Science World Journal Vol. 19(No 3) 2024 https://dx.doi.org/10.4314/swj.v19i3.8
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

MICROBIOLOGICAL EXAMINATION OF READY-TO-EAT COW HIDE (PONMO) SOLD BY STREET VENDORS IN MAKURDI, BENUE STATE, NIGERIA

*Tyona Ngodoo Magdalene, Ojowu Sally Ogbene and Akpa Joy Etele

Department of Microbiology, Joseph Sarwuan Tarka University, P.M.B. 2373, Makurdi, Benue State, Nigeria

*Corresponding Author Email Address: tyona.magdalene@uam.edu.ng

ABSTRACT
Cow hide, commonly known as ponmo, is an edible product derived from the skins of large animals such as cattle, camels, and buffaloes. Ponmo is a popular delicacy in various regions of Nigeria. However, the unhygienic production practices of ready-to-eat ponmo often lead to contamination and spoilage by microorganisms. This study aimed to assess the bacteriological quality of ready-to-eat cow hide (ponmo) sold by roadside vendors in Makurdi metropolis. Ten samples of ready-to-eat ponmo were collected from roadside vendors in five different markets: Wadata Market, Wurukum Market, North Bank Market, High-Level Market, and Modern Market. The samples were washed in distilled water, serially diluted and inoculated onto different media (Nutrient Agar and MacConkey Agar) using the pour plate method, followed by incubation at 37°C for 24 hours. The bacterial isolates were identified based on cultural, microscopic, and biochemical characteristics, revealing the presence of *Staphylococcus aureus*, *Escherichia coli, Bacillus* spp, and *Proteus* spp. *Staphylococcus aureus* was the most prevalent bacteria (46.67%), followed by *Escherichia coli* (35.56%). Statistical analysis indicated no significant difference ($P > 0.05$) in bacterial load among the samples, with total bacterial counts ranging from 2.50 x 10^5 to 2.75 x 10^5 CFU/g. The presence of these bacteria, some of which can cause food borne illnesses, highlights the public health risks associated with consuming ready-to-eat ponmo. This study underscores the need for enhanced monitoring of ready-to-eat products and increased public health education for both vendors and consumers.

Keywords: Cow Hide, Ponmo, Makurdi, Bacteria, Public Health.

INTRODUCTION

Cow hide, commonly known as ponmo in Nigeria, is a traditional meat product produced through the tenderization of cow skin (Okiel *et al.,* 2009). It is an integral component of many Nigerian dishes, particularly soups, where it is prized for its unique texture and flavor (Obiri-Danso *et al*., 2008). The preparation of ponmo involves several stages, including drying, singeing, scraping, boiling, and washing, which contribute to its distinct characteristics and coloration, ranging from off-white to brown depending on the animal source (Okiel *et al.,* 2009). Unprocessed ponmo is often transported from Northern to Southwestern Nigeria, making it readily available in various markets across the country.

Despite its popularity and cultural significance, ponmo is considered to have low nutritional value due to its processing, which diminishes its protein content and other essential nutrients (Obiri-Danso *et al.,* 2008). Nonetheless, its affordability makes it a staple among many, particularly in economically low communities (Adeyeye *et al*., 2015). The increasing demand for ready-to-eat ponmo, sold predominantly by street vendors, underscores its economic and culinary importance. However, this convenience comes with significant public health concerns.

Ready-to-eat ponmo, often sold by street vendors, is typically consumed without additional cooking, making it a potential vector for food borne pathogens (Oyewole and Ogundele, 2011). Vendors often operate in unsanitary conditions, lacking access to potable water and proper waste disposal, which exacerbates the risk of microbial contamination (Mamun *et al.,* 2013). Furthermore, these vendors usually have limited education and awareness of food safety practices, contributing to the potential for contamination with bacteria such as *Staphylococcus aureus, Escherichia coli,* and *Bacillus species* (Monday *et al*., 2014).

Street food vending, including the sale of ponmo, is widespread in developing countries and serves as a vital source of affordable nutrition and economic opportunity (Alimi, 2016). However, the safety of these foods remains a critical concern, particularly in the context of bacterial contamination, which can lead to food borne illnesses with serious public health implications (Rane, 2011). Given the lack of formal regulation and oversight in the sale of ready-to-eat ponmo, there is an urgent need for comprehensive microbiological evaluations to ensure its safety for consumers (Adeyeye *et al.,* 2015).

This study aims to assess the bacteriological quality of ready-to-eat cow hide (ponmo) sold by roadside vendors in Makurdi Metropolis. By identifying the prevalent bacterial contaminants and their potential health risks, this research seeks to inform better food safety practices and regulatory measures to protect public health.

MATERIALS AND METHODS

Experimental Design The study employed a completely randomized design (CRD).

Sample Collection A total of 10 ready-to-eat ponmo samples were collected from roadside vendors across five different markets in Makurdi Metropolis: Wadata Market, Wurukm Market, North Bank Market, High-Level Market, and North Bank Market. Each market provided two samples. The samples were labelled according to their collection site, packaged in sterile polythene bags, and transported to the Microbiology Laboratory at Joseph Sarwuan Tarka University, Makurdi for analysis.

Sterilization and Disinfection of Materials Workbenches were disinfected with 90% ethanol. Glassware was wrapped in aluminum foil, secured with masking tape, and sterilized in a hot air oven at 180°C for 1 hour.

Media The media used included:

1. Nutrient Agar
2. MacConkey Agar

Method of Preparation

Nutrient Agar Preparation: Nutrient agar was prepared by dissolving 28 grams of its dehydrated powder in 1000 mL of distilled water. The mixture was heated with continuous stirring until fully dissolved, covered with aluminum foil, and sterilized in an autoclave at 121ºC for 15 minutes. Post-sterilization, the media was cooled to 44ºC before being aseptically poured into Petri dishes.

MacConkey Agar Preparation: For isolating gram-negative enteric bacteria and differentiating lactose fermenters from non-fermenters, MacConkey agar was used. It provides essential nutrients through pancreatic digests of gelatin, meat, and casein peptones. Lactose monohydrate serves as the carbohydrate source. Crystal violet and bile salts inhibit most gram-positive bacteria, while sodium chloride maintains osmotic balance, and neutral red acts as a pH indicator. Agar solidifies the medium (Ayesha, 2022). To prepare, 49.53 grams of dehydrated MacConkey medium was dissolved in 1000 mL of distilled water. The solution was heated and stirred until dissolved, covered with aluminum foil, and sterilized by autoclaving at 121ºC for 15 minutes. The media was cooled to 44ºC before aseptically pouring into Petri dishes.

Preparation of Inocula/Serial Dilution: Ready-to-eat ponmo samples were ground into a semi-liquid form using a sterile electric blender. A stock solution was made by suspending 1 gram of the ground sample in 9 mL of sterile distilled water. This solution underwent ten-fold serial dilutions. Nine milliliters of sterile water were dispensed into five test tubes, labeled 10^-1 through 10^-5. One milliliter from the stock solution was added to the first tube (10^-1) and mixed thoroughly. This process continued to prepare dilutions up to 10^-5 using methods by Benson, **(**2002).

Total Heterotrophic Bacteria Count: The pour plate method for heterotrophic bacteria count followed Prescott *et al*.(2012). Diluted inoculum from the 10^-2 and 10^-4 tubes was mixed with molten nutrient agar and poured into Petri dishes. Plates were incubated at 37ºC for 24 hours (Geraldine et al., 2018). Bacterial counts were expressed as colony-forming units per gram (CFU/g) and calculated using formular by Benson (2002).

Isolation of Bacteria: Discrete colonies were identified following Cheesebrough (2005). Colonies were repeatedly sub-cultured by streaking on fresh media and incubated at 37ºC for 24 hours for growth. Pure cultures were then transferred onto slants for biochemical identification.

Identification and Characterization of Bacteria

Culture Characteristics

The bacteria colonies were identified based on the following: shape (circular, entire, rhizoid, punctiform), size, elevation (flat, raised, low convex, umbonate), colour (clourless, white, yellow, black, grey, pink), texture (dry, moist, viscid, muciod) and opacity (opaque, translucent, iridescent) to differentiate the microorganism following methods by Cheesbrough, (2006).

Gram Staining

Gram staining was carried out on the 24 hour old culture, a thin smear was made by placing a drop of water on a clean glass slide and a loopful of 24 hour old bacteria culture will placed into the drop of the water to make a thin film. The film was air dried and heat fixed by passing over a flame gently. It was stained with crystal violet solution for 60 seconds and was gently rinsed with distilled water to wash off excess stain. The smear was flooded with Lugols' iodine and was left to stay for 60 seconds and rinsed with water. Acetone was added to the smear for 5 seconds and rinsed with distilled water. The smear was counter stained with safranin and left for 60 seconds and finally rinsed with water. The smear was allowed to air dry at room temperature 27 ºC). Immersion oil was applied on the smear and the slide was examined microscopically for cell morphology following methods by Biyani *et al.,* 2013

Biochemical Tests

a. Catalase Test

A drop of 3% hydrogen peroxide (H_2O_2) was placed on a glass slide and an aliquot of 24 hours old bacteria culture was taken with wire loop and emulsified with the hydrogen peroxide on the slide. A positive test was indication by bubbling and frothing (Cheesbrough, 2006).

b. Coagulase Test

A clean slide was divided into 2 parts with grease pencil and a drop of physiological saline placed on each part under a specific condition. An aliquot 24 hours old bacteria culture was picked and emulsified in each drop of saline and mixed with human plasma using a sterile wire loop. The slide was held up and titled back and front for 1 minute. A positive test was indicated by clumping and agglutination (Cheesbrough, 2006).

c. Motility Test

The motility medium was incubated by making fine stab with a wire loop containing isolate to depth of 1-2cm short of the bottom of the tube. The inoculated medium was incubated at 35 ºC for 24-48 hours. For motile organism the line of inoculation will not be defined and the rest of the medium was cloudy. For non-motile organism, growth was restricted to the line of inoculation which came sharply defined, the rest of the medium remaining clear (Cheesbrough, 2006).

d. Indole Test

The bacteria isolates were inoculated into 5ml peptone water and left for 24 hours. After the three drops of kovac's indole reagent were added and shaken gently. A positive reaction was an indicative by the development of a red colour in the reagent layer above the broth within 1 minute while in the negative reaction, the indole reagent retained its yellow colour (Cheesbrough, 2006).

Science World Journal Vol. 19(No 3) 2024 https://dx.doi.org/10.4314/swj.v19i3.8
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

e. Simmon's Cirate Agar Test
The isolate was inoculated into a simmon citrate agar slant in a bottle and incubated for 24-72 hours at 37 °c. the development of a deep blue colour was an indicative of positive reaction (Cheesbrough, 2006).

Statistical Analysis
The data obtained was inputed in spread sheet and was imported to SPSS (21.0 versions) to complete frequencies and produce tables showing frequency counts and percentages means of the values of individual variables.

RESULTS
Table 1 showed the total viable counts of bacteria from ready to eat cow hide (ponmo) sold by roadside vendors in Makurdi Metropolis. Ready to eat cow hide (ponmo) obtained from Modern markets and high level had the highest counts (2.75 x 10^5 CFU/g) followed by samples obtained from North bank (2.50 x 10^5 CFU/g). Statistical analysis of data showed that there was no significance difference in the total viable counts of bacteria within the samples in different locations (P > 0.05).

Table 1: Total Viable Count of Bacteria from Ready to Eat Cow Hide (Ponmo) Sold by Roadside Vendors in Makurdi Metropolis

Samples Location	Total Bacteria Viable counts (CFU/mL)
Modern market	2.75 x 10^{5b}
Wadata	1.72. x 10^{5a}
High level	2.75 x 10^{5b}
Wurukum	2.30 x 10^{5ab}
Northbank	2.50 x 10^{5ab}
P- value	**0.076**

(P > 0.05)Values with the same case letters along the column are not significantly different

The cultural, microscopy and biochemical characteristics of bacteria isolates from ready to eat cow hide (ponmo) sold by roadside vendor in Makurdi Metropolis are recorded in Table 2. The result showed that four genera of bacteria were identified based on their distinct cultural, microscopy and biochemical characteristics. The bacteria identified include, *Staphylococcus* spp, *Bacillus* spp, *Escherichia coli* and *Proteus* spp.

Table 2: Cultural, Microscopy and Biochemical Characteristics of Bacteria Isolates from Ready to Eat Cow Hide (Ponmo) Sold by Roadside Vendors in Makurdi Metropolis

Colony Colour	Colony Shape	Morph	Grams Rxn	Cat	Cit	Urease	Indole	H_2S	Mot	Bacteria isolates
Pale	Circular	Cocci	-	+	-	-	-	-	-	*Escherichia coli*
Yellow	Circular	Cocci	+	+	+	-	-	-	-	*Staphylococcus* spp
Pale	Circular	Rod	-	+	+	+	-	+	+	*Proteus* spp
White	Irregular`	Rod	+	+	+	-	-	-	-	*Bacillus* spp

Key: + Positive, - Negative, Cat – Catalase, Rxn- reaction, Cit – Citrate, Mot – Motility

Table 3 presented the percentage prevalence of bacteria isolates from ready to eat ponmo sold by roadside vendor in Makurdi Metropolis are recorded. *Staphylococcus* spp had the highest prevalence (46.67%) followed by *Escherichia coli* (35.56%) while *Bacillus* spp had the lowest occurrence (6.67%). Ready to eat ponmo Sample from Modern market had the highest prevalence percentage (26.67%) of the isolates.

Table 3: Percentage Prevalence of Bacterial Isolates from Ready to Eat Cow Hide (Ponmo) Sold by Roadside Vendors in Makurdi Metropolis

Sample Locations	Isolates				
	Staphylococcus aureus	***Escherichia coli***	***Bacillus* spp**	***Proteus* spp**	**Total**
Northbank	4 (8.88%)	5 (11.11%)	1 (2.22%)	1 (2.22%)	11 (24.44%)
Wadata	3 (6.67%)	3 (6.67%)	0 (0.00)	0 (0.00)	6 (13.33%)
Modern Market	7 (15.56%)	2 (4.44%)	2 (4.44%)	1 (2.22%)	12 (26.67%)
High Level	5 (11.11%)	2 (4.44%)	0 (0.00)	1 (2.22%)	8 (17.78%)
Wurukum	2 (4.44%)	4 (8.88%)	0 (0.00)	2 (4.44%)	8 (17.78%)
Total	**21 (46.67%)**	**16 (35.56%)**	**3** (6.67%)	**5** (11.11%)	**45 (100%)**

DISCUSSION

The consumption of ready to eat ponmo is increasing on daily basis due to it appealing taste and affordability. However, ponmo can serve as a vehicle for transmission of pathogens when contaminated. In the findings of this study the bacterial isolated from ready to eat cow hide (ponmo) include: *Escherichia coli, Proteus* spp*, Staphylococcus* spp and *Bacillus* spp. The result of this finding are in line with the report of Olukitibil *et al.* (2017) on Antibiogram of Bacteria Isolated from Processed and Unprocessed Cow-Hide (*Ponmo*) in Ogbese Market.

In this study statistical analysis of data showed that there was no significant difference ($P > 0.05$) in the bacterial load among the cow hide (ponmo) samples. The total bacteria count ranges from 1.72. x 10^5 - 1.72. x 10^5 Cfu/g. The International Commission for Microbiological Specification for Foods (ICMSF, 1996) states that ready-to-eat foods with plate count between 0-10^3 is acceptable, 104-≤ 10^5 is tolerable and ≥10^6 is unacceptable. Therefore, all the ready to eat cow hide (ponmo) in this study are within the tolerable limit. However, the high level of bacteria load could be associated with inadequate handling and processing by vendors, contamination caused by storage facilities, either poor hygiene or poor quality of grains and water used.

The most prevalent bacteria in this study were *Staphylococcus* spp (46.67%) followed by *Escherichia coli* (35.56%). The result of this study was in conformity with the findings of Omorodion *et al*. (2022). The presence of *Staphylococcus* spp in cow hide (ponmo) may be due to the fact that these organisms are common contaminant of food especially from food handlers, environment and post process contamination (Bennett *et al.,* 2013). Outside the body, *Staphylococcus* spp can survive for long periods of time in a dry state making it one of the most resistant non-spore-forming pathogens. *Staphylococcus* spp are regarded as the main source of food contamination through direct contact or respiratory secretions (Bennett *et al.,* 2013).

The presence *Escherichia coli, Proteus* spp *and Klebsiella* spp in this study may indicates unsanitary conditions as well as dirty environment where the cow hide (ponmo) was processed and hawked. *Escherichia coli* indicate fecal contamination during production. Vendors mostly use untreated borehole and well water in the production of cow hide (ponmo). This untreated water can serve as a means of contamination during preparation (Kumar *et al.,* 2016). The presence of *Bacillus* spp in processed cow hide (ponmo) maybe due to contamination of raw materials and the subsequent resistance of spores to thermal and other manufacturing processes. During the cooling processes, spores may germinate, enabling *Bacillus* spp multiply in the food and produce high levels of the emetic toxin cereulide, depending on the strain(s) present (Wijnands, 2018).

Conclusion

The results of this findings demonstrated that bacteria associated with ready to eat cow hide (ponmo) include: *Escherichia coli, Proteus* spp*, Staphylococcus* spp and *Bacillus* spp. The most prevalence organisms was *Staphylococcus* spp (46.67%) followed by *Escherichia coli* (35.56%). The total bacteria count ranges from 1.72. x 10^5 - 1.72. x 10^5 Cfu/g. These bacterial associated with ready to eat cow hide (ponmo) are of public health significance, some of which have may results to food borne illnesses when consumed. This calls for improved surveillance system on ready to eat products and public health education as well as enlightenment of retailers and consumers of ready to eat cow hide (ponmo)

Recommendations

Based on findings of this study the followings recommendations are made:

- Food vendors should be educated on safety practices with a close supervision of ready-to-eat foods by relevant authorities to prevent food-borne illness.
- Good hygiene practices should be adhered to especially at slaughter houses and during preparation, including handling of ready to eat cow hide (ponmo) in other to avoid microbial contamination that may cause food borne illness.

REFERENCES

Adeyeye, S. A. O., Oyewole, O. B and Obadina, A. O. (2015). Safety of street-vended foods in developing countries: A review. *Comprehensive Reviews in Food Science and Food Safety*, 14(5), 420-434.

Alimi, B. A. (2016). Risk factors in street food practices in developing countries: A review. *Food Science and Human Wellness*, 5(3), 141-148.

Ayesha, F. (2022). Laboratory methods for microbiological analysis.

Cheesebrough, M. (2005). District Laboratory Practice in Tropical Countries. *Cambridge University Press.*

Benson, H. J. (2002). *Microbiological Applications: Laboratory Manual in General Microbiology*. 8th ed. McGraw-Hill.

Geraldine, O., Adetutu, A and Nwosu, J. (2018). Bacteriological assessment of street-vended ready-to-eat *foods. Journal of Food Safety*, 38(3),.

Mamun, M. A., Rahman, M. S., and Turin, T. C. (2013). Microbiological quality of selected street food items vended by school-based street food vendors in Dhaka, Bangladesh. *International Journal of Food Microbiology,* 166(3), 413-418.

Monday, P and Adesope, A. (2014). Bacteriological analysis of ready-to-eat food sold by street vendors in Federal University of Technology, Akure, Nigeria. *American Journal of Research Communication* 2(6), 101-120.

Obiri-Danso, K., and Okore-Hanson, A. (2008). The microbiological quality of street-vending meat products in a developing country: A case study of Kumasi, Ghana. *Journal of Food Protec*tion, 71(4), 1640-1644.

Okiel, R. A., Karrar, M. A and Eltahir, Y. I. (2009). Some physical and chemical characteristics of beef ponmo. *African Journal of Food Science*, 3(10), 279-283.

Oyewole, O. B and Ogundele, O. M. (2011). Methods of preparation and preservation of some Nigerian street foods as reported by the street food vendors. *Food and Public Health*, 1(4), 155-160.

Prescott, L. M., Harley, J. P., and Klein, D. A. (2012). Microbiology. McGraw-Hill Education.

Rane, S. (2011). Street vended food in developing world: Hazard analyses. *Indian Journal of Microbiology,* 51(1), 100-106.