Evaluation of dynamics and prevalence of microbial flora of soaked dry meats (Kundi and Ponmo) in Nigeria

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Abstract

The manner in which dry meat is prepared in under developed countries is highly unhygienic. Preserved meats (kundi and ponmo) were obtained from three food markets in Ibadan and were properly labelled. Samples cut into 50 grams were taken to the laboratory and soaked for 0, 6, 12, 18 and 24 hours. Water used for soaking and homogenised meat samples were cultured for Salmonella, Campylobacter, E. coli, total bacterial count (TBC), total coliform count (TCC). Data were subjected to analysis of variance (ANOVA). Comparison of microbial loads for all the parameters measured due to effects of soaking had no significant (p>0.05) differences on kundi and ponmo, and the water for soaking. There was a significant increase of microbial load for kundi water from $0.94 \times 10^5$ cfu/g at 0hr to $4.93 \times 10^5$ cfu/g at 24hr for total bacterial count(TBC) at p<0.05. The highest bacterial prevalence was the isolates from Escherichia coli in water for soaking Kundi, while the highest fungal prevalence was isolates from Aspergillus niger in water for soaking ponmo. The microbial load was lesser for ponmo than kundi and the soaking of the dried meat in water over a period of time reduced the microbial load in the preserved meat with the lowest microbial count found after 24hours. It is recommended that consumers of dried meat should endeavour to soak this meat for a minimum of 24hours before they are utilized.

Keywords: Microbial flora, food markets, bacterial prevalence
Evaluation of dynamics and prevalence of microbial flora of soaked dry meats

ponmo. La charge microbienne était moindre pour le ponmo que pour le kundi et le trempage de la viande séchée dans l'eau sur une période de temps a réduit la charge microbienne dans la viande conservée avec le plus faible nombre de microbes trouvés après 24 heures. Il est recommandé aux consommateurs de viande séchée de s'efforcer faire tremper cette viande pendant au moins 24 heures avant de l'utiliser.

Mots clés : Flore microbienne, marchés alimentaires, prévalence bactérienne

Introduction
Meat and other animal products contribute to diets due to their high nutritional qualities by supplying high quality protein, vitamins and minerals (Olusola and Omojola, 2010; Adeyeye, 2016). However, these high nutritional qualities make meat perishable and ideal for growth of spoilage microbes. Hence, various methods are employed to preserve meat products (Akhtar and Pandey, 2015; Adeyeye, 2016). Worldwide, meat can be preserved by freezing, refrigerating and thermal processing thoughsome traditional meat processed products are still popular especially in developing countries experiencing insufficient electric power supply. To preserve and add value to fresh meat, several food processing techniques such as boiling, frying, drying, and grilling are employed (Akhtar and Pandey, 2015; Adeyeye, 2016). Commonly preserved dried meats in Nigeria include Banda, Tinko or Kundi and Kilishi (Alonge, 1999; Vilar et al., 2000; Okaka et al., 2006; Adeyeye, 2016). Another common one is the processed raw hide (Ponmo), which is a by-product obtained from slaughter animal for leather production. Ponmo is obtained from the tenderization of hides of cattle in hot water followed by scrubbing with sharp object like blade or singeing and sun drying (Okiel et al., 2009). Ponmo and kundi are palatable, used as major delicacy and important food ingredient in the preparation of several stews in various cultures especially at several public outings, despite their less nutritional value (FAO, 2003; Obiri-Danso et al., 2008).

Traditionally dried meat microbiology involves a natural development of wild fermentation in which microbial successions occur, uniform salting over the entire surface is most critical to inhibit pathogens and spoilage organisms (Okaka et al., 2006). However, meat and meat products offer a highly favourable environment for the growth of pathogenic microbes such as Salmonella spp., Campylobacter spp., verotoxigenic Escherichia coli and Listeria monocytogenes, which will cause greatest risk of meat borne infections (Saucier, 2000; Ostry and Reprich, 2001; Fasanmi et al., 2017). Dried food including meat products have been increasingly reported to be involved in outbreaks and recalls due to the presence of food-borne pathogens (Bourdoux et al., 2016). Food-borne bacterial pathogen contamination may have been introduced at primary production (especially antibiotic resistant pathogens due to on-farm injudicious use of antibiotics), during and post-harvest levels - handling, processing, storage and transportation. The unregulated intake of antibiotics in food-producing animals has been associated with the development of antimicrobial-resistant bacteria; which can be transmitted from animals to humans either via direct contact, or consumption when such pathogens are passed down the food chain and the environment (FAO, 2014). Antibiotic use plays a major role in the emergence of antimicrobial resistance. Huge antibiotics use occurs in agricultural settings, especially in livestock production and slaughter animals, where little attention is focused (Landers et al., 2012). Ensuring
the safety of meat and meat products calls for control of foodborne pathogens at all points of the food processing chain; from the farm, inspection before and after slaughtering, to the handling, processing, preservation and storage of meat and the products until the time it is consumed (FAO, 2014). Therefore, this study was aimed to determine the microbial load dynamics of soaked dried meats over a space of time and the antibiotic resistance of the isolated bacteria.

Materials and methods

Materials
Incubator, colony counter, Kundi and Ponmo tissues, microscopes, culture media, cotton wool, refrigerator, wire loop, distilled water
Sample collection
Dry meat tissues (kundi and ponmo) were bought from three food markets (Bodija, Oje and Molete) located in Ibadan, Oyo State, Nigeria. The kundi and ponmo samples were put in an air-tight polythene bags separately and taken to the Microbiology laboratory of the Institute of Agricultural Research and Training (I.A.R.&T.), Moor Plantation, Ibadan.
Preparation of samples for microbial counts
At the laboratory, thirty samples of 20g each of kundi and ponmo tissues weighed in a Kern 572-33 weighing scale (Kern and SohnGmbh, Germany) were soaked in labelled sample bottles with 50 mL distilled water, all the materials used for sample collection were sterile. These samples were prepared for microbiological studies within 24 hours of collection, and soaked in water and analysed for 0hr, 6hr, 12hr, 18hr and 24hr duration of soaking. The samples (water samples for the respective periods) were analyzed for the presence of bacterial and Total Fungal Count (TFC). The same procedure was followed for the soaked tissues, ground using mortar and pestle and mixed with normal saline solution to make 10 millilitre and diluted serially into ten test tubes. One millilitre of inoculum was taken from the test tube using sterile pipette and poured into each sterile petri dish. Then 20 millilitre of sterile nutrient agar was poured into each sterile petri dish, distributed and mixed evenly throughout. All samples inoculated in the nutrient agar (Oxoid, USA) were incubated in an incubator (M e m m e r t i n c u b a t o r, BechickingSchwabach, Germany) at 37°C for 24 hours in order to get the viable bacterial count (Salmonella, Shigella, Escherichia coli), Total bacterial count (TBC), Total Coliform Count (TCC) and Total Fungal Count (TFC).
Interpretation of microbial growth
Petri dishes containing 30 - 300 colonies on nutrient agar plate were selected. These were counted using colony counter (Stuart Bibby Scientific, UK), enumerated and expressed in CFU/g/ml of meat and water samples from every soaked tissue.
Isolation of pathogenic bacteria from dry meat tissue and water
The pure colonies were obtained according to the method described by Clinical and laboratory standards institutes (CLSI (2005)). One nutrient agar plate, Mac Conkey agar plate, Salmonella-Shigellaagar plate and Eosin methylene Blue Agar plates (Oxoid ,USA) for each were streaked using a wire loop with inoculum got from the prepared meat samples and incubated in an incubator (M e m m e r t i n c u b a t o r, BechickingSchwabach,Germany) at 37°C for 24hours. The isolates obtained were observed for morphological and cultural characteristics in each of the cultured media for all the samples and biochemical tests.
Identification of isolate
The identification of the isolates Salmonella spp., Shigella spp. and Escherichia coli, was done following the procedure described by WHO (2003) and
Cheeseborough (2006) as follows:

**Morphological and cultural characteristics**

Incubated nutrient agar plates were examined for yellow and amber growth. Isolates were examined microscopically (Olympus Microscope, Zeiss 080600 Zeiss, Germany) using Gram staining procedure for Gram-positive Cocci in clusters. These suggested the presence of *Staphylococcus* species. The incubated EMB Agar plates (Oxoid, USA) were examined for characteristic dark centred greenish and translucent amber growth which suggest the presence of *Escherichia coli*, and translucent, amber colour or colourless growth which suggest the presence of *Salmonella* or *Shigella* species. The isolates were examined microscopically (Olympus Microscope, Zeiss 080600 Zeiss, Germany) using Gram-staining procedure for gram-negative straight short rods which suggested the presence of *Escherichia coli* and Gram-negative long rods which suggest the presence of *Salmonella* and *Shigella* species. The growth on Mac-Conkey plates (Oxoid, USA) were examined for pink colonies for *Escherichia coli* which is a lactose fermenter and amber colonies for *Salmonella* or *Shigella* species. The incubated *Salmonella Shigella* Agar plates (Oxoid, USA) were examined for colourless colony with black centred growth which suggest the presence of *Salmonella* species (hydrogen sulphite producing bacteria), pink growth which suggests the presence of *Escherichia coli* (lactose fermenter) and colourless colony which suggests the presence of *Shigella* species (non-lactose fermenter and non-hydrogen sulphite producing bacteria).

**Biochemical characteristics**

The biochemical activities were carried out according to the method described by Cheeseborough (2006) and Oyeleke and Manga (2008).

**Catalase production test**

A wire loop (sterile) was used to take a speck of growth from each plate of 24 hours growth and a suspension was made with sterile distilled water on a clean microscope slide. Few drops of hydrogen peroxide were added using a pipette. Positive result showed the evolution of gas bubbles (effervescence) while negative result produced no bubbles.

**Motility test**

The isolates were cultured in peptone water for 24 hours before the motility test was carried out. A drop of the suspension was placed on a glass slide and covered with a cover slip. The whole preparation was sealed using a petroleum jelly to prevent it from drying. The preparation was observed microscopically (Olympus Microscope, Zeiss 080600 Zeiss, Germany) for motile organisms using the X10 and X40 objectives.

**Coagulase test**

A small speck of growth from different plates were picked with a sterile loop and dropped on glass-slides. Few drops of plasma were applied on the inoculum and a smear was made and slides were rocked for 2 minutes. Positive result produced clumps while negative results produced no clumps.

**Oxidase test**

Oxidase test strips were used, by inserting it into a 24-hour broth culture of isolates and withdrawing and kept for 5 minutes for colour change. For Positive result, colour change from yellow to dark purple while negative result produced no colour change. Other biochemical tests carried out were citrate test, indole test, urease test, starch hydrolysis test, methyl-red test etc.

All identification tests were carried out according to validated standard operation procedures of Isenberg (2004) and Murray (1999).

**Isolation of pathogenic fungi from dry meat tissues and water for soaking**

The tissue and water samples were placed
on Sabouraud-glucose agar (SGA) supplemented with chloramphenicol for fungi. Petri dishes were incubated at 37°C for 48 - 72 h while the cultures were observed daily under a stereoscopic microscope for presence of fungal mycelia.

**Data analysis**

All data were subjected to analysis of variance (ANOVA) and Duncan multiple range test was used for post-hoc comparison of means found to be statistically significant (p < 0.05). Furthermore, Pearson's correlation coefficient analysis was carried out to determine the correlation between bacterial load and the soaking time for dried meat.

**Results**

Table 1 shows the comparison of microbial loads due to the effects of soaking preserved meats (kundi and ponmo). The variables measured for the different tissues and the water used for the soaking include total bacterial count, total coliform count. The TBC was the same for the kundi tissue, kundi water and ponmo water, while the TCC is the same for KDT and KDW. However, the TBC and TCC for KDT is greater than PT at p< 0.05. The *Escherichia coli* and *Salmonella* spp. count did not show any significant difference (p>0.05) among the tissues and their respective waters. *Shigella* count was highest in the kundi tissue and lowest in ponmo water at p<0.05. However, the total fungal count (TFC) is highest in ponmo tissue which is higher than the kundi tissue and water except for ponmo water, the least amount of TFC was observed in ponmo water at p< 0.05.

**Effects of the soaking duration of kundi meat on the microbial loads in water**

There was a corresponding increase in the microbial load with duration of soaking of the preserved tissue (kundi). It increases significantly from $0.94 \times 10^5$ cfu/g at 0hr to $4.10 \times 10^5$ cfu/g at 24hr for total bacterial count (TBC) at p<0.05. Similar trend was observed for TCC, *E.coli*, *Salmonella* spp. and *Shigella* spp and also TFC; all at p<0.05 (Table 2). The correlation between microbial load and duration of soaking is presented in Table 3. There is a direct correlation between the time of soaking and the microbial counts in the water used for soaking. As the time of soaking increases, there is a corresponding increase in the microbial load in the water.

**Table 1**: Comparison of microbial loads due to the effects of soaking preserved meats (kundi and ponmo)

<table>
<thead>
<tr>
<th>Variables (x 10^5 cfu/g)</th>
<th>KDT</th>
<th>KDW</th>
<th>PT</th>
<th>PW</th>
<th>±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBC</td>
<td>4.29^a</td>
<td>4.10^ab</td>
<td>2.75^b</td>
<td>3.05^ab</td>
<td>0.40</td>
</tr>
<tr>
<td>TCC</td>
<td>2.37^a</td>
<td>2.19^a</td>
<td>1.41^b</td>
<td>1.27^b</td>
<td>0.28</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1.36</td>
<td>1.11</td>
<td>0.86</td>
<td>0.89</td>
<td>0.19</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>0.70</td>
<td>0.86</td>
<td>0.65</td>
<td>0.60</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>0.57^a</td>
<td>0.40^b</td>
<td>0.35^b</td>
<td>0.22^c</td>
<td>0.11</td>
</tr>
<tr>
<td>TFC</td>
<td>0.81^b</td>
<td>0.77^b</td>
<td>0.95^a</td>
<td>0.44^c</td>
<td>0.13</td>
</tr>
</tbody>
</table>

abc mean values on the same row with different superscripts are significantly different (p< 0.05)


**Table 2**: Effects of the duration of soaking of kundi tissue on microbial loads in water

<table>
<thead>
<tr>
<th>Variables (x 10^5 cfu/g/ml)</th>
<th>Duration of soaking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0hr</td>
</tr>
<tr>
<td>TBC</td>
<td>0.94^c</td>
</tr>
<tr>
<td>TCC</td>
<td>0.51^d</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0.30^d</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>0.14^d</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>0.05^c</td>
</tr>
<tr>
<td>TFC</td>
<td>0.10^d</td>
</tr>
</tbody>
</table>

abc mean values on the same row with different superscripts are significantly different (p< 0.05)
Table 3: Pearson’s correlation between microbial loads and duration of soaking

<table>
<thead>
<tr>
<th></th>
<th>Time</th>
<th>TBC</th>
<th>TCC</th>
<th>E. coli</th>
<th>Salmonella sp</th>
<th>Shigella sp</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBC</td>
<td>0.80</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCC</td>
<td>0.83</td>
<td>0.93</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>0.98</td>
<td>0.87</td>
<td>0.94</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella sp</td>
<td>0.95</td>
<td>0.85</td>
<td>0.89</td>
<td>0.92</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shigella sp</td>
<td>0.83</td>
<td>0.82</td>
<td>0.92</td>
<td>0.92</td>
<td>0.83</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>TFC</td>
<td>0.87</td>
<td>0.75</td>
<td>0.82</td>
<td>0.84</td>
<td>0.89</td>
<td>0.85</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The highest bacterial prevalence was in isolates from *Escherichia coli* and this was seen in Kundi water, and the least prevalence was seen in different isolates for the different bacteria in tissues and water (Fig. 1a). The highest fungal prevalence was in isolates from *Aspergillus niger* and this was seen in ponmo water, while the least prevalence was seen in different isolates for the different fungi in tissues and water (Fig. 1b).

![Fig. 1a: Frequency of occurrence of bacteria isolated from dried meat tissues (kundi and ponmo) and water used to soak](image-url)
Meat has been tagged a perishable food, because of its ability to deteriorate within a short period of time if not properly handled, processed or preserved. Meat is rich in carbohydrate which the bacteria can utilize for energy; abundant amounts of proteins which putrefactive bacteria can utilize for energy and high water content that is essential for bacteria growth (Thomas, 2012). This was further corroborated by Kołozyn-Krajewska and Dolatowski (2012); the constituent of meat that makes it nutritious also causes its susceptibility to microbial infections.

The shelf life or keeping qualities of meat is prolonged if a combination of preservation methods is adopted; by so doing the meat quality assurance is increased in accordance with the measure of the microbial load assessment (Yousuf et al., 2008). The findings of this study however, shows that despite the application of preservation techniques (cooking and sun-drying), a number of harmful bacteria and fungi of public health importance were identified. This may be attributed to the stages in the processing and storage methods adopted for the dried meats (kundi and ponmo). The production processes involve the use of carcass, cutting, cooking for 15–30 min, drying and sun-drying or smoking for about 18–30 h, cooling, storage and packaging in sacks and jute/mat bags. Kundi is a stable product with a shelf-life of 6–12 months or even up to two years under ambient temperature (Idufueko, 1984; Okonkwo and Obanu, 1984). But their storage in jute bags may predispose to high humidity that will favour the growth of microbes by providing a conducive medium for their proliferation. The isolated bacteria that are of zoonotic importance were *Salmonella sp.*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Penicillium bispicatum*, and *Penicillium chrysogenum*.
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Clostridium sp., Escherichia coli, Bacillus sp., Pseudomonas sp., Micrococcus sp., Staphylococcus aureus. This is also similar to the findings of WHO (2007) and Biswas et al, (2011); which state that bacterial pathogens commonly identified with meat contamination and cause infections in humans include Salmonella sp, Campylobacter sp, Staphylococcus aureus, Escherichia coli, Listeria sp, Clostridium sp, Bacillus sp, and Vibrio sp.

All these findings are also in compliance with the findings of Clarence et al. (2009); that gram negative bacteria account for approximately 69% of the cases of bacteria food borne disease. Members of the gram negative bacteria such as the E. Coli are widely distributed in the environment contaminated food and water are the major sources by which bacteria are spread.

According to Fakolade and Omojola (2008), there was an increase in microbial load of organisms on dried meat samples after six months of storage due to moisture absorption from the environment. However, little or no information is available on the mycoflora of dried meat, hides and skin in Nigeria (Akano et al., 1983).

However, the fungi identified from the dried meat products (kundi and ponmo) include the following viz; Aspergillus niger, Fusarium compactum, Fusarium oxysporum, Fusarium sacchari, Penicillium chrysogenum, Penicillium citrinin and Penicillium oxalicum. This is also in tandem with the isolation of previous study carried out by Adeyeye (2016) on dried meats sampled from some commodity markets in Ibadan. It has been further proven that majority of these fungi are mycotoxigenic and the mycotoxin levels in the sun-dried meat (kundi and ponmo) samples is of public health concern and proper attention is needed for the control of quality. Adequate preservation and storage of these products before sales and consumption to prevent food poisoning must be ensured (De Saeger, 2011; Adeyeye, 2016).

Furthermore, when comparison was carried out between the microbial loads of the two dried meats (kundi and ponmo), it was observed that the kundi samples have higher values and higher degree of contamination. This can be attributed to higher level of crude protein of kundi which was determined through proximate analysis when compared with the ponmo samples (Adeyeye, 2016). Also, it has been established that microbes thrive better in highly proteinaceous media (Pathak, 2008); hence the higher microbial load in kundi than ponmo.

Literature search shows no publication on soaking time of preserved meat, so there is paucity of information on this subject matter, and this study probably is the first of its kind in this subject area. This study however, observed that by soaking of dried meat samples, the texture of the meat becomes soften, loose and there was reduction in the microbial loads of the meat sample irrespective of the type, while there was a corresponding increase in the microbial loads in the water used. As the dried meat stays longer in the water, there is also a corresponding increase in the microbes released from the tissue into the water. The study further shows that a correlation exists between the duration of soaking of the dried samples of meat (kundi and ponmo) and microbial loads. This may be due to the property of water as a universal solvent, in which it improves protein solubility and brine strength to inhibit microbial proliferation thereby improving the meat shelf life (Sebranek, 2015).

This study has established that despite subjecting the dried meat (kundi and ponmo) to preservatory treatments of heat and sun-drying, the microbial load which include bacteria and fungi that are of public health importance were isolated. It was...
observed further that the microbial load was lesser for ponmo than kundi and the soaking of the dried meat in water over a period of time reduces the microbial load in the meat (kundi and ponmo) with the lowest microbial count found after 24 hours. It is therefore recommended that consumers of dried meat (kundi and ponmo) in this part of the world, especially Nigeria should endeavour to soak dry meat for a minimum of 24 hours before they are used for food.

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