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Temporal changes in the microbial community of salted goat meat

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Abstract

Meat preservation by salting was common in Nigeria before widespread electrification replaced it with refrigeration. However, salting still has its place due to chronic nationwide power shortages. This study investigates the effect of varying salt concentrations on the microbial community of salted goat meat. Fresh goat meat was treated with salt (w/w 0 - 20 %) and air-dried. Proximate composition of all samples was determined. Microbial counts were monitored over five weeks, while recovered isolates were identified phenotypically. All treatments lowered bacterial counts compared to control, but raised fungal counts due to decreased MC from 64.5 % to 24.1 % and decreased bacterial competition. The mean counts were $\log_{10}$ 4.93 – $\log_{10}$ 5.34 and $\log_{10}$ 1.45 – $\log_{10}$ 3.8 for bacteria and fungi respectively. The 20 % salt concentration was most effective for decreasing the bacterial population to $\log_{10}$ 5.1 by Day 14 while 5 % treatment yielded the lowest fungal growths with $\log_{10}$ 2.52. The 20 % salt treatment however had the highest fungal counts. Microorganisms including *Staphylococcus aureus*, *Listeria* spp., *A. fumigatus* and *A. flavus* were recovered. While this study shows the potential for salting as a cheap and easy meat preservation method, possible mycotoxin contamination needs to be investigated.

Keyword: Chevon, fungi, bacteria, preservation, microbial counts

1. Introduction

The meat of the domestic goat (*Capra aegagrus* subsp. *hircus*) is known as chevon and is one of the most consumed meats in the world. It is an important part of food consumption and the main product of several traditional dishes in Mediterranean diet (Teixeira, 2003). The West African Dwarf goat is the most predominant breed of goat in Ilorin and other parts of South-Western Nigeria, and because of its distinctive taste, aroma and desirable chemical composition, it is increasingly consumed in Nigeria. Even though, the global consumption of goat meat is lower than consumption of beef (Madruga and Bressan, 2011), goats undoubtedly

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serve as a major source of red meat (Webb *et al.,* 2005), particularly in developing countries such as Nigeria.

The popularity of goat meat is subject to the common culture of communities and the forces of civilization (Webb *et al*., 2005). Chevon have been reported to contain high quality proteins, minerals, vitamins and fat (Iroha *et al.*, 2011; Francis *et al.*, 2015). The perception of consumers in the West is changing because of its low fat and cholesterol contents and favorable sensory characteristics (Webb *et al*., 2005; Madruga and Bressan, 2011). Globally, goat meat consumption increased during the past 20 years (Madruga and Bressan, 2011). With the growing annual per capita meat consumption and high meat export, the estimated number of animals slaughtered has increased from 2.5 million during 2009-2010 to 3.5 million during 2011-2012 (Singh *et al.*, 2014; TOI 2013). This type of meat is not opposing any religious belief and cultural aspects of food consumption.

Tissues from healthy animals are normally sterile however, slaughtering of animals in Nigeria is not commonly performed under hygienic conditions. Tissues can then be contaminated by microorganisms during dressing and cutting from the exterior of the animal such as its skin, hide and feet; from the faecal material in the intestinal tract during slaughter and from the hands, clothing and equipment of slaughter men (Ukut *et al.*, 2010*;* Adetunde *et al.*, 2011). Most fresh foods especially those of animal origin such as goat meat are particularly vulnerable to microbial invasion and food poisoning since meat is an ideal medium for growth of a number of microorganisms due to its nutritive value (Soriyi *et al.*, 2008). Raw meat may harbor many important pathogenic microbes such as *Escherichia coli, Clostridium perfringens,* faecal streptococci, *Klebsiella pneumoniae* as well as species of the following genera; *Salmonella, Shigella, Bacillus, Proteus, Staphylococcus, Salmonella* and *Listeria* according to various studies within and outside Nigeria (Zweifer *et al.*, 2008; Iroha *et al.*, 2011; Eze and Ivuoma, 2012).

In addition, the non-availability of clean water sources results in the use of contaminated water for dressing the birds. In addition, high humidity, ambient temperature, and poor handling practices pre-dispose raw meat to deterioration and contamination (Raji, 2006; Nnachi *et al.*, 2014). Goat meat is preserved in several ways including various refrigeration technologies notably: chilling to about 2 ^{o}C to 4^{o}C and freezing at around -20 to -55 ^{o}C; boiling then frying; use of ionizing radiation; chemical preservation; high hydrostatic pressure and advanced packaging techniques (Zhou *et al.*, 2010). Smoking, salting, drying and a combination of

salting and sun-drying are common and cheap traditional Nigerian preservation methods which require little expertise to employ. Salting and drying of meat is the oldest meat preservation method with evidence showing it dates back to at least 3000 BC (Taormina and Sofos, 2014). The preservative activity of salt is due to its osmotic effect whereby it draws out moisture from the meat, thereby slowing down or stops the metabolic activity of contaminating microorganisms. (Albarracín *et al*., 2011; Tobin *et al.*, 2012). Traditionally, in times of the availability of surplus meat, such as immediately following Eid festivals, left-over beef, mutton or chevon was salted and sun-dried for several days. The dried salted meat, which can keep for several weeks, is steeped in hot water to soften and de-salted, then used in stews and soups. The cured meat has a distinctive flavor and aroma and is considered a delicacy.

However, over time, salting as a means of preservation fell out of favor and was replaced with refrigeration which was a superior meat preservation technique. Considering the persistently worsening situation of power supply in Nigeria in recent decades and the high cost of alternative power sources such as generators, it is now almost impossible to store goat meat for long periods. Consequently, the use of salt for preservation needs to be revisited to ensure that surplus meat available in times of plenty can be held over longer periods.

2. Materials and Methods

Sample collection

Twenty samples of fresh, dressed goat meat were purchased from Ipata market in Ilorin, Kwara State, Nigeria. The goats were prepared in the usual, traditional way in the abattoir which involves slaughtering the goat and then singeing off the hair on the carcass. The samples were from the hind leg and included the skin, muscle and bone, as is usually served. Samples were aseptically collected in sterile plastic containers and transported on ice to the laboratory where they were analyzed immediately.

Preparation of samples

The meat sample was divided into five (5) portions of which 4 were treated with salt using a dry salting technique which included the massaging of dry salt all over the meat, including crevices. The samples were treated with 5 %, 10 %, 15 % and 20 % (w/v) salt concentrations, while a last sample was not salt-treated. All the samples were sun-dried for seven days on a metal gridiron and then kept in a cool dry atmosphere pending analysis.

Proximate analysis

The crude fibre, protein, moisture, ash and lipid contents of the meat samples were determined according to the AOAC (2000) methods. Carbohydrate content was determined using the estimation by difference method (Pearson, 1976) while the calorific values were determined by the Atwater factor method (Hunt *et al.*, 1987).

Isolation of microorganisms

Ten grams of each meat sample was homogenized in 90 ml of sterile saline, serially diluted and inoculated in duplicates onto nutrient agar (NA) and potato dextrose agar (PDA). The NA plates were incubated at 37 ± 2 ^{o}C for 24 hours while the PDA plates were incubated at 25 ± 2 ^{o}C for seven days. The colonies obtained were counted and the CFU/g transformed to Log_{10} values for easier computation. Isolates obtained were purified and stored at 4 °C on agar slants.

Isolates were also cultivated on mannitol salt agar (MSA) to isolate organisms that can tolerate 7.5 % - 10 % sodium chloride concentration and ferment mannitol. Only salt tolerant organisms will grow and a yellow zone around the colonies indicate an ability to ferment mannitol (Ayeni *et al.*, 2017). The colony colour of these isolates is useful for presumptive identification.

Characterization and identification of isolates

Fungal isolates were identified based on their colony morphology which include; shape, diffusable pigmentation, appearance, size, growth rate, elevation and margin. Microscopic examination was also carried out using a wet preparation of the isolate. Characterization of bacterial isolates was based on colony morphology, Gram's reaction and biochemical tests (Fawole and Osho, 2004).

Statistical analysis

The microbial counts were converted to Log_{10} values using the 2016 version of the Microsoft Excel software (Microsoft, California USA). The data was analyzed using one-way ANOVA for the five salt concentrations (SPSS, version 16.0).

3. Results and Discussion

Fresh meat is a nutrient-rich substrate that supports the growth of microorganisms and is highly susceptible to microbial contamination from a wide variety of physical, microbial, chemical and radiological agents (Soyiri *et al.*, 2008). Reports have shown that sodium chloride can be used as a preservative in addition to its flavoring properties (Taormina and Sofos, 2014). To mitigate the activities of microorganisms, traditional meat preservation can be accomplished by a combination of preservative methods. Salt works as a preservative mainly by lowering moisture content and thus water activity (a_w), and drawing water from cells of both the food and bacteria (Albarracín *et al.*, 2011). The principle of curing meat is that the salt combines with meat protein to lower the a_w value of the cured meats. In addition to sun-drying, Microbial spoilage of salted goat meat is greatly reduced because of the increase in osmotic pressure and decrease in a_w which is part of the intrinsic factors that aids microbial growth.

Visual examination

The meat shrunk in size and visual examination revealed a colour change from pink/red to hues of yellow, grey and brown (Plate 1), which may be attributed to the degradation of the myoglobin in the muscle cells over time. In addition, whitish patches were observed in the samples with higher salt concentration and this is due to the presence of dry salt crystals on the meat. This finding is similar to those of Tobin *et al.* (2012) who reported a deeper colour in low-salt frankfurter samples compared to the higher salt samples and that of Devatkal and Naveena (2010) who also found that salt addition resulted in decreased redness in salted meat. The control meat remained darker-coloured at the end of the experimental period than the salted meats, but had pungent odours, especially on day 3 (results not shown).

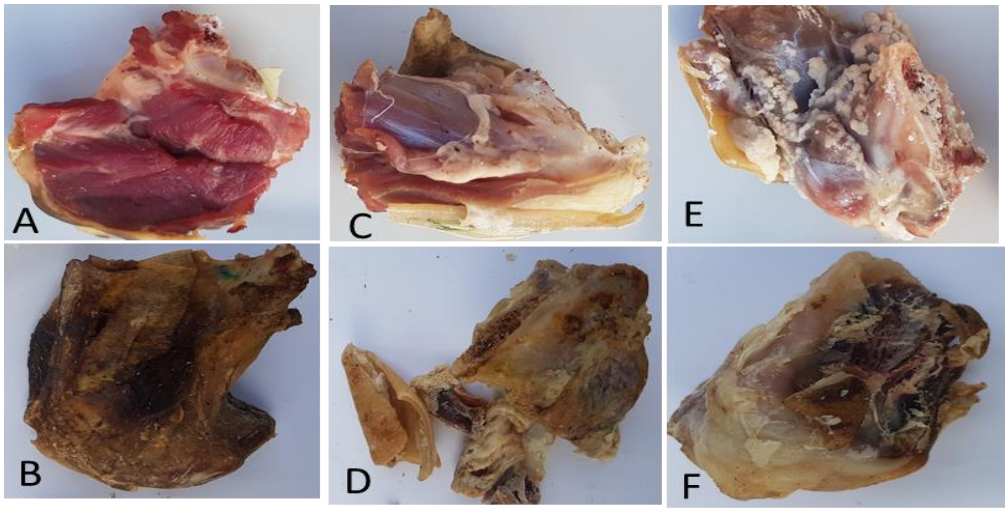

Plate 1: Physical appearance of chevon samples. Legend: *Top*: Fresh goat meat; *Bottom*: Chevon sun-dried for three weeks. A and B: unsalted chevon; C and D: chevon treated with 5 % salt; E and F: chevon treated with 20 % salt.

Proximate analysis

The proximate analysis of the chevon samples revealed that it was a rich meat sample with high protein and fat contents of about 20.8 % and 7.5 % respectively (Table 1).

Table 1: Proximate analysis of various goat meat samples.

Sample	Moisture %	Ash %	CHO %	Calorific Value KJ/100g	Total protein %	Crude lipid %	Crude fibre %
Fresh goat meat	64.45 ± 0.84	2.12 ± 0.02	4.76 ± 0.62	710.32 ± 58.23	20.75 ± 0.88	7.54 ± 2.21	0.38 ± 0.11
5 % salted goat meat	40.34 ± 0.57	7.11 ± 1.08	17.24 ± 1.28	1379.87 ± 33.38	28.32 ± 0.12	6.56 ± 0.25	0.43 ± 0.04
20 % salted goat meat	24.05 ± 0.89	12.53 ± 0.21	22.54 ± 2.33	1123.37 ± 23.09	36.81 ± 0.32	3.53 ± 1.48	0.54 ± 0.19
Unsalted (dried) goat meat	10.48 ± 0.28	4.83 ± 0.04	22.82 ± 0.53	1632.61 ± 8.30	50.56 ± 0.14	10.80 ± 0.51	0.51 ± 0.18

Values are averages of duplicate experiments ± SD

Microbial counts

Bacterial counts increased slightly in all samples with time (Figure 1) with the highest being in the unsalted meat even though there was no significant difference in the various treatments for much of the period. However at Days 1 and 28 of the experiment, the 20 % treatment yielded significantly fewer bacteria than the control which had the highest population, and the 5 %, 10

% and 15 % salted meat samples. The bacterial population trend was: 0 % > 5 % >10 % > 15 % > 20 %. The average bacterial counts rose from 4.82 $\log_{10}$ cfu/g to 6.31 $\log_{10}$ CFU/g while the fungal population of 3.83 $\log_{10}$ cfu/g declined to 1.45 $\log_{10}$ cfu/g over 4 weeks. These figures are similar to those reported by Eze and Ivuoma (2012) who analyzed fresh goat meat samples and reported that the total aerobic plate count was about 5.39 $\log_{10}$ cfu/g while fungal count was up to 3.56 ± 0.05 $\log_{10}$ cfu/g. The findings are also similar to the report of Ajiboye *et al.,* (2011) that bacterial counts increased in "tinko", a dried meat product, stored at room temperature.

Unlike the case with bacteria, the fungal counts initially increased then declined with time in all samples (Figure 1B). The control meat had lower fungal counts than the salted samples which may be because the fungi were out-competed by the higher bacterial population (Figure 1A). However, by Day 7, its fungal population became steady at $\log_{10}$ 2.28 while the salted 5 % treated sample became steady at Log_{10} 1.85. This indicates that lower salting levels may be better for controlling fungal contamination of goat meat. By Day 28 all the salted samples had lower and declining fungal counts than the control indicating that salting is slow to decrease fungal contamination of meat.

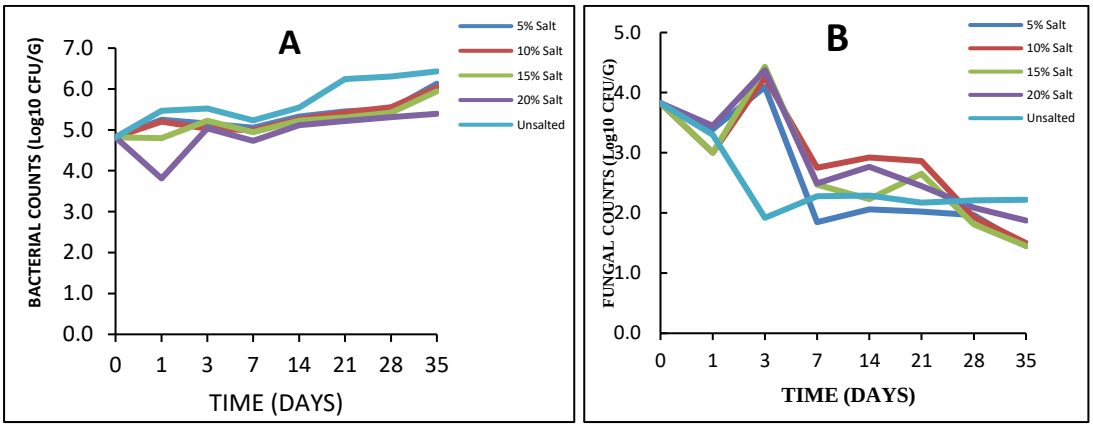

Figure 1: The effect of varying salt concentrations on bacterial (A) and fungal growth (B) in goat meat.

The phenomenon where the unsalted meat with the highest bacterial count also had one of the lowest fungal counts is due to its higher moisture content which favoured bacterial colonization while the dryer salted meats favoured fungal colonization. In the same vein, salting with 5 % was most effective at decreasing fungal populations by Day 7 because the salt may have been sufficient to eliminate the less halotolerant fungal species; but low enough to not decrease the a_w beyond the requirement of halotolerant species. In addition, the increase in the bacterial population (Figure 1A) introduced competition which discouraged the proliferation of fungi.

Microbial analysis

Microbial analysis yielded only three bacterial genera in the salted meat namely *Lactobacillus*, *Staphylococcus* and *Listeria*. It is thus deduced that the increased bacterial population observed over time (Figure 1A) was due primarily to the increase in the population of halotolerant bacteria mainly the staphylococci. Colonies that were mannitol-fermenting on MSA and were observed to be Gram-positive cocci in clusters microscopically were putatively identified as pathogenic *S. aureus* while the pink non-fermenting colonies were identified as *S. epidermidis.* This is similar to findings by Ratsimba *et al.* (2017) who isolated several staphylococcal species from "kitoza", a traditional Madagascar meat product. This observation is of significance because it indicates that these potentially pathogenic microorganisms can tolerate salting and thus persist in salted meats. Olaoye and Onilude (2009) and Essid *et al*. (2009) also isolated various lactobacilli from fresh beef and Tunisian traditional salted meat respectively.

The presence of the LABs may be through the production of antimicrobial compounds such as lactic acid, acetic acid and bacteriocins etc, inactivate pathogens (Tharrington *et al.*, 1992; Aymerich, 2000; Pieterse *et al.*, 2005; Perales-Adan *et al.*, 2017; Wemmenhove *et al.*, 2018) and this may explain the low bacterial diversity encountered. The findings in this study are also in concordance with reports that *Listeria* sp. can contaminate cured meats (Taormina and Sofos, 2014), and was isolated in cured meat products, occurring at 24.3 % and 36.8 % of raw- and dry-cured meat products respectively (Gomez *et al.*, 2015). This organism was however only encountered in the unsalted and the least salted (5 %) meat samples (Table 2) which indicates that salting at higher NaCl concentrations may be useful in avoiding the risk of *Listeria* contamination.

Table 2: Isolates recovered from the various salted meat samples.

Sample/ Organism	**Unsalted meat**	**5 % salted meat**	**10 % salted meat**	**15 % salted meat**	**20 % salted meat**
Putative *Listeria spp*	+	+	-	-	-
Staphylococcus aureus	+	+	+	+	+
Staphylococcus epidermidis	+	+	+	+	+
Lactobacillus sp	+	+	+	-	-
Aspergillus niger	+	+	+	+	+
A. flavus	+	+	+	-	-
A. terreus	-	+	+	-	-
A. versicolor	-	-	+	-	-
Rhizopus stolonifer	+	+	+	+	+

Among the yellow colonies isolated on MSA none gave a negative catalase test reaction which rules out the salt-tolerant *Enterococcus* sp as a potential isolate. Gram negative bacteria were also not recovered and this is because the high osmotic pressure selectively kills this group of bacteria (Taormina and Sofos, 2014). These points indicate a good hygiene standard of the salt-preserved meats.

The fungi observed included *Aspergillus niger, A. flavus, A. terreus, A. versicolor* and *Rhizopus stolonifer.* These organisms have been isolated from dried and salted meat (Roberts, 2004). Most fungi do not thrive in salty environments (El-Mougith, 1993) which explains the low diversity. However, the presence of particularly *A. flavus,* which has been reported to tolerate up to 10 % sodium chloride (El-Mougith, 1993) and *Aspergillus terreus,* suggests that the consumption of these salted meats could pose a potential health risk because they are mycotoxigenic and pathogenic species respectively (Risslegger *et al.*, 2017; Frisvad *et al.*, 2019).

4. Conclusion

Contaminating bacteria in meat cured with various concentrations of NaCl, increased while contaminating fungi decreased with time. The bacterial population of the 20 % salt-treated sample was the lowest while the highest was in the 5 % sample. Contrarily, the 5 % salt

concentration proved most useful at decreasing the fungal population while salting at higher concentrations proved slower. While the isolation of *Staphyloccoccus aureus* and *Listeria* sp was worrisome, these pathogens will be lost in the washing and cooking processes, if these processes are carried out properly. More research needs to be done to investigate mycotoxin production by *Aspergillus* species in salty meat and determine the safety of salting as an affordable and easy goat meat preservation technique.

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