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BACTERIOLOGICAL QUALITY OF FRESH AND SMOKE- DRIED OYSTERS SOLD IN CREEK ROAD MARKET, PORT HARCOURT, NIGERIA.

M. C. Maduka and S. N. Ibe
Department of Microbiology,
University of Port Harcourt
Port Harcourt, Rivers State, Nigeria.
E-mail: medilinechinny@yahoo.com, Tel.:+2348065176526

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ABSTRACT

This study evaluated the bacteriological quality of fresh and smoke-dried oysters (Crassostreagasar) from Okrika fishing port, sold at the Creek Road market, Port Harcourt, found to have high bacterial load. Smoke-drying significantly reduced total aerobic count by 3 log cycles ($p< 0.01$) from an average of $1.26x10^9$cfu/g to $1.42x10^6$cfu/g and average pH value from 6.51 to 5.69. Total coliform and Escherichia coli counts, indices of sanitary quality, were also high but not significantly different for the sample types ($p<0.05$). Average counts for total coliforms and E. coli were $5.78x10^8$cfu/g and $2.36x10^8$cfu/g for fresh oysters respectively compared to $4.1x10^7$cfu/g and $1.46x10^7$cfu/g for smoke-dried samples. Bacterial isolates found in fresh oyster were Klebsiella sp. Escherichia sp., Proteus mirabilis, Citrobacter sp., Micrococcus sp., Bacillussp., Staphylococccus sp. and Serratia sp. while E.coli, Streptococcus sp., Staphylococcus sp. and B.cereus predominated in dried oysters. The oyster meats did not meet the standards of International Commission on the Microbiological Specification of Foods (ICMSF) for raw oysters of $5x10^5$cfu/g for total aerobic count and 230/100g for E.coli using 5 sampling units and are therefore considered hazardous for export and consumption. However the pH of fresh oyster samples met the proposed standard for freshness of 6.2-5.9 while dried oysters did not.

Keywords: Fresh and dried Oysters, Bacteriological Quality, ICMSF, Port Harcourt

INTRODUCTION

Oysters are mollusks or bivalve seafoods, which are widely eaten as delicacies worldwide and specifically by people in the Riverine areas of Nigeria. The species *Crassostrea gasar* is harvested from the roots of mangrove trees where it is attached and becomes exposed at low tide. Oysters are considered as rich sources of vitamins, and minerals, low-calorific food source and high in protein (Ebenso, 2002). Oysters can be eaten fresh when harvested from known waters with low pollution and microbial contamination. The shelf life can be up to two weeks before spoilage sets in by fermentation of glycogen to produce lactic acid. Putrid meat has a pH of 5.2 and below compared to pH of 6.2 -5.9 for good quality meat (Jay, 1988).

Oyster meat can be preserved by freezing, sun or smoke drying, salting and canning. In the Niger Delta Region of Nigeria, drying is the preferred method of preservation. Fresh shell stock oysters are washed, steamed and shucked before the meat is extracted.

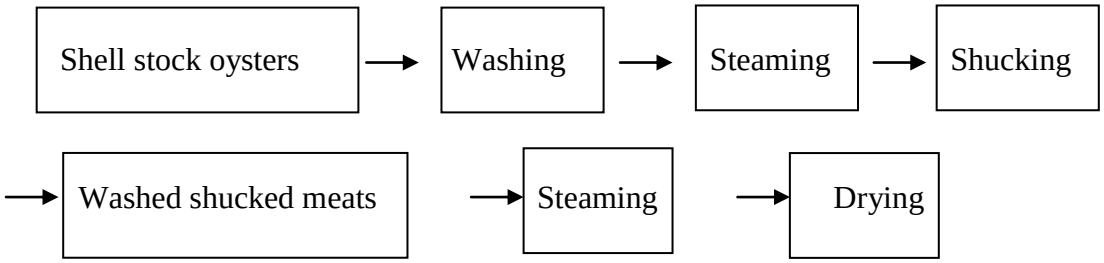
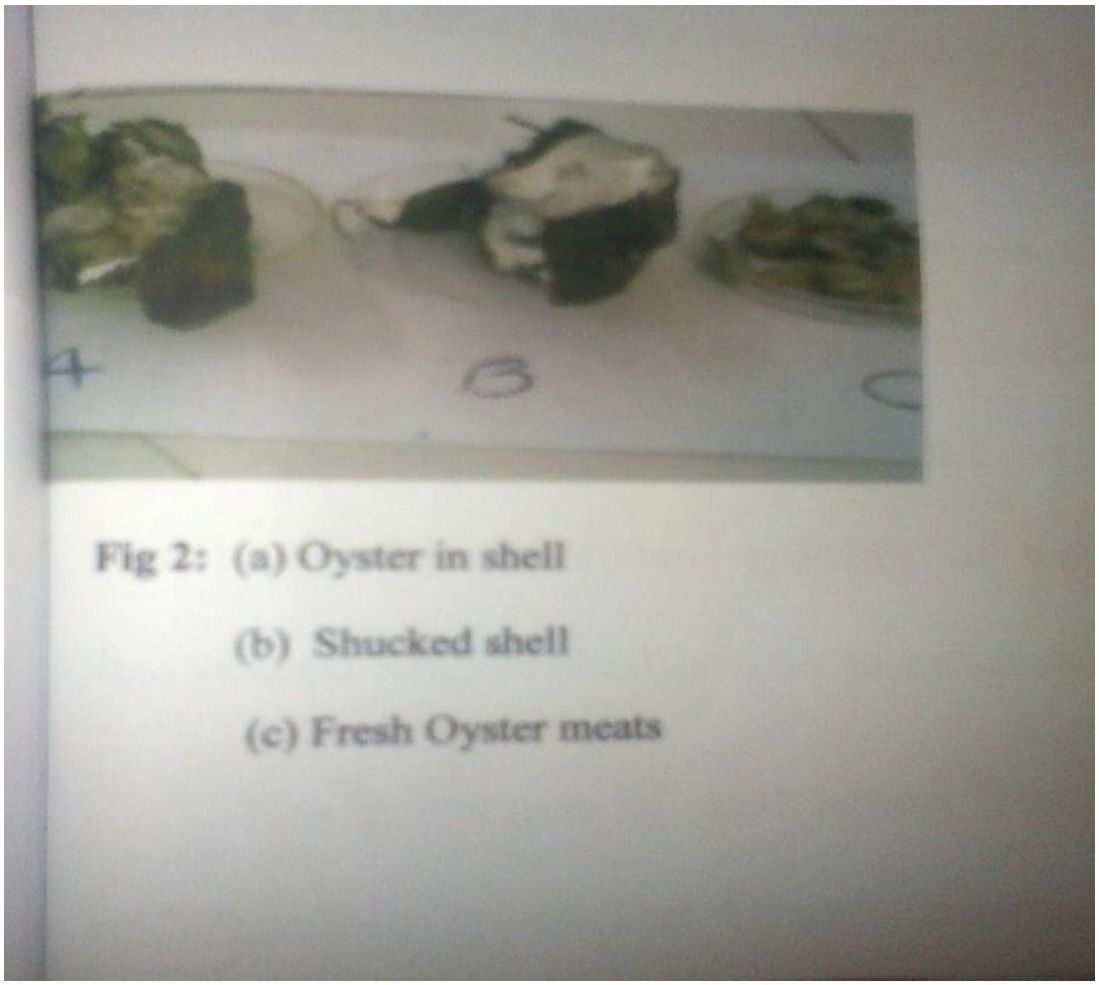


Fig.1. Flowsheet showing steps in oyster meat drying (FAO, 2013)



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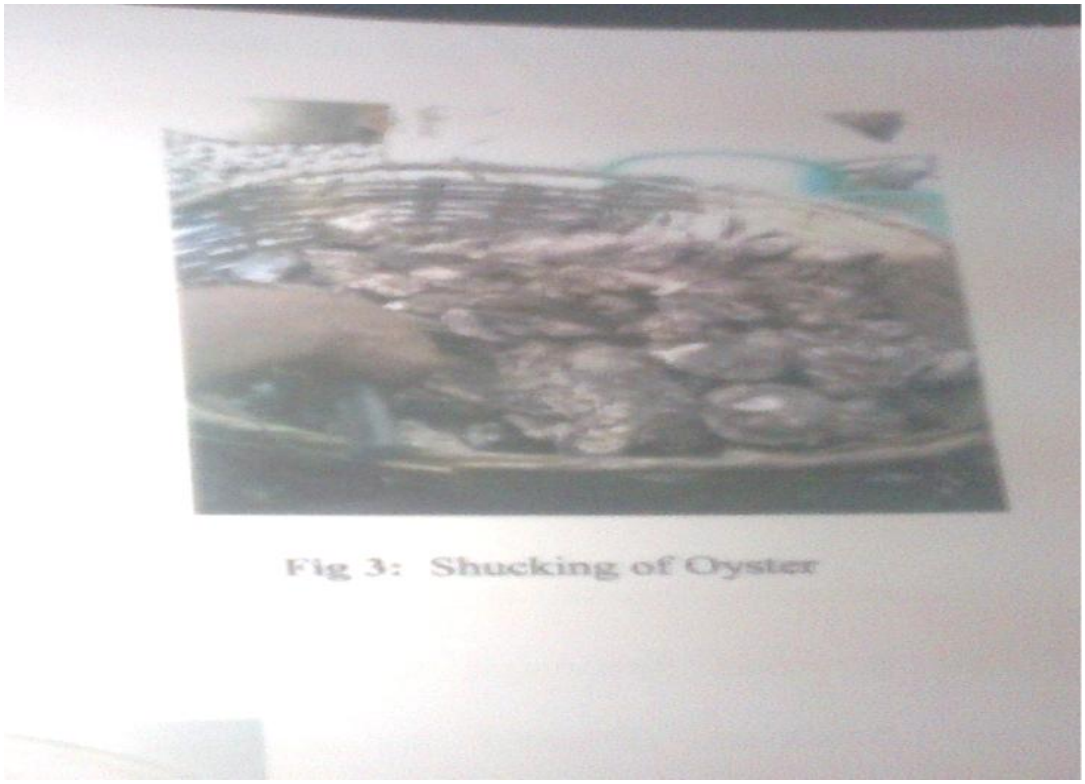

Fig 3: Shucking of Oyster

Fig 4: Dried Oyster

The microbial flora of sea foods including oysters depend on the quality of the harvesting water, handling and processing methods. Adebayo-Tayo et al. (2012), in a study of sea foods marketed in Uyo, AkwaIbom State, reported that the oysters were the most contaminated, with microbial load of 1.0 x 10^6 to 3.6 x 10^6cfu/g for bacteria and 2.0 x 10^6 to 5.7 x 10^6cfu/g for coliforms. Similarly, Odu et al. (2012) examined smoke-dried oysters obtained from four fishing ports and marketed in Port Harcourt and reported highest total counts in oyster meat from Bille of 7.18 to 7.2$\log_{10}$cfu/g and lowest count of 5.75 to 5.9$\log_{10}$cfu/g in oysters from Okrika fishing port. The coliform counts reported were however much lower, ranging from 14.2 to 41.0 MPN/g. Izuchukwu and Efiuvwevwere (2007) reported faecalcoliorm count of 10^4cfu/g of tail portions of prawns harvested from Port Harcourt Marine Creek. These samples did not meet the ICMSF standard for sea foods, of less than 5x10^5 cfu/g for total bacterial countand less than 230/100g for *E. coli*. The observations highlighted the public health implication of consuming contaminated oysters in Port Harcourt since various pathogens such as hepatitis A virus,*Vibrio* sp., *Salmonella* sp. and *Shigella* sp. have been reported as agents of oyster related food infection (CDC, 2006, ICMSF, 1998). This study was undertaken to compare the sanitary quality of fresh and smoke-dried oysters sold in Creek Road Market, Port Harcourt and thus evaluate the effect of processing using total microbial load, coliforms as indicator organisms and pH as physical parameter for freshness. In addition export of oysters will earn Nigeria some foreign exchange and local consumption will help Nigeria achieve the Millennium Development Goal (MDG) of protein sufficiency in the diet of citizens by year 2015.

MATERIALS AND METHODS

To get the smoke-dried oyster meat, the fresh oyster meat is washed and steamed before placing on a drying rack, covered with a clean net and exposed to sun or smoke from hard wood or saw dust (Figs. 1, 2&3). Washed meat is salted before sun drying or smoking in West Africa (Ihekeronye & Ngoddy, 1985). Smoking produces ready-to-eat oyster meats with light chocolate color, unique aroma and flavor (Fig.4).

Sample Collection

Five samples each of fresh and smoke-dried oysters from Okrika fishing port were purchased from Creek Road Market, Port Harcourt. Samples were transported in clean polythene bags to the laboratory within two hours for analysis of microbial load and pH.

Enumeration of Bacterial Contaminants

Enumeration of bacterial contaminants of fresh and dried oysters was carried out in duplicate using 10g samples. Samples were blended for 2 minutes in 90ml of sterile normal saline, in a sterile Moulinex blender. Ten-fold serial dilutions were made by transferring 1ml into 9ml of normal saline to obtain 10^{-2} to 10^{-7} dilutions (Hunt et al., 1976). The spread plate method was carried out by plating 0.1ml of the appropriate dilution on Nutrient agar for total aerobic count and on MacConkey agarfortotal and faecal coliform (*E.coli*) count. The plates were incubated at 35-37^{o}C for 24 hours for total aerobic count and total coliforms and incubated at 44.5^{0}C for faecal coliform count. The different types of colonies were counted and enumerated as cfu/g. Pink/red colonies on MacConkeyagar plates represented coliform bacteria.

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Identification of Isolates

Different colonial types were selected and sub-cultured for identification. The isolates were characterized and identified using various morphological tests (Gram staining, motility, spore staining) and biochemical tests including catalase, coagulase, urease, indole, methyl-red, Voges-Proskauer, citrate (IMViC), oxidase and sugar fermentation tests with glucose, sucrose, lactose, maltose and mannitol(Cowan, 1985, Treagan and Pulliam, 1982).Identification was confirmed using Bergey's Manual of Determinative Bacteriology (Bergey and Holt, 1993).

Determination of pH

pH measurement was carried out using 10ml of 1:10 dilution of oyster sample. The pH meter(Model ME 963-P, PyeUnican) was standardized using a buffer of pH 7, before measurement was made.

Statistical analysis

Using t-test, the data generated from this study was subjected to statistical analysis to determine the significance of differences in mean pH values and microbial levels of fresh and dried oyster samples.

RESULTS

Table 1 shows the pH, total aerobic count, total coliform and *E.coli*counts of 5 fresh oyster samples. Total aerobic count was high ranging from 1.22 x 10^9 to 1.31 x 10^9cfu/g with an average of 1.26 x 10^9cfu/g or log count/g of 9.1. Total coliform count was also high ranging from 5.4 x 10^8 to 6.0 x 10^8cfu/g, with an average of 5.78 x 10^8cfu/g or log count/g of 8.76.*E.coli* count was equally high ranging from 1.2x$10^8$to 3.1x10^8cfu/g, with an average of 2.36 x10^8cfu/g or log 8.37. Fresh oyster had an average pH value of 6.51.

Table 2 shows the pH values, total aerobic, total coliform and *E. coli*counts of 5smoke-dried oyster samples. Total aerobic count was 3 log cycles less than counts for fresh oysters and ranged from 1.37 x 10^6 to 1.48 x 10^6cfu/g, with an average of 1.42 x 10^6cfu/g or log count/g of 6.15.Total coliform count was high ranging from 3.3 x 10^7 to 4.7 x 10^7cfu/g with an average of 4.1 x 10^7cfu/g or log count/g of 7.61. *E. coli* count was from 8.0x10^6 to 2.4x10^7cfu/g with an average of 1.46x10^7or log count/g of 7.16. Dried oyster had an average pH of 5.69. There was a significant difference in total aerobic count and pH values for fresh and dried oyster samples ($p<0.01$) while there was no significant difference for total coliform and *E.coli* counts between the two sample types ($p>0.05$).

Table 3 shows the frequency of Bacterial isolates in fresh and dried oyster samples. It indicates that fresh oyster samples had both gram negative organisms namely, *Escherichia coli, Klebsiella* sp., *Enterobacte r*sp., *Proteus mirabilis*, *Serratia* sp., *Citrobacter* sp. and gram positive organisms namely, *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp. and few *Streptococcus* sp. Smoke-dried oyster on the other hand had mainly gram positive organisms, *Bacillus* sp., *Streptococcus* sp., *Staphylococcus* sp., and few gram negatives namely *E. coli* and *Serratia* sp.

Table 1: pH values, Total aerobic, Totalcoliform and *E.coli* counts of fresh oyster samples

Sample number	**pH values**	**Total Aerobic Count (cfu/g)**	**Total Coliform Count (cfu/g)**	***E.coli* Count (cfu/g)**
1	6.42	1.27×10^9	6.0×10^8	2.0×10^8
2	6.56	1.22×10^9	5.8×10^8	2.9×10^8
3	7.03	1.31×10^9	5.4×10^8	1.2×10^8
4	6.20	1.24×10^9	5.8×10^8	3.1×10^8
5	6.36	1.24×10^9	5.9×10^8	2.6×10^8

Table 2: pH values, Total aerobic, Total coliform and *E.coli* counts of dried oyster samples

Sample number	pH values	Total Aerobic Count (cfu/g)	Total Coliform Count (cfu/g)	E.*coli*Count (cfu/g)	ICMSF standards		
					pH	Total Bacterial Counts	E.*coli*
1	5. 60	1.42×10^6	4.4×10^7	2.4×10^7	6.2-5.9	$< 5.0 \times 10^5$cfu/g	<230/100g For 5 sampling units
2	5.63	1.37×10^6	3.3×10^7	1.0×10^7			
3	5.82	1.45×10^6	3.8×10^7	1.5×10^7			
4	5.60	1.48×10^6	4.7×10^7	1.6×10^7			
5	5.79	1.39×10^6	4.3×10^7	8.0×10^6			

Table 3: Frequency of Bacterial isolates in fresh and dried oyster samples

Isolate	Frequency/5 samples	
	Fresh	Dried
Bacillus sp.	5	5
E. coli	5	5
Klebsiella sp.	5	-
Enterobacter sp.	5	-
Citrobacter sp.	1	-
Proteus sp.	5	-
Micrococcus sp.	4	-
Serratia sp.	5	3
Streptococcus sp.	1	5
Staphylococcus sp.	5	5

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DISCUSSION

This study was done to show the bacteriological quality of fresh and smoke-dried oysters sold in creek road market, Port Harcourt, Nigeria. Bacteriological guidelines have the limit for raw molluscan shellfish contamination of not more than $5x10^5$ bacteria/g and less than 230 *Escherichia coli*/100g for oysters harvested from known unpolluted waters, using 5 sample units (Seafood Network Information Center, 2008; Canadian Food Inspection Agency, 2008).The observations from this study confirms reports that fresh oysters and mollusks accumulate high levels of bacteria resident in soil and water in addition to organisms from faecal pollution and other waste products. Oysters from less polluted waters or known waters may have high total counts but low faecal coliform or *E. coli* count. Reilly (1985) reported that most of the oysters tested in a USA survey had total bacterial count of 10^7 - 10^9 cfu/g and in Netherlands' survey count were one log scale lower. The Niger Delta oyster samples had total counts above 1.0 $x10^9$cfu/g with extremely high *E.coli* counts of 10^7-10^8cfu/g while no oysters in the USA study had *E.coli*in excess of 10/g and in Netherlands' study only 13% of samples exceeded the limit.

Houses in the Riverine areas of Nigeria lack proper toilet facilities and out- houses are used as toilets with faeces deposited directly into rivers, streams and swamps, hence the high level of coliforms and faecal coliforms in the oysters from Okrika. These indicator organisms imply that these oysters are hazardous to consume and the various gram negative bacteria isolated from fresh oysters including *E.coli*suggest that consumers could easily succumb to gastroenteritis, food intoxication and hepatitis A infection (CDC, 2006, ICMSF, 1998).

The study showed that smoke–drying reduced the total bacterial load by 3 log cycles but total coliform and *E.coli* levels, 10^6-10^7/g were still high and hazardous. Other workers (Odu et al., 2012,Adebayo-Tayo et al., 2012) also reported high bacterial load in smoked-dried oysters marketed in Port Harcourt and Uyo respectively. This is from the high initial level of bacteria in raw oysters followed by the use of contaminated water for washing steps and overlaying and use of unclean containers during processing and exposure of the product to flies in dirty market environment. Studies on tropical periwinkle by Odu et al., 2010, showed that laboratory shucked meat had low total counts of 10^2/g and no coliforms were detected compared to traditionally shucked meat with total count of log 8 to log 10/g. In the laboratory, sterile water and materials were used for processing. Since smoke-dried oyster and other ready-to-eat sea-food products are expected to have *E. coli* counts less than 40/g (Canadian Food Inspection Agency, 2008), it is recommended that the Food Inspection Section of NAFDAC (National Agency for Food and Drug Administration and Control) should start to regulate the harvesting, processing and storage of oysters and other sea-foods in Nigeria to make them safer for consumption and fit for export.

Salting and the heat from smoke-drying killed bacteria selectively enriching gram positive bacteria such as *Bacillus* sp. and *Staphylococcus* sp., which tolerate lower water activity and higher salt concentration as also reported by other workers (Odu et al., 2012). Spoilage of oysters is by lactic fermentation of glycogen hence the presence of streptococci in the smoked oysters.

It has been proposed that pH of 6.2-5.9 represents good microbial oyster quality, whereas pH of 5.2 and below implies putrid oyster meat (Jay, 1988). The observation that fresh oyster samples in this study with an average pH of 6.51 had high microbial counts, suggests that pH could be used for organoleptic quality of texture and freshness

but not necessarily for microbial quality. The lower pH of dried oysters of 5.69 definitely implied that fermentation had started before the smoke-drying process was embarked on. Thus oysters may appear fresh are microbiologically hazardous.

In conclusion, in Nigeria fresh oysters should be harvested from known unpolluted waters with low faecal contamination so as to meet set microbial standards. This will be possible if the quality of life of people in the Riverine areas is improved by ensuring the provision of toilets for houses and educating the populace on the health hazards of consuming faecally contaminated oysters and other sea foods. Processing, handling and storage of oysters should be monitored NAFDAC to prevent additional contamination.

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