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Microbial contamination associated with the processing of *tchachanga*, a roasted meat product

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This study aimed to assess the microbiological contamination and quality of *tchachanga*, a roasted meat braised product processed at traditional scale in Benin, West Africa. A survey was performed to collect samples of *tchachanga* and data related to hygienic conditions of the roasted meat processing environment. A total of 60 samples of *tchachanga* including skewers, roasted meat and seasoned wrapped meat were collected from different processing sites in Cotonou, Benin. The main food borne microorganisms involved were investigated using standard methods. Moreover, a follow-up of *tchachanga* processing was performed to identify contamination factors of the product. In this respect, samples were collected at certain steps during the processing, and various microorganisms such as coliforms, pathogenic *Staphylococcus* and *Salmonella* were traced. The number of mesophilic aerobic bacteria in different products ranged between 6.47±0.61 and 6.93±0.43 Log (cfu/g). Total coliforms, faecal coliforms and *Staphylococci* ranged from 1.59 to 4.79, 1.00 to 3.2 and 3.49 to 5.2 Log (cfu/g), respectively. No significant differences were observed in the microbial count of different types of *tchachanga* investigated, but different processing methods had significant changes in the microbial content of the samples as a result of the processing environments and the ingredients used. The presence of *Salmonella* sp. was observed in all products.

Key words: Street food, *Tchachanga*, meat, microbiology, quality.

INTRODUCTION

Food borne infection is a global health problem (Gong et al., 200; Si et al., 2006). It is a major cause of illness and death worldwide (Adak et al., 2005). According to Doyle and Evans (1999), food borne diseases result from ingestion of bacteria and toxins produced by microorganisms present in food. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin (Clarence et al., 2009). Food borne illness associated with the consumption of foods sold in open road side kiosks (street foods) has been reported in several places in Africa, India and elsewhere (Chumber et al., 2007; Estrada Garcia et al., 2004; FAO, 1988; Ghosh et al., 2007; Mosupye and Von Holy, 2000). Street foods consumption is frequently associated with diarrheal diseases which occur as a result of improper use of additives, the presence of pathogenic bacteria, environmental contaminants and disregard of good

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manufacturing and hygiene practices (Tambekar et al., 2008). Sellers are often poorly educated, unlicensed, untrained in food hygiene, and they work under crude unsanitary conditions with little or no knowledge about the causes of food borne disease (Barro et al., 2007). Among these street foods, those derived from meat products are of major concern. Indeed, by its composition, meat is an excellent environment for proliferation of microorganisms, particularly bacteria (Gill, 1998). Epidemiological studies have identified the consumption of poultry and its derived products as important risk factors for sporadic campylobacteriosis (Friedman et al., 2000). Similarly, a study conducted by Mosupye and von Holy (2000) on beef and chicken in street foods in Johannesburg (South Africa) revealed the presence of pathogenic bacteria in these foods. In Benin, one of the popular foods in the informal sector of street foods is roasted meat, locally known as *tchachanga*. It is largely preferred and consumed by adults and adolescents. *Tchachanga* products are diverse and include: Skewer, roasted meat and seasoned wrapped meat. Skewers are small pieces of meat seasoned and placed on wooden skewers and cooked on the embers of charcoal. Seasoned wrapped meat is small pieces of meat seasoned, wrapped in brown papers (papers, previously used in packaging cement material for construction) and cooked on the embers of charcoal. In the case of roasted meat, a sheep carcass is divided into four main parts, seasoned and roasted on the embers of charcoal.

The main steps of *tchachanga* processing are: Slaughter, dressing, evisceration, washing, cutting, seasoning, packaging and braising. Seasoning ingredients used are pepper, onion, salt and glutamate, usually bought from the market. The working personnel (the salesman) is mostly uneducated butchers who have received no training in food hygiene, and without sufficient financial resources. The products are therefore exposed to various environmental and microbiological hazards which make the safety of the product questionable. The present study was design to evaluate the microbiological status of *tchachanga* products sold as street food in Cotonou. The aim was to: (i) Investigate the environmental processing conditions in terms of hygiene and identify the contamination risks factors during the processing, and (ii) evaluate the main food borne micro-organisms in *tchachanga* samples collected from different processing areas. In addition, this work aimed at providing a better hygienic and quality management for *tchachanga* processing that will be free from microorganism contamination and thereby contribute positively to food safety improvement in Benin.

MATERIALS AND METHODS

Experimental design

The study was carried out in two phases in Cotonou, the capital and most populated city of Benin, West Africa. First, a survey was carried out among processors of *tchachanga* to gain insight into the current practices in food preparation and the potential risks associated with it. Samples were collected during this phase. Second, samples of *tchachanga* collected from different sellers in Cotonou were analysed for their microbiological status.

Survey

Preliminary to the survey, a rapid appraisal investigation was carried out to identify different production sites of *tchachanga* in Cotonou and to collect information related to the production environment and hygiene conditions. Data collected at this stage helped to classify the production sites, based on different criteria such as hygiene of the production area, location of the site in relation with environmental hygienic conditions of processing, etc.

During the survey, data were collected on 30 consumers and 30 producers. A questionnaire took into account frequency of consumption of *tchachanga* associated with health problems, and consumers' preference. The supply chain of *tchachanga* processing, the raw material and ingredients used, and all contamination risk factors associated with *tchachanga* processing were also recorded. After the survey, three processors were randomly selected for monitoring the *tchachanga* processing methods. The main risks associated with the processing was identified and analysed. Samples were collected for laboratory analysis.

Sample collection

Sixty (60) samples were collected for analysis in order to assess the overall microbial contamination of *tchachanga* sold in Cotonou. Samples were collected from 30 processors involved in the survey. Two samples were collected per processor, at different occasions. Three categories of samples were collected at the critical points of the processing: fresh sheep meat, seasoned wrapped meat and ready to eat products samples at rate of six samples per category. Samples of seasoning ingredients, water for preparation, twenty four and forty eight hours unsold products were also collected and analysed. Samples collected in sterile stomacher bag were kept in a cooler containing ice during transportation from production site to laboratory. Samples from three randomly chosen *tchachanga* were checked for microorganisms. Of particular interest were the following organisms: the mesophilic aerobic bacteria, total and faecal coliforms, *Salmonella* and *Staphylococcus aureus*.

Microbiological analysis

For all samples, 10 g was introduced aseptically in a sterile stomacher bag (Seward Medical, London, UK) and macerated for 2 min in 90 ml of sterile diluents [0.1% peptone (Oxoid L 37, Basingstoke, Hampshire, England), 0.8% sodium chloride (NaCl) with pH adjusted to 7.2] using a stomacher (Lab Blender, Model 400, Seward Medical, London, England). One milliliter (1 ml) of the suspension was serially diluted and used for enumeration of micro-organisms. Mesophilic aerobic bacteria were enumerated on plate count agar (PCA Oxoïd CM 0325, Basingstoke, Hampshire, England) incubated at 30°C for 72 h in accordance with standard NF ISO 4833. Coliforms were enumerated on violet red bile agar (VRBA, Oxoid CM107, Basingstoke, Hampshire, England) incubated at 37°C for 24 h for total coliforms and 44°C for 24 h for faecal coli. Pathogenic staphylococci were enumerated using the medium Baird-Parker (LAB 85), with addition of egg yolk tellurite (Oxoïd SR 0054C, Basingstoke, Hampshire, England) and the

Table 1. Microbial contamination of different samples of *tchachanga*.

Type of *tchachanga*	n[1] = 60	Mesophilic aerobic germs Log (cfu/g)	pH
Skewers	20	6.71 ± 0.53[a2]	6.20 ± 0.32[a]
Roasted meat	20	6.54 ± 0.58[a]	6.40 ± 0.36[b]
Packaged meat	20	6.77 ± 0.46[a]	6.20 ± 0.27[a]

[1]n = Total samples analysed. [2]Mean ± standard deviation; values with the same letter in the same column are not significantly different at 5%.

plates incubated at 37°C for 48 h in accordance with standard NF V08-014. The presence of *Salmonella* was investigated according to the standard of NF ISO 6579: 2002. The methods of Yang et al. (2003, 2004) were also used.

pH determination

For the determination of pH, 10 g of sample were taken and crushed. Twenty milliliter (20 ml) of distilled water are then added and the mixture is homogenized properly. The pH was measured using a pH meter (INOLAB 730) with glass electrode (AOAC, 1995),

Statistical analysis

Data were analyzed using with SPSS Inc. software (version 12.0). One-way analysis of variance was used to determine the significant difference between means, at level of α = 0.05. All data were mean values of samples of each category.

RESULTS

Hazards associated with the producing environment

In general, the hygienic processing conditions of *tchachanga* in Cotonou do not guarantee the safety of the product. This is mainly due to the ignorance of elementary hygiene rules by the processors/sellers. Factors favoring the contamination of *tchachanga* vary from one processor to another and include lack of basic hygiene by processor/seller, the preparation methods used, the equipments, the raw material, polluted processing environment, manipulation practices.

The use of polluted water

The water used in the preparation of *tchachanga* was obtained from wells around the production site, and was drawn in improperly cleaned container. Eighty seven percent (87%) of processors surveyed use that water. The remaining (13%) use tap water but the containers used to keep the water could be source of contamination because they are not well cleaned.

The use of raw material of questionable quality

Fifty four percent (54%) of processors practice illegal slaughter. This illegal slaughter of animals was done in very poor conditions, and therefore slaughter, dressing and evisceration takes place in a very unhygienic environment. In addition, the carcasses is transported under poor conditions from the slaughter house to processing place, including the use of dirty jute bags, dirty baskets and related accessories. It is also frequent to see the carcass handled by people wearing very dirty clothes.

The use of dirty equipments

The knives used are regularly cleaned with a cloth during the entire processing day and washed at the end of the workday. The cutting boards are made of wood, generally too old and rarely washed with water and soap. The surfaces of the boards are very rough and have crevices favourable for accommodating microorganism growth. The same observations were made for the metal grids used for roasting the meats. In addition, the products are sold in very unhealthy open and dusty environment. The products were not packaged properly and often handled with bare hands. They are not conserved in a manner that would inhibit infection or development of contaminants.

Microbiological characteristics of *tchachanga*

Tchachanga on sale in Cotonou city

The results of microbiological analysis showed that the mesophilic aerobic bacteria counts in all types of *tchachanga* were 6.71±0.53, 6.54±0.58 and 6.77±0.46 Log(cfu/g) for skewers, roased meat and seasoned wrapped meat, respectively (Table 1). The differences in these values were not statistically significant ($p>0.05$). The levels of microbial loads in the different *tchachanga* samples exceeded the recommended value of 5.48 Log (cfu/g). The pH values increased during processing from 5.61 to 6.2±0.32 for the fresh meat, and 6.2±0.27 and 6.4±0.36, for the skewers seasoned wrapped and the roasted meat, respectively.

Table 2. Effect of the environment of production/sale on the microbial contamination of *tchachanga*.

Areas of production/sale	n = 60	Aerobic mesophilic counts Log (cfu/g)
Dirty area	20	6.93 ± 0.43^{b1}
Moderately clean area	20	6.62 ± 0.44^{a}
Clean area	20	6.47 ± 0.61^{a}

n = Total samples analysed. [1]Mean ± standard deviation; values with the same letter in the same column are not significantly different at 5%.

Effect of the producing environment on product quality

The mesophilic aerobic bacteria counts in *tchachanga* are high in all the production areas. It was 6.47±0.61 in clean areas, 6.62±0.44 in moderately clean areas and 6.93±0.43 in dirty areas (Table 2). Furthermore, the mesophilic aerobic bacteria count in *tchachanga* provided from dirty areas was significantly higher ($p<0.05$) than those provided from clean and fairly clean areas.

Effect of ingredients and processing step on product quality

Table 3 shows the results of microbiological analysis on fresh meat, water and seasoning pepper used during *tchachanga* processing. All samples collected had a high microbial count and were contaminated with coliforms, staphylococci and *Salmonella*. None of the samples analysed was contaminated with *Staphylococcus aureus*. The microbial count of meat increased after cutting, washing, seasoning and wrapping for the seasoned and wrapped meats. From fresh meat to seasoning and wrapping, mesophilic aerobic bacteria, total coliform, faecal coliform and staphylococci counts (Logcfu/g) were 7.28±0.63, 4.06±0.92, 2.77±0.95 and 4.77±1.06 in fresh meat respectively, rose to 7.65±0.59, 4.79±1.32, 3.20±1.09 and 5.20±0.56 in seasoned wrapped meat respectively. Significant differences ($p<0.05$) were observed between the mean microbial counts in fresh, seasoning and wrapped meats. After cooking, a significant decrease of microbial count was observed in the ready to eat product. Indeed, the mean microbial count decrease to 6.71±0.48, 1.59±1.43, 1.00±0.87 and 3.49±1.81 for mesophilic aerobic bacteria, total coliforms, faecal coliforms and staphylococci, respectively (Table 4). In addition, *Salmonella* sp. was detected at every steps of processing, even after cooking.

Effect of shelf life on the quality of products

The *tchachanga* produced was rarely sold in one day. The remaining product was kept to be sold the following days and the shelf-life depends on the type of product and consumer preference. Generally, roasted meat was sold within 24 h after processing (53.33% of processors surveyed). The skewers and seasoned wrapped meat were retained for 72 to 96 h (26.66% of processors surveyed). During this period, the unsold products are wrapped in brown papers (papers, previously used in packaging cement material for construction) and undergo heat treatment before being sold again. For this study, samples of roasted meat, skewers and seasoned wrapped meat freshly produced as well as one and two days old unsold products were collected after heat treatment for analysis. As shown in Table 5, the mesophilic aerobic bacteria counts of skewers, roasted meat and seasoned wrapped meat increased during storage from 6.26±0.24 at 0 h to 6.97±0.21 at 48 h in roasted meat, 6.35±0.23 at 0 h to 7.06±0.17 at 48 h in skewers and 6.71±0.5 at 0 h to 8.46±0.41 at 48 h in seasoned wrapped meat. The mesophilic aerobic bacteria counts of seasoned wrapped meat was significantly higher ($p<0.05$) than those of roasted meat and skewers after the same storage time.

DISCUSSION

This study shows that *tchachanga* produced at traditional scale in Benin contains a high number of microorganisms; this could probably be due to the poor hygiene practices observed during *tchachanga* processing. Improper handling and hygiene lead to the contamination of ready-to eat food and this eventually affects the health of consumers (Adebolu and Ifesan, 2001; Dunn et al., 1995; Okonko et al., 2008; Omemu and Bankolé, 2005).

Due to the fact that different types of *tchachanga* are produced under the same hygienic conditions and manufacturing practices, no significant difference was observed among microbial loads of the three types of the product. For the same processor, roasted meat, skewers and seasoned wrapped meat prepared from the same meat source, using the same water source, same equipment and seasoning ingredients, the microbial loads were not significantly different. If the materials used for the production are contaminated or of poor quality, this

Table 3. Main microorganisms associated with fresh meat, water for preparation and seasoning pepper for *tchachanga* processing.

Sample	Microorganisms sought Log (CFU / g)						
	Aerobic mesophilic germ	Total coliform	Faecal coliform	Staphylococci	*Salmonella*[2]	*Staphylococcus aureus*	pH
Fresh meat (n=6)	7.28 ± 0.58[1]	4.06 ± 0.84	2.72 ± 0.87	4.77 ± 0.97	Present	Absent	5.61 ± 0.08
Seasoning pepper (n=6)	5.61 ± 0.59	2.83 ± 0.56	1.35 ± 0.77	3.97 ± 0.93	Present	Absent	5.97 ± 0.15
Water for preparation (n=6)	5.19 ± 0.65	3.39 ± 0.68	2.44 ± 0.57	3.66 ± 0.84	Present	Absent	-

n = Number of samples analysed. [1]Mean ± standard deviation. [2]Search in 25 g of product. -, not determined.

Table 4. Effect of processing steps on microbial counts from the meat.

Sample	Microorganisms sought Log (CFU/g)						
	Aerobic mesophilic germs	Total coliform	Faecal coliform	Staphylococci	*Salmonella*[2]	*Staphylococcus aureus*	pH
Fresh meat (n=6)	7.28 ± 0.63[a1]	4.06 ± 0.92[a]	2.72 ± 0.95[a]	4.77 ± 1.06[a]	Present	Absent	5.61 ± 0.08[a]
Seasoned and wrapped meat (n=6)	7.65 ± 0.59[b]	4.79 ± 1.32[b]	3.2 ± 1.09[b]	5.2 ± 0.56[a]	Present	Absent	5.97 ± 0.15[b]
Packaged meat after cooking (n=6)	6.71 ± 0.48[c]	1.59 ± 1.43[c]	1.00 ± 0.87[c]	3.49 ± 1.81[b]	Present	Absent	6.14 ± 0.28[c]

n = Number of samples analysed. [1]Mean ± standard deviation; values with the same letter in the same column are not significantly different at 5%. [2]Search in 25 g of product.

could negatively influence the quality of the final product. The pH values of the products increase during the processing. As the investigated products are proteins-rich materials, the rise in pH was possibly due to the formation of nitrogenous basic compounds as a result of microbial proteolytic activity. The increase in pH is a common phenomenon observed during animal or vegetable rich-proteins processing (Achinewhu and Oboh, 2002; Anihouvi et al., 2007). It was reported that the pH slightly increase due to the microbial activity of *Bacillus* sp. during the alkaline fermentation of African locust beans (*Parkia biglobosa*) to produce some African fermented food condiments such as *afitin*, *iru*, *sonru* (Azokpota et al., 2006; Parkouda et al., 2009), *dawadawa* (Odunfa, 1985), *soumbala* (Ouoba et al., 2003) or *nététu* (N'dir et al., 1994; N'dir et al., 1997). In addition, Horner (1997) has reported that fish usually presents a pH higher than that found in meat, which rises during storage as a result of the release of NH_3 and amines during protein degradation. The pH of roasted meat was significantly higher than that of skewers and seasoned wrapped meat. As opposed to skewers and seasoned wrapped meat for which the fresh meat is cut into small pieces, the whole sheep carcass was used for roasted meat preparation. It is therefore probable that the transfer of heat inside the meat does not efficiently take place. The higher moisture content of roasted meat provided favourable conditions for microorganisms to grow.

Significant differences observed between microbial loads of *tchachanga* from different production areas were probably due to the effects of the vectors of contamination such as air, dust and flies which are abundant in these unhealthy production areas. The increase of microbial count in unsold products indicates that conservation methods, including heat treatment applied to the products are not sufficient to inhibit the growth of

Table 5. Effect of storage on the microbial contamination of *tchachanga*.

Retention period after production (h)	Aerobic mesophilic counts Log (CFU/g)		
	Roasted meat (n = 6)	Skewers (n = 6)	Packaged meat (n = 6)
0	6.26 ± 0.24[a1]	6.35 ± 0.23[a]	6.71 ± 0.5[a]
24	6.97 ± 0.21[b]	6.75 ± 0.19[b]	7.63 ± 0.39[b]
48	-	7.06 ± 0.17[c]	8.46 ± 0.41[c]

n = Number of samples analysed. [1]Mean ± standard deviation; Values with the same letter in the same column are not significantly different at 5%.

growth of micro-organisms. The significant differences observed between the microbial counts of seasoned wrapped meat, skewers and roasted meats after storage are probably due to the effect of the packaging of seasoned wrapped meat. This packaging might be one of many reasons why the packaged product does not adequately receive enough heat to prevent further microbial contamination. The main factors having the greatest influence on the growth of microorganisms in meat and meat products are the storage temperatures, moisture and oxygen availability (Forest et al., 1985).

The high microbial load of fresh meat is due to unhygienic practices from the slaughter of animals to meat processing. Indeed, animals are slaughtered in a highly polluted environment which contains a lot of garbage, faeces, viscera and flies. In addition, the contamination by air and dust during transportation of meat from the slaughterhouse to the production site (preparation of *tchachanga*) created good conditions for the proliferation of micro-organisms. The transport of the meats from the slaughterhouse is not done under refrigerated or frozen conditions and the meats were often carried under unhygienic conditions in containers such as jute bags, re-usable brown papers (cement-packaging papers), bowls. Also, it was observed that after evisceration, the meat is exposed to ambient air for 2 to 3 h before handling. Meat is considered as an important source of proteins, essential amino acids, B complex vitamins and minerals which offer favorable environment for the growth of pathogenic bacteria. The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughterhouses and retail establishments (Abdalla et al., 2009; Gill, 1998).

Salmonella are present in viscera, lymph nodes and sometimes in the muscles when animals are slaughtered during periods of acute or sub acute disease (Leclerc and Mossel, 1989). Its presence in meat can be explained by inadequate evisceration or slaughter of sick animals. *Salmonella* can also come from the working environment and equipment, which are used in handling both meat and offals. According to the study of Bhaskar et al. (2004), *Salmonella* could contaminate foods through polluted water, or sewage and/or soil, handling of food by infected workers, vendors and consumers in the market place. The presence of *Salmonella* in the ready to eat food represents a great hazard for the consumer. These pathogens are often responsible for gastroenteritis, food poisoning, typhoid, and paratyphoid fever (Gledel, 1996).

The increase in microbial count observed between fresh and processed meat can be attributed to the various manipulations, the quality of water used during the processing, the quality of seasoning ingredients. Laboratory analysis of water and seasoning ingredients showed that they were of poor quality (Table 3). This increase in microbial count could also come from the material (cement-packaging paper) used to package the products; the packaging materials host coliforms and other pathogens (FAO, 1988). In fact, tissue from healthy animal are sterile however, it has been pointed that during slaughter, dressing and cutting, microorganisms came chiefly from the exterior of the animal and its intestinal tract as well as from knives, cloths, air, carts and equipment in general. External contamination of meat is a constant possibility from the moment of bleeding to consumption (Lawrie, 1984). The factors that affect microbial growth in the meat intrinsic properties (physical and chemical properties of meat) as well its extrinsic (environmental factors) properties should be taken into account (Rombouts and Nout, 1994).

Conclusion

Due to the high level of pathogenic microbial contamination, *tchachanga* produced and sold in Cotonou should not be considered as safe products. Poor hygiene and bad food manufacturing practices have been observed during *tchachanga* processing. Consequently, there is a need to educate tchachanga processors/sellers on good hygiene and manufacturing practices.

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