amadi, 2015) using a calibrated pH meter (model Jenco 6177, USA).

The TMA contents of the respective triplicate samples were determined as described by Malle and Poumeyrol (1989). The determination involved use of Kjedahl distillation unit 2100 (Foss, Sweden).

Statistical analysis

The data generated for different quality characteristics were analysed using Analysis of variance (ANOVA) software of SPSS version 15 and the significance of the mean differences determined at $p<0.05$.

Results and Discussion

Microbial quality of oyster samples as influenced by critical control points

The populations of the various microbial groups differed significantly with critical control points (CCPs) as shown in Table 1. The highest total viable counts (TVCs) of 6.73 $\log_{10}$ cfu/g and 6.43 $\log_{10}$ cfu/g were observed in un-iced oyster samples on arrival in the laboratory (i.e about 4 hr following harvest) and those immersed in tap water before ambient temperature storage for 48hr respectively (Table 1).

In contrast, samples subjected to other CCPs exhibited lowest significant counts with those immersed in 10 ppm calcium hypochlorite alone as well as those subjected to 10 ppm calcium hypochlorite prior to refrigerated storage for 48hr (Table 1). Similarly, the lowest coliform counts were 1.49 $\log_{10}$ cfu/g and 1.41 cfu/g involving oyster subjected to 10 ppm calcium hypochlorite treatment alone and those immersed in 10 ppm calcium hypochlorite then followed by refrigerated storage for 48hr (Table 1). Comparable trends as observed in TVCs and coliform counts were also found in *Staphylococcus* spp counts (Table 1).

However, some variations with respect to effects of CCPs on *Salmonella* spp and *Vibrio* spp counts were observed (Table 1). Evidently, these microbial population variations reflect the impacts of CCPs in oyster processing which confirm that certain conditions or treatments favour the development or growth of microorganisms and at the same time, inhibit the development or growth of others. These are termed the intrinsic, processing and extrinsic factors (1CMSF 1980; Gould, 1989; Banwart, 2004) and they play critical roles in microbial food safety and spoilage.

For example, refrigeration temperature is critical for control of growth and activity of microorganisms hence the lower the temperature, the lower the microbial population (Banwart, 2004) as evidenced in this work (Table 1). However, most mesophilic microorganisms do not grow below 10^0C.

Consequently, they are not often a problem in refrigerated foods but some mesophiles are psychrotrophic in nature and are capable of growth in refrigerated foods (Banwart, 2004). Thus, the critical control measures concerning microbial safety of oysters should be applied in conjunction with refrigeration temperature. Additionally, the National Shellfish Sanitation Program (Banwart, 2004) established a microbiological criterion of total viable counts for shellfish (including oysters) ranging between 4.70 and 6.0 $\log^{10}$ cfu/g.

Evidently, 4 out of the 9 treatments of the samples subjected to CCPs viz, (a) un-iced oysters, (b) iced oysters, (c) iced, immersed in tap water and stored at ambient temperature for 48hr as well as (d) those immersed in 10 ppm calcium hypochlorite before storage for 48hr at ambient temperature (Table 1) are unacceptable since samples subjected to those treatments had TVCs which exceeded the recommended limit of 4.>0 log cfu/g to 6.0 $\log^{10}$ cfu/g (Banwart, 2004).

The antibacterial benefits exhibited by samples subjected to CCPs involving calcium hypochlorite treatment may be attributed to the formation of hypochlorous acid and disruption of several vital functions of the microorganisms (Dumani *et al.,* 2016; Wikipedia: https://en.Wikipedia.org/wiki/calcium_hypochlorite). But the high populations of *Salmonella* spp. and *Vibrio* spp. especially in samples stored at ambient temperature (Table 1) clearly indicate the potential microbial hazards of these samples

to consumers being good sources for transmission of these pathogens (Jay, 2000). Furthermore, these microorganisms have been reported to increase to hazardous numbers when exposed to high temperatures (Miget 2010). Therefore, the need for implementation of cold- chain food supply to reduce the risks of microbial growth has been demonstrated as evidenced by the present results (Table 1).

Microorganisms isolated from oyster samples as influenced by critical control points and storage temperatures

Several bacterial genera were isolated from the oyster samples and they varied with the critical control points (Table 2). The most diverse bacterial genera (6) occurred in virtually all the samples (un-iced, iced, cleaned/washed and shucked, iced and immersed in tap water (control) and those subjected to 10 ppm calcium hypochlorite before refrigeration ($4-6^0$C) storage for 48hr (Table 2).

In contrast, only 4 bacterial genera were isolated from shucked oysters immersed in 10 ppm calcium hypochlorite (as CCP) (Table 2). Nonetheless, irrespective of the CCP applied, *Bacillus* spp. occurred in all treatments (Table 2). Thus, these results corroborate the ubiquitous nature of *Bacillus* spp. including their psychrotrophic, mesophilic and thermophilic characteristics coupled with variations in pH of their growth ranging from acidic to alkaline with most growing at pH 6.5-7.5 (Banwart, 2004) which is comparable to the pH values of the oyster samples.

The relative low population and frequency of occurrence of *Staphylococcus* spp. in samples treated with calcium hypochlorite (Tables 1 and 2) suggest their sensitivity to chlorine compounds as previously reported (Dumani *et al.,* 2016; Banwart, 2004).

pH and TMA contents

The effects of critical control points (CCPs) on pH of oysters are shown in Table 3. Limited pH variations were observed with the highest (7.07) occurring in samples immersed in 10 ppm calcium hypochlorite and refrigerated for 48hr and those immersed in tap water before refrigeration storage for 48hr (pH 6.95) while the lowest (pH 6.25) occurred in un-iced and shucked as well as those immersed in tap water prior to ambient temperature storage for 48hr (pH 6.21) (Table 3).

The marginal variations in pH associated with the CCPs/treatments are similar to the findings by others (Mudoh *et al.,* 2014; Cao *et al*., 2009) which showed slight decreases in pH of oysters stored under different low temperatures. Oysters contain relatively high carbohydrate content (Cao *e tal*. 2009) hence prone to fermentative process (Jay, 2000).

Therefore, decrease in its pH is deemed to be an indication of on-set of spoilage. Thus, the samples having pH values of 6.30 and below (Table 3) are considered to be in the process of spoilage and are generally unacceptable (Cao *et al.,* 2009).

The TMA contents varied with CCPs resulting in maximum contents (45.84mg N/100g) in samples immersed in tap water and stored at ambient temperature for 48hr followed with those immersed in 10 ppm calcium hypochlorite before ambient temperature storage for 48hr (37.65mg N/100g) (Table 3).

In contrast, the lowest contents (1.28mg N/100g) occurred in samples immersed in 10 ppm calcium hypochlorite only or those immersed in 10 ppm calcium hypochlorite and stored at refrigeration temperature for 48hr (Table 3).

TMA is a good indicator of seafood (including oyster) freshness or spoilage (Efiuvwevwere and Amadi, 2015; Cao *et al.,* 2009). The higher the value, the lower the quality. It is evident from the results (Table 3) that samples subjected to 10 ppm calcium hypochlorite or immersed in tap water prior to ambient temperature storage were spoiled having exceeded the TMA limit of acceptability (10-15mgN/100g) which apparently must have been exacerbated by the high ambient temperature (Oruwari and Efiuvwevwere 2016; Jay, 2000).

Correlation of parameters and their coefficients

Table 4 shows the correlation values for the several correlated variables. The pH either correlated poorly or negatively against the microbial groups but showed strong negative correlation (r=-7185) between pH and *Staphylococcus* spp. counts (Table 4). On the other hand, TMA content correlated positively (r=0.5221) against *Salmonella* spp. counts. Significantly positive correlation (r= 0.9909) was observed between total viable counts and coliform counts (Table 4). Similarly, microbial groups showed strong correlations such as TVCs against *Vibrio* spp. (r=0.9861) and coliforms vs *Vibrio* (r= 0.9772) (Table 4).

These variations in correlations between variables clearly indicate the impacts of interplay among parameters such as the effects of calcium hypochlorite and storage temperatures on some of the microbial groups. Thus, their growth behaviour became altered and could not be closely correlated as would have been the case. Consequently, the use of such microbial group to predict the growth/behaviour of another group became complex and highly unpredictable as was reported earlier (Edberg and Smith, 1989).

Percentage frequency of occurrence of bacteria isolated from oyster samples as influenced by critical control points (CCPs)/treatments and storage temperatures

Figures 2a and 2b show the percentage frequency of bacteria isolated from un-iced and iced oyster samples respectively. Whereas *Bacillus* spp. (26%) and *Staphylococcus* spp. (22%) dominated the un-iced samples, *Pseudomonas* spp. (15%) and *Vibrio* spp. (21%) were the most dominant microorganisms in the iced samples (Figure 2b).

This differential in microbial prevalence may be attributed to impact of bacterial growth temperatures which corroborate some earlier findings which indicated that *Bacillus* spp. mostly exhibit both psychrotrophic and mesophilic growth characteristics (Ozbay *et al.,* 2018; Chen *et al.,* 2016; Montanhini and Neto, 2015; Banwart, 2004). In contrast, *Pseudomonas* spp and *Vibrio* spp are predominantly psychrophilic /pschrotrophic in nature and this partly explains their prevalence in the iced-stored samples (Figure 2b).

The dominance of the cleaned/washed and shucked samples by *Bacillus* spp (34%) and *Staphylococcus* spp (30%) (Figure 3) clearly indicates their ability to survive and thrive in more adverse conditions since they are Gram positive organisms and moreso, *Bacillus* spp are aerobic, wide-spread in nature and spore-formers (Jay, 2000).

On the contrary, only four bacterial genera dominated by *Bacillus* spp. (65%) and *Staphylococcus* spp. (24%) occurred in shucked oysters subjected to 10 ppm calcium hypochlorite only (Figure 4a). This further confirms the survival of Gram positive flora and spore-formers in unfavourable conditions (Figure 4a). This also confirms that calcium hypochlorite is an inhibitory agent to microbial growth (Dumani *et al.,* 2016). On the other hand, samples subjected to tap water as control showed most diverse bacterial profile consisting of six bacterial genera dominated by both Gram positive and Gram negative flora (Figure 4b).

The effect of pH is likely to have played a role because of the pH intrinsic property of oyster (Mudoh *et al.,* 2014; Jay, 2000). Figure 5a shows the percentage frequency of occurrence of bacteria isolated from oysters subjected to CCPs involving application of 10 ppm calcium hypochlorite before refrigeration storage for 48hr. The high prevalence of *Pseudomonas* spp (40%) could be attributed to the favourable low temperature of growth associated with these microorganisms as psychrophiles (Chen *et al.,* 2016; Jay, 2000).

Similarly, the immersion of the samples in tap water (control) also resulted in *Pseudomonas* spp. (43%) being the most dominant organism under refrigeration storage (Figure 5a).

This further corroborates the impact of refrigeration temperature on growth of *Pseudomonas* spp. regardless of additional control measures. However, when the samples were subjected to 10 ppm calcium hypochlorite prior to ambient temperature storage for 48hr as critical control point, *Bacillus* spp drastically increased to 51% and followed by *Staphylococcus* spp (19%) (Figure 6a).

Thus, the commercial/traditional practice of immersing oysters in tap water before ambient temperature storage exacerbated the bacterial profile thereby increasing the dominance of *Staphylococcus* spp to 41% and enhanced the microbial hazards as well as potential risks to consumers.

Table.1 Total viable counts, coliforms, Staphylococcus spp; Salmonella spp. and Vibrio spp. counts ($\log_{10}$cfu/g) of oyster samples as influenced by critical control points (CCPs)/ treatments and storage temperatures Microbiological quality ($\log_{10}$cfu/g)

Samples/CCPs/Treatments	TVCs	Coliforms	Staphylococcus	Salmonella	Vibrio
Uniced oysters	6.73a	5.07b	4.54a	4.83a	4.57C
Iced oysters	5.56b	4.61c	4.37b	4.51b	4.20d
Iced + cleaned/washed + shucked	3.95c	2.23e	1.99e	2.54e	2.38g
Iced + 10ppm Ca(OCl)$_2$	2.59f	1.40g	1.16g	2.12f	2.04h
Iced+ tap water (control)	3.53d	1.74f	1.51f	2.72e	2.88f
Iced + tap water+48hr Refrigeration temperature	3.45d	2.30e	2.06d	3.35d	3.18e
Iced + 10 ppm Ca(OCl)$_2$ +48hr Refrigeration temperature	2.93e	1.41g	1.42f	1.43g	2.84f
Iced+tap water +48hr Ambient temperature storage	6.43a	5.55a	4.10b	4.98a	5.80a
Iced + Ca (OCL)$_2$ + 48hr Ambient temperature storage	5.28b	4.00d	3.76c	4.00c	5.09b

Ca(OCl)$_2$ = Calcium hypochlorite; 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 oyster samples as influenced by critical control points (CCPs)/treatments and storage temperatures

Samples/CCPs/Treatments	Microorganisms Isolated
Un-iced fresh oysters	*Bacillus* spp., *E. coli, Pseudomonas spp., Salmonella spp, Staphylococcus spp, Vibrio spp.*
Iced fresh oysters	*Bacillus* spp
	E. coli
	Pseudomonas spp., *Salmonella spp, Staphylococcus* spp, *Vibrio* spp
Iced fresh oysters +cleaned/washed +shucked	*Bacillus* spp., *E. coli, Pseudomonas* spp, *Salmonella* spp, *Staphylococcus* spp, *Vibrio spp.*
Fresh shucked oysters + 10 ppm Ca(OCl)$_2$	*Bacillus* spp., *E.coli, Salmonella spp, Staphylococcus* spp
Fresh shucked oysters + tap water (control)	*Bacillus* spp.,*E.coli, Proteus* spp, *Salmonella spp, Staphylococcus* spp, *Vibrio* spp.
Fresh shucked oysters + 10 ppm Ca(OCl)$_2$+ 48hr Ref.	*Bacillus* spp., *E.coli, Pseudomonas* spp, *Salmonella spp, Staphylococcus* spp, *Vibrio* spp.
Fresh shucked oysters + tap water +48hr Ref.	*Bacillus* spp., *E.coli, Proteus* spp, *Pseudomonas* spp, *Salmonella spp, Vibrio* spp.
Fresh shucked oysters + 10 ppm Ca(OCl)$_2$ + 48hr Amb.	*Bacillus* spp., *E. coli, Salmonella spp, Staphylococcus* spp, *Vibrio* spp.
Fresh shucked oysters + tap water + 48hr Amb.	*Bacillus* spp., *E.coli, Salmonella spp, Staphylococcus* spp, *Vibrio* spp.

Ca(OCl)$_2$ = Calcium hypochlorite, Ref = Refrigeration storage; Amb. = Ambient temperature storage

Table.3 pH and Trimethylamine (TMA) contents of oyster samples as influenced by critical control points (CCPs)/treatments and storage temperature

Samples/CCPs/Treatments		
	pH	TMA(mgN/100g)
Un-iced fresh oysters	6.25_e	3.30_c
Iced fresh oysters	6.30_e	3.64_d
Iced + cleaned/washed + shucked oysters	6.28_e	3.23_e
Fresh shucked oysters + 10 ppm Ca(OCl)$_2$	6.56_d	1.28_f
Fresh shucked oysters + tap water (control)	6.60_d	3.42_e
Fresh shucked oysters + 10 ppm Ca(OCl)$_2$ + 48hr Ref.	7.07_a	1.06_f
Fresh shucked oysters + tap water + 48hr Ref.	6.95_b	4.34_c
Fresh shucked oysters + 10 ppm Ca(OCl)$_2$ + 48hr Amb.	$6.83c$	37.65_b
Fresh shucked oysters + tap water + 48hr Amb.	6.21_e	45.84_a

Ca(OCl)$_2$ = Calcium hypochlorite; Ref. = Refrigeration temperature; Amb. = Ambient temperature storage. Mean values of triplicate determinations in columns of pH and TMA having different letters are significantly ($p<0.05$) different

Table.4 Correlation between physico-chemical parameters (pH and TMA), microbial groups and among the microbial groups in oyster samples as influenced by critical control points (CCPs)/treatments

Correlated variables	Correlation values (r[*], r[**])
pH versus TMA	0.4552
pH versus Total viable counts (TVCs)	-0.4693
pH versus coliforms	-0.4701
pH versus *Staphylococcus* spp counts	-0.7185**
pH versus *Salmonella* spp counts	0.2198
pH versus *Vibrio* spp counts	-0.3704
TMA versus TVCs	-0.3463
TMA versus coliform counts	-0.3493
TMA versus *Staphylococcus* spp counts	-0.2630
TMA versus *Salmonella* spp counts	0.5221*
TMA versus *Vibrio* spp counts	-0.2990
TVCs versus coliform counts	0.9909**
TVCs versus *Staphylococcus* spp counts	0.1635
TVCs versus *Salmonella* spp counts	-0.1770
TVCs versus *Vibrio* spp counts	0.9861**
Coliform counts versus *Staphylococcus* spp count	0.1742
Coliform counts versus *Salmonella* spp count	-0.1710
Coliform counts versus *Vibrio* spp counts	0.9772**
Staphylococcus spp count versus *Salmonella* spp counts	-0.1227
Staphylococcus spp counts versus *Vibrio* spp counts	0.0353

Correlation values (r[*] and r[**] = 0.01 and 0.001 level of significance respectively). Correlation coefficients are based on overall mean of 9 determinations of 3 replicates (n=27)
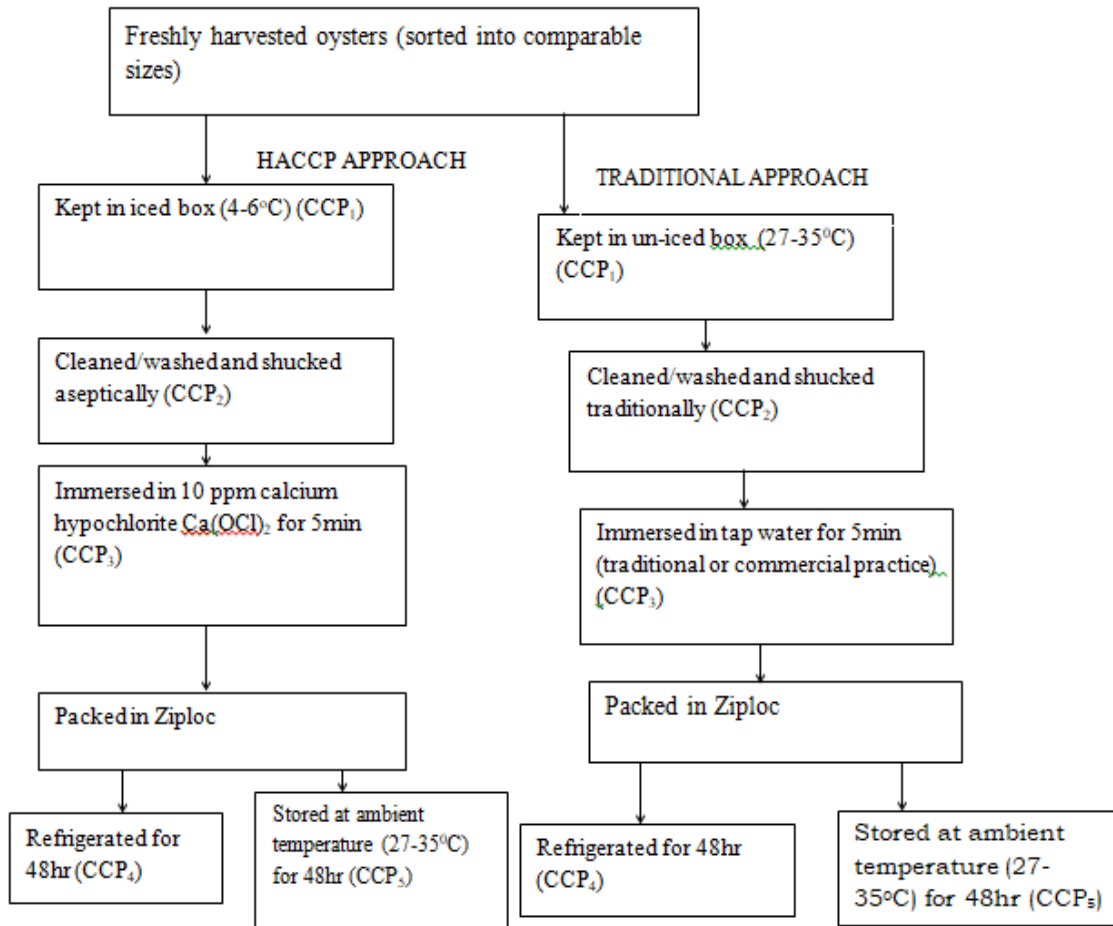
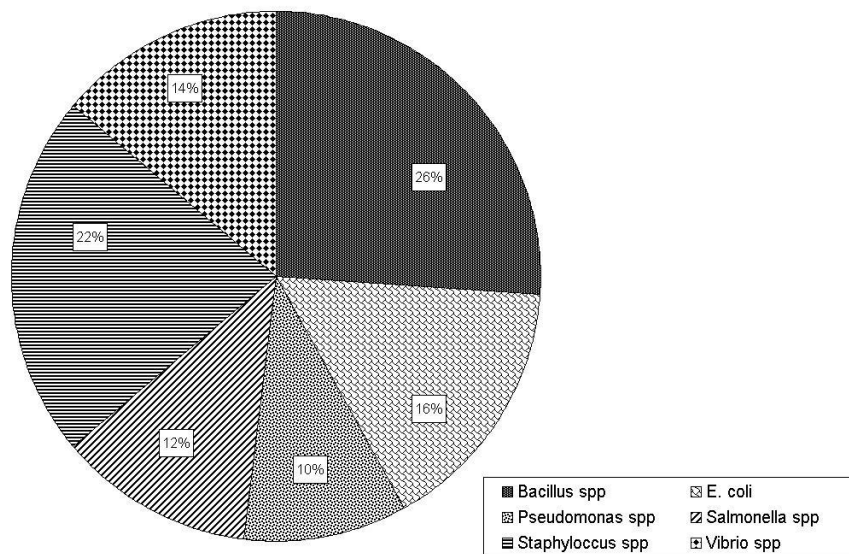**Figure.1** The critical control points (CCPs) used during the processing of oysters

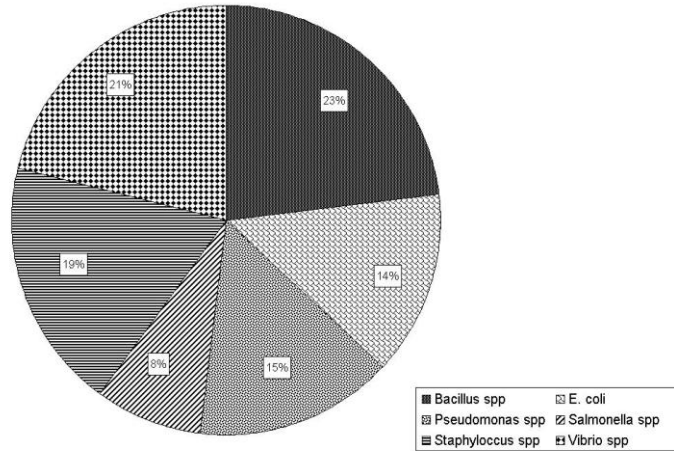**Figure.2a** Percentage frequency of occurrence of bacteria isolated from un-iced oyster samples

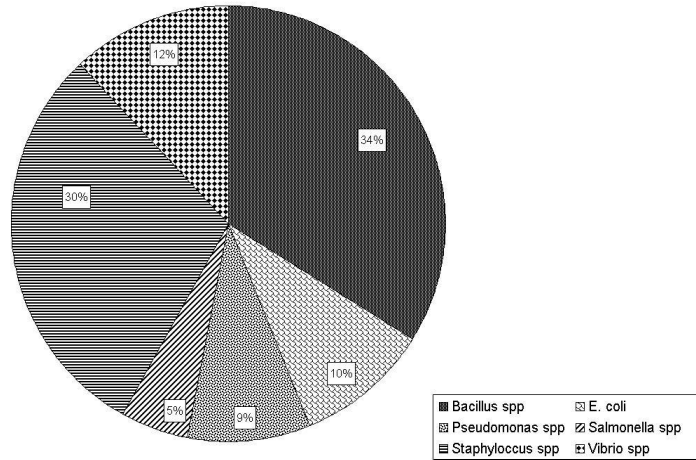**Figure.2b** Percentage frequency of occurrence of bacteria isolated from iced oyster samples

Figure.3 Percentage frequency of occurrence of bacteria isolated from iced, cleaned/washed and shucked oyster samples
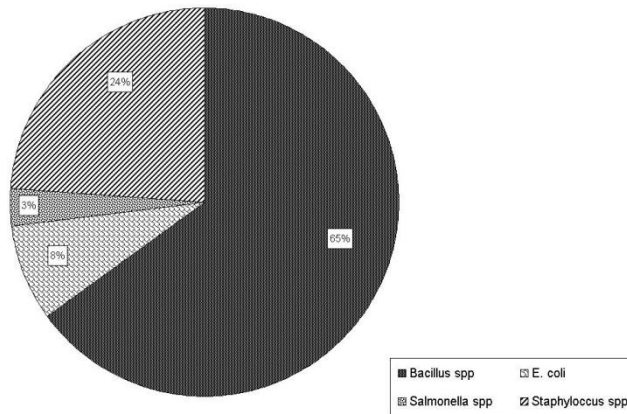

Figure.4a Percentage frequency of occurrence of bacteria isolated from iced, shucked oyster samples immersed in 10 ppm calcium hypochlorite
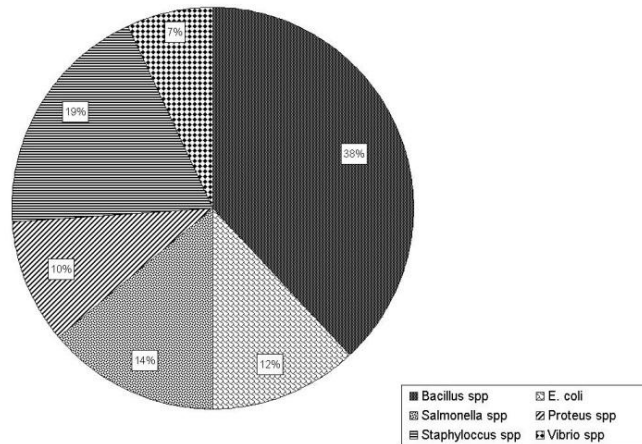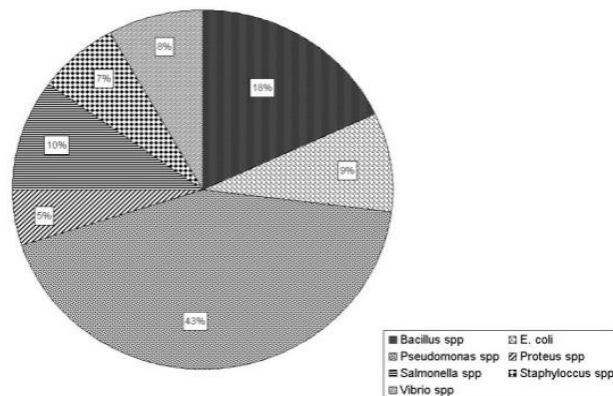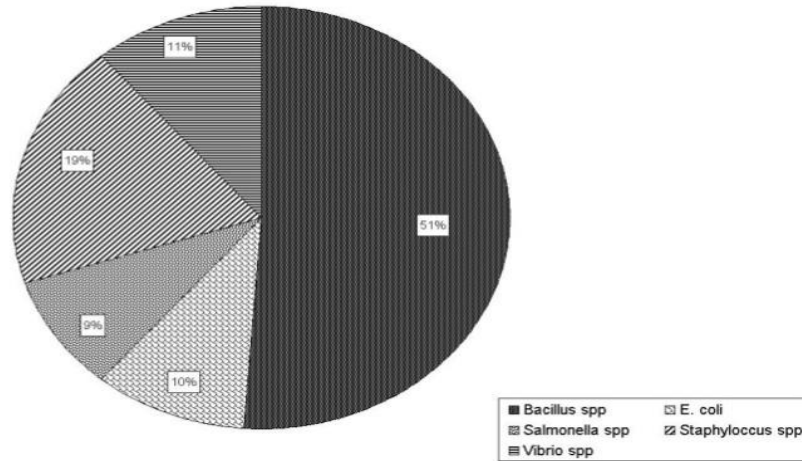
Figure.4b Percentage frequency of occurrence of bacteria isolated from iced, shucked oyster samples immersed in tap water (control)
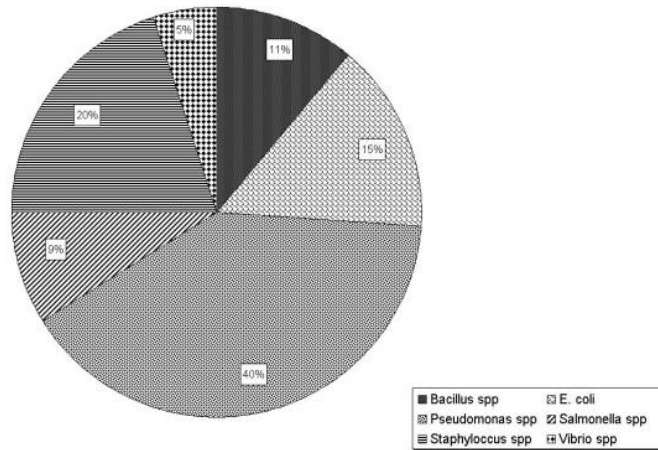
Figure.5a Percentage frequency of occurrence of bacteria isolated from iced, shucked oyster samples immersed in 10 ppm calcium hypochlorite prior to refrigerated storage for 48 hr

Figure.5b Percentage frequency of occurrence of bacteria isolated from iced, shucked oyster samples immersed in tap water prior to refrigerated storage for 48hr

Figure.6a Percentage frequency of occurrence of bacteria isolated from iced, shucked oyster samples immersed in 10 ppm calcium hypochlorite prior to ambient temperature storage for 48 hr
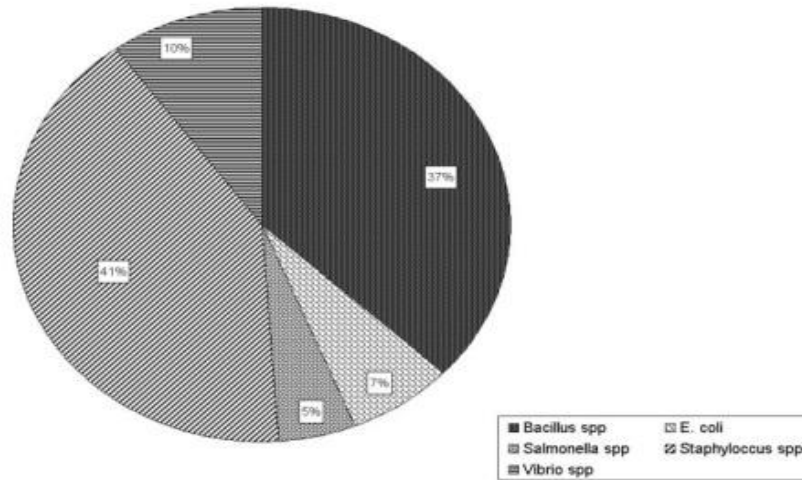

Figure.6b Percentage frequency of occurrence of bacteria isolated from iced, shucked oyster samples immersed in tap water prior to ambient temperature storage for 48 hr.

It is therefore evident that irrespective of the CCPs (such as application of calcium hypochlorite), the impacts of temperature of storage appears to override those of some other critical control measures particularly when the effects of refrigeration storage are compared with the ambient temperature storage. These effects are underscored when the Gram positive bacteria isolated from refrigerated samples are compared with ambient temperature stored samples. For example, Figure 5a versus Figure 6a clearly illustrates the shift in bacterial profile from ratio of Gram positive to Gram negative flora with 31:69 (i.e 0.45) versus 70:30 (i.e 2.33). Thus, this clearly shows the dramatic increase of approximately 5-fold in Gram positive organisms which may be attributed to effect of microbial growth temperature.

The microbiological safety of oysters has been shown to be enhanced by application of critical control points involving use of calcium hypochlorite in conjunction with

refrigeration storage (4-6°C). Transportation of oysters following harvest in an un-iced polythene box adversely resulted in significant microbial population increase. Irrespective of transportation of oysters in iced box and application of calcium hypochlorite, storage at ambient temperature of 27°C-35°C led to significantly high microbial populations exceeding recommended population of approximately $5\log_{10}$ cfu/g with concomitant undesirable physico-chemical parameters (pH and TMA).

Whereas strong positive correlations were observed in some parameters, others showed negative or poor relationships. Thus, they could not be used as indicators for prediction and this may be attributed to the application of critical control points. Overall, whereas critical control points are important in processing of oysters (including other foods), adequate consideration should be given to combination of CCPs application with temperature of storage being a major impact factor.

Acknowledgements

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