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ISOLATION AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH HAWKED SUYA MEAT

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ABSTRACT

This study was aimed at determining the micro biological quality of Suya sold within Federal Polytechnic Nekede Owerri, Imo State Nigeria. Six Suya samples were purchased from six vendors within the study area. Standard microbiological methods were adopted in the determination of the microbial load, isolation and characterization of the bacteria from the suya meat. Total viable bacterial counts recorded ranged from 4.0 x 10^3 cfu/g to 8.4 x 10^4 cfu/g while total coliform counts were not recorded in all the samples. Bacterial species isolated were; *Pseudomonas* species, *Micrococcus* species, *staphylococcus* species, *Corynebacterium* species and *Bacillus* species. The environment where suya are prepared, together with the suya processors and equipment could be sources of microbial contamination. There is need to ensure personal hygiene among the suya processors.

Keywords: isolation, inoculation, safety, coliform, viable, colony.

INTRODUCTION

Suya (Hausa Language for roasted meat) is a popular spicy, smoked, or roasted street meat in Nigeria and other countries surrounding northern Nigeria like Chad, Sudan and Niger (Agence, 2012). Hausa is one of the three major ethnic groups in Nigeria. In northern Nigeria where over 80% of Nigeria's cattle rearing occurs, suya production and consumption is about the main nutrition source. Generally, meat including suya is excellent in supplying high quality protein, vitamins and minerals salts such as iron and zinc (Egbebi and Seidu, 2014).

Suya, Kilishi, balangu, kundi, and dambu nama are all Hausa Language for processed, smoked, roasted or dried meat are also very popular meat products eaten in Northern Nigeria. The purpose

of processing operations of meat to produce these products is to preserve and increase the shelf-life in addition to improving the palatability and food value of the meat. The consumption of suya, kilishi, balangu, kundi and dambu nama has extended to other parts of the country (Eke *et al.*, 2014).

In Nigeria, suya sales in cities and small towns are prominent. Suya is prepared basically from boneless meat of animals (Egbebi and Seidu, 2014). Muscles meat of almost any kind can be dried to increase its keeping quality. When food materials are dried or roasted, there is loss of moisture. This reduces the water activity (a_w) of the food thereby preventing some bacteria from forming spoilage association. In suya preparation, use of lean meat is necessary since fat becomes rancid during the drying process (Huda *et al.*, 2010).

'Suya' or 'tsire', is an important food products that provide valuable animal protein in the diet of millions of Nigerians. 'Suya' and 'kilishi' are made by roasting the spiced, salted slices/strips of meat (usually beef). 'Kilishi' differs from 'suya' in that the two-stage sun-drying process proceeds roasting. Consequently, 'kilishi' has much lower moisture content (6-14%) than 'suya' (25-35%). Smoking and sun-drying are used to preserve a wide variety of Nigerian fresh water species of fish including *Clarias, Gymnarchus, Chrysicthys, Citharinus, Alestes, Hydrocynus* and *Tilapia* (Norrung *et al.*, 2009).

Suya is however the most popular as its consumption has extended to other parts of the country (Eke *et al.*, 2014). In big cities and small towns, Suya vendors have become very prominent with their grill stands becoming very busy from about midday until late at night. It is gradually making its way into elite circles where it has become a delicacy served at parties. The preparation process carried out under largely unhygienic conditions and the risk of contamination is very high. The fact that there are sporadic cases of gastroenteritis and symptoms of food infection after consumption of Suya indicate that the product indeed constitutes a food safety risk (Segev, 2012).

In developing countries, despite the apparent dearth of sustainable disease surveillance and reporting, it is widely known that cholera; salmonellosis, Campylobacteriosis, shigellosis, typhoid, brucellosis, poliomyelitis, and

Escherichia coli infections are prevalent (WHO, 2009). Diarrheal diseases are a major cause of morbidity and mortality in children where at the age of five, on average, the children suffer 2 – 3 episodes of diarrhea per year. Even though epidemiological evidence on outbreaks of food borne diseases is scarce, there are indications that foods could be contaminated to unsafe levels at the point of consumption with air flora and other microorganisms from handlers, equipment/utensils and the raw material itself (Siri-Tarino *et al.*, 2010).

Developing countries face with high incidences of food poisoning outbreaks, with obvious economic consequences. While food borne diseases remain an important public health problem worldwide, one of the most significant food safety hazards is associated with foods from animals (Kivi *et al*., 2007; Maripandi and Al-Salamah, 2010).

Meat is a major source of protein and an important source of vitamins for most people in many parts of the world, thus they are essential for the growth, repair and maintenance of body cells which is necessary for our everyday activities. Meat could be traced back to human history, then when primitive men use raw flesh of dead animals. But as man developed, he domesticated wild animals. Beef have been the major supply of meat in Nigeria as a result of extensive and semi-intensive cattle production system in Nigeria by Fulani and Hausa people of the northern Nigeria (Umoh, 2004).

Suya meat is a boneless lean meat of mutton, beef, goat or chicken meat staked on sticks, coated with sauces, oiled and then roasted over wood using a fire from charcoal. It is a traditionally processed meat product and is usually not done with strict hygiene condition because they are still done locally. It is served hot and sold along streets, at clubs, picnics centers, and restaurants and within institutions. Suya meat is one of the intermediate moisture products that are easy to prepare and highly relished. Suya meat is a spicy skewered meat which is a popular food item in various parts of Nigeria (Eke *et al*., 2014).

It is traditionally prepared by the Hausa people of northern Cameroon, Nigeria, Niger, and some parts of Sudan (where it is called agashe). Suya is generally made with skewered beef, ram, or chicken (AFP, 2012). The thinly sliced meat is marinated in various spices which include peanut cake, salt, vegetable oil and other flavorings, and then barbecued (Egbebi and Seidu, 2014). The possible sources of contamination are through slaughtering of sick animals, washing the meat with dirty water, handling by butchers, contamination by flies, processing close to sewage or refuse dumps environment, spices, transportation and use of contaminated equipment such as knife and other utensils (Igyor and Uma, 2005).

MATERIALS AND METHODS

Collection of Samples

Suya meats used in this study were bought from Suya sellers within Federal Polytechnic Nekede, Owerri Imo State and were taken to the laboratory for suya preparation. A prepared suya was bought from a suya seller which was used to compare the prepared one for hygienic conditions.

Sterilization of Materials

All the glass wares used for the experiment were sterilized using the laboratory hot air oven at a temperature of 160 ^{0}C for 1 hour while media was autoclaved at a temperature of 121^{0}C for 15 minutes at 15psi. Wire loop was sterilized over burning flame and allowed till its red-hot, while glass spreader was sterilized by dipping into 70 % ethanol and passing over Bunsen flame. The media used in this study; Nutrient agar, Simmon citrate agar, peptone water and Triple sugar ion agar were prepared according to manufacturer's instructions and sterilized using the autoclave at a temperature of 121^{0}C at 15psi for 15minutes and were allowed to cool to a temperature of 45^{0}C and about 20 millilitres was poured into sterile

petri-dishes. The plates were allowed to cool and set for inoculation.

Microbiological Analysis of the Suya

Serial Dilution

The serial dilution method as described by Falegan *et al* (2017) was adopted. Ten grams (10g) each of the suya samples were weighed and mashed in a laboratory type mortar and pestle into a paste. Thereafter, each mashed sample was added into different clean sterile beakers containing ninety millilitres (90ml) of sterile water (stock). The beakers were shaken at intervals for 30 minutes. Nine (9) millilitres of sterile water was added into six (6) sets of test tubes. One millilitre from each of the stock was transferred to the first test tubes containing nine millilitres of sterile water using sterile pasteur pipettes for viable counting. The test tubes were shaken and one millilitre was transferred to the second test tubes. This procedure was continued to the last test tubes and one millilitre was discarded from each of the last test tubes.

Inoculation of Samples

The spread plate technique as described by Cheesbrough (2010) was used in the inoculation of the plates. 0.1 millilitre aliquot of the serially diluted samples was pipette from each of the test tubes labelled 10^{-3} and was dropped onto the different media in the plates. A sterile bent glass rod was used to spread the aliquot evenly on the media. The plates were labeled accordingly. The inoculated plates were inverted and incubated in the incubator at a temperature of 37^{0}C for 24 hours.

Microbial Plate Count

After the incubation of the different plates, the different colonies formed on the media were counted using the digital colony counter. The total population of the colonies was expressed as colony forming unit per gram (CFU/g).

Purification and Preservation of Isolates

After the various colony counts, bacterial isolates were pick with a wire loop based on their morphological appearances. The picked colonies were sub cultured onto freshly prepared nutrient agar plates to obtain pure cultures. They were further incubated for 24h at 37^0C. After incubation pure cultures were stored in McCartney bottle in a refrigerator at 4^0C. Fungal isolates were sub cultured onto freshly prepared Sabouraud dextrose medium.

Gram Staining Techniques

A smear of each of the bacterial isolates was made and fixed by air drying. The smears were then covered with crystal violet stain for 60 seconds and rapidly washed off with water thereafter. The smears were then covered with Lugol's iodine for 60 seconds and washed off with water. The smears were decolorized with acetone alcohol and washed off after 10 seconds. The smears were finally flooded with safranin for 2minutes and washed- off with clean water. The back of the slides were then wiped and placed in a draining rack for the smear to dry before they were viewed with x 10 oil immersion objective lens (Cheesbrough, 2010).

Gram positive bacteria gave purple coloration while gram negative bacteria gave pinkish coloration.

Identification of Bacterial Isolates

The methods described by Cheesbrough (2010) and Ochei and Kolhatkar (2010) were adopted in the biochemical tests reactions of the bacterial isolates.

Catalase test

Three millilitres (3ml) of hydrogen peroxide was poured in a test tube. A colony of test organism was taken with sterile wooden or glass rod and immersed into hydrogen peroxide solution. Generation of bubbles indicated oxygen production. If bubbles were produced, the organism was catalase-positive. However, if bubbles were not produced, the organism was catalase-negative.

Oxidase test

A piece of filter paper was placed in Petri-dish and 3 drops of freshly prepared oxidase reagent were added. Using a sterile glass rod, a colony of

test organisms was removed from a culture plate and smeared on the filter paper. Oxidase-positive organisms gave blue color within 5-10 seconds, and in oxidase-negative organisms, color did not change.

Citrate test

A bacterial colony was inoculated in Simmons citrate agar and incubated at 35 ^{0}C to 37 ^{0}C for 18 to 24 h. Thereafter, development of blue color was observed. Citrate positive showed that growth was visible on the slant surface and the medium became an intense blue while Citrate negative showed trace or no growth was visible and no color change occurred.

Indole test

Test bacterial colony were inoculated in peptone water and incubated at 37 ^{0}C for 24-28 h. Thereafter, 0.5 ml of Kovac's reagent was added. Positive test showed pink colored ring was observed after addition of reagent. Negative test showed no color change after reagent addition.

Motility Test

The semi-solid agar of nutrient agar used for this study. The media was prepared in slants and the organisms were inoculated by stabbing technique. Zig-zag growth along the line of stabs indicated a positive result while none indicated a negative result.

Coagulase Test

A drop of distilled water was placed on each end of a slide for each of the test organisms. Thereafter a colony of each of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was then-added to one of the suspension and mixed gently for each of the test organism. Clumping within 10 seconds was an indication of positive test while none was an indication of a negative test.

Sugar Fermentation Test

Each colony of the different test organisms were inoculated onto sterile agar slopes of triple sugar iron agar using stab inoculation. After this, the inoculated, agar slopes were incubated at 37°C for 24 hours. The different colors of the slopes and butts in addition to the presence of gas production and hydrogen sulphide (H_2S) blackening was an indication of the type of bacteria present.

RESULTS AND DISCUSSIONS

Results

The results of this study are shown in table 4.1 to table 4.6. Table 4.1 shows the results for the microbial loads of the Suya samples used in this study. Table 4.2 shows the results for the identification and characterization of the bacterial isolates. Table 4.3 shows the results for the identification and characterization of the fungal isolates.

Table 1: Microbial loads of the suya samples

Samples	TVBC	TCC	TFC (cfu/g)
A	4.0×10^3	NG	NG
B	8.4×10^4	NG	2.0×10^3
C	1.2×10^4	NG	1.0×10^3
D.	1.8×10^4	NG	NG
E	6.2×10^4	NG	4.0×10^3
F	6.0×10^3	NG	NG

Keys: A – F = suya samples used

Cfu/g = colony forming unit per gram

NG = no growth

TVBC = Total Viable bacterial counts

TCC = Total coliform counts

TFC = Total fungal counts

The table above shows the results for the microbial loads of the different suya samples used in this study. Six (6) suya samples labeled A to F were used in this study. Total viable bacterial counts recorded ranged from 4.0 x 10^3 cfu/g to 8.4 x 10^4 cfu/g. there were no coliform counts recorded in all the six (6) suya samples used in this study. Total fungal counts recorded ranged from 1.0 x 10^3 cfu/g to 4.0x10^3 cfu/g. Three samples out of the six samples used had no fungal counts

Table 2: Identification and characterization of the bacterial isolates from the suya

Morphological Characteristics	Gram reaction	Oxidase test	Indole test	Spore test	Catalase test	Citrate test	Coaguase test	Motility test	S	FT			
S	B	G	H_2S	Possible bacteria									
Milkish, flat, rhizoid-like dry-surface colonies	Gram positive rods in short chains	-	-	+	+	-	-	-	Y	Y	+	-	*Bacillus* species
Bluish-green, flat, non-mucoid colonies	Gram negative rods in diploids	+	-	-	+	-	-	+	R	R	-	-	*Pseudomonas* species
Milkish, raised, non-mucoid colonies	Gram positive rod	-	-	-	+	-	-	-	No Reaction		-	-	*Corynebacterium* species
Yellowish, raised, non- mucoid colonies	Gram positive cocci in clusters	-	-	-	+	-	+	-	No Reaction		-	-	*Staphylococcus* species
Milkish, raised, non-mucoid circular colonies of about 3mm in size	Gram positive cocci in pairs	-	-	-	+	-	-	-	No Reaction		-	-	*Micrococcus* species

KEY: - = Negative + = Positive S = color of slope B = color of butt G = Gas production H_2S = Hydrogen sulphide production (blackening) R = Reddish coloration (alkaline production) Y= Yellow coloration (Acidic production) SFT= Sugar fermentation test

The table above shows the identification and characterization of the bacterial isolates from the suya samples used in this study.

DISCUSSION

Meat basically contains all the nutrients necessary for microbial growth and metabolism, making it susceptible to microbial continuation. This study was undertaken to assess the microbiological quality of some suya meat sold within Federal Polytechnic Nekede, Owerri, Imo State. The results of this study are shown in Table 1 to Table 2.

From table 1, the microbial loads of the suya meat samples were shown. Total viable bacterial counts recorded ranged from 4.0 x 10^3 cfu/g to 8.4 x 10^4 cfu/g. there was no coliform count recorded. Total fungal counts recorded ranged from 1.0 x 10^3 cfu/g Nwakanma *et al.* (2015) reported total viable counts ranging from 1.9 x 10^3 cfu/g with suya meat sold in Enugu metropolis. They reported total coliform counts ranging from 1.1 x 10^3cfu/g to 3.0 x 10^3 cfu/g. The suya meat samples used in this study had no coliform counts as reported in their study.

According to the standard of international commission of Microbiological safety for foods (ICMSF, 1998), the suya samples are within the satisfactory limit for consumption.

Table 2 shows the results for the bacterial isolates from the suya meat samples. They include; *Pseudomonas aeruginosa*, *Staphylococcus* species, *Micrococcus* species, *Corynebacterium* species and *Bacillus* species. Edema *et al.* (2008) reported the presence of *Bacillus cereus*, *Staphylococcus aureus*, and *salmonella* species from some ready-to-eat suya

They were; *Pseudomonas aeruginosa*, *Micrococcus* species, *Corynebacterium* species, *Staphylococcus* species and *Bacillus* species

samples. Similarly, Osho (2004) reported the presence of *Pseudomonas aeruginosa, Klebsiella* species, *Proteus* species and *Enterococcus* species from suya samples.

The presence of these bacteria could be traced. The presence of *Micrococcus* species could be as a result of poor air quality within the area where these suya samples are displayed for sale. The presence of *bacillus* species, *Corynebacterium* species and *Pseudomonas aeruginosa* could be from the slabs and trays, soil, dust, sticking and utensils used in preparing the suya samples (Edema *et al.,* 2008). Left over samples kept at ambient temperature for too long could be risky since this could encourage the growth of the pathogens to hazardous levels

The results of this study have shown that the environment where suya are prepared together with the suya processors and equipment could be sources of microbial contamination. There is need to ensure personal hygiene among the suya processors.

CONCLUSION

Suya is a meat product and meat supplies essential nutrients to humans, the preparation, display and handling of suya should be done in a hygienic manner so as to avoid microbial contamination.

The results of this study have shown that the environment where suya are prepared together with the suya processors and equipment could be sources of microbial contamination. There is need to ensure personal hygiene among the suya processors.

RECOMMENDATIONS

1. There should be education of suya processors on the hazards associated with unhygienic practices during suya preparations.
2. There is need to ensure good hygienic practices during preparation of suya.

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