Bacteriological assessment of a salted pork product (*Unaminung*) sold in Calabar metropolis

Akpan, I. P.

Animal Product Processing Unit Department of Animal Science, University of Uyo

DOI : https://doi.org/10.56293/IJASR.2022.5416

IJASR 2022
VOLUME 5
ISSUE 4 JULY – AUGUST **ISSN: 2581-7876**

Abstract: The study was carried out to evaluate the microbiological quality of *Unaminung*sold at watt market in Calabar,Nigeria.Samples of Commercial Ui (CUi) from three processors (P1, P2, P3) were assessed for Total Bacteria Count (TBC, log cfu/g) and Total Fungi Count (TFC, log cfu/g)using standard procedures.The bacteria and Fungi isolates were also characterized and identified.The TBC of 3.36 ±0.02 (P2) was higher than 3.17±0.20 (P3) and 3.07±0.11(P1).The bacteria isolated were identified as*Bacillus spharius, Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus avium, Aerococcusviridans.* while the fungi isolate*were Verticilliumalbo-atrum*, *Rhyzopusstolonifera, Penniciliumcitrinum, Candida pseudotropicalis, Candida tropicalis, Saccharomyces estuary.*The results revealed that the hygienic condition of the meat was within acceptable standard for human consumption. There is need for the processors to be educated on proper handling and personal hygiene to avoid cross contamination during processing.

Keywords: Total bacterial count; Total Fungi count; Microbial quality; Unaminung; Calabar

1. Introduction

Meat products are derivatives of different species of meat used as food. They include sausages of all kinds, jerky, deli meat, pate,dried salted meat and cooked meat. They are among the food vended on the streets for human consumption. Street vended foods as described by WHO are ready-to-eat foods and beverages that are prepared and/or sold by vendors and hawkers on the streets or other similar places for immediate consumption or consumption at a later time without further processing or preparation (Buscemi*et al.,* 2011). A variety of meat products exist on Nigerian streets, restaurants, canteens, drinking joints and other public places such asSuya, roasted chicken, chevon, mutton and skewed snail, cooked, roasted pork. A typical pork product exists in Calabar metropolis known as *Unaminung*. It is a pork product whose popularity is dwindling and it's almost extinct from the market. It is usually pre-salted, cooked and presented with an accompaniment known as edita- iwa which is boiled cassava chips. It is highly relished by the efiks and usually presented as a dessert before the main meal is served. A notion exists that street vended foods are often regarded as unsafe for consumption.This is because there is possibility of them being exposed to various forms of contamination at every stage of handling. Mridha, 2013, stated that safety of food chain can be broken by both contamination and adulteration of food in different steps of the cycle. Some factors such as insufficient roasting /heating duration, uneven temperature distribution and exposure to unhygienic environments enhance infection and contamination (Ekanem, 1998). Unsafe food causes many acute and life-long diseases, ranging from diarrheal diseases to various forms of cancer. It has been reported that people who indulge in the consumption of street vended food sometimes suffer from food borne diseases like cholera, diarrhea typhoid fever and gastroenteritis (Rane, 2011). Unhygienic practices during food preparation and storage generate conditions that allow the growth and transmission of food borne pathogens and other microorganisms which may cause food poisoning (Angelillo*et al.,* 200).Itis commonly suggested that microorganisms can enter meat preparation from raw materials, processing environment, equipment, and handlers which can have a significant impact on the microbiological status of the end-products (WHO, 1999).

It has been observed that most ready-to-eat meat products and meat are often displayed in Nigeria markets under poor hygienic conditions and hence contaminated by various microorganisms (Faparusi, 1981).

Microbiological quality of food indicates the amount of microbial contaminants it has, ahigh level of contamination indicates low quality of food storage and its handling more likely to transmit diseases (Oranusi et al., 2013). Bacterial

count in prepared food and water is a key factor in assessing the quality and safety of food. It also reveals the level of hygiene adopted by food handlers in the course of preparation of such foods. Ologbobo*et al.,* 2010, opined that consumers are not aware of the risk they are exposed to on consumption of street vended food.It is pertinent to educate both consumers and processors on safe food handling. This study therefore assessed the hygienic quality of *Unaminung*sold in Calabar metropolis.

2. Materials and methods

2.1 Experimental site:

The experiment was carried out at the Department of Animal Science laboratory, University of Uyo.

2.2 Sample Collection and Experimental Design

The ready-to-eat *Unaminung*was purchased from three different processors at Watt market and immediately transported to Animal Science laboratory at University of Uyo for the various analyses. The design of the experiment used was the completely randomized design (CRD) with the products from each processor representing a treatment and was replicated three times.

2.3 Microbiological analysis

Microbial analysis was carried out immediately after obtaining *Unaminung* from the processors to determine the Total Bacterial Count (TBC) and Total Fungi counts (TFC) and also to identify the microorganisms present in the samples.

2.4 Estimation of microbial densities

Microbial loads were assessed by the pour plate method reported by Harigan and McCane (1990). Using standard microbial techniques, logarithmic dilution of the samples was conducted down the dilution gradient to the third factor (10^{-3}) in sterile water using a 1ml pipette. A sample of 1 gram was homogenized in 9ml of sterile water to form the aliquot. 1ml of the aliquot (sample supernatant) was pipetted and mixed in 9ml of sterile water in another test-tube and shaken vigorously thereafter 1ml of the desired dilution was plated out in duplicate set on nutrient agar amended with cycloheximide (Nystatin) at 100uml to prevent fungal growth (Essien*et al.*, 2006). The plates were nurtured at 37^{o}C for 24-48 hours. Discrete microbial populations after incubation were enumerated, studied and recorded as colony forming unit per gram (cfu/g) of meat. The desired diluent was also plated out in duplicate sets on sabouraud dextrose agar sets to which streptomycin at 30uml to prevent bacterial growth. The plates were nurtured at 28-30^{o}C for 5-7 days. Microbial Colonies after incubation were counted and enumerated.

2.5 Characterization and Identification of bacterial and fungi isolates isolates

Bacterial isolates were characterized and identified based on their cultural and morphological as well as microscopic examination and biochemical characteristics following the methods described by Holt *et al.*, (1994) and Cowan, (1985). The biochemical tests conducted to assist in the identification include- Gram stain, catalase test, urease production, coagulase test, oxidase test, indole production test, citrate test, methyl red test, Motility,VogesProskauer, starch hydrolysis and sugars fermentation.

Fungal isolates were characterized and identified as describe by Fawole andOso(2004). The identification was based on cultural, morphological and vegetative as well as reproductive features.

2.6 Statistical analysis

Data gathered from the experiment was subjected to Analysis of Variance using SAS (1999) package.

3.0 Results

3.1 Microbial load (log cfu/g) of the commercially available *Unaminung*samples

The microbial load of the commercially available *Unaminung* samples are shown in Table 1. The Total Bacterial Counts from the three sources varied significantly from each other. Products from Processor 2 had the highest counts (3.36 ± 0.02) followed by products from Processor 3 (3.17± 0.20) and products from Processor 1 (3.07± 0.11). Total fungal counts (TFC) also varied across the sources significantly. Products from Processor 3 had the highest counts (2.87 ± 0.02) followed by products from Processor 2 (2.72 ± 0.02) and products from Processor 1(2.60 ± 0.04).

Table 1: Microbial load (log cfu/g) of the commercially available *Unaminung*samples

Parameters	Processor 1	Processor 2	Processor 3	SEM
TBC	3.07± 0.11^{c}	3.36 ± 0.02^{a}	3.17± 0.20 ^{b}	0.44
TFC	2.60± 0.04^{c}	2.72 ± 0.02^{b}	2.87 ± 0.02^{a}	0.39

^{abc}Means with different superscripts are significantly different ($p<0.05$) TBC: Total bacterial count; TFC: Total fungal count * Acceptable limit: < 7 log cfu/g

3.2 Incidence of bacterial species in commercially available *Unaminung*

Table 2 shows the occurrence of microbiological organisms in the commercially available *Unaminung* samples. A total of twelve (12) bacteria were isolated from the *Unaminung* samples from the three processors. *Staphylococcus epidermidis*and *Aerococcusviridans*were the most commonly isolated bacteria occurring three times each at 25% followed by *Bacillus spharius, Staphylococcus aureus, Streptococcus avium*which occurred twice each at 16.67%. *Staphylococcus aureus* and *streptococcus avium* were absent in products from Processor 1 but present in Processor 2 and 3 respectively. A reverse trend is seen in *Bacillus spharius* which occurred in Processor 1 and 2 but absent in Processor 3.

Table 2. Incidence of bacterial species in commercially available Unaminung

Samples	Bacillus spharius	Staphylococcus epidermidis	Staphylococcus aureus	Streptococcus avium	Aerococcusviridans
Processor 1	+	+	-	-	+
Processor 2	+	+	+	+	+
Processor 3	-	+	+	+	+
Total	2	3	2	2	3
% Occurrence	16.67	25	16.67	16.67	25

+ = Positive present; - = Negative absent

3.3 Incidence of fungal species in commercially available *Unaminung*.

Table 3 shows the occurrence of fungal species in commercially available *Unaminung* sold in Calabar metropolis,*Candida pseudotropicalis, Candida tropicalis, Saccharomyces estuary* occurred twice each at 20% in samples from Processor 2 and 3 respectively. *Verticilliumalbo-atrum*and*Penniciliumcitrinum*occurred once each in samples from Processor 3 and Processor 1 respectively. *Rhyzopusstoloniferа*occurred twice in samples from Processors 1 and 3 at 20% and absent in samples from Processor 2.

Fungal species	Verticilliumalbo-atrum	Rhyzopusstolonifer	Penniciliumcitrinum	Candida pseudotropicalis	Candida tropicalis	Saccharomyces estuary
Processor 1	-	+	+	-	-	-
Processor 2	-	-	-	+	+	+
Processor 3	+	+	-	+	+	+

Total	1	2	1	2	2	2
% occurence	10	20	10	20	20	20

Table 3.Incidence of fungal species in commercially available *Unaminung*
+ = Positive present, - = Negative absent

3.4 Morphological and biochemical characteristics of bacterial isolates

The morphological characteristics biochemical characteristics and names of bacterial species in the sample are presented in Table 4. The bacteria identified were *Bacillus spharicus*, *Staphylococcus epidermidis*, *Staphylococcus aureus,Streptococusavium* and *Aerococcusviridans.*

Table 4. Morphological and biochemical characteristics of bacterial isolates

+ = positive reaction, - = negative reaction, Ag = acid with gas production, Ao = acid, no gas production, 00 = no acid, no gas, VP = VogesProskaur

	Cell shape	Gram stain	Catalase	Coagulase	Motility	Methyl Red	Citrate	Urase	Spore	VP	Oxidase	Glucose	Lactose	Maltose	Manitol	Xylose	probable Organism
1	Rod	+	+	-	+	+	+	+	+	-	+	00	00	00	00	Ao	*Bacillus spharicus*
2	Cocci	+	+	-	-	+	-	-	-	+	-	Ag	Ag	Ag	Ag	00	*Staphylococcus epidermidis*
3	Cocci	+	+	+	-	+	-	+	-	+	-	Ag	Ag	Ag	Ag	00	*Staphylococcus aureus*
4	Cocci	+	-	-	-	-	-	-	-	-	-	Ao	00	00	00	00	*Streptococcus avium*
5	Cocci	+	-	-	-	+	+	-	-	-	-	Ao	Ao	Ao	Ao	00	*Aerococcusviridans*

4.0 Discussion

Microbial load in the samples were considerably low. The Total bacterial count and the Total fungal counts were within the permissible level of microbial standards (6 log cfu/g of sample) in cooked meat products as reported by Jay (1996). The reason for the low count could be attributed to the salt and heat application through the salting and cooking processes. Salt is known to be a preservative because it reduces the water activity of foods. It could be noted that water activity is the amount of unbound water that is available for microbial growth and chemical reaction in a food medium. Salt is able to decrease water activity through the association of sodium and chloride ion with water (Fennema, 1996). Addition of salt to meat also cause microbial cells to lose water through osmosis thereby inhibiting growth (Davidson, 2001). Salt also limits the solubility of oxygen to some microorganisms, deter enzymes activities in the cells and force cells to spend energy to eliminate sodium ions from the cell which also inhibits growth (Shelef and Seiter, 2005). The variations in Total Bacterial and Fungal counts obtained in this study could be attributed to the utensils, equipment, handling and processing methods used by the processors of *Unaminung*. This view corresponds with the findings of Igene*et al.* (1988) which stated that quality variation in suya exist from one processor to another due to unstandardized methods of preparation leading to inconsistent product quality.The presence of *Staphylococcus aureus* may be caused by application of salt to the meat during processing as affirmed by Gilbert and Harrison (2001) who reported that meat preserved with salt encourages the growth of

Staphylococcus aureus. Boles *et al.* (2000) also stated that *Staphylococcusaureus* requires about 6.5% Sodium Chloride for growth and is usually found in salty meat products. However, contamination of food with *Staphylococcus spp*.are mainly through physical contact as it is a normal flora of the human skin (Gilbert and Harrison 2001). *Staphylococcus spp*.are part of the normal human flora frequently found in the nose, respiratory tract, and on the skin. The prevalence of *Staphyloccocusepidermidis* may be due to fact that they are transmitted by the carrier (processors) (Cheesbrough, 2000; Mankee, 2003). They are responsible for a number of common infections. *Bacillus spp* are soil microflora, vegetation and food. They are usually implicated in food borne diseases. When ingested, it causes gastrointestinal illness with nausea, vomiting and diarrhea. It is also linked to serious infection in host whose immune system has been compromised to cause septicemia and endophthalmitis which can lead to vision loss (McDowell *et al.,* 2021). *Aerococcusviridans* are airborne present in the environment.

5.0 Conclusion

The microbial quality assessment of *Unaminung*sold in Calabar metropolis showed that the salted pork was less contaminated and within the satisfactory limits. The bacteria isolated were *Staphylococcus epidermidis*, *Aerococcusviridans, Bacillus spharius, Staphylococcus aureus and Streptococcus avium.* The highest occurring bacteria were*Staphylococcus epidermidis*and *Aerococcusviridans*while the *fungi isolated were Candida pseudotropicalis, Candida tropicalis, Saccharomyces estuary,Verticilliumalbo-atrum*and*Pennicilium, Rhyzopusstolonifera. Candida pseudotropicalis, Candida tropicalis, Saccharomyces estuary were the highest occurring fungi.* The organisms isolated are possibly from cross contamination and the processing environment.Hence personal hygiene and environmental sanitation during preparation and handlingof *Unaminung*by processors are encouraged to produce wholesome meat products for consumption.

References

1. Angelillo, I.F.; Nunzia, M.A.V.; Greco, R.M.; Rito, D. HACCP and Food Hygiene in Hospital: Knowledge, Attitudes, and Practices of Food Services Staff in Calabria, Italy. Infect Control Hosp Epidemiol 2000. 22(6):pp.363-369. DOI: https://doi.org/10.1086/501914
2. AOAC.1999. Official Method of Analysis of the Association of Official Analytical Chemists International. 16th Ed. 5th Revision. Washington.
3. Boles, J. A., Rathgether, B. M., and Shand P. J. 2000.Staphylococcus in salted meat product.*Journal of Meat Science* 55: 220-231.
4. Cheesebrough, M. 2000. Water quality Analysis: in Cheesebrough, M (Ed) district Laboratory practice in Tropical Countries part II, London: Cambridge University Press. Pp.144-156.
5. Cowan, S. T. and K. J. Steel, 1981. Manual for the Identification of Medical Bacteria. 2nd Edn., Cambridge University Press, Cambridge, UK
6. Ekanem, E. O. (1998). The Street Food Trade in Africa: Safety and Socio-Environmental Issues. Food Contr. J. 9 (4); 211-215.
7. Faparusi, S. I. (1981) Identification of microorganisms from khundi, a smoked meat. Journal of Food Technology 44: 581-582.
8. Fawole, M. O. and Oso B. A. 2004.Laboratory manual in Microbiology.1st Ed, New Spectrum Books publisher, Ibadan, Nigeria.
9. Fennema, O. R. 1996. Food chemistry. 3rded. New York: Marcel Dekker; 1996.
10. Gilbert, U. and Harrison, A. 2001.Occurrence of enterotoxin producing Staphylococcus aureus in meat market in Nigeria.*Journal of Food Infection* 56: 25-35. 17.
11. Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T. 1994. Bergey's Manual of Determinative Bacteriology, 9th Edn., William and Wilkins, Baltimore, pp 565-596
12. Igene, J. O., Farouk M. M., and Akanbi, C. 2009 Preliminary Studies on the quality and drying Characteristics of "suya". *Nigeria Food Journal* 7: 29-38.
13. Jay, J. M. 1996. Modern food microbiology (5th Ed.).Wolters Kluwer Law & Business. New York: Chapman and Hall.
14. Mankee, A., Ali, S., Clin, A., Ludalsing, R., Khan, R., Mohamed, F., Reheman, R., Sooknanam, S., Tota-Maherraj, R., Simeon, D., Adesiyun, A. A. 2003. Bacteriological quality of doubles sold by street vendors in Trinidad and the attitude, knowledge and perceptions of the public about its consumption and health risk.*Food microbiology* 20: 631-639.

15. Mcdowell, R. H., Sands, E. M.., Friedman, H. 2021. Bacillus cereus (Updated 2021 sep 16). In: StatPearls (Internet) Treasure Island (FL): StatPearls Publishing; 2021 Jan-Available from: https://www.ncbi.nlm.nih.gov/books/NBK459121/
16. Mridha, Z. U. (2013). Food safety-A global concern. Bangladesh J. Med.Biochem.4(2):3-5.
17. Rane, S. (2011).Street Vended Food in Developing World: Hazards Analyses, Indian J Microbiol 2011. 51(1): pp.100-106. DOI: https://doi.org/10.1007/s12088-011-0154-x
18. SAS. 1999 – Statistical Analysis System Institutes. Users guide. SAS Institute Inc. Cary N. C
19. Shelef, L. A. and Seiter, J. 2005. Indirect and miscellaneous antimicrobials. In: Davidson P. M., Sofos J. N, Branen A. L., editors. Antimicrobials in Food. 3rd ed. Boca Raton, Florida, USA: CRC Press; 2005. pp. 573-598
20. WHO, 1999. *Strategies for implementing HACCP in small and/or less developed businesses.* Geneva: World Health Organisation, WHO/SDE/FOS/99.7; 1999.