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Full Length Research Paper

Evaluation of microbial hazards associated with the processing of *Suya* (a grilled meat product)

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***Suya* is a popular spicy roasted meat product in Nigeria. Ready-to-eat *Suya* samples were collected from six '*Suya* spots' serving at least 240 consumers in 6 selected cities within south-western Nigeria. On-line process monitoring and sampling during processing was used to identify the Critical Control Points and evaluate microbiological hazards. Microbiological analyses of 144 samples of *Suya*, processing water, meat processing slabs, utensils, spices and raw meat revealed contamination with potential pathogens such as *Bacillus cereus*, *Staphylococcus aureus*, salmonellae and aflatoxigenic molds (*Aspergillus flavus* and *Aspergillus parasiticus*) from utensils and hands of the producers during slicing, staking onto sticks, spicing and holding at ambient temperature (28 ± 2°C). Aerobic mesophilic counts were in the order of 10^5 cfu with the highest value (7.17) observed in the packaging material and the lowest value (1.47) observed in the processing water. The raw meat samples and processing slabs recorded counts in the same range (above 5.5). Occurrence of such organisms in ready-to-eat food constitutes a food safety issue which calls for urgent response in the education of *Suya* producers on the hazards, Critical Control Points and the importance of personal hygiene and clean environment. Critical limits for the Critical Control Points identified in this study are proposed.**

Key words: Aflatoxin, critical control points, grilled meat, HACCP, microbiological hazards, *Suya*, food safety.

INTRODUCTION

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 pre-occupation and a major source of livelihood for the people. This leads to the production of ready - to - eat beef products such as *Suya, Kilishi, Balangu* and *Kundi* are very popular street foods. *Suya* is however the most popular as its consumption has extended to other parts of the country (Inyang et al., 2005). 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 is prepared basically from boneless meat of animals. (Abdullahi et al., 2004). 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 (Odusote and Akinyanju, 2003; Inyang et al., 2005). 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 (FAO/WHO, 2003). 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.

Osho (2004) evaluated the bacteria contamination of *Suya* processed in Abeokuta South western Nigeria and found up to 10^3 cfu / g enterobacteriaceae in 40% of the

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samples collected; more than 10^4 cfu / g aerobic mesophiles including *Staphylococcus aureus* in all collected samples. Inyang et al. (2005) also evaluated the bacterial quality of *Suya* sold in Markurdi, Northern Nigeria and concluded that feacal coliforms were the main bacterial contaminants although they occurred within acceptable limits (less than 10^4), although the total bacterial counts were more than 10^6 cfu / g. The focus of research on this food product has been narrowed to bacterial contaminants alone. In addition no attempt has been made to reduce the microbiological food safety risks associated with this popular protein food product. This is not good enough in a part of the world where protein intake in the diet is very low and the consumption of products such as *suya* should be encouraged. The objectives of this study therefore were to determine the microbiological quality of ready-to-eat *Suya*, evaluate the critical control points (CCPs) during the processing of *Suya* and determine the likely microbial hazards associated with this food product with the aim of improving its quality.

MATERIALS AND METHODS

Sampling procedure

Samples of *Suya* used in this study were obtained from six *Suya* spots in South-western Nigeria (Nigeria is located in the tropical zone of West Africa between latitudes 4°N and 14°N). The survey was conducted over a period of 8 months between November 2005 and June 2006, and a total of 144 samples were collected. A total of 24 replicate samples were collected from each location. From each of the sites, ready-to-eat *Suya* samples were purchased and transported to the laboratory in sterile bags packed in insulated containers with ice packs. Analyses were usually carried out within 6 h after sampling. Where immediate microbiological evaluation was to be delayed, the samples were refrigerated at 4°C and analyzed within 24 h of collection (Abdullahi et al., 2004). Two centers were later selected for the evaluation of critical control points during *Suya* preparation. Selection was based on willingness to participate without incentive. *Suya* samples were taken at different stages of processing for microbiological evaluation. In addition, samples or swabs of processing water, meat preparation slabs, cutting utensils, stacking sticks, spices and raw meat were taken as appropriate. All experimental determinations were made in triplicate.

Microbiological analyses

Ten grams (or ml) of each sample for microbiological evaluation were aseptically transferred into 90 ml of 0.1% sterile peptone water, shaken thoroughly and appropriate dilutions (up to 10^5) prepared for microbiological studies (Harrigan and McCance, 1976). Total viable counts (aerobic mesophiles) were made on Plate Count Agar (PCA, Oxoid, U.K.) while fungal counts (mould/yeast) were made on acidified Potato Dextrose Agar (PDA, Oxoid, U.K.). PCA plates were incubated at 37°C for 24 h while the plates for fungal counts were incubated at 25°C for 72 h. Coliforms were isolated using MacConkey broth and Eosin Methylene Blue agar. A 10^{-1} dilution of each sample was enriched in tetrathionate broth (Difco), incubated at 37°C for 6 h before inoculation on *Salmonella-Shigella* agar (Oxoid) for isolation of salmonellae. *Bacillus cereus* was isolated on mannitol/eggs yolk/poly myxin agar (MYP). MYP was prepared using peptone (Oxoid, U.K.), meat extract (Oxoid, U.K.), D-mannitol, sodium chloride, phenol red, agar-agar, egg yolk (Oxoid, U.K.) and polymyxin B sulphate (Pfizer). The plates were incubated at 32°C for 24 h (Gallenkamp Economy Incubator - Size 2). Gram-positive rods with halo zone of egg yolk precipitation were confirmed using standard test procedures (Sneath et al., 1986). All catalase positive, motile organisms with ellipsoidal spores and positive V-P reaction were confirmed as *B. cereus*. *Staphylococcus* strains were isolated on Mannitol salt agar and Baird-Parker medium for *S. aureus*. The coagulase and catalase tests were used to differentiate *S. aureus* from other staphylococci (Harrigan and McCance, 1976).

Determination of pH and moisture content of *Suya*

The pH of the *Suya* samples was determined using a pH meter (Mettler-Toledo, Essex M3509 Type 340) while moisture content was determined by the methods of A.O.A.C (1990) on dry matter basis.

Aflatoxin production by isolated moulds

The moulds isolated from the *Suya* samples and other samples taken during processing were grown on Yeast Extract Sucrose (YES) medium made by mixing 2 g yeast extract and 20 g sucrose in 78 ml of distilled water. The medium was sterilized at 121°C for 15 min, allowed to cool to about 45°C before pouring into sterile Petri dishes. The plates were allowed to set before stab inoculation of the mould isolates and incubation at 27°C for 3 days, after which the plates were examined under UV light (365 nm). On visual observation, the emission of a blue fluorescence indicated the production of aflatoxin in the medium by the isolates (Davis et al., 1987; Onilude et al., 2005).

Evaluation of critical control points (CCPs)

The critical control points (CCPs) during processing of beef into *Suya* were evaluated by on-line monitoring of the production process. The CCPs were identified as presented in Figure 1 and microbiological hazards associated with these points were confirmed with results of microbiological analyses.

Statistical analyses

The data generated were subjected to statistical analyses using SPSS 11.0 for Windows. Means that were statistically different at 95% confidence level were separated by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Moisture contents and pH values of *Suya* samples examined in this study ranged from 40.17 to 57.17% and 8.28 to 9.07 respectively as presented in Table 1. The reason for the observed significant differences in moisture contents and pH values among the locations is unknown. The pH values however, fell within the alkaline range for raw meat (Frazier and Westhoff, 1986). Using bi-variate correlation at 0.05 significance level, positive correlations were observed between moisture content and numbers of coliforms/salmonellae. This indicates that

Table 1. Microbial counts (10^5 cfu per g / ml), pH and moisture contents of *Suya* samples from selected locations

Location	pH	Moisture content (%)	Aerobic mesophiles	Fungi (yeast and molds)	Coliforms	Staphylococci (% *S. aureus*)	*Bacillus cereus*	Salmonellae (% *Salmonella)*
I	8.54^{d}	54.93^{e}	1.40^{bc}	0.16^{d}	0.20^{bc}	1.17b	0.10^{a}	0.07^{ab}
II	8.28^{a}	40.17^{a}	0.07^{a}	0.02^{a}	0.12^{a}	0.53^{a}	ND	ND
III	8.36^{b}	49.13^{b}	0.21^{a}	0.10^{bc}	0.13^{a}	0.57^{a}	ND	0.03^{ab}
IV	8.43^{c}	53.23^{c}	1.11^{b}	0.18^{d}	0.24^{c}	1.33^{b}	0.07^{a}	0.17^{b}
V	9.07^{f}	54.10^{d}	2.22^{d}	0.15^{cd}	0.21^{bc}	1.67^{b}	0.07^{a}	0.13^{ab}
VI	8.62^{e}	57.17^{f}	1.73^{c}	0.09^{b}	0.16^{ab}	1.20^{b}	0.03a	0.10^{ab}

Mean values followed by different superscripts within columns are significantly different by Duncan's multiple range test ($P \leq 0.05$)

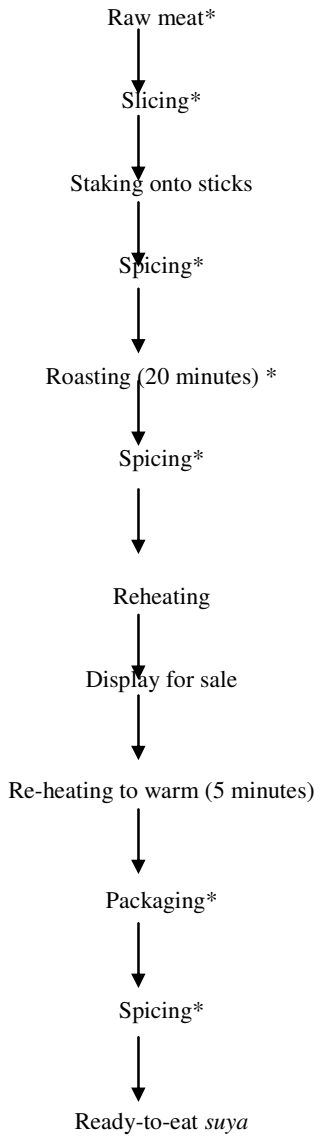

Figure 1. Flow diagram for *suya* preparation indicating CCPs and microbial hazards.
Key: Processing steps marked with * indicate CCPs.

exposure to higher temperature for a longer time during roasting could help reduce the numbers of these groups of microorganisms which constitute food safety risk in *Suya* and related foods.

Table 1 also shows the counts of the different microbial groups enumerated. Aerobic counts ranged from 0.07 to 2.22 x 10^5 colony forming units (cfu) per gram of *Suya*. Inyang et al. (2005) recorded comparably similar values for total plate counts in *Suya* samples in the order of 10^5 and 10^6 (cfu /g) and stated that the values placed the *Suya* samples consumed in Nigeria within satisfactory limit according to the International Commission of Microbiological Standards for Foods (ICMSF, 1978). However, these values place the *Suya* samples examined in this work in the acceptable but not satisfactory range under the Public Health Laboratory Service guidelines for the bacteriological quality of ready–to- eat foods sampled at the point of sale (PHLS, 2000). The numbers of coliforms and *B. cereus* rendered the samples unsatisfactory according to the same guidelines. *B. cereus* was however, not isolated in samples from two locations while no salmonellae were isolated from only one location. Detection of *Salmonella* in the order of 10^5, *S. aureus* and *Bacillus* species at >20 and >10^3 cfu/g respectively by PHLS guidelines rendered the samples wherein they were found of unacceptable quality (PHLS, 2000). Microbial counts of samples from the different locations were significantly different except for counts of *Bacillus cereus* and salmonellae. *Micrococcus*, *Bacillus* and *Streptococcus* were among the organisms identified as aerobic mesophiles, an observation in agreement with the findings of Osho (2004). *Pseudomonas, Klebsiella, Proteus* and *Enterococcus* species were also isolated. *E. coli* and *S. aureus* were isolated from all *Suya* samples. This was very significant from a food safety point of view as similar trends had been observed (Inyang et al, 2005). Three species of yeast were identified: *Candida, Saccharomyces* and *Rhodotorula* species while molds found were *Aspergillus flavus, Aspergillus niger* and *Aspergillus parasiticus* among the aspergilli. Other molds isolated were identified as *Penicillium, Trichoderma, Rhizopus* and *Mucor* species. *A. flavus* and *A. parasiticus* isolated from all the *Suya* and mixed spices used at all

Table 2. Counts of microbial groups (10^5 cfu per g / ml) in some samples taken during processing from two locations selected for Critical Control Point evaluation

Sample	Location	Aerobic mesophiles	Fungi (yeast and molds)	Coliforms	Staphylococci (% *S. aureus*)	*Bacillus cereus*	Salmonellae
Processing water	I	1.47^{a}	0.01^{a}	1.53^{d}	0.23^{ab}	ND	0.01^{a}
	II	1.80^{a}	0.01^{a}	1.30^{cd}	0.10^{a}	ND	0.02^{a}
Processing slab	I	5.57^{c}	0.24^{b}	1.20^{c}	2.43^{f}	0.03^{ab}	0.27^{b}
	II	5.67^{c}	0.32^{b}	1.07^{c}	2.07^{e}	0.03^{ab}	0.27^{b}
Mixed Spice	I	3.42^{b}	1.43^{d}	0.47^{b}	0.37^{b}	0.02^{ab}	0.43^{b}
	II	3.50^{b}	1.27^{c}	0.37^{b}	0.37^{b}	0.02^{ab}	0.37^{b}
Raw meat	I	5.73^{c}	0.01^{a}	2.57^{e}	1.80^{d}	0.07^{b}	1.97^{d}
	II	5.60^{c}	0.01^{a}	2.77^{e}	1.53^{c}	0.03^{ab}	1.73^{c}
Packaging material	I	7.10^{d}	3.37^{e}	ND	0.01^{a}	ND	ND
	II	7.17^{d}	3.60^{f}	ND	0.01^{a}	ND	ND

Mean values followed by different superscripts within columns are significantly different by Duncan's multiple range test ($P \leq 0.05$)

the locations were confirmed to be toxigenic strains. The spices used were particularly heavily contaminated with the aflatoxigenic mold species identified.

Considering the possibility that the presence of the researcher might affect the routine food handling procedures (Lucca and Torres, 2004), online monitoring was done first with the researcher unnoticed and then with the awareness and consent of the processors. Observation did not appear to influence their handling processes as there was little or no difference in total counts of groups of microorganisms investigated (results not shown). *Suya* processors were all middle-aged illiterate men without any kind of formal education. None of them had any training in food preparation which is necessary and important for hygienic handling of foods (FAO, 1999) especially for a nutritious food like *Suya* made from a substrate that is also an excellent culture medium for a wide variety of microorganisms. Personal hygiene of *Suya* processors was observed. All used bare hands to handle both food and money simultaneously. Personal hygiene is imperative because humans are the largest sources of contamination in food (Marriot, 1985) and any food handler who observes other forms of hygiene but not personal hygiene will definitely contaminate food. All *Suya* processors prepared the *Suya* at their wooden stalls located by the roadsides. The surroundings were considered unhygienic given that garbage and dirty wastes littered the food processing environment with open gutters nearby, all of which attracted houseflies. None of the processors was observed to wash their raw meat before *Suya* preparation. Slabs and trays used for cutting and sticking were inadequately cleaned. Utensils made of plastic, metal or enamel were washed only once and the water used repeatedly until obviously cloudy and dirty. Water was not readily available at the locations so processors usually ferried water from home or nearby water sources (wells or pipe-borne water) hence it is used economically. Even then the water used contained a significantly high coliform count in the order of 10^5 cfu/ml while the processing slab and raw meat both had counts of aerobic mesophiles above 5×10^5 cfu / g (Table 2).

Prepared *Suya* samples were not covered but left exposed to flies and dust during display. They were kept at ambient temperature and the re-heating temperature of less than 70°C was not sufficient to destroy pathogens (Table 3). According Bryan, Bartleson and Christopherson (1981), under appropriate temperature, emetic and diarrhoeagenic toxins could be elaborated especially if the product is held at ambient temperature for a reasonably long period of time. While there is no documented case of food infection or intoxication resulting from consumption of *Suya*, cases of acute intravascular hemolysis following ingestion of *Suya* have been reported by Odusote and Akinyanju (2003). The roasting period for *Suya* is on the average 45 min at about 70°C. This time and temperature regime may not be sufficient to destroy all the vegetative cells and heat-resistant spores of bacteria especially if the meat is heavily contaminated with enteric bacteria (Bryan, 1988). Heat treatment of food such as roasting not only improves the taste, smell, appearance and digestibility of the food, it also reduces the number of microorganisms, improves keeping qualities by inhibiting moulds, yeast and bacteria that promote decay and infection. Thus, heat treatment is a practice aimed at improving the overall safety of food. In many cases *Suya* is not consumed immediately after preparation. It is held at ambient temperature for more than 5 hours before serving. The temperature of the meat after reheating is about 50°C. This time and temperature regime was not adequate for destroying all vegetative bacterial cells. Holding *Suya* at ambient temperature for too long could be risky since this could encourage the growth of the pathogens to hazardous levels (Table 3).

Packaging material used were old newspapers collected from various sources, some of which had off-

Table 3. Evaluation of Critical Control Points (CCPs) and identification of microbial hazards in the preparation of *Suya* from beef

Step	Hazard	CCP	Critical Limit
Purchase of raw materials	Microbiological	Yes	Wholesome raw meat, food grade spices with low microbial load.
Storage of raw materials	Microbiological	Yes	Refrigeration of meat, keep spices dry in sealed clean containers
Slicing	Microbiological, Physical	Yes	Use of potable water, clean utensils and slab Hand washing practice
Staking onto sticks	Microbiological and Physical	No	Use of good quality, clean sticks. Careful handling
Spicing	Microbiological, Chemical	Yes	Storage of spices in good condition to avoid contamination and multiplication of microorganisms
Roasting	Microbiological, Chemical	Yes	Exposure to higher temperature for a longer time to destroy pathogens and reduce moisture level and fat contents quickly
Display at ambient temperature	Microbial re-contamination	No	Holding in an air-tight containers to reduce re-contamination with air flora and contact with insects
Reheating	Chemical	No	Reduce exposure to smoke to prevent formation of aromatic hydrocarbons which are potential carcinogens. Hot air oven recommended for re-heating
Serving	Microbiological, Chemical	Yes	Careful handling, personal hygiene, use of clean food grade wrappers with low microbial load. Hand washing practice.

odors with high mould counts and aerobic spore-forming bacilli above 3 x 10^5 and 7 x 10^5 cfu/g respectively. Only two of the processors confessed to having leftovers which they store at ambient temperature and warm the next day mixing with fresh batches.

This study has shown that *Suya* is prepared and sold under largely unhygienic and un-safe conditions thereby constituting a food safety risk, in this case microbiological, to the numerous and ever-increasing consumers. Many compelling motivations are driving the use of HACCP, but four of the most prominent driving forces are that HACCP (1) is focused on food safety, (2) is science-based, (3) relies on preventive controls rather than retrospective end-product testing, and (4) focuses control on those food safety hazards that are reasonably likely to occur. In essence, HACCP requires food processors to understand the safety hazards associated with the food, the process, as well as distribution and marketing conditions, and to use appropriate controls so that any identified hazard(s) is prevented, eliminated, or reduced to acceptable levels. The HACCP concept has not been applied to the processing and vending of meat products in Nigeria. This study therefore recommends that the critical limits suggested for *Suya* in this study be applied in the processing of the product, as is the focus of ongoing attempts by the authors.

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