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Microbial analysis and factors associated with contamination of ready-to-eat chili pepper sauce in Buea municipality, Cameroon

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Chili peppers sauce is a dietary complement largely consumed in Cameroon. It is consumed in a powder or wet (pepper sauce) form or directly introduced into cooked food. In this study, the microbiological quality of chili pepper sauce used as food complement in the Buea municipality was assessed. The study was an observational and cross-sectional study involving 70 chili pepper sauce samples from food vendors. The samples were cultured on Salmonella-Shigella agar, violet red bile agar, plate count agar and the colonies isolated were enumerated and identified using the Enterosystem 18R. Factors associated with microbial count were identified using a multiple linear regression model. Bacteria isolate from chili pepper sauce were mainly *Entrobacter cloacae* (31.57%), *Citrobacter freundii (*15.78%) and *Klebsiella pneumonia* (15.78%) and other Enterococcal species*.* Factors associated with bacteria count were: age of the vendor, number of customers served, types of food and food storage conditions (covering, heating, type of storage containers). Chili pepper sauce used as food complement in Buea Municipality were contaminated with Enteric microorganisms and may represents a potential public health hazard to consumers. The presence of these microorganisms from chilli pepper sauce could result from poor handling.

Key words: Chili pepper sauce, contamination, enteric bacteria, health hazard.

INTRODUCTION

Foodborne diseases are important cause of morbidity and mortality, and a significant impediment to socioeconomic development worldwide. Billions of people are at risk and millions fall ill every year; many die as a result of consuming unsafe food (Kirk et al*.,* 2015). Unsafe food poses a global health threats and creates a

vicious cycle of diarrhoea and malnutrition, threatening the nutritional status of the most vulnerable. Every year 220 million children contract diarrheal diseases and96 000 die as a result of food contamination (Kirk et al., 2015). Where food supplies are insecure, people tend to shift to less healthy diets and consume more "unsafe foods" in which chemical, microbiological and other hazards pose health risks (WHO, 2017). The incidence of infections transmitted through food has remained largely unchanged over years (Marder et al., 2018). Foods may become contaminated by infected food handlers, water and soil or from fecal contamination.

Chili peppers are dietary complements largely consumed worldwide especially in several parts of Africa and particularly in Cameroon. This high consumption is certainly due to its taste and its nutritional benefits. Chili pepper has characteristic taste and pungency (Yazdizadeh et al., 2013). The red peppers are a source of Vitamin A, C and Folate (Davis et al., 2016). Vitamin A helps in the vision while vitamin C is a strong anti-oxidant (Yang and Xu, 2016). Fresh peppers have high quantities of ascorbic acid and carotenoid pigments such as β-carotene with pro-vitamin A activity which have proven to be effective at scavenging free radicals (Deepa et al., 2006).

In Cameroon, several peppers species have been domesticated and the most widely consumed peppers include the Chili peppers (hot and sweet peppers of the genus Capsicum), bell pepper (green pepper), Penja white pepper (*Piper nigrum*) and the Jalpeno pepper. In the Buea municipality, chili pepper is used on a daily basis to complement various foodstuffs, for example, "Eru", "Ndole", Roast fish, "Fish roll", "Scotch egg", boiled egg, chicken, "accra banana", pork, "soya", snail etc. It is a habit in Cameroon to consume foodstuff with chili pepper complement. Chili pepper is consumed fresh, dry (dusty), stewed, stir fried or moist as a dietary complement to increases flavour of most foodstuffs. However, moist pepper sauce (especially those prepared using additional ingredients such as garlic, ginger, tomatoes, onion, green spices) may provide a very conducive environment for the growth of microorganisms. Street foods sold in Buea are mostly prepared and processed manually by vendors and sold to the public. Most at times the safety requirements are ignored or not known by the vendors. These practices raise some doubts on the safety and the microbial quality of such food complement. Previous studies have shown that foodborne illnesses constitute a major health problem associated with food sold to the public (Tabashsum et al., 2013). Street vended chili sauces in Mexico were shown to be faecally contaminated, harboring sufficient enterotoxigenic *Escherichia coli* to cause an infection (Estrada et al., 2002). In this study, the microbiological quality of chili pepper sauce used as food complement in the Buea municipality was assessed and factors predisposing to contamination were investigated.

MATERIALS AND METHODS

Study design

The study was an observational and cross-sectional study in which pepper sauce samples used as food complement were randomly selected from food vendors and analysed in the laboratory for their microbiological content. Data on the sociodemographic characteristics as well as the handling and preservation of the pepper sauce was also collected.

Settings

The study was carried out in Buea, the head quarter of the South West Region of Cameroon from May to July 2018. Buea is located on the eastern slopes of the mount Cameroon and lies between latitudes 4°12'N and longitudes 9^0 12' E with surface area of 870 km^2. The total population estimated by the health population denominators (2017) revealed 169,745 inhabitants. The population is mainly made up of students and civil servants with a high density in the university surroundings. The samples were collected in Molyko, Mile 17 Motor Park and Muea market. The choice of these areas for sample collection was motivated by the fact that these areas are very prolific in restaurants, mobile and stationed food vendors.

Participants

The study population was food vendors operating in the Buea Municipality. Only those food vendors that sell foodstuff with pepper sauce supplements were approached for sample collection. A total of 70 chili pepper sauce samples were collected from 70 food vendors at the University of Buea campus and layout, Mile 17 Motor Park, Malingo and Muea market.

Sample collection

All food vendors in the study site who sold food stuff with chili pepper sauce as complement and who gave their consent were recruited into the study. Samples were collected from vendors selling boiled egg, roasted fish, snails, pork "Accra banana" "Gateau" (fish roll, fish pie, Scotch egg) and chicken. These samples were served into sterile sample collection tubes by the vendors themselves with the same utensil they use to serve their customers. A questionnaire was administered to the vendor to obtain demographic information, food types sold, mobility of the vendor, preparation, handling and preservation of pepper sauce and the number of times the sauce was heated. Observations were also made on the environmental and hygienic conditions of the food vendor. The pepper sauce samples were transported to the laboratory in a cool box for analysis.

Laboratory processing of samples

One millilitre of the pepper sauce was diluted in peptone water to obtain a uniform distribution of the micro-organisms before inoculation. A loop full of the homogenized samples was inoculated by streak plate method on Salmonella-Shigella (SS) Agar, Violet Red Bile (VRB) Agar, and the plates were incubated at 37°C for 24

Table 1. Demographic characteristics of the vendor population enrolled in the study.

Variable		Frequency	Percentage
Gender	Female	46	65.7
	Males	24	34.3
Age group (years)	11 - 20	26	37.1
	21 - 30	26	37.1
	> 30	18	25.7
Education level	Higher	2	2.9
	Primary	9	12.9
	Secondary	59	84.3

h after which they were observed for bacterial growth. In order to determine the total bacteria count, a 10-fold serial dilution was carried out on the samples and 0.1 ml of the 10^{-3} dilution was inoculated by the spread plate technique onto the Plate Count Agar (PCA) and incubated at 37°C for 24 h. Colonies were counted and recorded as colony forming units (CFU/ml).

A Gram stain was carried out for each of the colonies that grew on the plates and observed under the light microscope at 100X Objective. Colonies that were Gram negative were sub-cultured on Nutrient Agar and incubated at 37°C for 24 h to obtain pure cultures used for bacteria identification. Bacteria were identified using the Enterosystem 18R, which identifies Gram negative, Oxidase negative Enterobacteria of clinical significance. In this kit, identification is based on biochemical tests performed in wells containing specific culture media. The test kit employs 18 standardised and miniature biochemical tests. Sample collection and laboratory processing was done under aseptic conditions to prevent contamination which could introduce biases in the study.

A culture was considered positive for bacteria when growth was observed on any of the culture media used. A culture was considered positive for enteric microorganisms when positive growth was observed on VRB or SS agar and negative when no growth was observed on any of the media used in culturing. The independent variables or predictors were the demographic characteristics of food vendors and pepper handling.

Statistical methods

Demographic data from food vendor, data on the handling of pepper as well as laboratory culture results were entered in a template developed in MS Excel and analysed in SPSS version 21.0. A generalized linear regression model was fitted to investigate the relationships between vendor demographic characteristics, handling of pepper and bacteria count in chilli pepper sauce. The response bacterial count was approximated to normal distribution and normality checked before fitting the model. All tests were performed at a 5% significant level.

Ethical consideration

The protocol of this study was approved by the Faculty of Health Sciences Institutional Review Board (Ref. 2018/0241/UB/ SG/IRB/FHS) of the University of Buea, Cameroon. Prior to data collection, the purpose of the study was clearly explained to the participants. A verbal informed consent was obtained from adults whereas for the minors, a written inform consent was obtained from the guardian or parents.

RESULTS

Demographic characteristic of food vendors

Table 1 shows the demographic characteristics of food vendors from whom pepper sauce samples were collected. There were more females (65.7%) than males (34.3%). The mean age of vendors was 25. 84 ± 10.56 of which 37.1% were of ages 11 – 20 years, 37.1% of ages 21 – 30 years and 25.7% were 30 years and above. Most of the vendors had attained secondary education level (84.3%).

Characterisation of the foodstuff from which pepper sauce was collected

Table 2 shows the site of collection and the various foodstuffs from which the pepper sauce was sampled. A great proportion of samples were collected within the University of Buea Layout (37.1%), followed by Malingo street (27.1%), Mile 17 motor park (21.4%) and Muea market (14.3%). The chili pepper sample collected were complements of roasted fish (20%), snails (15.7%), "gateaux" (12.9%), boiled egg (11.4%), pork (10%), chicken (11.5%) and others. A greater proportion of the food handlers were stationary (67.1%).

Pepper sauce handling conditions

Of the 70 respondents, 52 (74.3%) reported that they prepare the pepper on a daily basis. However, 11 (15.7%) of them reported that they prepare the pepper within 2-days interval and 7 (10%) reported doing it weekly. For the heating of pepper sauce during the sale period, 30 (42.9%) did not heat pepper sauce at all. For those who heated pepper sauce, 29 (41.4%) heated it once whereas 3 (4.3%) heated it thrice during the sale period. At the time of sample collection, 9 (12.9%) pepper samples were hot and the rest were cold (87.1%). Most of the pepper sauce were stored in plastic containers

Table 2. Characteristics of the food stuff from which pepper sauce were sampled.

Variable		Frequency	Percentage
Site of food collection	Malingo	19	27.1
	Mile 17	15	21.4
	Muea Market	10	14.3
	University Layout	26	37.1
	"Accra Banana"	6	8.6
	Boiled Egg	8	11.4
	Chicken	8	11.5
	Eru	4	5.7
Food types	Fish	14	20.0
	Gateaux	9	12.9
	Pork	7	10.0
	Snails	11	15.7
	Soya	3	4.3
Mobility of vendor	Mobile	23	32.9
	Station	47	67.1

(54.3%), followed by food storage jar (22.9%), dishes (14.3%) and pots (4.3%). Some of the storage containers were covered (58.6%) while others were not (41.4%) (Table 3).

Isolation and enumeration of bacteria from pepper

The percentage bacterial growth on the various culture media was as follows: On PCA, 95.7%, 68.6% on SS agar and 74.3% on VRB agar. The bacterial colony count on PCA agar ranged from 1×10^3 to 3×10^5 CFU/ml.

Bacterial speciation

Table 4 shows the bacteria identified in chili pepper sauce using the Entrosystem 18R kit. A total of 20 isolates randomly selected from different culture media were identified using the Enterosystem 18R. The most prevalent bacterial identified were *Entrobacter cloacae* (31.57%), *Citrobacter freundii* and *Klebsiella pneumonia* (15.78%). Other bacterial identified were *Serratia liquefaciens, Enterobacter alvei, Enterobacter aerogenes, Proteus mirabilis, Salmonella spp.* and *Escherichia coli*.

Factors associated with bacterial count in pepper sauce

It was observed that the bacterial count was significantly associated with the vendor's age, types of food, pepper storage and handling conditions (Table 5).

For the food types, bacterial count was significantly low in pepper sauce used to sell pork (p=0.041) compared to cow meat. Pepper stored in flask and plastic containers had significantly (P< 0.001) lower bacterial count than that stored in pots. Pepper stored in uncovered containers had a significantly higher bacterial count compared to pepper store in covered containers (p<0.001). Similarly, the bacterial count was significantly higher (p<0.001) in pepper heated once or two times compared to pepper sauce heated 3 times per day. There was a significant positive relationship between the bacterial counts and the number of persons requesting for pepper (p=0.001) and a negative relationship (P<0.001) between the age of the vendor and the bacterial count. The microbial count decreased with the increase in the age of the vendor.

DISCUSSION

Seven species of entrobacteria were isolated from chili pepper sauce including *Enterobacter* spp. *(E. aerogenes, E. alvei, E. cloacae), P. mirabilis, C. freundii, S. liquefaciens, E. coli, K. pneumonia* and *Salmonella* ssp. Similar organisms were isolated in food in previous studies (Powers et al., 1975, Filiz, 2001). The bacterial isolates were all members of the *Enterobacteriocea* family since the bacteria identification kit used in this study was for Enterobacteriocea. This group of organisms includes several species of bacteria that cause primary infections of the human gastrointestinal

Table 3. Characteristics of the pepper sauce at the time of sample collection.

Variable		Frequency	Percent
Food sold in a clean environment	No	16	22.9
	Yes	54	77.1
Periodicity of pepper preparation	Daily	52	74.3
	Two days	11	15.7
	Weekly	7	10.0
Types of storage container	Bottle	3	4.3
	Dish	10	14.3
	Food jar	16	22.9
	Plastic container	38	54.3
	Pot	3	4.3
Pepper sauce covered	No	29	41.4
	Yes	41	58.6
Heating of pepper sauce during sale period	No	30	42.9
	Yes	40	57.1
No. of times pepper sauce is heated per day	One time	29	41.4
	Two times	8	11.4
	Three times	3	4.3
Pepper temperature at time of sample collection	Cold	61	87.1
	Hot	9	12.9

Table 4. Bacterial species isolated from pepper cultures.

SN	Species	Media	Frequency	%
1	*Enterobacter aerogenes*	VRBA	1	5.26
2	*Enterobacter alvei*	VRBA	1	5.26
3	*Entrobacter cloacae*	VRBA/SSA	6	31.57
4	*Proteus mirabilis*	SSA	1	5.6
5	*Citrobacter freundii*	VRBA/SSA	3	15.78
6	*Serratia liquefaciens*	VRBA	2	10.52
7	*Escherichia coli*	SSA	1	5.26
8	*Klebsiella pneumonia*	SSA	3	15.78
9	*Salmonella species*	SSA	2	10.52

SSA, Salmonella Shigella Agar; VRBA, Violet Red Bile Agar.

tract infections including septicaemia, pneumonia, meningitis and urinary tract infections. The presence of enteric bacteria in food may indicate faecal contamination probably as a result of poor hygiene practice by the food handlers. These findings clearly indicate that chili pepper sauce used as food complement may represent a potential public health hazard to the consumers. Bacterial counts of up to 3×10^5 per millilitre of chilli pepper sauce are indications of the load of bacterial people are exposed to when consuming the pepper

sauce. The fact that only few vendors heat pepper after preparation could account for the high bacterial count observed in some samples. In fact the bacterial count was significantly associated with number of times pepper was heated. Pepper samples that were heated a least three times a day had less bacterial counts than those that were not heated at all or once per day after preparation. But the fact that these heated samples were still contaminated probably suggests that these pepper sauces were improperly handled after heating. This raises

Table 5. Relationship between food vendors demographic characteristics, food types, pepper handling and bacterial count.

Variable		Coefficient	Standard error	Wald Chi-Square	P-value
Age of food vendor	Age	-2.46	0.631	15.21	0.000
No. of customer requesting pepper per day	Pepper request	1.24	0.253	24.05	0.000
Site of sample collection	Malingo street	46.58	17.80	6.85	0.009
	Mile 17 motor park	31.96	22.54	2.01	0.156
	Muea market	38.13	26.59	2.06	0.152
	University layout	0	.	.	.
Food types	Accra banana	-87.10	48.60	3.21	0.073
	Boiled egg	-46.01	36.43	1.60	0.207
	Chicken	15.08	41.09	0.14	0.714
	Eru	8.59	49.08	0.03	0.861
	Fish	-23.86	47.29	0.26	0.614
	Gateaux	22.78	41.21	0.31	0.580
	Pork	-126.33	38.14	10.97	0.001
	Snails	-97.71	51.85	3.55	0.059
	Soya	0	.	.	.
Types of storage container	Bottle	-171.42	34.14	25.21	0.000
	Dish	-28.24	27.14	1.08	0.298
	Flask	-196.15	25.62	58.64	0.000
	Plastic container	-113.921	25.77	19.54	0.000
	Pot	0	.	.	.
Pepper sauce covered	No	37.89	10.57	12.86	0.000
	Yes	0	.	.	.
No. of time pepper was heated per day	One time	177.04	25.14	49.61	0.000
	Two times	195.85	26.42	54.96	0.000
	Three times	0	.	.	.

a general hygiene problem as far food handling is concerned

This study also investigated the factors that accounted for high bacterial count in pepper sauce. It was observed that the bacterial count was associated with demographic characteristics of the vendors and handling of food. The bacterial count was associated with education level of the vendors. More educated vendors are much more aware of food hygiene compared to less educated vendors. This result is in line with those of findings of Ackah et al. (2011), and Feglo and Sakzi (2012) in Ghana, which reported that low levels of education among food vendors contributed greatly to the increase of food contamination.

This studied revealed that the type of storage containers affects bacterial count. The microbial count was significantly higher in opened containers than closed ones. Covering of pepper sauce had an impact on the bacterial count but not on the contamination since samples from covered and uncovered were equally contaminated with the only difference being the number

of CFU counted in both situations. Covering of pepper therefore did not prevent contamination of the sauce. The process of pepper preparation as reported by vendors involves boiling which may kill potential microbial contaminants. It is likely that contamination of pepper observed in this study occurred during post handling process. The presence of faecal contamination indicators like *E. coli*, *Klebsiela* and *Salmonella* species could be as a result of poor hygiene and/or poor handling of the food. This is in line with findings of Gallo et al. (1992), who reported faulty food handling, especially storage of food at improper temperatures for long periods of time. Ghosh (2009) also reported an increase prevalence of microbial contamination as a result of poor hygiene and sanitation practices.

A greater proportion of the vendors heated pepper at least once per day while others did not. Among those who heated pepper, the frequency of heating per day also varied (once, twice, and thrice). The frequency at which the pepper was heated had an influence on the

microbial count of the pepper. Samples that were heated three times a day had significantly low bacterial counts than those heated once or twice a day. This finding is in line with a study by Schwab et al. (1982).

The type of storage container also played an important role in chili pepper sauce contamination. Bacterial counts were higher in pepper stored in pots and dishes compared to pepper stored in bottles. Environmental and personal hygienic as well as food handling practices of the vendor can increase the level of microbial contamination of food. This study revealed viable bacterial counts with values ranging from 1×10^3 to 3×10^5 CFU/ml. The Centre for Food Safety and EHD Microbiological Guidelines for ready to eat food (Centre for Food Safety, 2014) classified food into satisfactory, borderline and unsatisfactory based on bacterial load in food. Based on these guidelines, the viable bacteria count revealed by this study makes the pepper sauce unsatisfactory for consumption.

Chili pepper sauce used to complement all sampled food types were contaminated. The only difference between the various food types was the bacteria count which varied significantly from one food type to another with highest counts observed in pepper sauce used to complement chicken and gateau, whereas the least count was observed in pepper sauce used as complement for pork and snails.

Conclusion

More than 95% of pepper samples examined were contaminated with bacteria. Microorganisms isolated from chili pepper sauce include: *Enterobacter* spp*. (E. aerogenes, E. alvei, E. cloacae), P. mirabilis, C. freundii, S. liquefaciens, E. coli, K. pneumonia* and *Salmonella* ssp. The presence of theses microorganisms in chili pepper sauce suggest food contamination which could be potentially cause disease in humans. The age and level of education of the vendor, handling of pepper sauce after preparation (heating, covering and storage) were factors that significantly influence bacterial count. The identification of bacteria in this study was limited to enteric microorganisms. Further studies that will check a wider scope of microorganisms will give a clearer picture of the microbial contamination of this highly consumed food complement.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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