

Microbiological Safety and Proximate Composition of Suya Stored at Ambient Temperature for Six Hours from Maiduguri, Northern Nigeria.

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Abstract

Microbiological and Proximate analyses were performed on suya to ascertain its safety. Total viable count (TVC), *E. coli*, *S. aureus*, *Salmonella*, *Shigella* and yeast and mould were determined to measure its microbiological quality. Mean and range of these microbial groups varied. Range count of the microbes was of the order log 0.0 – 8.08 CFU/g. Variation also occurred in distribution and % frequency of different microorganisms isolated from suya samples with average % of 64, 62, 47 and 31 for *E. coli*, *S. aureus*, *Salmonella*, *Shigella* respectively. The overall mean value for TVC was log₁₀ 6.69cfu/g, whereas *E. coli*, *S. aureus*, *Salmonella*, *Shigella*, yeast and mould were log₁₀ 4.33, 5.65, 4.17, 3.43 and 3.95 CFU/g of suya respectively. There is statistically significant difference ($P < 0.05$) between *S. aureus* and other organisms. *Shigella* and *Salmonella* were significantly ($P < 0.05$) lower than *E. coli* and *S. aureus*. Proximate composition of the suya showed variation in crude protein, crude fat, ash and moisture content. We concluded that suya sold in the area were microbiologically unsafe and recommended improved hygiene in the process and distribution line.

Key words: Suya, microbial safety, Nigeria, *Salmonella*, *Shigella*, *S. aureus*.

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 (Inyang *et al.*, 2005). 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 (Kramiliah *et al.*, 1973).

Meat is ideal for the growth of a wide range of spoilage bacteria (May *et al.*, 2003) which accounts to a great extent why it is perishable. Its high ultimate pH generally makes it very susceptible to microbial growth even under the best handling or manufacturing conditions and practice (Hedrick *et al.*, 1994).

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

The processing operations of meat to produce these products preserve and increase the shelf life in addition to improving the palatability and food value of the meat. The consumption of suya, klishi, balangu, kundi and dambu nama has extended to other parts of the country (Inyang *et al.*, 2005). In Nigeria, suya sales in cities and small towns are prominent.

Suya is prepared basically from boneless meat of animals (Abdullahi *et al.*, 2004). 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. Suya preparation process in Nigeria lacks hygienic control and the risk of foodborne infections is very high. Some researchers elsewhere had noticed sporadic cases of gastroenteritis and symptoms of infection after consumption of suya which indicated that the product indeed constitute a food safety risk (Odusole *et al.*, 2003; Inyang *et al.*, 2005).

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Microbiological quality assessment of suya is to this respect very critical even though epidemiological evidence on outbreaks of suya borne diseases is scarce, there are indications that suya could be contaminated to unsafe level at the point of consumption with air flora and other microorganisms from handlers, equipment, utensils like trays, spoons and knives. However there is no research stating the microbiological quality of suya from this area. The aim of this study was to determine the microbiological quality and safety of suya; a dried meat product very popular in northern Nigeria.

Material and Methods

Suya samples. Ready-to-eat suya samples were purchased from five popular markets which represented the major areas where suya is produced and sold within Maiduguri Metropolis. Purchased samples were transported to the Microbiology Laboratory in sterile bags and allowed a period of 6h at ambient temperature before analyses. The holding of the suya samples for 6h at ambient temperature before analyses was because it is almost a general practice of most suya processors to keep the product exposed under such condition before sales. Sampling lasted from December, 2009 to February, 2010. The preparation process of suya involves the de-fattening of the meat. The raw lean meat is sliced on a slab or table top. This is subsequently staked into sticks and spiced followed by roasting for about 20 minutes. Thereafter, the product is spiced again followed by a brief reheating for about 2 minutes. The ready-to-eat product (suya) is displayed for marketing on table tops or on trays where they could be street-vended. All suya processors prepare the suya at their wooden stall located by the road sides. Two samples were taken from each market in every sampling and ten samplings times were carried out. The total samples for the study were 100 from the five markets.

Microbiological analyses. 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 5minutes in a sterile 250ml screw-capped bottle followed by further decimal dilutions to up to 10^{-6} concentrations. A 0.1ml quantity of appropriately diluted sample was used to inoculate freshly prepared media by spread-plating method Media were sterilized by autoclaving at 121°C for 15min except DCA which involved only boiling over gauze. In all cases of colony counts, the resulting colonies following inoculation and incubation were counted using digital colony counter (Labtech, New Delhi, India).

Total Viable Count. Nutrient Agar (NA) (Biotech Lab. Ipswich, UK) sterilized by autoclaving at 121°C for 15 minutes was inoculated with a 0.1ml quantity of appropriately diluted suya sample, surface plated and

incubated at 37°C for 24 h. Emerging colonies were counted using digital colony counter (Labtech, New Delhi, India) and multiplied by the dilution factor. Counts were expressed as log CFU/g.

Yeasts and moulds counts. Yeasts and mould counts were carried out on Potato Dextrose Agar (PDA) (Lab M. Ltd, Bury Lancashire BL9 6As, United Kingdom) plates. PDA medium sterilized by autoclaving as described previously; containing Chloramphenicol were inoculated as above. Incubation was at 28°C for 5d. Colonies were thereafter counted using a colony counter as stated above. Counts were expressed as log CFU/g.

Isolation and enumeration of *E. coli*. Eosine Methylene Blue Agar (EMBA) (Himedia Laboratories Pot Ltd, India) was used for the isolation and enumeration of *E. coli*. EMBA plates prepared following autoclaving were inoculated as described previously and incubated at 37°C overnight after which typical colonies with greenish metallic sheen were subjected to biochemical tests for *E. coli*. Positive indole test described below was used to identify *E. coli* from the colonies showing the metallic sheen. Colonies were counted as described before and expressed as log CFU/g.

Isolation and enumeration of *S. aureus*. Medium employed for the isolation and enumeration of *S. aureus* was Baird Parker Medium (BPM) (Lab M. Ltd, Bury Lancashire BL9 6As, United Kingdom). BPM medium was sterilized by autoclaving at 121°C for 15minutes. Sterile medium was inoculated as above and incubated at 37°C for 48h. Greyish-black or black colonies with or without a halo were presumptively identified as Staphylococci as recommended by Macfaddin (1977) and coagulase test was carried out to further characterize *S. aureus*.

Isolation and enumeration of *Salmonella* and *Shigella* sp. Desoxycholate Citrate Agar (DCA) (Park Scientific Limited, Moulton Park, Northampton) was used for the isolation and enumeration of *Salmonella* and *Shigella* spp. One millilitre quantity of homogenized suya saline mixture was each inoculated into 9ml of pre-enrichment broths (tetrathionate and selenite cysteine) and incubated at 37°C as recommended by Macfaddin (1977). DCA plates were inoculated with 0.1ml of the pre-enrichment broth 24h growth and incubated at 37°C overnight. Typical colonies with black centres were identified as *Salmonella* spp on DCA according to Macfaddin (1977). Pinkish colonies were identified presumptively as *Shigella* on DCA and subjected to further biochemical testing.

Biochemical identification of the isolates. The biochemical tests for the identification of the isolates were the citrate utilization, indole, methyl-red, Voges-proskauer, triple sugar iron (TSI), urease, oxidase, coagulase and catalase tests. Cowan and Steel (1965) and Cheesbrough (2004) procedures were used for these biochemical tests. The indole production test involved the inoculation of sterile peptone water with the test organism followed by incubation at 37°C for 48h. Kovacs' reagent (0.5ml) was

thereafter added and the culture shaken for 1min. A red colour in the reagent layer indicated the presence of indole. Methyl red and Voges-Proskauer were also demonstrated according to Cowan and Steel (1965) with red colouration for positive methyl red and yellow colour for negative. A positive reaction for Voges-Proskauer was indicated by a strong red colour. The citrate utilization test involved heavy inoculation of tryptose-citrate medium from a 10-12h culture. The inoculated tubes were placed in 40°C water bath. After 90 minutes, 1 drop of 0.05% aq. bromothymol blue was added to each tube. A positive test was shown by blue colouration, whereas a green colour showed a negative result. The catalase test was conducted by immersing the cultured isolate on a clean oil-free glass slide, followed by the addition of few drops of hydrogen peroxide solution. An immediate effervescence showed positive test, whereas negative test was shown by no visible reaction. A yellow butt with a pinkish slant region and a trace of black precipitate indicating H₂S production was positive for TSI test. Colours for positive oxidase and urease were dark purple on a paper within 10s and reddish colouration respectively.

Chemical analyses. The pH of various samples of suya was determined using a pH meter (WPA pH Meter, India) after standardization with pH 4, 10 and 7 buffers (BDH England). Crude protein of the samples was determined with macro-Kjeldahl procedure following the method described by AOAC (1990). Crude fat of 10g of samples was determined with soxhlet procedure and total ash obtained by igniting 10g sample at 600°C using muffle furnace (Laboratory electric furnace, typOH-857R, England) according to the protocol of Pearson (1976). Crude fibre was determined according to the procedure of AOAC (1990). Moisture content was determined by weighing 10g of suya sample and drying to constant weight in an oven at 80°C; and reweighing the sample. The moisture content was determined as the difference in weight between the fresh sample and the oven-dried sample. This was expressed as percentage (%) of the total weight of the sample.

MC = LWS / WFS x 100; where MC = Moisture content (%), LWS = Loss in weight (of sample) and WFS weight of fresh sample.

Statistical analysis. Data were analyzed by Multiple-Sample Comparison using STATGRAPHICS Centurion XVI Version 16.1.05 (32-bit). When the F-test in the ANOVA was significantly ($p \leq 0.05$) different between the means, Multiple Range Tests were conducted to tell which means were significantly different from others.

Results

The holding of the suya samples for 6h at ambient temperature before analyses was informed by the fact that most of the vendors keep this ready-to-eat meat product at such temperature in exposed trays or table tops or slabs for longer time before sales. Upon sales, the product may be warmed near amber fire. The warming temperature of about 50°C for a period of time between 1-5min would not have reduced any significant level of microbial contaminant; hence the consumers go home not conscious of the public health implication. The fact that suya is not consumed immediately after preparation but held at ambient temperature for more than 5h before serving has previously been reported (Edema et al., 2008).

Microbiological analysis of suya consumed within Maiduguri metropolis revealed that the highest total viable counts (TVC) was log 8.08 CFU/g; *Escherichia coli* count was highest at log 5.48 CFU/g; Staphylococcal count was highest at log 6. CFU/g; *Salmonella* species count was highest at log 5.70 CFU/g; *Shigella* count was highest at log 4.60 CFU/g and yeast and mould count was highest at log 4.30 CFU/g (Table 1).

Mean *S. aureus* of log 6.25 CFU/g was higher than 0.53×10^5 to 1.67×10^5 obtained by Edema et al. (2008), values of the same organism from the other markets were however similar. Staphylococcal count of Abdulahi et al. (2006), highest at log₁₀ 3.96 CFU/g was also lower than our present count. The value of total viable counts (TVC) up to log 8.08 CFU/g was also slightly higher than 0.07 to 2.22×10^5 CFU/g (Edema et al., 2008) and results in the order 10^5 to 10^6 obtained for total aerobic count (Inyang et al., 2005). The range of *E. coli* was also slightly higher than log₁₀ 4.08 CFU/g previously obtained (Abdulahi et al., 2006) for coliforms and 3.8×10^1 CFU/g (Raji, 2006).

Table 1. Minimum and maximum values of count of microorganism isolated from different markets in Maiduguri (expressed as log cfu/g of suya)

Market	<i>E. coli</i>	<i>S. aureus</i>	<i>Salmonella sp.</i>	<i>Shigella sp.</i>	Yeast and mould	TVC
Bama Road	0.0 – 5.48	0.0 – 4.03	0.0 – 4.48	0.0 – 4.53	0.0 – 3.64	0.0 – 8.08
Kano Road	0.0 – 4.90	0.0 – 6.25	0.0 – 4.90	0.0 – 4.30	0.0 – 4.40	0.0 – 7.30
Gamboru	0.0 – 4.78	0.0 – 6.23	0.0 – 4.78	0.0 – 4.60	0.0 – 5.00	0.0 – 7.07
Baga Road	0.0 – 4.70	0.0 – 6.12	0.0 – 4.78	0.0 – 4.48	0.0 – 3.91	0.0 – 7.12
Damboa Road	0.0 – 4.85	0.0 – 6.22	0.0 – 5.70	0.0 – 4.30	0.0 – 5.36	0.0 – 7.22

Table 2. Distribution and % frequency of occurrence of different microorganisms isolated from suya samples

Market	<i>E. coli</i>	<i>S. aureus</i>	<i>Salmonella sp.</i>	<i>Shigella sp.</i>
Bama Road	13 (65)	11(55)	9(45)	8(40)
Kano Road	14(70)	15(75)	10(50)	6(30)
Gamboru	12(60)	13(65)	8(40)	7(35)
Baga Road	14(70)	11(55)	11(55)	4(20)
Damboa Road	11(55)	12(60)	9(45)	6(30)
Overall % mean	64	62	47	31

Figures in parenthesis are % occurrence. In each market, 20 samples were analysed

Table 3. Mean microbial counts (counts were expressed as log 10cfu/g) and mean pH of samples of suya from different markets in Maiduguri.

Market	<i>E. coli</i>	<i>S. aureus</i>	<i>Salmonella sp.</i>	<i>Shigella sp.</i>	Yeast and mould	TVC	pH value
Bama Road	4.86	6.13	3.72	3.77	3.48	7.33	7.10
Kano Road	4.22	5.62	4.35	3.48	3.95	6.69	6.80
Gamboru	4.19	5.31	4.15	3.64	4.19	6.37	8.00
Baga Road	4.16	5.53	4.13	3.20	3.85	6.36	7.60
Damboa Road	4.22	5.66	4.48	3.26	4.30	6.70	6.90
Overall mean	4.33	5.65	4.17	3.47	3.95	6.69	

TVC = Total viable count. There is statistically significant difference ($P < 0.05$) between *S. aureus* and the other bacteria; and also between *S. aureus* from Bama and other markets. *E. coli* and TVC from Bama were also significantly different from that from other markets.

Results obtained for highest *Salmonella* count of log 5.7 CFU/g was slightly higher than 1.97×10^5 reported previously (Edema *et al.*, 2008). *Shigella* count highest at log 4.60 CFU/g was very significant in terms of human health standpoint. This is true since *Shigella* is needed in comparatively small dose to initiate infection in a healthy individual. However *Shigella* was not isolated from all the suya samples (Table 2).

The highest yeast and mould count from the different markets ranging from 3.64 to 5.36 CFU/g slightly contradicts the yeast and mould count highest at log₁₀2.49 CFU/g reported previously (Abdulahi *et al.*, 2006), it however supports the value of the order 10^4 to 10^5 CFU/g obtained by other authors (Edema *et al.*, 2008).

The reason for the high level of these organisms isolated from suya could be environmental, handling contamination issue and holding time. The number of these organisms in public health perspective is high indicating that they may be all involved in one form of foodborne problem or the other. Public Health Laboratory Service Guidelines for the bacteriological quality of ready-to-eat foods at the point of sales considers a food unacceptable if the level of *Salmonella* and *S. aureus* are in the order $> 10^5$ CFU/g and $> 10^3$ CFU/g respectively (PHLS, 2003). Jablonski and Bohach (Jablonski and Bohach, 1997) noted that dissemination of *S. aureus* from humans to food can occur by direct contact, indirectly by skin fragments, or through respiratory tract droplet nuclei. Since many suya processors do not take cognizance of microbial contamination *visa vis* the health implications of eating a contaminated product, during processing the suya, they could use unwashed hand

in handling the suya, could sneeze or cough into the product. The use of dirty hands, table tops and dirty processing and other unhygienic practices lead to increased microbial level. The presence of *Shigella* in ready-to-eat food calls for alarm as < 10 cells could cause infection depending on the health status of the consumer. *E. coli* is used to assess the sanitary quality of food products. Therefore its presence in suya in large number is a problem. Presence of *E. coli* is not surprising since other authors had reported post processing contamination from producers, water, utensils and animal in the environment (Oranusi, 2003). The air and soil also contribute to the increased contamination. Air and most importantly soil is the reservoir of a wide range of microbes. Where there is relax in hygiene standard as was the case with most processing environments where sampling took place, exaggerated increase in the microbial level occurs. Yeast and moulds are known to cause spoilage of food products. Therefore when they are available in significant number, they lead to huge economic loss in addition to the pathogenic species causing health dangers.

Mean values of the organisms (log CFU/g of suya) showed means as high as 7.33 for TVC; 4.86 for *E. coli*; 6.13 for *S. aureus*; 4.48 for *Salmonella species*; Lower mean values were associated with *Shigella* count. On the whole, the lowest mean value of log 3.02 CFU/g of suya was got on *Shigella spp.* from Baga road market. The overall mean value for TVC, *Escherichia coli*, *S. aureus*, *Salmonella species*, *Shigella* and yeast and mould were (log CFU/g of suya) 6.69, 4.33, 5.65, 4.17, 3.34 and 3.95 respectively (Table 3).

There is statistically significant difference ($P < 0.05$) between *S. aureus* isolated from Bama road and other market and also between *S. aureus* isolated from the markets and other organisms. *Shigella* and *Salmonella* were significantly ($P < 0.05$) lower than *E. coli* and *S. aureus* in Bama market. The reason for these differences could be linked to the meat quality, processing and dispensing operation and standard of hygiene. Some of the markets were closer to waste dumpsites and gutters more than others, hence had more organisms.

The value of Staphylococcal count up to 75% in kano road market and least distribution and % frequency of occurrence of 55% (Table 3) demands a public health attention. Distribution and % frequency of occurrence of different microorganisms isolated from suya samples ranged from 55 to 70% which also calls for health attention. Proximate composition of suya bought from different markets within Maiduguri metropolis shows variation in crude protein, crude fat, ash and moisture content (Table 4).

Table 4. Proximate Composition results of suya from different markets in Maiduguri

Market	Crude Protein	Crude Fat	Ash	Moisture content
Bama Road	33.42	23.77	2.40	36.65
Kano Road	35.10	20.90	1.80	39.09
Gamboru	30.58	26.10	2.31	38.80
Baga Road	28.33	28.26	2.86	35.00
Damboa Road	34.21	21.66	2.43	37.20

Results were mean of triplicate sample readings

The highest % protein of 35.10% was recorded in samples from Kano road market and the least of 28.30% was from Baga road samples. Fat, ash and moisture content were highest at 28.26%, 2.86% and 39.09% respectively. The lowest values were 20.90%, 1.80% and 35.00% respectively. The % protein content of the samples agreed with 31.8% (Abdulahi *et al.*, 2006). Also the values obtained (Abdulahi *et al.*, 2006) for fat and ash level which respectively were 23.7% and 2.4%, agreed closely with that obtained in some of the markets.

The differences in the proximate composition of suya could be either from the status of the animal used in its production or the processing technology. Suya could be made from beef, mutton, or goat and most mammalian muscles meat. There is however fish, chicken and kwadra (Hausa name for a kind of small bird) suya popular the study area. The status of the animal also determines its proximate composition. Animals facing severe malnutrition or suffering one disease or the other before slaughter would not have the same nutrient composition as a healthy animal.

The pH values of the suya samples from the different areas were in the order of 6.8 to 8.0 (Table 2) as opposed to the pH range of 8.28-9.07 reported (Edema *et al.*, 2008). The pH of food sample determines the number and kind of

contaminating organisms. The pH values between 6.8 to 8.0 is ideal for the growth of most pathogenic bacteria including *E. coli*, *S. aureus*, *Salmonella*, *Shigella* isolated in this study. Moisture content values of 35.00 to 39.09% were lower than that obtained by Edema *et al.* (2008) who reported between 40.17 and 57.17 (Edema *et al.*, 2008) and higher than 23.29 obtained for kundi another kind of dried meat (Fakolade and Omojola, 2008) in some markets. Moisture content contributes significantly to the microbial flora of food samples (Prescott, 2002). It is therefore possible that the reason for the high level of microbial growth is because of high moisture contents of the samples.

Conclusion

Organisms capable of endangering human lives were isolated from the suya samples and in numbers that could likely cause health problems in healthy individuals. The practice of preparation and distribution of suya in open places where there is no emphasis to the hygiene standard leads to exaggerated increase in the proliferation of microorganisms especially where the suya sellers are themselves unhygienic. Proper hygiene in the process-line and the processing environment of suya is recommended to prevent the likely detrimental health implications to consumers.

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