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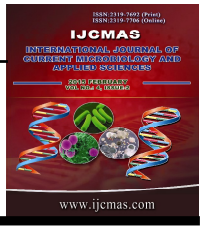

Original Research Article

Microbiological quality and safety of street meat-food sold in Soudano Sahelian zone of Cameroon

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A B S T R A C T

Street-vended meat samples were purchased from street food sellers in five major town from soudano-sahelian zone of northern Cameroon. Standards methods of microbiological control were used with the aims of determining their sanitary status. Total aerobic microflora, Staphylococcus aureus, Bacillus cereus, Salmonella, *Escherichia coli*type 01 non-0157:H+ *Escherichia coli*strain and Yeast and Mould moulds were checked. The mean aerobic counts and E.coli, of roasted beef meat, fried pork meat and roasted chicken for all street-vended meat samples from mobile food sellers (MFS), and stationary food seller (SFS) were not significantly different from one to another. However, all the counts were as much as the permissible level of count (3.0 log10/g) for cooked foods. The mean of *Staphylococcus aureus*, Salmonnella sp, where found to be higher for Mobile Food Sellers (MFS), than for Stationary Food Seller (SFS). Yeasts and moulds species were only found in "kilichi" a sun-dried beef meat samples [5.5 (□0.5) log10 spores/g]. Of 200 samples collected, 60 (30%) were contaminated with E.coli, 46 (23%) were contaminated with Bacillus cereus, 38 (19%) with Staphylococcus aureus, 30 (15%) with Salmonella sp. and 10 (5%) with yeast and moulds. Seven (26.92%) of the 26 coagulase positive *Staphylococcus aureus* isolates tested produced enterotoxin A (SEA). Listeria monocytogenes, *Vibrio cholerae* and *Yersinia enterocolitica* were also tested for in the street-vended meat samples but not detected. Based on the relatively low bacterial counts and comparatively low incidence of foodborne pathogens, the quality and safety of street-vended meat products analysed in this study was considered to be acceptable. However measures must be taken to better ameliorate their quality.

K e y w o r d s

Meat,
Street-foods,
Soudano-sahelian,
Ready-to-eat meat,
Street-vending,
Food safety

Introduction

In the soudano-sahelian zone of cameroon, there are many types of ready-to-eat foods which are commonly sold by street vendors. Like in many developing countries, those ready to eat foods are a common part of urban lifestyle due to high unemployment, low salaries, limited work opportunities and limited social programmes (Bryan *et al.*,

1988; Mensah, 2002). Street vendors provide an essential service to people from all economic level of life by selling to them complete meals, refreshing drinks and snacks. These streets foods are important economic factor, as they provide source of inexpensive, nutritious meals to a large number of peoples (Mosupye and Von Holy, 1999). Street foods currently make significant contribution to the food intake of large segments of most African countries (Edwards, 1985). Street vending is a means of income generation by less educated peoples. Most of such foods are prepared daily from a variety of ingredients to suit local taste and demand. The most important in terms of quantity of those ready-to-eat foods in the soudano-sahelian zones of Cameroon are ready-to-eat meat products. Ready-to-eat meat products comprise mainly roasted and fried beef, pork or chicken meats locally called: "Soya" (roasted beef meat), "Rôti de porc" (Fried pork meat), "Poulet braisé" (roasted chicken meat) and "kilichi" (sun-dried beef meat). This ready-to-eat meat products, are prepared and sold in the streets for immediate consumption or for consumption at a later time without further processing or preparation (Dawson and Canet, 1991; Mosupye, & Von Holy, 2000). In Cameroon, and other African countries, where street-food vending is common, there has been little information regarding the incidence of street-food in general and especially ready-to-eat meat products, related diseases. This has raised many concerns because the conditions under which street vendors operate are usually unsuitable for the preparation and selling of food-meat (Ekanem, 1998; Muinde, & Kuria, 2005, Olayinka, *et al.,* 2008). In most cases foods are usually not effectively protected from dust and flies which may harbor foodborne pathogens and safe food storage temperatures seems difficult to maintain (Bryan *et al.,* 1988; Ekanem 1998; Von Holy, & Makhoane 2006). There are thus, potential health risks associated with initial contamination of raw foods with pathogenic bacteria as well as subsequent contamination by vendors during preparation and through post-cooking handling and cross-contamination (Bryan *et al.,* 1988, Oliver *et al.,* 2009). The objective of this paper is to investigate the safety and quality of artisanal ready-to-eat street-vended meat locally produce and collected from several typical vendors surrounding major point of road transit of major towns from soudano-sahelian zone of Cameroon.

Materials and methods

Study area and Sampling procedure

The study area, soudano-sahelian zone of Cameroon, was divided into five zones/Divisions. Each zone had a population of no less than 350 hawkers selling in the market, motor park and by the roadside (Edderai, & Dame, 2006). These street food sellers are generally divided into three categories based on their method of sales (WHO, 1992): mobiles food sellers (MS) are those that prepare food at home and then carry around for sale, stationary food sellers without shelter (SWS) which prepare their food in open air under the tree or by the roadside and sell at the spot and stationary food sellers with shelter (SS) which prepare full meals in the open and serve on tables in stalls as in restaurants. In this study we preferred dividing them in two categories: The first group, mobiles food sellers (MFS) which prepare meat at home or in the open air under the tree or by the roadside and then carry around for sale in headed metallic thanks on heated wheelbarrow, or wrap in paper cartons. Stationary food sellers with or without shelter (SFS) which prepare their meat in open air under tree or by the roadside and sell at the spot on tables in

stalls as in restaurants. Street-vended meat namely roasted beef meat "soya", fried pork meat "Rôti de porc", roasted chicken "Poulet braisé" and sun-dried beef meat "kilichi" samples (200 in total) were collected from each vendor over 12 replicate survey from May to July 2013. Only samples which temperatures were under 45°C were collected because some of the street-vended foods were always cooked and held at high temperatures and a pilot previous study revealed consistently low bacterial pathogens (results not shown). Samples of about 250 g of each ready-to-eat meat were collected using the vendor utensils and transferred into sterile Whirl-Pak bags (Nasco, USA). All samples were kept on ice during transportation to the laboratory for analysis on the same day.

Sampling preparation and analysis

A 20 g amount of each food sample was mixed with 180 ml peptone-saline [0.1% peptone (Oxoid) + 0.85% NaCl (Biolab)] and homogenised for 2 min in a Blinder 200 stomacher this mix solution where considered as the 10^{-1} dilution. Ten-fold serial dilutions were then carried out.

The spread plate technique was used to prepare duplicate aerobic plate count, (Morton, 2001). *Bacillus cereus* was isolated on mannitol/eggs/ yolk/ polymyxin B. sulfate agar (Pfizer) as describe by Baer *et al.*, (1976). The plates were incubated at 32°C for 24 hours and re-incubated for another 24 hours where there was no visible growth. All gram-positive rods with halo zone of egg yolk precipitation were confirmed using spore stain, catalase test, citrate test, Voges-Proskauer (V-P) test and motility. All organisms with central or ellipsoidal spore, motile, with positive V-P reaction were considered *Bacillus cereus*. *Salmonella* strains were enriched in tetrathionate broth (Oxoid), by incubating at 37°C for 6 hours before being streaked for isolation on Salmonella-Shigella agar (SSA) medium (Oxoid). Representative non-lactose fermenting colonies were streaked on triple sugar iron Agar (TSI) and urease agar slants. The mean number of colonies counted for all count types was expressed as log colony forming units (CFU). *Escherichia coli* type 01 was detected by adding 1 ml of the 10^{-1} dilution to 10 ml of each of two single Strength MacConkey Broth (Oxoid) tubes, and incubating at 37°C for 24 hours. A HEC ™ kit was also used to detect *E.coli*. A loopful from each culture showing production of acid and gas was transferred to Brilliant Green Bile Broth (Oxoid) and Trypptone Water (Oxoid) tubes which were then incubated at 44°C for 24 hours. The production of gas in the Brillant Green Bile Broth and a positive indole reaction in the Tryptone Water indicated the presence of *E.coli* 1 (SABS, 1975). Yeast and Mould moulds were determined in Potatoes dextrose Agar by plating 0.1 ml of appropriates dilutions, and incubating at 37°C for 72 hours. *Staphylococcus* strains were isolated on Baird Parker Agar (BP Oxoïd) incubated at 37°C for 48 h and typical colonies stored on nutrient agar slants at 4°C for Biochemical studies.

Biochemical tests

Coagulase and haemolysin production by the staphylococcal strains and Enteroxin detection: The tubes coagulase test was done on an overnight culture in nutrient broth using human phase and incubated at 37°C. The procedure describe by Baer *et al.*, (1976) and test interpretation of Sperber and Tatini (1975) were used. Sterile broth was added as negative control. The ability of Staphylococcal isolates to produce haemolysin was determined on human blood agar plates. Interpretation of the haemolytic pattern was as described by Freney, *et al.*,

(1999). Coagulase positive Staphylococcus isolates were grown on Brain Heart Infusion Agar (Oxoid) plates using the cellophane over agar method of Robbins *et al.,* (1974).The growth was harvested with 2.5 ml of 0.1 ml disodium hydrogen phosphate (0.1 mg Na_2HPO_4), centrifuged at 2000rpm and supernatant used for enteroxin A microslide gel double diffusion assay (Macmillan, 2014).

Statistical analysis

All statistical analysis were computered, by one-way analysis of variance and student's *t*-test of S-Plus 2000 program where appropriate, to determine if significant differences exist between mean counts. The multiple range test was carried out to determine the level of significance (Montgomery, *et al.* 2009).

Results and Discussion

Mean values for the different microbial counts

The mean aerobic counts and *E.coli*, of roasted beef meat, Fried pork meat and roasted chicken for all street-vended meat samples from mobile food sellers (MFS), and stationary food seller (SFS) were no significantly different from one to another (P>0;05). However, all the counts were higher than the permissible level of count (5.0 $\log_{10}$/g) for cooked foods.

Foodborne pathogens

Of 200 samples collected, 60 (30%) were contaminated with *E.coli*, 46 (23%) were contaminated with *Bacillus cereus*, 38 (19%) with *Staphylococcus aureus*, 30 (15%) with *Salmonella sp*. and 10 (5%) with yeast and moulds. The mean of *Staphylococcus aureus*, *Salmonella sp*, where found to be higher for Mobile Food Sellers (MFS), than for Stationary Food Seller (SFS): The sun-dried beef meat sold by Mobiles Food Sellers (MFS) had a significantly higher *Staphylococcus aureus* mean count (3.7±0.2 $\log_{10}$/g) than that sold by Stationary Food Sellers with or without shelter (SFS) (1.3±0.2 $\log_{10}$/g) ($p<0.05$). Similarly, the Fried pork meat sold by Stationary Food Seller (SFS) and Mobile Food Sellers (MFS) had *Salmonella sp* count that is significantly different from each other ($P<0.05$) with the fried pork meat sold by SFS having no *Salmonella sp.* isolated, were that from MFS having wide hight amongst of *Salmonella sp* (2.4±0.7 $\log_{10}$/g) (Table 2). However, all the counts were lower than the permissible level of counts (5.0 $\log_{10}$/g) for cooked ready-to-eat foods (Speck, 1976).

Of the 26 coagulase positive *Staphylococcus aureus* isolates tested, 17 (53.12%) were haemolytic of which 12 were α- haemolysis and 5 β-haemolysis using human blood and 7 (26.92%) produced enterotoxin A (SEA) (Table 3). 15 (46.87%) haemolitic coagulase positive *Staphylococcus aureus* were isolated from Mobiles Food Sellers (MFS), were only 2 (6.24%) come from Stationary Food Sellers with or without shelter (SFS). We only noticed that 5 *Staphylococcus aureus* isolates from Mobiles Food Sellers (MFS) samples, produced enterotoxin A (SEA) when only 2 isolates from Stationary Food Sellers with or without shelter (SFS), produced enterotoxin A (SEA) (Table 3).

Listeria monocystogenes, *Vibrio cholerae* and *Yersinia enterocolitica* were also tested for in the street-vended meat samples but not detected. A non-0157:H^+ *Escherichia coli* strain were each found in one fried pork meat sample; this were detected only on MacConkey-Sorbitol agar plates and not detected in the same sample by using HEC ^{TM} kit.

High incidences of foodborne pathogens can be attributed to cross contamination from environmental source and to handling by the vendors during holding (Bryan *et al.,* 1988; Fenlon, *et al.*,1996 ; Gorman, *et al.*,2002). The environmental condition under which street vendors worked, as well as their food-handling practices, is no different from those observed in other countries (Bryan *et al.*,1997, Bryan *et al.*,1998; Kusumaningrum, *et al.,* 2003). In most cases, running water is not available at the vending sites and hand and dishwashing are usually done in one or more buckets or pans of water, sometimes without soap. The water is not only used for dish washing, but also for cleaning of the meat preparation areas and for hand washing by vendors or their customers before and after eating. Vendors also commonly washed their hands in the dish water when returning from toilets or ablutions (main town of the region are predominantly Moslems). This is reflected by high bacterial counts as well as the relatively high incidence of *E. coli*, and *Salmonella sp.* in stationary ready-to-eat meat samples. Waste water and garbage are discarded in streets providing food and harbourage for insects and rodents. Food are usually not effectively protected from dust and flies witch may harbour foodborne pathogens and safe food storage (Förster, *et al.,* 2007). Additional bacterial contamination of raw materials may occurred at the vending sites during cutting and chopping (Mosupye, & von Holy, 2000). In this regard, it was observed that raw meat and poultry as well as ingredients from "kilichi" production (groundnuts paste, special spices…etc) were cut, chopped using the same materials without in-between cleaning, resulting in cross-contamination between different raw materials and products. Literature survey, indicate that *Bacillus spp*, and *Staphylococcus spp* are usually found in the environment and on people's hands (ICMSF, 1998; Kampf, & Kramer, 2004 ; Oliver, *et al.,* 2009).

In the present study, from around collected 200 samples, 60 (30%) were contaminated with *E.coli*, 13 (6.5%) were contaminated by *Bacillus cereus*, 20 (10%) with *Staphylococcus aureus*, 32 (16%) with *Salmonella sp*. and 7 (3.5%) with yeast and moulds. *Bacillus cereus*, and *Staphylococcus aureus* counts as high as 7.0 log cfu/g each, were reported in meat ball samples in a study carried out in Zambia (Bryan *et al.,* 1997). The fact that some of these pathogens especially *Salmonella sp* were only detected following enrichment may be an indication that they were present in the food at low levels. In the same idea, the fact that the non-0157: H^+ *E. coli* strain was only isolated by the conventional method and not the HEC ^{TM} kit, may be an indication of high specificity of the HEC ^{TM} kit for *E. coli* 0157: H7. Because this *E. coli* strain was not further characterised to determine its serotype, it was not possible to in indicate how its presence might affect the safety of the food. However, several non-0157 strains of *E.coli* are reportedly capable of producing toxins harmful to humans (Louie *et al.,* 1998; Moxley, 2004).

The predominance of *Bacillus* isolates on all products could be related to the presence of spores in the raw materials like meat, and ingredient (spices, onion, pepper,…etc). These heat-resistant spores may have survived cooking while vegetative bacteria were eliminated. *Staphylococcus spp*. common environmental bacteria and non-0157: *E. coli* could thus have been introduced into the food after cooking through cross-contamination, for instance from utensils, vendors hands when touching food, dishcloths, or the water during dish washing or hand washing.

The stationary ready-to-eat meat sellers start cooking operation at about 08:00PM when the meat (beef and pork) is available and sell between 10:00PM to 23:00PM in the night depending on the meat product. The mobile ready-to-eat meat sellers begin preparation procedures at the same time but sell between 10:00PM to 18:00PM (This concern especially "Soya" and "Kilichi"). Street-vended meat from mobile ready-to-eat meat sellers is prepare only in the morning and held at ambient temperatures over street selling. As a result, theses street-vended meat had higher bacterial counts than those from Stationary Food Sellers with or without shelter (SFS) which continuously reheat their products. In addition, ready-to-eat meat from Stationary Food Sellers with or without shelter (SFS), were mostly serve with vinegar and consequently contained lower bacterial count, due to acidification effect of vinegar (Rodrigo, *et al.*,1999; Karapinar, & Gönül, 1992). In the light of the observed activities during preparation and handling procedures of the raw materials at the vending sites, it was not surprising that these bacterial groups were predominant in mobile ready-to-eat meat sellers than these of stationary ready-to-eat meat sellers for all types of samples. Bacterial counts in the street ready-to eat meat analysed in here is as much as than those reported in similar studies carried out elsewhere (Bryan *et al.*, 1997). A study carried out in the Dominican Republic reported aerobic mesophilic counts of between 5 and 9 log cfu/g in street-vended fried fish, chicken, beef, meat stews and rice (Bryan *et al.*, 1988). Aerobic mesophilic counts as high as 8 log cfu/g were reported for street-vended rice and mesophilic counts as high as 7 log cfu/g for cooked ground meat sold on the streets in Pakistan (Bryan *et al.*, 1992). In Zambia and Nigeria, aerobic mesophilic counts of 9 log cfu/g were reported for street-vended meat, chicken, and meat balls (Ekanem, 1998, Bryan *et al.*, 1997). However, other study carried out in Nigeria and Ghana, bacterial counts of street food samples were lower and did not exceed 4.7 log cfu/g (Umoh *et al.*, 1999). Studied conducted in other countries reported also high counts of bacterial pathogens, like in Pakistan where amongst of *Staphylococcus aureus*, *Bacillus cereus* and *E.coli* found were respectively 5.0, 5.0 and 6.0 log cfu/g in street-vended foods (Bryan *et al.*, 1992). However bacterial counts of these, ready-to-eat meat, especially those from stationary ready-to-eat meat sellers, are lower than those found in literature for other street-foods (Bryan *et al.*, 1997, 1998, Ekanem, 1998), this could be the result of high cooking temperatures, which exceed 80°C and the continuous cooking process and therefore, are adequate to kill vegetative forms of most bacteria, including foodborne pathogens. Bacterial spores could, however be expected to survive these temperatures (Bryan *et al.*, 1992). Although some of theses meat product are served with vinegar and onions which are reported to have inhibitory effects on bacterial growth (Bryan *et al.*, 1992; Entani *et al.*, 1998)

Based on theses results, it could be concluded that, the quality and safety of all street-vended meat analysed in this study was considered to be acceptable regarding other streets foods. This could be attributed to adequate cooking and /or short holding times which apparently compensated for the observed shortcomings in environmental and personal hygiene as well as food preparation practices which led to cross contamination. Consequently, this study highlighted that the production of relatively safe street-vended foods with low bacterial counts is possible in Africa even under poor conditions of environmental and personal hygiene and lack of sanitary facilities, provided attention is paid to the relevant critical control points.

Table.1 Aerobic Plate count and Yeast and Mould count of street ready-to eat meat by category of sellers

Samples	Microbial count (in X log_{10}/g)			
	Mobiles Food Sellers (MFS)		Stationary Food Sellers with or without shelter (SFS)	
	Aerobic Plate count	Yeast and Mould	Aerobic Plate count	Yeast and Mould
Roasted beef meat, n=80	6.1±0.9	nd	5.9±1.9	nd
Sun-dried beef meat, n=60	5.7±1.2		5.3±0.7	
Fried pork meat, n=40	7.3±1.4	nd	6.8±1.3	nd
Roasted chicken, n=20	5.6±1.7	nd	5.1±0.6	nd

nd: not detected; n: samples number; Mean yeasts and moulds species were only found in sun-dried beef meat samples and never exceeded 7.5 (±0.5) log_{10} spores/g.

Table.2 Presence of *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* of street ready-to eat meat by category of sellers

Samples	***Bacillus cereus***		***Staphylococcus aureus***		***Escherichia coli***	
	Mobiles Food Sellers (MFS)	**Stationary Food Sellers with or without shelter (SFS)**	**Mobiles Food Sellers (MFS)**	**Stationary Food Sellers with or without shelter (SFS)**	**Mobiles Food Sellers (MFS)**	**Stationary Food Sellers with or without shelter (SFS)**
roasted beef meat n=115	2.1±0.1	1.8±0.6	nd	nd	3.3±0.4	2.8±0.7
sun-dried beef meat n=80	3.7±0.2	2.0±0.9	1.3±0.2	1.8±0.7	5.1±0.2	3.2±0.5
Fried pork meat n=70	3.3±0.4	2.4±0.7	1.0±0.1	nd	4.1±0.3	3.4±0.7
roasted chicken n=35	2.6±0.7	1.0±0.4	1.1±0.7	nd	2.6±0.7	1.2±0.6

Table.3 Enterotoxigenicity of coagulase positive staphylococcal isolates from street ready-to eat meat by category of sellers

Source of Isolate	Number of samples contaminated			
	Mobiles Food Sellers (MFS)		Stationary Food Sellers with or without shelter (SFS)	
	Haemolitic positive	SEA positive	Haemolitic positive	SEA positive
roasted beef meat n=115	nd	nd	nd	nd
sun-dried beef meat n=80	7	3	2	1
Fried pork meat n=70	5	1	nd	1
roasted chicken n=35	3	1	nd	nd
Total= 26 coagulase positive	15 haemolitic	5 produced enterotoxin A	2 haemolytic	2 produced enterotoxin A

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