International Journal of Pathogen Research

5(4): 105-111, 2020; Article no.IJPR.64759
ISSN: 2582-3876

Microbiological Evaluation of Laboratory Prepared Suya and Vendor Samples in Ilaro, Ogun State Nigeria

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/IJPR/2020/v5i430155
Editor(s):
(1) Dr. Rafik Karaman, Al-Quds University, Palestine.
Reviewers:
(1) Marcel Muengula-Manyi, Université de Kinshasa, Democratic Republic of Congo.
(2) Heinrich du Plessis, South Africa.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64759

Original Research Article

Received 28 July 2020
Accepted 30 November 2020
Published 31 December 2020

ABSTRACT

Aims: Suya is a popular spicy roasted meat product in Nigeria. The aim of the work is to investigate the microbial safety and effect of *Lactobacillus species* on some pathogens when used as a biopreservative on suya.
Methodology: Five suya group samples were used. Two samples were prepared in the laboratory, one of them was inoculated with *Lactobacillus plantarum* while the other was un-inoculated. Three other group samples coded C, D and E were bought from different vendors at different locations in Ilaro. All the samples were subjected to microbial analysis using standard method.
Results: The result of microbiological analysis shows that significant difference exist in all the samples. Total viable counts of the samples ranged from 16.0 ±2.5 x 10^3 cfu/g to 50.0 ±1.5 x 10^3 cfu/g, coliform counts ranged from 8.0±3 x 10^3 cfu/g to 44 ±3.0 x 10^3 cfu/g, *Staphylococcus* counts ranged from 10.0 ± 2.0 x 10^3 cfu/g to 45.5 ±3.5 x 10^3 cfu/g, and fungi counts ranged from 10.0 ± 2.5 x 10^3 cfu/g to 34.02 ±4.0 x 10^3 cfu/g, while Salmonella had no growth. The prepared samples harbored *Streptococcus* spp*., Pseudomonas* spp. *and Staphylococcus* spp. Four bacteria and three fungi species were isolated from vendor suya meat. The isolates were *Staphylococcus* spp*., Pseudomonas spp., Escherichia coli and Streptococcus* spp*., while the fungi were Rhizopus spp, Penicillium* spp. *and Saccharomyces* spp.
Conclusion: The sample inoculated with *Lactobacillus plantarum* had the lowest microbial count; however, the isolation of potential pathogens from suya samples is of public health significance.

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Keywords: Microbial; evaluation; suya; Lactobacillus sp; vendors.

1. INTRODUCTION

Meat is a nutritious, protein-rich food which is highly perishable and has a short shelf-life unless preservation methods are used. Spoilage by microbial growth is the most important factor in relation to the keeping quality of meat. In most developing countries, including Nigeria, fresh meat forms a significant proportion of meat intake [1]. It is either eaten cooked or processed into other forms to avoid spoilage. The main causative factor of spoilage has been linked to unavailability of necessary storage facilities and favorable ambient temperature that usually prevail in developing countries that are in tropical regions [2]. Research findings have suggested that there is increasing attention on the use of naturally occurring metabolites produced by selected lactic acid bacteria (LAB) to inhibit the growth of spoilage microorganisms [1,2,3]. These authors have demonstrated the potential of LAB cultures as bio-preservatives during processing and preservation of many forms of meat products. Lactic acid bacteria growing naturally in foods produce antimicrobial substances such as lactic and acetic acids, diacetyl, hydrogen peroxide and bacteriocins [2,3].

Suya is a spicy, barbecued, smoked or roasted meat product. It originated from the Hausa people of northern Nigeria, where rearing of cattle is an important preoccupation and a major source of livelihood for the people [4]. The production of ready - to – eat beef products such as *Suya, Kilishi, Balangu* and *Kundi* are very popular street foods [4]**.** *Suya* is, however, the most popular as its consumption has extended to other parts of the country [5]. 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. *Suya* when being sold is usually packaged in old newspaper, also most of the stages for processing the product, the materials used, the handlers and the environment where it is processed and sold can serve as sources of contamination to the product [6]. Suya is prepared from boneless meat of cattle [7] and is usually prepared under 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 this product may indeed constitute a health and food safety risk [5,8]. Lactic acid bacteria have been employed in the preservation of food materials for many centuries. The preservative activity of LAB is due to their ability to produce acid (low pH), hydrogen peroxide and bacteriocins [9]. The aim of this study was to evaluate the microbial safety of suya sold in Ilaro, Nigeria and to determine the effect of LAB on the microbial safety of laboratory prepared suya.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fifteen (15) samples of suya used in this study were obtained randomly from three suya vendors at three locations namely Orita, Express, and Ikosi in Ilaro area of Ogun state, Nigeria. The samples were immediately wrapped in sterile aluminum foil to prevent contamination and then transported to the laboratory for microbial analysis. Samples from two groups containing five (5) sticks each were prepared in the laboratory, All samples from one of the groups was inoculated with *Lactobacillus plantarum* which is used for biological preservation, while the other one which served as the control sample was not inoculated.

2.1.1 Preparation of Suya from meat

Fresh meat sample was purchased from a meat shop in Ilaro, placed in a sterile container and transported to the laboratory for preparation for Suya meat.

2.1.2 Preparation of suya ingredients

The spices used in preparing the ingredients were purchased from a specialized spice market. These included ginger (*Zingiber officinales)*, alligator pepper (*Afromomum melegueta*), black pepper (*Piper guineense*) and red pepper (*Capsicum fructescens*). Other constituents were groundnut cake powder (*Arachis hypogea*), salt (*Sodium chloride*), and seasoning (*Monosodium glutamate*). The spices and other constituents were milled individually and mixed together in a specific proportion as described by [10,11].

2.2 Processing of Suya

Suya was produced from meat using a modified traditional method [3]. The meat was washed in 5% sterile saline water and cut into pieces 25g each and hung on suya stick (two pieces). The ingredients were spread on a flat tray and each stick of meat was pressed on the ingredient to be properly soaked into the meat. The 10 sticks of meat were labeled, while about 5-10 ml of groundnut oil was sprinkled on each meat stick before roasting and then divided into 2 parts. All the labeled sticked meats were arranged round a glowing smokeless fire made from charcoal. The sticked meats were positioned at a distance of 22-23 cm from the center of fire for 20mins and the products were intermittently turned. The first part of suya meat was first cooled and then inoculated with *Lactobacillus plantarum* culture (10^7cfu/g) and incubated at 35°C (Sample A). The second part (Sample B) which was the control was not inoculated, additional groundnut oil was sprinkled on the meat, while roasting continued [12]. All the suya samples were allowed to cool down and then packaged using aluminum foil [13].

2.3 Microbiological Analyses

Total viable counts were carried out using Nutrient agar (Oxoid, Ltd England). Ten grammes of ready-to-eat suya was weighed, cut into pieces using sterile pair of scissors and added to 90ml of sterile saline. In each isolation protocol, suya saline mixture was homogenized by hand shaking for 5 minutes in a sterile 250 ml screw-capped bottle followed by further dilutions up to 10^{-5}. A 0.1ml quantity of appropriately diluted sample was pour-plated in duplicate and incubated at 37°C. Media were sterilized by autoclaving at 121°C for 15 min.

2.4 Enumeration of Fungi

Yeast and mould were grown on acidified potato dextrose agar (PDA, Oxoid, UK). In serial dilution preparation, 10g of sample was aseptically transferred into 90ml of diluted water and homogenized by vortex. Subsequent serial dilutions up to 10^5 were made [14]. The enumeration of microorganisms in the samples was done by the pour plate technique. Fungi were counted after 5 days at 28°C.

2.5 Isolation and Enumeration of *Staphylococcus sp.*

Baird Parker Medium (BPM) was used for the isolation and enumeration of *Staphylococcus* sp and sterilized by autoclaving at 121°C for 15 minutes. A 0.1ml quantity of appropriately diluted Samples were pour plated using BPM and incubated at 35°C for 48 hrs. Greyish-black or black colonies with or without a halo were presumptively identified as *Staphylococcus* and coagulase tests were carried out for further characterization.

2.6 Isolation and Enumeration of *Salmonella* sp

Bismuth sulphite agar was used for the isolation and enumeration of *Salmonella spp*. A 0.1ml quantity of homogenized suya saline mixture was each inoculated on sterile medium and incubated at 35°C for 48 hrs.

Identification of isolates: Growth of organisms was identified according to their morphological characteristics and reactions to biochemical test such as gram reaction, catalase, oxidase, coagulase, spore, indole, motility, urease,sugar fermentation test etc [15,16].

2.7 Data Analysis

The data generated were subjected to statistical analyses using SPSS 17.0 software for windows. Means were separated by Duncan's Multiple range tests [17].

3. RESULTS AND DISCUSSION

The result of the microbial analysis of suya vendor samples and laboratory samples are presented in Table 1. Total plate count of the samples ranged from 16.0 to 50.0 x 10^3 cfu/g, coliform counts ranged from 8.0 to 44.0 x 10^3 cfu/g, *Staphylococcus* counts ranged from 10.0 to 45.5 x 10^3 cfu/g, and fungi counts ranged from 10.0 to 34.0 x 10^3 cfu/g.

Meat basically contains all the nutrients necessary for microbial growth and metabolism, making it susceptible to microbial contamination. In view of the microbial quality of meat and meat products, proper hygiene must be ascertained to ensure safety from infection after consumption of such products and to promote quality assurance. Of the samples purchased from the three processing vendors in Ilaro town, Ikosi samples had the lowest total viable count 38.5 x 10^3 cfu/g, while Express samples contained the highest total viable counts of 50.0 x 10^3 cfu/g (Table 1). The counts were within the ready to eat limit of ICMSF of x10^5 cfu/g. This might be due to the exposure of the Suya meat to air and

water contamination after roasting. The total viable counts of the vendors sample were in line with the findings of Orogu and Oshilim [18]. The sample inoculated with LAB had the lowest microbial count unlike the uninoculated samples. This is in agreement with the findings of Adesokan et al. [13] who reported that Suya samples inoculated after grilling had a low microbial count during the storage period compared to those samples inoculated before grilling and the control. This is also in agreement with the finding of [19] who preserved minced goat meat using *Lactococcus lactis var. lactic* biovar *diacetylactis*.

In a recent study by [1], the potential of selected species of *Pediococcus* as biological preservatives in the extension of shelf life of fresh beef in Nigeria was investigated. The authors reported that the LAB strains used were able to effect preservation of the meat products, for few days before spoilage started to set in. In a similar study, Olaoye and Dodd [20] reported the extension in shelf life of *tsire*, a traditional Nigerian stick meat, after treatment with bacteriocinogeic cultures of *Pediococcus*.

The characterization and identification results are presented in Table 2. The isolates were identified as *Staphylococcus, Pseudomonas, Streptococcus and Escherichia coli*. The most frequently isolated organism was *Pseudomonas species.* The fungi isolate were *Rizopus* spp *Penicillium* spp and *Saccharomyces spp.* These results were in consonance with the report of [21], which stated that microbiological analysis of meat samples in Awka urban of Anambra State, indicated contamination of meat samples with various bacterial species including *Staphylococcus aureus,* and some enteric bacteria. Gilbert Harrison [22] also affirmed that meat preserved with a certain amount of salt may also allow the growth of *Staphylococcus aureus* whereas, the presence of some members of the family of *Enterobacteriacea* are due to contamination from intestine of slaughtered animals.

Four bacteria and three fungi were isolated from the suya samples in view of the unhygienic condition of meat handling in Nigeria, the organisms isolated in this study are the organisms usually implicated in meat spoilage. The presence of *Staphylococcus* species agrees with the report of cross contamination from meat handlers during processing, since it is normal flora of the skin [23]. Raw meat is usually carried on the body by butcher in Nigeria due to lack of education and coliform may be introduced from the water used for washing the meat. This is also in agreement with the report of Umoh [23], which speculated that the presence of *Escherichia coli* may arise from the use of non–portable water during washing of raw meat. The meat also showed presence of *Pseudomonas aeruginosa*, which usually occurs in soil, vegetation and surfaces of plants, humans and animals [24]. *Streptococcus* spp*, Staphylococcus*, *Escherichia coli* and *Pseudomonas* species were also isolated, which is in agreement with the findings of [25,26] .

Table 1. Microbial analysis of samples of suya obtain from vendors in Ilaro and laboratory samples (x10^{3}cfu/g)

*Samples	Total plate count (cfu/g)	Coliform count (cfu/g)	*Staphylococcus* count (cfu/g)	*Salmonella* count (cfu/g)	Fungi count (cfu/g)
A	16.00±2.5^c	Nil	10 .00±2.0^c	Nil	Nil
B	25.00±5.0^a	15.00±3.0^a	18.50±2.5^b	Nil	17.51±3.5^b
C	50.00±1.5^d	40.01±3.0^a	45.54±3.5^a	Nil	18.52±2.5^c
D	48.01±5.0^a	36.50±2.5^b	39.00±1.0^{ab}	Nil	34.02±4.0^a
E	38.53±3.5^b	44.00±3.0^a	32.51±1.5^d	Nil	24.52±2.5^c

**Sample A (laboratory sample inoculated with Lactobacillus plantarum)*
Sample B (laboratory sample uninoculated (control))
Sample C (express sample)
Sample D (orita sample)
Sample E (ikosi sample)
Mean on the same column with different superscript are significantly different at p<0.05

Table 2. Morphological and biochemical characteristics of bacteria isolated from Suya samples

Suspected Microbes	Shape	Gram reaction	Catalase	Oxidase	Coagulase	Motility	Urease	Indole	Spore	Suc	Glu	Gal
Staphylococcus sp	Cocci	-	+	-	+	-	+	+	+	+	+	+
Streptococcus sp	Cocci	+	+	-	+	-	+	+	-	+	+	+
Pseudomonas sp	Rod	-	+	-	+	+	+	+	-	+	+	+
Escherichia coli	Rod	-	+	-	-	+	+	-	-	+	+	-

The presence of molds could have come from contaminated spices used and wrapping with contaminated wrap before serving [27]. Edema et al. [25] found that suya are kept at ambient temperature and the re-heating at temperatures of less than 70°C is not sufficient to destroy all the vegetative cells and heat resistant spores of bacteria, especially if the meat is heavily contaminated with enteric bacteria [26]. In general, the major sources of microbial contamination of suya meat appear to be handling by butchers and the use of contaminated water and equipment. So control of suya meat contamination can be achieved if aseptic techniques are employed during preparation of suya and also the use of LAB helps to reduce the microbial count thereby prolonging its shelf life.

4. CONCLUSION

This research study showed that the microbial load of suya samples obtained from vendors was higher than the laboratory prepared samples. The few pathogens isolated from vendor suya indicate that the standards of preparation and preservation have not improved much over the years and facilities used for preparation are not sterile. Aseptic techniques should be adequately employed in the meat industries so as to reduce microbial load of meat and its products for safe consumption by consumers and thus prevent food-borne diseases or infections. In addition, the microbial count of the suya samples inoculated with a specific LAB culture had the lowest microbial count compared to the uninoculated samples or vendors samples. Hence, the use of LAB as a bio-preservative needs further investigation.

5. RECOMMENDATION

There is need to educate processors and consumers on good sanitary practices during processing, displaying and sales of the product and the possible danger of contaminated products. Proper animal husbanding, hygienic slaughter, adequate meat inspection, proper meat transportation sanitation of utensils and equipment, portable drinking water and proper storage of meat should all be employed to reduce microbial contamination.

ACKNOWLEGEMENT

The author wish to express her appreciation to Azeez Lateef Olatunji for his immense contribution to this research work for assisting in the laboratory analysis.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/64759