

## Bacteriological Contaminants of Ready-to-Eat Yellow Achu Soup Sold in Bamenda Municipality

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<p><b>Corresponding Author</b> Nodem Falonne Flavie</p> <p>Pinnacle University Institute of Health and Technology Bamenda/University of Bamenda</p> <p><b>Article History</b></p> <p>Received: 21 / 12 / 2024                  Accepted: 06 / 01 / 2025                  Published: 08 / 01 / 2025</p>	<p><b>Abstract:</b> Street foods have become a major concern for public health due to their high implication in the increased incidence of food-borne diseases reasons likely due to the unawareness of most vendors on food safety and hygiene or failure to follow proper food handling and personal hygiene practices. Yellow achu soup is a palm oil/water mixture partially emulsified using Kanwa or nikih. It remains one of the most sold and highly appreciated soups used for the consumption of achu in the North-West and West region of Cameroon surely due to its traditional value and cherished taste. However, this soup is usually eaten without pasteurization therefore it is likely to be susceptible to many contaminants. This cross-sectional study carried out at Bamenda from February to March 2024 aimed to determine the bacterial contaminants of ready-to-eat yellow achu soup sold in Bamenda municipality. To achieve this, 45 yellow achu samples were collected in some randomly selected Bamenda I, II, and III restaurants using sterile containers and administered questionnaires. The samples were then aseptically transported to the Science for Life Foundation Laboratory where they were immediately inoculated onto blood agar and MacConkey agar respectively. The culture plates were then incubated at 37°C for 24 hours and bacterial isolates were identified using conventional bacterial identification techniques. Out of the 45 samples collected, 37 samples were positive for the bacteria giving a percentage contamination of 82.2%. The following organisms were identified: <i>Salmonella</i> spp (46.6%), <i>Escherichia coli</i> (11.6 %), <i>Shigella</i> spp (4.7 %), <i>Klebsiella</i> spp (4.7%), <i>Enterobacter</i> spp (2.3%), <i>staphylococcus aureus</i> (23.2%), and coagulase-negative staphylococci spp (7%). It was therefore recommended that the Minister of Public Health should organize training seminars to educate street vendors on food safety and hygiene.</p> <p><b>Keywords:</b> Foodborne Diseases, Bacteria Contamination, Sample Inoculation</p>
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### 1.1 Background of the studies

Consuming contaminated food can result in a variety of acute and chronic ailments, ranging from diarrheal sickness to various forms of cancer <sup>[1]</sup>. According to WHO estimates, 2.2 million people die each year from foodborne and waterborne diarrheal illnesses combined, with 1.9 million of those deaths occurring in children <sup>[2]</sup>. In many regions of the world, there is still

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a chance of severe food poisoning outbreaks connected to street foods, with microbiological contamination being one of the biggest issues <sup>[1]</sup>. Street meals are known to pose a serious danger to health due to food-borne diseases, with the risk largely varying depending on the food's kind, preparation, and storage <sup>[3]</sup>. Yellow achu soup being usually eaten without pasteurization, it is therefore likely to

be susceptible to many contaminants. This seminar write-up presents a research proposal that aims to detect and identify the microbiological contaminants on this soup and the possible factors affecting this contamination.

Foodborne illnesses are those brought on by consuming food or water tainted by poisons, pathogenic microbes, or physical or chemical factors [4]. Although it is challenging to assess the prevalence of food-borne illness worldwide, it has been stated that diarrheal illnesses claimed the lives of 2.1 million people in 2000 alone [5]. Consuming contaminated food can result in a variety of acute and chronic ailments, ranging from diarrheal sickness to various forms of cancer [1]. According to WHO estimates, 2.2 million people are killed each year by food-borne and water-borne diarrheal illnesses combined, with 1.9 million of those deaths occurring in children [2]. In many regions of the world, there is still a chance of severe food poisoning outbreaks connected to street foods, with microbiological contamination being one of the biggest issues [1]. Street meals are known to pose a serious danger to health due to food-borne diseases, with the risk largely varying depending on the food's kind, preparation, and storage [3].

An occurrence where two or more persons have a similar illness after consuming the same tainted food is known as a foodborne disease outbreak [6]. Nausea, vomiting, cramping, diarrhea, and fever are the symptoms that most frequently follow the use of tainted food or water. The amount and kind of microbe or toxin that was inadvertently consumed have a direct impact on the degree and intensity of these symptoms [7]. Despite advancements in food preservation and cleanliness methods, foodborne illnesses continue to rank among the world's most significant public health issues [8].

A local earthy substance called kanwa, also known as nikih (liquid extract from the ash of plantains or banana peelings), partially emulsifies (stabilizes) the palm oil/water emulsion that makes up yellow achu soup, or "Nah poh" [9]. In the North West and West Region of Cameroon, it is still one of the most popular and well-liked soups for achu consumption [10].

## 1.2 Statement of the problem

Due to the dearth of essential infrastructure and services, as well as the difficulties in managing the numerous street food vendors' operations due to their diversity, mobility, and transient nature, street foods are thought to pose a serious risk to public health [53]. The application of a precise scientific approach to this very serious issue of public health and safety has been severely hampered by a general lack of factual knowledge about the epidemiological significance of many foods sold on the street, poor street vendor knowledge of basic food safety measures, and inadequate public awareness of the hazards posed by certain foods.

Meat, dairy products, poultry, cooked foods like achu, garri, rice, and egusi soup, as well as prepared foods like [54] and water fufu, eru, and djamajama, are the main foods that cause poisoning in the North-West Region of Cameroon.

Achu's yellow soup is typically prepared at room temperature, which increases its vulnerability to bacterial infection, particularly if proper precautions are not done. Additionally, the safety and cleanliness of both customers and vendors are critical to the livelihood of restaurant patrons as well as their health [54]. The

majority of these food vendors don't observe personal hygiene procedures and know very little about food safety. *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter*, and *S. aureus* are among the diseases that vendors may have and then transmit to customers. The most significant way that germs can spread from the hands of food handlers to the food is through their feces, nose, and skin [56]. Therefore, it is imperative that the safety of these street foods be investigated.

## 1.3 Research questions

1. What are the specific types of bacterial contaminants present in ready-to-eat yellow achu soup sold in Bamenda Municipality?
2. Which bacterial species are most commonly found in samples of ready-to-eat yellow achu soup collected from restaurants in Bamenda Municipality?
3. How does vendors' knowledge and adherence to Food safety and cleanliness procedures correlate with the presence and levels of bacterial contamination in ready-to-eat yellow achu soup sold in Bamenda Municipality?

## 1.4 Research hypotheses

### 1.4.1 Null Hypothesis (H<sub>0</sub>):

- There are no significant bacteria contaminants present in ready-to-eat yellow soup in Bamenda Municipality.
- Statistical Test: Descriptive statistics and qualitative analysis (such as frequency distributions, percentages) can be used to identify and categorize the types and prevalence of bacteria contaminants found in the yellow soup samples.

### 1.4.2 Null Hypothesis (H<sub>0</sub>):

- There is no specific prevalent bacteria species in ready-to-eat yellow soup in Bamenda Municipality.
- Statistical Test: Inferential statistics, specifically chi-square test or Fisher's exact test, can be used to determine if there is a significant difference in the prevalence of different bacteria species in the yellow soup samples. These tests will help in identifying whether certain bacteria are more prevalent than others.

### 1.4.3 Null Hypothesis (H<sub>0</sub>):

- There is no correlation between vendors' knowledge on food safety and hygiene and the presence of bacteria in their soup.
- Statistical Test: Pearson correlation coefficient (for continuous variables, such as knowledge scores and bacterial counts) or Spearman's rank correlation coefficient (for ordinal or non-parametric data) can be used to assess the strength and direction of the correlation between vendors' knowledge on food safety and hygiene and the presence of bacteria in their yellow soup samples.

## 1.5 Research objectives

### 1.5.1 General objective

- To investigate the bacteriological contaminants present in ready-to-eat yellow achu soup sold in Bamenda Municipality,

### 1.5.2 Specific objectives

- To determine the types and levels of bacterial contamination present in samples of ready-to-eat yellow

achu soup obtained from various restaurants in Bamenda Municipality.

- To identify the most prevalent bacterial species found in the ready-to-eat yellow achu soup samples collected from Bamenda Municipality.

**1.6 Significance of the research study**

1. **Public Health Impact:** This study aims to identify and quantify bacterial contaminants in a popular local dish, providing valuable data on potential health risks associated with its consumption. Understanding the types and levels of contaminants can inform health authorities and vendors about necessary precautions and interventions to safeguard consumer health.
2. **Food Safety Improvement:** By highlighting the prevalent bacteria and their sources in yellow achu soup, the research can contribute to improving food safety practices among vendors and restaurants. Recommendations stemming from the findings can lead to enhanced hygiene protocols, better storage conditions, and improved handling practices to reduce contamination risks.
3. **Policy and Regulation:** Findings from this study can inform local regulatory agencies and policymakers about the specific challenges and risks posed by bacterial contamination in street foods like yellow achu soup. This may lead to the development or revision of food safety regulations tailored to street vendors, thereby promoting safer food practices and minimizing the incidence of foodborne illnesses.
4. **Research Gap Bridging:** This study addresses a gap in current literature regarding the bacteriological profile of yellow achu soup specifically in Bamenda Municipality. By contributing new data and insights, it serves as a foundation for future research endeavors aimed at further understanding and mitigating foodborne risks associated with similar street foods.

**2.0 Literature Review**

**2.1 Food Safety**

Demand for safe food with less chemical additions has increased, and there is a lot of interest in substituting natural items that are safe for the environment and the host in place of these chemical compounds [11]. The demand for natural food additives is rising as people become more aware of the need to limit artificial components in food. Lecithin and gum Arabic are two examples of natural food emulsifiers that are hampered by processing and climate. Consequently, the use of biosurfactants derived from microorganisms in the food business has enormous potential [12].

**2.2 Different ingredients used in yellow achu soup**

**2.2.1 Palm Oil**

The Momo division in Cameroon's Northwest produces the majority of the country's palm oil [13]. About 90% of Cameroonians' edible oil needs are met by palm oil [14].

Red palm oil is consumed by 80% of Cameroonians, with artisanal mills producing an estimated 30% of it [13]. Because it is less expensive and has been a long-standing dietary habit, palm oil

is widely utilized in its crude form (crude palm oil) for food uses [14]. It has nutritional benefits since it is a good source of carotenoids, vitamin E, and some vital fatty acids. Vitamin E has long been recognized for its nutritional and physiological benefits, which include decreasing cholesterol, protecting against atherosclerosis, having antioxidant properties, and having anticancer effects [15,16,17]. Furthermore, Crude Palm Oil (CPO) is a significant meal that may be used to treat vitamin A insufficiency due to its high pro-vitamin A carotenoid concentration [17].

Studies reveal that there are still issues with the safety and quality of CPO, despite the fact that it is highly advantageous for human consumption [14]. First of all, the traditional production methods used to extract palm oil are carried out by people who are either ignorant of the microbiological consequences of inadequate sanitation and storage practices or have little to no awareness of contemporary aseptic production procedures [18,19].

Second, deterioration of oil quality due to improper storage conditions remains a major public health concern because different packaging containers used under different storage conditions exacerbate oil spoilage [20]. Environmental microbial contamination, raw materials, and equipment used for processing, storing, and distributing oil can also contribute to or exacerbate deterioration [19,18].

Since CPO is commonly utilized as a key ingredient in food, animal feed, and traditional medicine formulations, its microbiological purity is crucial [17]. The microbial burden can be minimized by heating and frying the oil. However, CPO is frequently eaten raw, such as in cold-prepared yellow soup, which is one of the main meals taken by the locals in Cameroon's Northwest Region. This is concerning because it may lead to health issues for consumers [18].



**Fig.1: palm oil**

**2.2.2 kanwa or nikh (emulsifying agent)**

In West Africa, Kanwa is frequently used, especially in Nigeria, Ghana, and Cameroon [9]. Kanwa is used as an antacid stomachic in the gastrointestinal tract to relieve flatulence and constipation [21]. Kanwa stimulates the liver as well. Kanwa dilates blood vessels to improve renal blood flow and causes alkaline diuresis, or greater urine, in the renal system. Kanwa acts as an expectorant in the respiratory system by inducing the production of respiratory mucosa.

It is common to find plantain peelings being dried around the kitchen of most women in the West and North West Region of Cameroon as it is their main source of nikh used in the preparation of this soup. Thirty-five women specialized in the preparation of this soup were interviewed in Bafoussam, West Region of Cameroon prior to this research work where the spices were obtained and their responses revealed that nikh is a better

emulsifier than kanwa. They made it clear that they use kanwa only when they run out of their stock of nikh in the preparation of this soup. Studies carried out by Tchiégang and Mbougueng<sup>[10]</sup>, and Abdou<sup>[22]</sup> as an attempt to valorize the spices used in the production of this soup did not take into account the chemical emulsifiers or stabilizers such as kanwa or nikh used in its preparation. It has been proven that accumulation of high levels of kanwa in the body damages some organs and tissues<sup>[23]</sup>.



**Fig 2: Kanwa**

### 2.3 Some Spices of yellow soup

More than nineteen (19) different spices are used for the preparation of nah poh<sup>[10]</sup>. These spices with different chemical and biochemical composition confers to this soup some important nutritional and therapeutic functions such as induction of lactation, revitalization, antibacterial activity, detoxification effects, not living out its social and cultural values<sup>[22]</sup>. Previous studies on the spices used for the preparation of this soup showed that they are a source of many essential nutrients and possess health benefits<sup>[24]</sup>.

However, many West and Central African ethnic groups employ them primarily as medicinal plants and as spices in traditional meals. Every meal has unique qualities that set it apart from the others. When spices are properly combined, they contribute significantly to a meal's flavor and taste from a sensory perspective. It has also been demonstrated that spices and sensory qualities are related<sup>[25]</sup>. Because each spice has a specific component or compounds that stimulate a particular sensory receptor or provide a cross-modal effect due to the many sensory modalities, it is necessary to determine and employ the proper type and quantity of each entity in the mixture<sup>[26]</sup>.

#### 2.3.1 *Monodora myristica* (Ehuru)

Morphologically, *Monodora myristica* (*M. myristica*) is a perennial plant of the Annonaceae or custard apple family of flowering plants (Burubai et al., 2009).

*M. myristica*'s nutritional value focuses on how well it works as a condiment due to its flavorful fragrance<sup>[27]</sup>. The seed's essential oil is added to dishes to enhance flavor<sup>[28]</sup>. In soups, sauces, canned goods, and meal preparations, the oil adds a valuable flavor<sup>[29]</sup>. The oil could be utilized as margarine, edible fat, or salad oil that is high in linolenic acid (Achinewhu et al., 1995). Its seeds are widely used as a spice to add flavor and thickness to food<sup>[30]</sup>. Numerous writers have noted that unsaturated fatty acid-containing oils, particularly linoleic and oleic acids, can be utilized to reduce plasma cholesterol<sup>[31]</sup>. Consuming *M. myristica* oil may help prevent coronary heart disease due to its high content of unsaturated fatty acids<sup>[32]</sup>. The entire seed, along with its seed coat, is either ground and added to

West African stews or soups as a seasoning or ground and used to flavor cakes and pastries in a manner similar to that of nutmeg<sup>[33]</sup>.



**Fig 3: *Monodora myristica* (Ehuru)**

#### 2.3.2 *Afrostryax lepidophyllus* (kontry onion)

Equatorial and tropical Africa are home to the Huaceae family of plants, including *Afrostryax lepidophyllus*<sup>[34]</sup>. In Congo, this herb is used in traditional medicine to treat gastrointestinal disorders and as an antiseptic<sup>[35]</sup>. The seeds of this plant have long been used as a spice in the Central African Republic. Furthermore, pharmacological investigations conducted by a number of researchers have demonstrated the intriguing qualities of *A. lepidophyllus* seed extracts. found Afrostryaxthioside A, Afrostryaxthioside B, and Afrostryaxthioside C exhibited antifungal activity. According to the research of<sup>[37]</sup>, seed extracts have antioxidant qualities. Additionally, research conducted by<sup>[38]</sup> demonstrated the insecticidal activity of *A. lepidophyllus* seed extracts<sup>[39]</sup> demonstrated that the essential oil of *A. lepidophyllus* seeds lowers free radicals (DPPH and ABTS) and assessed the oil's cytotoxicity against a panel of cancer cell lines. The purpose of this work is to analyze the phytochemical composition of *A. lepidophyllus* seed extracts and then assess their biological activity.



**Fig 5: *Afrostryax lepidophyllus* (kontry onion)**

#### 2.3.3 *Peper nigrum* (Black pepper)

*Peper nigrum* (Black pepper) is one of the most commonly used spices and considered as "The King of Spices" due to its trade in the international market<sup>[40]</sup>.

*Peper nigrum* exhibits diverse pharmacological activities like antihypertensive and anti-platelets<sup>[41]</sup>, antioxidant, antitumor<sup>[42]</sup>, antipyretic, analgesic, anti-inflammatory, antidiarrheal, antispasmodic, hepato-protective<sup>[43]</sup>, antibacterial, antifungal, anti-thyroids, anti-apoptotic, anti-spermatogenic, insecticidal and larvicidal activities etc. Piperine has been found to enhance the therapeutic efficacy of many drugs, vaccines and nutrients by increasing oral bioavailability by inhibiting various metabolizing enzymes<sup>[44]</sup>.



Fig 6: Black pepper

## 2.4 Preparation of yellow soup

### 2.4.1 Ingredients

- Lime stone / cooking soda / kangwa or Nikki. Nikki is an extraction of fireside grey dust and mix with water to extract soda liquid to use for the preparation of this soup.
- Palm oil, cow skin/canda, Maggi cubes, salt, pepper(optional), composes achu spices, water.

### 2.4.2 Procedure

- Boil your assorted meat like cow meat, canda, strips, smoked meat and garden eggs. Season the meat with salt pepper and bouillon cubes.
- Extract stock from your delicious assorted meat and set aside. Inside your hot stock add lime stone to dissolve.
- In a saucepan heat up palm oil for maximum 1 mins. Just give it a light heat not too much. This process is very optional.
- Add appropriate quantity of water and add the dissolved lime stone slowly while monitoring color change until you get consistent yellow color.
- In a blender, salt, cubes and composed achu powder spice and add to the soup; mix well and add your meat or canda. The soup is ready to serve.



Fig 7: yellow achu soup ingredients



Fig 8: Ready to eat yellow achu soup

## 2.5 Factors contributing to the contamination of ready to eat food.

### 2.5.1 Food types, method of preparation and the manner in which the food is served.

Certain microbial types are associated with particular foodstuffs, depending on their chemical composition and physical factors such as pH and water content. Acidic foods such as fruits, for example, tend to favor the growth of fungi rather than bacteria. Often, food spoilage organisms come from the same source as the food, for example soil on vegetables, or meat exposed to intestinal contents following slaughter. Others are introduced as contaminants during transport, storage or preparation. Among the most commonly found spoilage organisms are a number of human pathogens, including *Pseudomonas*, *Salmonella*, *Campylobacter* and *Listeria* [45]. Street food is often prepared by the vendors at home or at the road side where the food is being sold [45].

Before serving, the dish is not cooked to high temperatures once it has been prepared [46]. Due to their poor construction, the roadside selling sites are more susceptible to dust and smoke contamination [45]. In some cases, there is no portable water available in the food vendors' area of business, so they fetch water from their homes. Typically, this water is insufficient for food preparation and dishwashing, and sellers fail to adequately wash new food. Mensah pointed out that street food vendors may contribute to the spread of food-borne illnesses since they lack formal education and are ignorant of safe food handling practices [47]. While certain food varieties are partially covered, others are left exposed. Fruits and roasted foods like plantains and plums are sold outdoors, which raises the possibility of food contamination because they are being sold on a roadway where pollutants are present in high concentrations [47].

### 2.5.2 Knowledge on personal and food hygiene.

The use of fast food, whose preparation is outside the consumer's control, has given food safety concerns a broader scope in recent years. People now consume more meals outside of their homes due to their hectic lifestyles. A significant amount of ready-to-eat food is sold on the streets in developing nations. Food-borne infections are inevitable if this food is not handled hygienically or stored at the proper temperature [48].

Every member of the family may participate in the purchasing of ingredients, meal preparation, and cooking for street food businesses [45]. They offer a vital service to employees, customers, tourists, students, and others with modest means by supplying snacks, full meals, and refreshments at comparatively inexpensive costs. After using the restroom, food workers' unwashed hands have been shown to transfer germs to food products [46]. Since *E. coli* is present in both human and animal digestive tracts, its presence in ready-to-eat food is typically seen as a sign of fecal contamination. This in turn raises the possibility that there are other pathogenic species, such as bacteria from the genera- *Salmonella*, *Shigella*, and *Campylobacter*, viruses like Hepatitis A, or protozoa like *Toxoplasma* [49].

Additionally, *Staphylococcus* is naturally present in humans and may be isolated from a healthy human population [50]. Hence failure to regularly wash hands while serving food can easily lead to contamination of the food served. *Salmonella* species

and Campylobacter are easily transferred from chicken to the hands of food handlers given that poultry is their natural habitat [49]. If this contaminated hand is left unwashed the food handler will contaminate the food they serve and consequently the consumer. A study in Santa Fe de Bogota, Colombia revealed that over 30% of a group of food handlers examined were carriers of pathogenic microorganism including Salmonella typhi, Staphylococcus aureus, Salmonella enteritis, and Shigella [51].

### 2.5.3 Vendor's knowledge of sources and mode of transmission of foodborne pathogens

A majority of street food vendors are unaware of the food regulations and lack proper training in food-related illnesses. Their lack of knowledge on the source of foodborne infections and how they can be transmitted explains why the above mention bacteria type are isolated from ready to eat food. A study carried out in Nairobi, Kenya among food vendors between the ages of 20 – 25 years upon their interview revealed that 62% of the food vendors interviewed had primary education and below, 36.3% had secondary education while only 1.3% had tertiary education [52]. Because of vendor's ignorance of the sources of food contamination and their mode of transmission, instead of using hand towels and handkerchief to swipe sweaty face and running nostrils respectively, the hands of these food vendors are being used, they can also be seen picking their nostrils with their hands even when serving food. It is a common practice to find vendors sneezing, coughing and even talking over food which can leads to contamination of the food they serve.

## 3.0 Materials and Methods

### 3.1 Materials

- **Equipments:** Microscope, incubator, autoclave, refrigerator, measuring glass Petri dishes measuring cylinder, weighing boot, clean round flasks, bijoux bottles, dishes, Bunsen burner, clean glass slides, staining rack, wire loop, droppers, gas lighters, culture bottles.
- **Reagents:** Crystal violet, lugol iodine, absolute alcohol, diluted carbolfushin, clean water, normal saline, hydrogen peroxide, human plasma, human fresh whole blood, MacConkey and nutrient agar, peptone water, triple sugar Iron agar, Kovac's reagent.

### 3.2 Methods

Description of the study area The research was conducted within the Bamenda city as follows:

The sample was collected from a few Bamenda I, II, and III restaurants in Bamenda town, which is located in the Mezam division of Cameroon's North West region. The city is located at 5.95° North latitude, 10.16° East longitude, and 1472 meters above sea level. It is also known as Abakwa with about two million habitants and is located 366 km from the Cameroonian capital Yaoundé. Comprising the towns of Mankon, Nkwen, and Bamendakwe, it is a cosmopolitan metropolis with a cool climate brought on by vegetation, with residents from all over the nation and its bordering areas.

### 3.3 Research design

- A cross-sectional study design was used to carry out this research.

### 3.4 Target Population

The study aims to recruit the following participants:

- Food Vendors: Individuals who sell yellow achu soup in restaurants and street food stalls in Bamenda Municipality.
- Consumers: Individuals who frequently purchase and consume yellow achu soup from these vendors.

### 3.5 Study sample

- Ready to eat Yellow achu soup sold in the various restaurants of Bamenda I, II and III.

### 3.6 Sampling technique

- The Random sampling technique was used.

### 3.7 Sample size and research duration

- A total of fifty (50) ready to eat yellow soup samples was collected in the various restaurants of Bamenda I, II, and III. And this research was carried out from April to May 2024.

### 3.8 Recruitment Strategies

- To ensure a diverse and representative sample, the following strategies were employed.

#### 3.8.1 Outreach to Food Vendors

- Direct Visits: Researchers visited restaurants and street food stalls in Bamenda I, II, and III to inform vendors about the study. This face-to-face interaction helped build trust and encouraged participation.

#### 3.8.2 Outreach to Consumers

- Survey Distribution: Distribute surveys to consumers who frequent these food establishments. Surveys were brief and designed to gather information on their eating habits and willingness to participate in further study activities.
- Community Events: Attend local community events and gatherings to distribute information about the study and recruit participants. This included market days, health fairs, and cultural events.

### 3.9 Inclusion and Exclusion Criteria

#### Inclusion Criteria:

- Food vendors who sell yellow achu soup.
- Consumers who frequently eat yellow achu soup.
- Participants must be at least 18 years old.
- Willingness to provide informed consent.

#### Exclusion Criteria:

- Individuals who do not sell or consume yellow achu soup.
- Individuals under the age of 18.
- Unwillingness to provide informed consent.

### 3.10 Informed Consent Process

- Participants were provided with detailed information about the study, including its purpose, procedures, risks, and benefits. They were given an opportunity to ask questions and were required to sign an informed consent form before participating.

### 3.11 Data Collection Tools

- Questionnaires: Administered to both vendors and consumers to collect demographic information, food safety knowledge, and eating habits.
- Sample Collection: Obtain samples of yellow achu soup from vendors for laboratory analysis.

### 3.12 Data collection procedure

- The vendors were given a questionnaire. Using the serving spoons that the food vendors used, the food samples were gathered and placed in clearly marked, sterile pee cups. To avoid contaminating the meal, care was taken. After being put in a transportation flask, the samples were brought to the lab for examination.

### 3.13 Preparation of culture media

- For examination of bacteria contaminants, two culture media were used: MacConkey Agar and Blood Agar.

### 3.14 Sample processing

- After collection, the samples were carefully transported to the Laboratory and the following were performed:

#### 3.14.1 Sample identification

- Once in the Laboratory, the Sample was first identified, a labelling code was given to each sample and it was registered in the appropriate book.

#### 3.14.2 Inoculation of culture plates

##### Upon arrival at the microbiology unit

- PPEs were put on, and hair confined
- Culture plates were removed from the fridge and allowed to obtain room temperature
- Working surface was decontaminated using the decontaminating solution
- Samples were arranged on the working surface according to the order of inoculation
- The dried culture plates were removed, labelled with the sample codes and date of inoculation
- A tiny amount of sample was transferred to the agar plate (Blood agar and MacConkey agar) using a sterile inoculating wire loop, and the yellow soup was thoroughly mixed by shaking it close to the lit Bunsen burner. The plate was then infected using the streaking method such that distinct colonies formed.

#### 3.14.3 Incubation conditions

- The inoculated plates were then incubated at 37°C for 24hrs in an incubator for possible growth.
- After 24hrs, the plates were removed from the incubator and read for possible growth.
- The morphology of the isolates was examined both macroscopically and microscopically by means of post culture gram staining.

#### 3.14.4 Gram staining

##### Principle

- When bacteria are stained with primary stain crystal violet and fixed by the mordant, the gram positive bacteria because of their cell wall made up of thick layer of peptidoglycan, they will retain the primary stain and

appear purple in color while gram negative bacteria because of their thin layer of peptidoglycan, they will be decolorized and appear pink in color (counterstain).

##### Procedure

- Working close to the flame of a lighted Bunsen burner, an applicator stick was used to pick up a colony from the culture plate which was mixed with one drop of normal saline and a smear was made on a well labelled clean grease free microscope slide and allowed to air dry.
- The smear was air dried and heat fixed by passing the slide 2-3 times on the flame.
- The slide was placed on the staining rack and the smear covered with crystal violet using a dropper and allowed to stain for 1 min then rinsed with clean water.
- Lugol's iodine was then applied on the smear for 1 min and rinsed.
- Alcohol was applied for 10 seconds and immediately rinsed with water.
- Lastly, diluted carbolfuchsin was applied for 1 min and rinsed with water.
- After blotting and letting the slide air dry, it was examined under a microscope using an immersion oil ( $\times 100$ ) objective.

#### 3.14.5 Triple sugar Iron test

##### Principle

- This test is based on the ability of the organism to reduce glucose, sucrose, lactose and produce hydrogen sulphite.

##### Procedure

- TSI agar was prepared following manufacturer's instructions and dispersed aseptically in sterile bijoux bottles and allowed to solidify in a slopping form.
- Using a straight wire loop, an isolated colony was removed from the MacConkey culture plate and stabbed right in the center of the media in the bijoux bottle.
- The inoculated bijoux bottles were then incubated at 37° for 24 hours.

##### Interpretation

- An alkaline/acid (red slant/yellow butt) reaction: It is indicative of dextrose fermentation only.
- An acid/acid (yellow slant/yellow butt) reaction: It indicates the fermentation of dextrose, lactose and/or sucrose.
- An alkaline/alkaline (red slant, red butt) reaction: Absence of carbohydrate fermentation results.
- Blackening of the medium: Occurs in the presence of  $H_2$ .
- Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of  $CO_2$  and  $H_2$ ).

#### 3.14.6 Biochemical tests

- After gram staining the following tests were carried out for identification of the isolates:

##### ➤ Catalase test

- Both gram negative bacilli and gram positive cocci bacteria isolated from both MacConkey and blood agar

were tested for the presence or absence of the enzyme catalase.

**Principle of catalase test**

- The test is based on the breakdown of hydrogen peroxide into water and oxygen by the enzyme catalase when a small amount of the test organism that produces catalase is emulsified in hydrogen peroxide resulting in rapid elaboration of bubbles of oxygen.

**Procedure**

- A drop of hydrogen peroxide was placed on clean glass slide using a dropper
- Using a sterile stick, a fresh isolated colony was taken from the culture plate and placed on the drop of hydrogen peroxide
- The mixture was macroscopically examined for the presence or absence of air bubbles

➤ **Coagulase slide test**

- This test was performed only on gram positive cocci that were positive for the catalase test to differentiate between pathogenic *staphylococci* (*S. aureus*) and nonpathogenic ones.

**Test principle**

- Coagulase is an enzyme protein that is thermostable thrombin-like substance, which converts fibrinogen to fibrin resulting in clotting or clumping of cells.

**Procedure**

- A drop of plasma was placed on a clean glass slide.
- Using a wire loop, a well isolated colony was taken from the culture plate and emulsified with the drop of plasma.
- The mixture was macroscopically observed for the presence or absence of agglutination

➤ **Indole test**

- This test was carried out for all gram negative bacilli isolated on MacConkey agar to differentiate indole producing from non-indole producing bacteria

**Test principle**

- The test is based on the ability of the organism to produce indole from the amino acid tryptophan through the use of an enzyme called tryptophanase resulting in color change.

**Procedure**

- Peptone water broth was prepared following manufacturer's instructions (25g of powder in 500mls of distilled water).
- The medium was then dispensed in small sterile bijoux bottles, labelled and autoclaved at 121°, 15 bounds for 15minutes.
- An isolate was then taken from a 24 hours cultured plate of MacConkey (gram negative bacilli) and sub cultured in the peptone broth using a sterile wire loop.

- The inoculated bottles were then incubated at 37° for 24 hours.
- Following incubation, 3 The culture broth was supplemented with drops of Kovac's reagent..
- A favorable outcome was noted by the appearance of pink layer at the top of the broth; a negative result appears yellow.

➤ **Motility testing**

- This test was performed in order to differentiate motile from non-motile bacteria. A drop of sub cultured colony from peptone water using a Pasteur pipette was placed on a slide, covered with a cover slide and observed under ×10 objective after 2 minutes.

**3.15 Limitations of the Study**

1. **Sample Size:** The study is limited by its sample size, which includes a restricted number of samples collected within a specific timeframe. This may affect the generalizability of findings to the entire population of yellow achu soup vendors in Bamenda Municipality.
2. **Sampling Bias:** There is potential for sampling bias as the study relies on a random sampling technique, which may not fully represent the diversity of vendors and soup preparation practices across different locations within Bamenda Municipality.
3. **Laboratory Constraints:** The availability of resources and equipment in the laboratory imposed limitations on the types of bacterial analyses and tests that can be performed, potentially affecting the comprehensiveness of the microbial profile obtained.
4. **Time Constraints:** The duration allocated for data collection and analysis limited the depth and scope of the study, particularly in capturing seasonal variations or long-term trends in bacterial contamination levels.

**3.16 Delimitations of the Study**

1. **Focus on Bacterial Pathogens:** The study specifically focused on bacterial contaminants in yellow achu soup and did not encompass other potential contaminants such as viruses, parasites, or chemical contaminants.
2. **Geographical Scope:** The study was delimited to Bamenda Municipality in the North West Region of Cameroon, Therefore, results could not apply to other areas or nations with different culinary practices or environmental conditions.
3. **Methodological Approach:** The research employed microbiological methods such as culture and identification techniques for bacterial analysis, omitting molecular or genomic analyses that could provide additional insights into microbial diversity.
4. **Timeframe:** The study was delimited to a specific timeframe for data collection and analysis, which restricted the ability to capture temporal variations or changes in contamination levels over longer periods.

**3.17 Ethical Considerations**

1. **Informed Consent:**
  - **Purpose:** Participants (both food vendors and consumers) were fully informed about the research objectives, procedures, potential risks, and benefits before their involvement.



- **Procedure:** Written informed consent were obtained from all participants. For illiterate participants, verbal explanations were provided in the presence of a witness, and their consent were documented by the witness.
  - **Voluntary Participation:** Participation in the study were entirely voluntary, and participants had the right to withdraw at any stage without consequences.
- 2. Confidentiality and Anonymity:**
- **Data Handling:** All data collected, including personal information and research findings, were kept confidential and stored securely. Data were only accessible to authorized researchers involved in the study.
  - **Anonymity:** Participants' identities were protected, and any published results will not include personally identifiable information to ensure anonymity.
  - **Beneficence and Non-maleficence:**
  - **Participant Safety:** Measures were taken to minimize risks to participants during sample collection and handling. Proper sanitation and safety protocols were followed to prevent any harm or discomfort.
  - **Benefit to Participants:** The research aimed to contribute to public health knowledge and food safety improvements, potentially benefiting both participants and the wider community.

**3.18 Approval and Permissions:**

- **Ethical Approval:** The research protocol, including the study design and informed consent procedures, were reviewed and approved by the Pinnacle University Institute of Health and Technology Bamenda Ethics Committee and the Regional Delegation of Public Health, North West Region.
- **Permissions:** Prior permission were obtained from relevant authorities, such as restaurant owners and local authorities, before collecting samples or conducting surveys in their establishments.

**3.19 Disclosure of Findings:**

- **Dissemination:** Research findings were shared with participants, the Pinnacle University Institute of Health and Technology Bamenda, the Ministry of Public Health, and other relevant stakeholders. Results were presented in a clear and understandable manner, respecting the participants' contribution to the study.

**Conflict of Interest**

All researchers disclosed any financial, professional, or personal relationships that could influence the study's outcomes. Transparency and impartiality guided decision-making, ensuring that participant welfare and research integrity remain paramount. The Pinnacle University Institute of Health and Technology Bamenda Ethics Committee oversaw the process, evaluating disclosures and providing guidance to mitigate conflicts. These efforts aimed to uphold ethical standards, maintain credibility, and safeguard the reliability of findings concerning food safety in the community.

**Timeline**

- ✓ **Week 1:** Develop recruitment materials
- ✓ **Week 2:** Began outreach to food vendors and consumers; distributed informational materials.

- ✓ **Week 3:** Conducted direct visits to food vendors and attended community events.
- ✓ **Week 4:** Followed up with interested participants to obtain informed consent and scheduled sample collection.

**4.0 Results**

Out of the forty- five (45) samples analyzed, thirty-seven (37) samples showed growth on MacConkey and blood agar and the following results were obtained:

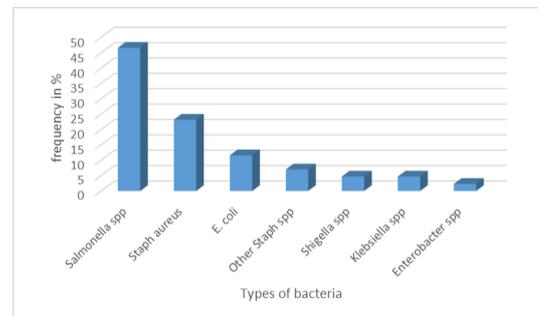
**4.1 Hygienic knowledge of vendors on food safety and correlation with significant bacterial growth**

Out of the 45 vendors sampled, 30(66.6%) said that they have heard about food safety (either from friends, school or internet) and that they followed proper food handling and adequate personal hygiene; out of these 30 samples there were bacterial growth on 22 samples (73.3%) and 8 samples (26.6%) were sterile or had no significant bacterial growth while 15 vendors (23.4%) said they have no knowledge about food safety and hygiene and all these samples (100%) had significant bacterial growth.

Also, out of the 45 vendors, 42(93.3%) mostly used *Afrostyrax lepidophyllus* (kontry onion), *Monodora myristica* (Ehuru), *pepper nigrum* (Black pepper) and *Tetrapleura tetraptera* as spices for the preparation of their soup while 3(6.6%) used no spices. Finally, all the 45(100%) vendors sampled used nikh for their achu soup preparation.

**Table 1: Prevalence of each bacterial isolated.**

Locality	Total sample collected	Positive samples	Frequency (%)
<b>Bamenda I</b>	15	11	73.3
<b>Bamenda II</b>	15	14	93.3
<b>Bamenda III</b>	15	12	80
<b>Total</b>	<b>45</b>	<b>37</b>	<b>82.2</b>



**Figure 9:** Bar chart showing the frequency in % of each bacteria isolated.

**Table 2:** Macroscopically, microscopically and Biochemical tests results with suspected organisms.

Macroscopy of colonies	Gram reaction	TSI test	Catalase	Coagulase	Indole	Motility	Possible organism
Small whitish on BA	Gram + cocci	N/A	+	+	N/A	N/A	Staph aureus
			+	-	N/A	N/A	Other Staph spp
Pinkish, non-mucoid on MCA	Gram - bacilli	Acid slant/Acid butt gas+, H <sub>2</sub> S-	+		+	+	E. coli
			+	N/A	-	+	Enterobacter spp
Pinkish, mucoid on MCA	Gram - bacilli	Acid slant/Acid butt gas+, H <sub>2</sub> S-	+	N/A	-	-	Klebsiella spp
Colorless on MCA	Gram - bacilli	Alkaline slant/Acid butt gas-, H <sub>2</sub> S-	+	N/A	-	-	Shigella spp
		Alkaline slant/Acid butt gas+, H <sub>2</sub> S+	+	N/A	-	-	Salmonella spp

**KEY**

- = negative

+ = positive

N/A = not applicable

**5.0 Discussion**

A total of 45 samples of ready to eat yellow achu soup were collected in the various restaurants randomly selected in Bamenda I, II and III and were analyzed. The morphological and biochemical characteristics were recorded. Out of the 45 samples, 37 were positive giving a percentage contamination of 82.2%. This high prevalence of contamination agrees with the research done by Hefnawy et al (2009) after what he noticed that there is an increasing rate of food poisoning in recent years; about 20 million worldwide reported including 11 million in Africa.

The following bacteria were isolated from the positive samples: *Salmonella spp* (46.6%) *Staphylococcus aureus* (23.2%), *Escherichia coli* (11.6%), *coagulase negative staphylococci spp* (7%), *Shigella spp* (4.7%), *Klebsiella spp* (4.7%), and *Enterobacter spp* (2.3%). The results show that *salmonella spp* is the most prevalent bacteria with the prevalence of 46.6%; this correlates with the results found by Much et al (2007) who cited despite this wide variety of etiologic agents, the *Salmonella spp.* stands out as a common microorganism, being responsible for 76% of foodborne disease outbreaks in Austria.

Correlating the hygienic knowledge of the vendors on food hygiene and safety with the presence of pathogenic bacteria isolated in their food, the results shows that the prevalence of bacterial contaminants amongst those who had no knowledge about food safety and hygiene is higher (100%) than the prevalence amongst those who have heard about food safety and hygiene and (73%). This goes in the same direction with the research done by Rane et al (2011) who cited that inadequate knowledge of street vendors about basic food safety measures poses risks of spreading food borne diseases within the community. Still, this elevated prevalence amongst those who said they have knowledge about

food hygiene and safety may be explained by the fact that either the knowledge was not enough or they don't actually put the knowledge they have into practice; this goes in the same direction with the study carried out in India by Bhattarjya et al(2014) which showed that despite the fact that street food vendors have minimum level of education and food safety knowledge, many such vendors were not following minimum safety and personal hygiene practices . But this is contrary to the study developed in Nigeria by Okojie et al (2014) which showed that food vendors had good food handling practices and that street food vending sites were sanitary.

**6.0 Conclusion**

At the end of this research work, in which the main objective was to determine the bacteriological contaminants of ready to eat yellow achu soup sold in Bamenda municipality, the results obtained show that most(82.2%) of the yellow soup sold in this locality is contaminated with the following bacterial: *Salmonella spp*, *Staphylococcus aureus*, *Escherichia coli*, *coagulase negative staphylococci spp*, *shigella spp*, *Klebsiella spp* and *Enterobacter spp*. Amongst which *Salmonella spp* Was the most prevalent (46.6%) bacteria. This is likely due to the unawareness of some food vendors on food hygiene and safety or the failure to follow proper personal hygiene practices and inadequate food handling by other vendors. From this work it can be concluded that most of the ready to eat yellow achu soup sold in Bamenda municipality is not safe for consumption.

**7.0 Recommendations**

**7.1 To the Ministry of Public Health**

- The ministry of public health should organize training seminars to sensitize street food vendors on food safety and food hygiene for better health.
- To see into it that the regulatory bodies put in place to monitor the hygienic conditions and practices of food vendors are effective.
- Make available a constant water supply at commercial eateries.

## 7.2 To the school

- Similar studies should be carried out in other areas or same area with a broader scope of microbial aspect such as evaluation of parasitic and fungal contamination.

## 7.3 To food vendors

- All food vendors should endeavour to practice personal and good food hygiene such as washing of hands regularly when serving food, avoid talking over food, cook food properly, put on clean clothing, go for regular medical check-up etc.

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