

Research Article

Microbial Quality of Soups Served With "Tuo-Zaafi" Vended in the Central Business District of the Tamale Metropolis

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Abstract

Food safety have become topical across the globe, especially in developing countries owing to an increase in street food vendors and the non-enforcement of laws governing establishment and operation of food vendors. This research determined the microbiological quality of soups served with "tuo-zaafi" marketed in the Central Business District of the Tamale Metropolis. A total of 30 samples were collected, preserved, transported and analysed in the Spanish Laboratory of the University for Development Studies. The presence, levels and antibiotics resistivity of bacteria in soup samples were determined. *Staphylococcus aureus* recorded the highest (83.3%) in soup sample with levels ranging from 0 to 9.2×10^4 cfu/ml. *E. coli* was detected in 76.7% of the soup with levels varying from 0 to 8×10^4 cfu/ml and *Salmonella* spp. (63.3%) levels ranged from 0 to 9.6×10^4 cfu/ml. *Shigella* spp. was present in 73.3% of samples with levels ranging from 0 to 9.5×10^4 cfu/ml. Mean microbial counts in "Ayoyo" and Dry Okra soups across the study area were 1.19×10^4 cfu/ml and 1.83×10^4 cfu/ml, respectively. Contamination varied significantly (p<0.001). *E. coli, Salmonella* spp. and *Shigella* spp. were more resistant to antibiotics whiles *Staphylococcus* aureus was more unsatisfactory and unsafe for consumption and posed a potential health hazard to consumers. The contamination was attributed to improper hygiene, handling and environmental conditions. The Food and Drugs Authority should strictly impose laws and ensure compliance to food safety measures.

Keywords: Soups; Ayoyo; Dry Okra; Tuo-zaafi; Tamale; *Staphylococcus* aureus; *Escherichia coli*; *Salmonella* spp; *Shigella* spp

Background

Food is an energy source which provides the needed nutrients for the human body to withstand diseases. The certainty in the safety of food supplied to customers is essential [1] and it is aimed at preventing food contamination and food poisoning [2]. Also many consumers attach great significance to hygiene whiles choosing a food vendor and its location [3]. Nevertheless, everyone has had an unpleasant encounter with foodborne illnesses in the past as a result of consuming contaminated foods. Due to this, food safety has become topical across the globe in recent times owing to the widespread of foodborne illnesses from poor handling. The food service industry has however been mostly associated with and has recorded a large number food safety issues and the international food trade has also been damaged by the increasing concerns of food safety and quality standards [1].

The business of food vending is very popular in the world especially in many evolving countries such as Ghana and has substantially reduced unemployment by offering great business opportunities through its sales as well as adequately influencing the financial system of many developing countries [3,4]. Many people satisfy their nutritional requirements by relying on the services of these vendors. It was reported by Mensah et al. [5] that, most market women in Accra rely on street foods to feed their children and this is attribute to the busy schedules of the mothers which prevent them from cooking for their children when they are busy at work. Estrada-Garcia, et al. [6] further reported that about 120,000 people were in the food vending business in Mexico.

Despite the numerous advantages of the food vending business, it still remains a serious issue and a challenge of health concern to experts and international bodies due to the unhygienic ways in which these foods are handled [7]. This is because, the outbreak of foodborne illnesses does not impact negatively only on people's health but also on the economic activities and output leading to economic loss which adversely affect countries nationwide [8,9]. In Ghana \$69 million USD

is spent yearly to deal with the outbreak of foodborne diseases [10]. United State also spends \$152 billion dollars on this same problem yearly [11]. The activities of these vendors leading to the contamination are mainly due to negligence and the non-enforcement of laws governing food safety and food vending establishments.

Food is fragile hence can be contaminated at any stage during its production, processing or cooking by bacteria. Most Bacteria associated with food contamination in general are, *Shigella* spp, *Salmonella* spp, *Staphylococcus aureus*, *Bacillus*, *Escherichia coli* among others and the most predominant bacteria in Ghanaian foods are *Enterobacter* spp., *Escherichia* spp., *Klebsiella* spp., and *Citrobacter* spp [12].

Soups served with tuo-zaafi (a local meal prepared from maize flour) thus "ayoyo" and dry okra are cooked with raw vegetables and meat which have the potential of getting contaminated if they are not properly washed and cooked. Meat is known to be a great source of proteins which aims at regulating body tissues and organs. They are needed to help the body fight bacteria and viruses however the microbial contamination of these meats has become a serious challenge deserving a greater attention.

The contamination may be from the water used in washing and cooking, production and processing sites, the utensils and equipment used. A study by Gorman et al. [13], detected *E. coli, Campylobacter, Salmonella*, and *Staphylococcus aureus* on napkins, aprons, counter

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tops, oven handles and chopping boards. Saba and Gonzalez-Zorn [12], further detected in chicken, beef, pork and fish.

Research conducted in Ghana on bacteria contamination recorded unwholesome levels. A review by Yeleliere, et al. [14], observed various levels of bacteria in foods in some capital cities in Ghana. Abakari et al. [15] and Amoah [9] on the other hand recovered various levels of bacteria from sampled salad in Tamale and Kumasi. Among these bacteria recovered were *Escherichia coli, Shigella* spp, *Salmonella* spp, *and Staphylococcus aureus* among others. Mensah et al. [5] analysed 511 foods in Accra and reported the presence of *Shigella sonnie*, *Staphylococcus aureus, and E. coli* and *Bacillus cereus*.

Though a number of studies have been done in the Tamale metropolis on non-homemade foods, not much have been done on the microbiological quality of soup accompanied with "tuo-zaafi" sold at food joints in Tamale Metropolis. This study therefore seeks to find out the microbial quality of soup served with "tuo-zaafi" in the central business district in Tamale and to provide information on the safety of soup consumed at food joints.

Materials and Methods

Study area

The study was conducted in the Tamale Metropolis specifically the Central Business District. It is located in the heart of the Tamale Metropolis where almost all the major business activities take place. The capital city of the Northern region and the fourth largest city in Ghana. The Metropolitan area covers a total area of about 750 km² and lies geographically between longitudes 00°51′12′′ and 0.85333° west and latitudes 09°24′27′′ and 9.40750° north. The Metropolis has a total landmass of 646.90180 km. As a result of the availability of vast agriculture lands, the rural communities take advantage and engage massively in agriculture and thus agriculture has been a major economic activity and serves as a food basket for the Metropolis (Figure 1).

Sample collection

Stratified Random sampling and Convenient sampling were implored based on the location of the food joint. The study area was divided into four zones (Zone 1-4) in order to obtain representative samples. Zone 1 included areas such as (Gumbihini, Moshie Zongo, Park and Garden and its environs). Zone 2 included (Tishigu, Taxi run, Main Bolgatanga station, Changyili), also zone 3 included (Transport Yard, Timber Market, Nyohini) and, zone 4 constituted (Zhongli, Aboabo, Sabonjida). In total, thirty (30) soups samples ("ayoyo" and dry okra) vended on the street in the Central Business District of the Tamale Metropolis were collected from January, 2016 to March, 2017 between 9:00 am to 11:00 am using stratified random sampling procedure. Based on the number of tuo-zaafi vendors in each zones demarcated, eight (8) samples each were taken from both zone 1 and 2 and seven (7) samples each were taken from zone 3 and 4. The samples were aseptically kept in ice chest with ice and transported for microbial analysis at the Spanish Laboratory complex of the University for Development Studies, Nyankpala campus, Ghana processed within 2-4 hours for microbial analysis.

Microbial analysis and culture conditions

The various media were prepared as indicated by the manufacturer's protocol. The media used in this analysis included, Nutrients Agar (Oxoid CM003), Plate Count Agar (Oxoid CM0325), Xylose Lysine Deoxycholate Agar (Oxoid CM0469), Levine Eosin-Methylene Blue Agar (Oxoid CM0069) and Mannitol Salt Agar (Oxoid CM0085). They were used for the growth, isolation, maintenance and sensitivity test of bacteria.

Preparation of soup samples

Twenty-five milligrams (25 ml) of each soup sample was measured into a sterile bag under a laminar flow hood (Labcaire, UK). 225 ml of peptone water was mixed with the 25 ml soup in each sterile bag (Plate 3.2). A uniform mixture was obtained by constantly shaking



and simple "hand massaging". Ten (10)-fold serial dilutions were also carried out at four levels. Specifically, 0.1 ml each of 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴ were taken aseptically under the laminar flow hood and inoculated on a solidified Levine Eosin-Methylene Blue Agar and Mannitol Salt Agar. One milligram (1 ml) of each the solution were then pipetted into a 9 ml Rapaport solution and 0.1 ml was taken from each of the 10 ml solution and inoculated on a solidified Xylose Lysine Deoxycholate Agar. The inoculated plates were then inverted and incubated at 37°C for 24 hours. After the incubation period, bacteria colonies were then identified based on colony colour. A growth of large, greenish shiny colonies was depicted as Escherichia coli on Levine Eosin-Methylene Blue. Growth of pink red colonies with black centres were identified as Salmonella spp and pink red growth without black centres were depicted as Shigella spp. on Xylose Lysine Deoxycholate Agar and the golden yellow growth were depicted as Staphylococcus aureus on Mannitol Salt Agar.

Antibiotic resistivity test

Kirby Bauer Disk Diffusion Method was done. The antibiotic disks used included, Tetracycline (TE₃₀), Ampicillin (AMP), Amoxillina+Acid Clavulanic (AMC), Amoxicillin (AMX), Ciprofloxacin (CIP₅), Gentamicin (GM₁₀), Ceftazidime (CAZ₃₀), Cefprozil (CPR₅), (CHI₃₀), Chloramphenicol (CHL₃₀), Metronidazole (MTZ), Oxacillin (OX₅), Penicillin (PEN), Cefoxitin (CXT), Clindamycin (CLN), Erythromycin (ERY) and Tretacilina (TET). A solidified Nutrient Agar plates were spread with bacteria and paper disks of antibiotics were added and incubated for 24-48 hrs. After incubation, the zones of inhibition were measured and recorded.

Data analysis

Microsoft excel software and SPSS version 22 were used for descriptive statistics thus finding means, standard deviation, developing graphs, tables, frequencies and Percentages. GenStat (ANOVA) was also used to determine the differences among the microbial counts and levels of "ayoyo" and dry okra and the significant differences of mean microbial counts among the four zones.

Result

Prevalence of bacteria isolated in the soup

"Ayoyo" and Dry Okra go through a series of handling and preparation before they are served to consumers. The present study shows variation in bacteria presence and levels in the soups obtained from the Central Business District of Tamale which was attributed to different hygienic practices by the vendors. Out of the 30 soup samples collected, 25 (83.3%) samples recorded positive occurrence of *Staphylococcus aureus* (Table 1). 23 (76.7%) samples recorded positive results for *Shigella* spp whilst 19 (63.3%) samples that recorded positive occurrence of *Salmonella* spp (Table 1).

The mean count of bacteria in the soup samples

The isolates obtained and the mean bacterial count of the various

Bacteria	No. of Samples(+)	Percentages (%) 76.7	
Escherichia coli	23		
Salmonella spp	19	63.3	
Shigella spp	22	73.3	
Staphylococcus aureus	25	83.3	

 Table 1: Summary of prevalence of bacteria isolated on the 30 soup samples collected.

bacteria in the soup samples were expressed as Cfu/ml. Table 2 presents the results of bacteria isolates with their mean values and standard deviation. *Escherichia coli* count from the soup samples ranged from 0 to 8×10^4 cfu/ml with a mean of $3.71 \times 10^4 \pm 2.39 \times 10^4$ cfu/ml (Table 2). *Salmonella* spp. count from the soup samples ranged from 0 to 9.6 $\times 10^4$ cfu/ml with mean $2.11 \times 10^4 \pm 2.77 \times 10^4$ cfu/ml (Table 2). The table also shows *Shigella* spp count ranged from 0 to 9.5×10^4 cfu/ml and a mean of $4.14 \times 10^4 \pm 2.7 \times 10^4$ cfu/ml (Table 2). And *Staphylococcus aureus* count ranged from 0 to 9.2×10^4 cfu/ml with a mean of $3.79 \times 10^4 \pm 2.22 \times 10^4$ cfu/ml.

A summary of the comparism in terms of bacteria mean counts and levels across the zones are shown in Figure 2 below. Zone 2 recorded the least *Salmonella* contamination with a mean of 1.61×10^3 whiles Zone 4 recorded the highest *Salmonella* contamination with mean of 3.93×10^4 (Figure 2). In terms of *E. coli* contamination Zone 3 recorded the highest with mean count of 5.11×10^4 and Zone 1 recorded the least with a mean count of 2.69×10^4 cfu/ml. *Staphylococcus aureus* contamination recorded the higest mean count of 4.85×10^4 cfu/ml in Zone 3 and recorded the least mean count of 2.70×10^4 cfu/ml.

Statistically, *Salmonella* spp., *Staphylococcus aureus*, *Shigella* spp and *E. coli* contamination varied significantly (p<0.05) across the sample site (p<0.001) and the venders (p<0.001) (Figure 2).

Mean of microbes in "Ayoyo" and Dry okra soups

The total mean of microbes in "Ayoyo" and Dry Okra soups are shown in Figure 3. The total microbes in ayoyo recorded the highest with a mean count of 1.91×10^4 cfu/ml whiles the total microbes in dry okra recorded the least with a mean of 1.83×10^4 cfu/ml.

Antimicrobial disks on bacteria isolates

The bacteria and their percentage of inhibitions are shown in Figure 4. The zones of inhibitions recorded ranged from 6 to 35 mm. *Shigella* spp. recorded the highest zones of inhibitions with ciprofloxacin recording the highest diameter of 35 mm. *Staphylococcus*

Bacteria	Minimum (cfu/ml)	Maximum (cfu/ml)	Mean (± StD) cfu/ml
Escherichia coli	0	8×10 ⁴	$3.71 \times 10^4 \pm 2.39 \times 10^4$
Salmonella spp	0	9.6×10⁴	2.11×10 ⁴ ± 2.77×10 ⁴
Shigella spp	0	9.5×10⁴	4.14×10 ⁴ ± 2.7×10 ⁴
Staphylococcus aureus	0	9.2×10⁴	3.79×10 ⁴ ± 2.22×10 ⁴

StD: Standard Deviation

Table 2: Microbiological assessment of soup samples collected.



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aureus recorded the second highest of inhibition zones which 30 mm. Ciprofloxacin recorded the highest inhibition zone on *E. coli, Shigella* spp, *Stapylococcus aureus* and *Salmonella* spp. *E. coli* was resistant to 80% and susceptible to 20% of the antibiotics (Figure 4). *Salmonella* spp was resistant to 60% and susceptible to 40% of the antibiotics (Figure 4). *Shigella* spp. and *Staphylococcus aureus* were resistant to 65% and 59% of the antibiotic and susceptible to 35% and 41%.

Discussion

Occurrence of bacteria isolates in soup samples

The variation and high load in presence and occurrence of bacteria in this study may be attributed to different hygienic practices and improper observation of hygienic measures. It may also be associated to the use of contaminated water in the washing and cooking of raw materials and the environmental conditions at the both preparation and retailer outlet. This was confirmed by observation which revealed that, most vendors use water stored in tanks, from dams, and boreholes which could have been contaminated with faecal matter.

Escherichia coli: The presence *E. coli* in food samples indicates the faecal coliforms contamination and poor hygiene [16]. Though some strains are non-pathogenic and harmless, other strains are highly infectious and capable of causing diseases such as diarrhoea, dehydration and death [17,18]. Adams and Moss [19] established that, *E. coli* can cause diarrhoea in children. In a related study, Saba and

According to the Centre for Food Safety [21] Guidelines, *E. coli* counts <20 are regarded as satisfactory for consumption, *E. coli* counts of 20 to $\leq 10^2$ are regarded as being on the borderline whereas *E. coli* counts >10² are considered unsatisfactory. Per this guideline, the twenty-three (23) samples representing 76.7% that detected positive *E. coli* contamination were categorised as unsatisfactory and unsafe for consumption whilst the remaining 7 samples that recorded negative occurrence were considered satisfactory.

The study further revealed that there was significant difference in the means of *E. coli* between the vending site and vendors (p=0.001). This suggests that *E. coli* contamination was influenced by the location in which the samples were taken, the vendors from which the samples were taken and environmental conditions in which the foods are exposed to.

Salmonella spp: Salmonella spp. is implicated in most food borne diseases because from microbiological and epidemiological evidence even a small numbers of pathogens in foods have the potential of causing illness [22]. The present study detected the presence of *Salmonella* spp. in soup in the business district of Tamale. The detection of *Salmonella* spp may be due to poor hygiene practices [16] and cross contamination of food. the was reaffirmed by observations which revealed that, most of the vendors wash their hands in a basin of water which may get contaminated due to the number of people who use the same basin of water in washing their hands after visiting the toilet and after handling raw food. Also most of the vending premises were not kept tidy. Observation further revealed that, some of the vendors use the same ladle to serve different kind of soups at the retailer outlet.

Centre for Food Safety [21] stipulated that *Salmonella* spp. not detected in 25 g in foods are satisfactory whilst *Salmonella* spp. detected in 25 g are unsatisfactory. Per these guidelines, all the 63.3% soup samples that detected the positive *Salmonella* spp contamination were considered as unacceptable for consumption.

There was significant difference in the mean of *salmonella* spp across the zones and vendors (p=0.001). These indicate that *Salmonella* contamination was dependent on both the location and the vendors themselves. It also suggests that environmental conditions of the zones may be different.

Shigella spp: Shigella spp. contamination occurs in foods that are subjected to processing by hand. The bacterium is the third food borne bacterial pathogen mostly implicated in most reported diarrhoea cases [23]. Shigella spp. was detected in soup samples obtained from the business district. This is indicating improper hygiene practices, presence of flies, the use of contaminated water for washing and cooking food. Observations revealed that, flies were present in almost all the vending location. The flies may be a source of contamination because they might have rested on faecal matter and can transfer the bacterium from the faecal matter to the food.

The Centre for Food Safety [21] stipulated that, *Shigella* spp. not detected in 25 g in foods are categorized as satisfactory for consumption whilst *Shigella* spp detected in 25 g are unsatisfactory. Following this guideline, the 73.3% samples that showed the presence of *Shigella* were considered as unsatisfactory and unsafe for human consumption. The present study was in contrast with a similar study by Yeboah-Manu et

al., [24] who did not detect *Shigella* in any of the foods sampled on the university of Ghana campus.

The study further established that the mean of *Shigella* spp varied significantly across the zones (p=0.001). This implies that different factors such as environmental conditions may have contributed to the contamination in the vending location.

Staphylococcus aureus: The presence of *Staphylococcus aureus* in food indicates a greater health risk for consumers. *Staphylococcus* contaminations in food commonly occur when there is contact with food handlers' hands [21]. The present study identified the presence of *Staphylococcus aureus* in soup in the business district of Tamale.

Out of the thirty (30) soups collected, 83.3% were under the unsatisfactory category whereas 16.7% were under the satisfactory category. This was reaffirmed when compared to the guidelines provided by the Centre for Food Safety (2014), which states that *Staphylococcus aureus* counts <20 in foods are satisfactory for consumption, counts of 20 to $\leq 10^4$ are on the borderline and counts >10⁴ are unsatisfactory for human consumption.

Personal observations revealed that, most of the food handlers do not put on hair net or wear gloves when cooking or serving the food and they also blow air into the rubbers to open them up when packaging the food and also handlers do not wash their hand after handling money.

The study also established that the mean of *Staphylococcus aureus* varied significantly across the zones (p=0.001). This indicates that different factors including environmental factors may have contributed to the contamination at the vending location.

Occurrence of microbes in "Ayoyo" and dry okra soups

The high load of microbes in both samples could be attributed to unhygienic practices employed by these vendors. This was reaffirmed by observations which revealed that most of the vendors did not have a proper means of checking the temperature of their soups which could lead to the soups especially the meat being undercooked.

According to the Centre for Food Safety [21], Guidelines for Cooked Foods, Microbial counts $<10^2$ are described as Satisfactory, counts of 10^2 to $\le 10^4$ are on the borderline and counts $\ge 10^5$ are unsatisfactory. Per these Guidelines, all the 15 samples for both "ayoyo" and dry okra are classified as been on the borderline. This indicates that if the factors influencing the microbes in the soups are improved, there is a high probability that the food would be in the satisfactory category and if they are not improved, there is another high possibility that it would be in the unsatisfactory category (Table 3).

Interpretation of inhibition zones on bacteria isolates

Kirby Bauer disc diffusion method of antibiotic sensitivity was done as per Clinical and Laboratory Standards Institute (CLSI) and Vet lab supply guidelines using a wide panel of antibiotics. Per the results obtained, *E. coli, Salmonella* spp, *Shigella* spp and *Staphylococcus aureus* was Resistant to Ciprofloxacin and Susceptible to Gentamycin.

The resistance of the bacteria to the antibiotics means they can live and multiple in the presence of the antibiotics. This may be attributed to misuse and over exploitation of the antibiotics thus the more one uses them, the more resistant they become. According to the Centre for Disease Control and Prevention [25], bacteria becomes resistance by reducing the number of ways available for drugs diffuse through, by setting up molecular blockades and neutralizing the antibiotics rendering them harmless to the bacteria. For instance, some bacteria have adopted strategies to resist penicillin by producing enzymes called beta-lactamases that chew up penicillin.

Another strategy is to pump the antibiotics outside of the bacteria before it causes any harm. They do this by the use energy from ATP to power pumps that shoot antibiotics out of the cell. Many antibiotics also work by attaching to their target and preventing it from interacting with other molecules inside the cell. However, some bacteria change their outer structure so that the antibiotic will have no way of recognizing, attaching or binding to the bacteria they are designed to kill [25].

The susceptibility of bacteria to the antibiotics indicates that the bacteria cannot live or multiple while the antibiotics are present. The susceptibility may be attributed to the fact that the antibiotic is either properly used or not well known [26].

Conclusion

The occurrence of foodborne illness is increasing each day due to contamination by microbes. The study therefore sought to determine the microbial quality of soups sold by food vendors and the hygienic practices observed by food vendors in the Central Business District of the Tamale Metropolis. In this study, *Escherichia coli* and *Salmonella* spp were present in 76.7% and 63.3% of the soup samples with total mean of 3.71×10^4 cfu/ml and 2.11×10^4 cfu/ml respectively. *Staphylococcus aureus* was present in 83.3% of soup samples with total mean count of 4.14×10^4 cfu/ml whereas *Shigella* spp was detected in 73.3% of soup collected with a total mean count of 3.79×10^4 cfu/ml. Also statistical analysis showed that the mean counts of *Salmonella* spp., *E. coli, Staphylococcus aureus* and *Shigella* spp. varied significantly across of the study area.

Criterion	Satisfactory	Borderline	Unsatisfactory: Potentially injurious to health /or unfit for human consumption
Escherichia coli O157(and *other Shiga toxin-producing E. coli (STEC))	n.d. in 25 g	N/A	Detected in 25 g
Salmonella spp	n.d. in 25 g	N/A	Detected in 25 g
Shigella spp	n.d. in 25 g	N/A	Detected in 25 g
Staphylococcus aureus and other coagulase-positive staphylococci	<20	20 - ≤10 ⁴	>104
Hygiene indicator organism	Satisfactory	Borderline	Unsatisfactory: potentially injurious to health
Escherichia coli	<20	20 to <10 ²	>10 ²
Food Category	Satisfactory	Borderline	Unsatisfactory
Foods cooked immediately prior to sale or consumption	<10 ³	10³ to <10⁵	≥10 ⁵

*n.d.: not detected, * N/A: Not Applicable

Table 3: Guidelines on the interpretation of results for particular foodborne bacterium in ready-to-eat food in general (cfu)/ml.

The study further revealed the mean of microbes in "Ayoyo" was 1.91×10^4 cfu/ml whiles the mean count of microbes in Dry okra was 1.83×10^4 cfu/ml. The study further revealed that the bacteria isolated were resistant and susceptible to certain antibiotics with *Staphylococcus aureus* being more susceptible.

The present study revealed that soups served with T.Z preferably "Ayoyo" and Dry Okra sold in the Business District of Tamale indicates a potential health risk to consumers in terms of microbial quality. This is evident that the soups recorded bacteria contamination which made the soup unsatisfactory and unwholesome for human consumption. The contamination was attributed to unhygienic food preparation, improper food handling, cross contamination at the preparation and retailer sites as well as conducive environmental condition.

Based on the findings of this study, the following are recommended:

- The Food and Drugs Authority should strictly enforce laws and ensure compliance to food safety measures as well as providing training on hygiene and food safety to food vendors in order improve the quality standards and protect consumers in the Business District of Tamale.
- 2. Further research should be carried out on how the siting of food establishment influences the microbial quality of the soup.

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