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Research Article

Microbial Quality Assessment of Processed Meat Product (Tsire) Sold Within Wudil Town, Wudil Local Government Area, Kano State, Nigeria

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Abstract

Microbial quality assessment of Tsire meat products sold within Wudil town was conducted between February-April 2018. A total of fifteen (15) samples were purchased from five different spots and analyzed microbiologically using the pour plate method. The total aerobic plate count on nutrient agar ranged from $1.4x10^4$cfu/ml to $2.95x10^5$cfu/ml. The coliform count using most probable number technique (MPN) ranged from 3cfu/g-240cfu/g. Total fungal count (yeast and mold) on Sabaroud dextrose agar with *Chloramphenicol* (as control) ranged from $1x10^3$cfu/ml-$8x10^3$cfu/ml. The bacteria isolated include *Staphylococcus aureus, Escherichia coli, Shigella spp*, and *Salmonella* spp. The fungal isolates obtained from this study are *Penicillium spp,* and *Aspergillus niger*.

The percentage of occurrence of bacterial isolates was highest in *Staphylococcus aureus* (43.5%), *Shigella spp* (21.7%), Salmonella spp (21.7%) and *Escherichia coli* (13.0%) recorded the lowest. The percentage of occurrence of fungi isolated from the Tsire samples was highest in *Aspergillus niger* (66.7%), and *Penicillium spp* (33.3%). It is concluded that the occurrence of such organisms indicates contamination of the Tsire meat samples. Hence, proper care in the course of preparation and handling of Tsire meat needs to be established. Educating the meat handlers on the issue of food safety and public health will reduce the rate of contamination of the Tsire meat.

Keywords: Aerobic plate count; Coliforms count; Microbial quality assessment; Tsire; Wudil

Introduction

Meat which refers to meat flesh, skeletal muscles, connective tissues or fat and others than meat flesh, including brain, heart, liver, kidney, pancreas, spleen, thymus tongue and tripe that is used as food, excluding the bone and bone marrow and it contains high biological value protein and important micronutrients that are needed for good health throughout life [1]. Meat as a source of animal protein is consumed heavily in Nigeria and is also recommended by nutritionists as a major source of protein for growing children, the convalescent, the expectant mothers and the aged. Meat contains an abundance of all nutrients required for the growth of bacteria, yeasts and molds and an adequate quantity of these constituents exist in fresh meats in available form.

Processed meat is any meat which has been modified in order to either improve its taste or extend its shelf life. Methods of meat processing include salting, smoking, curing, and fermentation. Processed meat is usually composed of pork or beef, but also poultry, while it can also contain offal or meat by-products such as blood. Processed meat products include bacon, kebabs, sausages, corned beef, beef jerky, and canned meat-based sauces. Meat processing includes all the processes that change fresh meat with the exception of simple mechanical processes such as cutting, grinding or mixing [2]. According to the guidelines of good manufacturing practices, the level of total microbiological contamination of raw meat and raw meat preparations, it is commonly suggested that microorganisms can enter meat preparation like sausages from meat, spices, and other ingredients, as well as from processing environment, equipment, and handlers that can have a significant impact on the microbiological status of the end-products [3].

Meat is highly nutritious and rich in proteins, consequently serves as a good substrate for microbial growth [4]. Tsire is a roasted boneless meat of beef, goat or mutton that is grilled around a glowing charcoal fire in which the meat pieces are stacked on wood sticks, spiced with peanut cake powder, spices, vegetable oil, salt or other flavorings [5]. Tsire has become very popular as a street delicacy of several countries, particularly those in West Africa. It is processed and sold along streets often under unhygienic conditions. The prepared Tsire when sold are usually packaged in newspapers or nylon bags. Most of the stages of Tsire preparation,

materials used in its preparation and packaging, the handlers and the surrounding environment can serve as source of contamination of the product [6]. 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 [7]. Microorganisms depend on nutrients from meat or meat products for survival. Pseudomonas aeruginosa synthesizes its vitamin and so cause spoilage even in a medium without vitamins. Staphylococcus aureus requires about 6.5% of sodium chloride for growth and is usually found in salty meat products.

Tsire meat due to its chemical compositions and characteristics is a highly perishable food. This provides an excellent medium for the growth of many microorganisms that can cause infection in man and also lead to spoilage and economic loss. The possible sources of contamination may be through slaughtering of sick animals, washing the meat with dirty water, handling by butchers, contamination by flies, through processing done close to sewage, during transportation, use of contaminated equipment such as knife and other utensils; and addition of unclean spices. This work is aimed at assessing the microbial quality of commercially processed meat product (Tsire) sold within Wudil town, Wudil Local Government Area of Kano State.

Materials and Methods

Sample collection

A total of fifteen samples of Tsire meat were purchased, three (3) samples each from five (5) different spots within Wudil Town. Purchased samples were collected and labeled as A, B, C, D and E and then placed in a sterile bag. The samples were then transported to the Microbiological Laboratory of Kano University of Science and Technology, Wudil. Microbiological analyses were processed immediately to avoid further contamination.

Sample preparation

One gram (1) of the Tsire meat sample was weighed, cut into pieces using a sterile knife and then aseptically introduced into 9ml of distilled water; it was properly shaken and was used as stock. Several dilutions were achieved up to 4 fold (10-4) for each prepared sample using 1ml from stock homogenate and 9ml of sterile distilled water for serial dilution. This was carried out in order to obtain discrete colony [8].

Bacterial isolation and determination of total viable counts

Nutrient Agar (NA) was the media used for determining the total viable count because it is a supportive medium for the growth of non-fastidious microorganisms. The media was prepared using the manufacturer's preparation manual, followed by sterilization and then cooled to about 45 °C. 1ml of the appropriately diluted meat sample was placed at the centre of the Petri-dish followed by pouring of the nutrient agar using the pour plate method. It was allowed to solidify for some minutes and then incubated at 37 °C for 24 hours. Emerging colonies were counted and expressed as log cfu/ml using the following formula as described by Rukayya et al. [9], Thus; N=n/vd, Where N=the number of colony per gram of sample, n=number of colonies counted, v=volume of inoculums used and d=dilution factor. Discrete colonies were purified by sub-culturing into Nutrient agar plates and Mac Conkey agar plates were subsequently identified using standard methods [10]. Discrete colonies were inoculated aseptically on the agar plates with a wire loop using streak method, and then the plates were incubated at 37 °C for 18-24 hours. After 24 hours, the plates were observed for growth of isolates [10]. The isolates were characterized and identified based on their cultural characteristics and biochemical reactions.

Test for coliforms using three tubes (most probable number) techniques

Aseptically, 10g of the Tsire meat sample was weighed and transferred into 90ml of sterile lactose broth which served as stock. Decimal dilution of 1:10-1 to 1:10-3 was prepared by adding 1ml of the previous dilution (stock) to 9ml of the sterile lactose broth. Three (3) replicate tubes containing durham tubes and lactose broth per dilution were prepared with 1ml of the previously prepared 1:10, 1:100, and 1:1000 dilutions. The tubes were then incubated for 24 hours at 35 °C and were observed for gas production. Negative tubes were incubated for an additional 24hours. All tubes showing gas within 48±2hours were recorded. The number counted was referred to as Most Probable Number (MPN) for the three tubes dilution [10].

Bacteria identification

The distinct colonies that develop in the pure culture plate were observed for the morphological and cultural characteristics including the nature of margin, elevation, shape, colour and transparency and Gram staining [10-12] and set of Biochemical Characterization i.e. indole test, Methyl-Red test, Vogues-Proskauer test and Citrate utilization test, catalase test, coagulase test and oxidase test by standard method given by Sherman [11] and Holt et al. [12].

Isolation of yeast and molds

The media of choice used was Sabaround Dextrose Agar (SDA) containing Chloramphenicol (50mg). The SDA was prepared and sterilized following the manufacture procedure manual. After sterilization, the media was allowed to cool to 45 °C and poured into the Petri dish containing 1ml of the inoculated sample using pour plate method then allowed to solidify for some minutes and incubated at 28 °C for 5days [10,11]. Emerging colonies were then counted, calculated and expressed as log cfu/ml and further identified through microscopy.

Characterization of fungi

The Fungi were identified using the Lactophenol cotton blue technique [10]. A drop of Lactophenol cotton blue was placed on clean grease free. A straight wire loop was used to pick the

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organism colony and teased on the drop. A cover slip was placed on the Lactophenol cotton blue and examined under×40 objective lens to check for the structure of the organism [10].

Result

The total aerobic plate count, total fungal count, and coliform count (MPN) of the samples was presented in Table 1. The result showed that sample A1 had the highest total aerobic plate count with $2.95x10^5$, while the least total aerobic plate counts were recorded in sample E2 with $1.4x10^4$. The result also indicated that sample A2 had the highest total fungal counts of 8x103 while all samples in C recorded the least total fungal counts of $1x10^3$. Additionally, for coliforms count, sample A1 was the highest with 240 coliform counts and sample E2 recorded the least coliforms counts of 3. However, the result showed that the samples were within the acceptable WHO standard limit.

Table 1: Result of total aerobic plate count, total fungal count, and coliform count (MPN) of the Tsire meat samples analyzed. TPC: Total Aerobic Plate Count; TFC: Total Fungal Count.

S/N	Sample	TPC	TFC	Coliform count	Conformity with Standard Limits
		(CFU/ml)	(CFU/ml)	(MPN/g)	
1	A_1	$2.95X10^5$	$5X10^3$	240	Pass
2	A_2	$9.2X10^4$	$8X10^3$	150	Pass
3	A_3	$1.19X10^5$	$5X10^3$	240	Pass
4	B_1	$8.7X10^4$	$2X10^3$	43	Pass
5	B_2	$7.7X10^4$	$1X10^3$	21	Pass
6	B_3	$6.2X10^4$	$1X10^3$	23	Pass
7	C_1	$1.10X10^5$	$1X10^3$	21	Pass
8	C_2	$7.8X10^4$	$1X10^3$	14	Pass
9	C_3	$8.6X10^4$	$2X10^3$	14	Pass
10	D_1	$1.98X10^5$	$2X10^3$	210	Pass
11	D_2	$1.63X10^5$	$1X10^3$	210	Pass
12	D_3	$6.5X10^4$	$4X10^3$	150	Pass
13	E_1	$2.8X10^4$	$2X10^3$	9	Pass
14	E_2	$1.4X10^4$	$1X10^3$	3	Pass
15	E_3	$6.8X10^4$	$1X10^3$	9	Pass

Table 2: Morphological and biochemical properties of bacteria isolated from Tsire meat samples collected from Wudil Town. Key: 1=Catalase, 2 =Coagulase, 3=Citrate, 4=Indole, 5 =Methyl red, 6=Voges-proskeur.

Isolates	Colonial Morphology	Gram Reaction	1 2 3 4 5 6	Organism
A	Small circular colonies with black centre	Gram negative rods	+ - - - + -	Salmonella spp

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B	Small circular colonies without black centre	Gram negative rods	+ - - - + -	Shigella spp
C	Golden yellow colonies	Gram positive cocci	+ + + - + +	S. aureus
D	Green metallic sheen	Gram negative rods	+ - - + - -	E. coli

Table 3: Morphological and microscopic description of fungal isolates.

Isolates	Colonial Morphology	Microscopy
A	Dark brown to black pigmentation	Numerous mycelia conidiophores are black spherical to oval, produced in chain.
B	Colony has shades of green with white	Single-celled conidia produced with brush-like spore-bearing structures.

Table 4: Occurrence of bacterial isolates in Tsire samples from Wudil Town. -=Absence of isolates, +=Presence of isolates.

S/N	Samples	*S. aureus*	*E. coli*	*Shigella*	*Salmonella*
1	A_1	-	+	+	-
2	A_2	+	+	-	+
3	A_3	+	-	+	-
4	B_1	-	-	-	-
5	B_2	+	-	-	-
6	B_3	+	-	-	-
7	C_1	+	-	+	+
8	C_2	-	-	-	-
9	C_3	-	-	-	-
10	D_1	+	+	+	+
11	D_2	+	-	+	-
12	D_3	+	-	-	+
13	E_1	_	-	-	-
14	E_2	+	-	-	-
15	E_3	+	-	-	+

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	Total	10	3	5	5
	Percentage (%)	43.5	13	21.7	21.7

Table 5: Occurrence of fungi isolates across Tsire samples in Wudil town. -=Absence of isolates, +=Presence of isolates.

S/N	Sample	Aspergillus Niger	Penicillium Spp
1	A_1	–	–
2	A_2	+	–
3	A_3	+	+
4	B_1	–	–
5	B_2	–	–
6	B_3	–	–
7	C_1	–	+
8	C_2	+	–
9	C_3	+	–
10	D_1	–	–
11	D_2	–	–
12	D_3	+	+
13	E_1	+	–
14	E_2	–	–
15	E_3	–	–
	Total	6	3
	Percentage (%)	66.7	33.3

The morphological, microscopic description and biochemical characterization of four (5) different bacteria isolated from the fifteen different Tsire samples was presented in Table 2. The bacteria identified were *Salmonella spp*, *Shigella spp*, *Staphylococcus aureus* and *Escherichia coli*. The morphological and microscopic description of two different fungal species which were isolated from the samples was presented in Table 3. The species identified were *Aspergillus niger*, and *Penicillium spp*. The distribution of the isolated bacteria was presented in Table 4. The result showed that a total number of Twenty three (23) bacteria were isolated from the Tsire samples, *Staphylococcus aureus* was the most common bacteria isolated with 10 (33.3%), followed by Shigella 5 (21.7%) and *Salmonella spp* with 5 (21.7%) while *Escherichia coli* had 3 (13.0%). The distribution of the isolated fungi was presented in Table 5. The result indicated that *Aspergillus niger* was the most common fungi isolated with 6 (66.7%) while the least was *Penicillium spp* with 3 (33.3%).

Discussion

This study shows that the Tsire meat analyzed contained a high number of microorganisms, this could be due to possible source of contamination such as, through slaughtering of sick animals, washing the meat with dirty water, handling by butchers, contamination by flies processing close to a refuse dump environment, spices, transportation and use of contaminated equipments such as knife and other utensils [13-16]. There are lots of factors affecting the growth of microorganisms on meats, these factors include temperature, pH, water availability, presence of nutrients, moisture, acidity (intrinsic factors), gaseous requirement and atmosphere of storage (extrinsic factors) as stated by Nester et al. [17]. The variations in aerobic plate counts, fungal counts as well as in the coliform counts recorded in this study could be due to the materials and different treatment/processes used by the producers and sellers of the Tsire meat. This observation is in line with the findings of Igene et al. [18] which stated that the quality of suya (Tsire) produced by the processors varies from one producer to another due to lack of standard method of preparation that would ensure consistent product quality. The aerobic plate counts ranged from 1.4x104 to 2.95x105 colony forming units (cfu) per ml. Inyang et al. [19] recorded comparably similar values for total plate counts in suya (Tsire) samples in the order of 105 and 106 and stated that the values were within satisfactory limits according

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to the International Commission of Microbiological Standards of Foods [20].

In this study, four different bacteria were isolated and identified which are *Staphylococcus aureus*, *Salmonella spp*, *Shigella spp* and *Escherichia coli* and similar findings of some of these bacteria have been earlier reported by several workers [21-31]. Bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp*, and *Shigella spp* observed in this study are of public health importance as they have been found to be related to various diseases of man such as gastroenteritis. These findings strongly agrees with the publications of FAO/WHO [32] which stated that in developing countries such as Nigeria, Cholera, *Salmonellosis*, *Brucellosis*, *Shigellosis* and *Colibacillosis* are prevalent due to the feeding habit of people. The presence of *Staphylococcus aureus* and some enteric bacteria in the present study is in consonance with the findings of Gilbert & Harrison [16] who reported that meat preserved with salt permit the growth of Staphylococcus aureus whereas, the presence of some members of *Enterobacteriaceae* is due to contamination from the intestines of slaughtered animals. Additionally, Staphylococcus aureus requires about 6.5% Sodium Chloride for growth and is usually found in salty meat products [33]. Many healthy people carry Staphylococcus aureus as a normal flora of the skin [16]. However, foods commonly implicated in *Staphylococcal* food poisoning are those that have been contaminated via physical handling and then subjected to time/temperature abuse [34].

The presence of *Escherichia coli* may be due to the use of non-portable water during washing of raw meat as stated by Umoh [35]. Bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp*, and *Shigella spp* are of public health importance they have been found to be related to various diseases of man such as gastroenteritis. In the present study, *Staphylococcus* aureus was recorded as the most frequently isolated bacteria. This is in conformity with the results of Egbebi et al. [36], Tijjani et al. [37] Lawrence et al. [27], Nwakanma et al. [38], Muhammad et al. [28] Ananias et al. [30] and Falegan et al. [31] who also recorded *Staphylococcus aureus* as the most frequently isolated bacteria and highest percentage occurrence. However, this result is in disagreement with the findings of Moshood et al. [8]; Manyi et al. [39] and Samuel et al. [26] and Uze et al. [25] who identified *Bacillus cereus*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella species*, and *Bacillus subtilis* and *Enterobacter aerogenes* respectively, as the most frequently *isolated bacteria*.

Additionally, it was observed in this study that *Escherichia coli* was the least isolated bacteria, and this is in agreement with the findings of Nwakanma et al. [38]; Lawrence et al. [27] and Falegan et al. [31] respectively. However, these findings were in disagreement with the findings of Edema et al. [23]; Ogbonna et al. [24]; Samuel et al. [26] as well as that of Ananias et al. [30] respectively, who reported *Salmonella spp*, *Shigella spp*, *Streptococcus pyogenes*, *Pseudomonas spp* and *Staphylococcus aureus* and *Lactobacter species* respectively as the least bacteria isolates recorded. The findings from this study shows that the level of hygienic practices of the Tsire meat processing by the sellers affect the level of contamination of the meat, even though the results are within the range of satisfactory and marginal when compared with the guidelines of microbiological examination of ready-to-eat food samples. The critical control points of contamination of Tsire meat are purchase of raw materials, storage of raw materials, slicing, spicing, roasting and serving [23]. Therefore, control of contamination can be achieved if aseptic ways of preparation are observed.

Conclusion

The microbial quality assessment of Tsire meat sold within Wudil town showed that the Tsire meat was contaminated but within the satisfactory and marginal limits. The bacteria isolated include *Staphylococcus aureus*, *Escherichia coli*, *Shigella spp*, and *Salmonella spp*. The fungal isolates obtained from this study are *Penicillium spp*, and *Aspergillus niger*. The percentage of occurrence of bacterial isolates was highest in *Staphylococcus aureus* (43.5%), *Shigella spp* (21.7%), *Salmonella spp* (21.7%) and *Escherichia coli* (13.0%) recorded the lowest. The percentage of occurrence of fungi isolated from the Tsire samples was highest in *Aspergillus niger* (66.7%), and *Penicillium spp* (33.3%). It is concluded that the occurrence of such organisms indicates contamination of the Tsire meat samples. Hence, proper care in the course of preparation and handling of Tsire meat needs to be established.

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