

## Research Paper



## Detoxification of bacterial toxins in soured soups using activated charcoal

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### Article Info

#### Article History:

Received: 17 May 2024

Revised: 25 July 2024

Accepted: 02 August 2024

Published: 18 September 2024

#### Keywords:

Detoxification

Bacterial Toxin

Soured Soups

Activated

Charcoal

Food safety



### ABSTRACT

A study to evaluate the use of activated charcoal to detoxify bacterial toxins in soured soups was carried out. An activated charcoal solution was prepared. Three different types of soups (okra, banga, and Kalabari native (Odu-Fulo) were used in this study. The soups were prepared and left for 48 hours at ambient (room) temperature for them to get soured. Five microliters (5 ml) of each soured soup sample were homogenized, and 1 ml from the stock was used for serial dilutions. The pour-plate method and streaking techniques were used to isolate and identify the bacteria present. The lateral flow assay device, Biothreat Alert test strip, and Enzyme Immunoassay (EIA) kits were used to detect the bacterial toxins, while activated charcoal was used to test the detoxifying effectiveness of the bacterial toxins present. The result showed that *Lactobacillus* sp, *Bifidobacterium* sp, *Streptococcus* sp, *Pediococcus*, sp *Leuconostoc* sp *Bacillus cereus*, *Clostridium perfringens*, *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, *Salmonella* sp, *Shigella* sp, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Clostridium botulinum* were associated with the soured soups, *Bacillus cereus* toxin, and *Staphylococcus aureus* exotoxins were detected in the native soup, *Clostridium botulinum* toxin in banga soup, and none were found in the okro soup, and also indicated the effectiveness of activated charcoal solution in absorbing (removing) the bacteria toxins in soured soups.

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## 1. INTRODUCTION

Preservation of soups is one of the main issues that rural communities in Nigeria face. Many times, soups get soured or spoiled due to a lack of appropriate preservation methods, especially in rural areas where electricity supply is sparse. When soups are not properly preserved, they are wasted and thrown away, causing more difficulties for the less fortunate and possibly even leading to starvation and economic burden, but when not thrown away and consumed, they cause food poisoning and other illnesses (NIFS, 2022). The bacteria in the soups, through their metabolic activities, produce poisonous substances that will make the soups sour and, therefore, render the soups unfit for consumption. In order to still make the soups safe for consumption, it is then required to look into the soured soups' bacterial contamination and remove the poisons. Many tribes and cultures in Nigeria make use of red palm oil and onions to restore and recover their sour soups. Many adopted the use of charcoal as a preservative, which means dipping the pot of soup into a bowl of water. Some preferred continuous heating of the soured soup as a way of making it safe to be consumed, and a piece of charcoal was dropped inside the soup. This, it was believed, would remove all the bad taste and smell from the soup and bring it back to its original taste, where slices of onion and red palm oil, crayfish, and seasoning cubes like Maggi were added to restore it. The practice is still in place in some localities today [1].

The methods used to prepare, handle, and store food to make sure it is wholesome and suitable for eating by humans determine how safe a soup is [2]. The majority of sour-soup-borne illnesses are brought on by *Staphylococcus aureus*, *Clostridium botulinum*, *Campylobacter* sp., *Bacillus cereus*, enteropathogen sp., *Escherichia coli*, and *Vibrio parahaemolyticus* [3]. The symptoms of these illnesses include diarrhea, vomiting, abdominal cramps, and nausea. A common cause of food-borne sickness is the presence of specific bacteria or their toxins, which are harmful proteins generated by the bacteria.

Both *Clostridium botulinum* and *Staphylococcus aureus* possess the capacity to produce toxins in food, which may lead to symptoms upon ingestion [4]. The ability is contingent upon the specific environment and cell populations being considered. *Clostridium perfringens* may create a toxin in food; however, a substantial number of cells are required for this to occur. The presence of a high cell concentration is often seen in the advanced stages of food decay [5]. Both ingesting pre-formed toxins in food and swallowing cells from the bacteria *Bacillus cereus* may lead to symptoms [6]. *Bacillus cereus*, *Clostridium botulinum*, and *Staphylococcus aureus* are the only bacterial species acknowledged as major contributors to the type of food poisoning known as intoxication [7]. All of these bacteria have the ability to cause illness by releasing toxins into the food. There is a lack of understanding about the microorganisms responsible for the deterioration of Nigerian soups. Several academics have shown that *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Clostridium botulinum*, and *Campylobacter jejuni* are significant microbes responsible for food deterioration and food-borne illnesses [8], [9].

Food contamination caused by bacteria can be dangerous since it occasionally looks quite normal, even when it is extremely polluted. Additionally, it is typically not until a food poisoning outbreak occurs and a laboratory analysis identifies the causative agent that the presence of highly dangerous toxins and bacterial spores is discovered. [10] States that many bacterial species produce poisons. According to him, *Clostridium botulinum* produces a toxin that is maybe the most lethal chemical known to date. The spores of *C. botulinum* exhibit a high level of resistance to temperatures of 100°C, maintaining viability for around 6 hours [11]. Despite the rapid denaturation of the botulinum toxin at 100°C, the extended cooking duration would render the poison inert, even in the presence of *C. botulinum* in the soup. He said that *Clostridium perfringens* has the ability to survive high temperatures when in spore form, tolerate boiling, and then need 8–10 hours in a heated soup to transition back to an active state and proliferate to around 10 per milliliter [12]. Upon consumption, the substance would resume the process of sporulation, leading to the production of an enterotoxin in the gastrointestinal tract around 7–12 hours later. This would then cause diarrhoea [13]. They added, that this toxin is distinct from that of *Staphylococcus aureus* in that its growth and toxin production, if not controlled, can lead to food poisoning upon consumption. The organism may be quickly inactivated with heat treatment since it does not create spores.

However, the toxin is very resilient to boiling and is likely to persist in the soup. They emphasized that some other spore-forming species, such as *Bacillus cereus*, might withstand the heat and subsequently,

under extended favourable conditions, proliferate and produce poisons. Nevertheless, when heated, non-spore-forming microbes, including Salmonella, Escherichia coli, Yersinia, Listeria, Klebsiella, Shigella, Campylobacter, Vibrio, and streptococci, will undergo denaturation [14]. A food microbiologist, food-borne bacteria secrete toxins into the food environment. Ingesting these harmful substances would lead to food illness since they often taint food. The bacteria would be eliminated by heat, leading to the bursting of their cells and the subsequent release of their poisons into the surrounding environment. The toxins would thus remain present in the food product. Most infections may be effectively eliminated by temperatures over 150°F (66°C); however, the toxins they produce may not always be completely eradicated or altered in structure. Despite extensive cooking and reheating, the toxins will persist [11], [15].

## 2. RELATED WORK

[16], [17] Evaluated detoxification of bacteriotoxins in sour soups using red oil, heat, and onion and indicated that bacterial toxins detected in the sour soups included Bacillus cereus toxins, Clostridium botulinum toxins, and Staphylococcus aureus toxins and that the test materials were not able to detoxify the sour soups.

Investigated “the correlation between dominant bacteria and primary metabolites during the fermentation of sour soup. They found that fermentation led to a decrease in the presence of native bacterial strains, with lactic acid bacteria (LAB) becoming the dominant microbes and this shift in microbial composition resulted in changes in the relative abundance of bacteria and variations in the types and quantities of metabolites.

The objective was to examine and identify bacterial toxins and thermal stability of enterotoxins in food present in food sellers and some vegetables found in market and revealed that all the soup samples that were tested had different levels of bacterial toxins and growth, ranging from  $1.0 \times 10^5$  to  $3.0 \times 10^6$  cfu/ml.

[8] Assessed the bacteria in recently spoiled ofe-okwu soup, isolated several strains of Bacillus, and hypothesized that these microbes could generate toxins in the soup. Found that Bacillus cereus spores exhibited resilience to the sterilization process, as they remained intact and maintained stability for a minimum of three years at temperatures as high as 00°C.

[11] Examined Clostridium botulinum toxin inactivation in selected soured soups in South-South region of Nigeria and revealed that Clostridium botulinum neurotoxin was denatured at a temperature of 100°C. Also, the serological activity of the toxins treated with palm oil was unaffected, whereas, the joint treatment with heat and palm oil remained denatured.

The aim of the study was to use activated charcoal solution to denature bacterial toxins in soured soups. The objectives were to;

- Investigate (test) some soured soups for contamination with Bacillus cereus toxins, Clostridium botulinum toxins, and Staphylococcus aureus toxins.
- Ascertain the effect of activated charcoal on Bacillus cereus toxin in soured soups.
- Ascertain the effect of activated charcoal on Clostridium botulinum toxin in soured soups.
- Ascertain the effect of activated charcoal on Staphylococcus aureus toxin in soured soups.

## 3. METHODOLOGY

Three different types of soups (okra, banga, and Kalabari native (odu-fulo) were used for this study. All the ingredients used in preparing the soups were purchased from Creek Road main market, in Port Harcourt City Local Government Area of Rivers State, Nigeria. The ingredients were dried fish, clams, shrimps, periwinkles, pepper, palm oil, crayfish, onion bulbs, seasoning cubes, salt, banga (palmfruit), offor, okra, pumpkin leaves, fresh fish, and beef. The ingredients used to prepare the various types of soups were thoroughly washed and the soups prepared according to the different methods adopted by the natives [18].

- **Isolation of Bacteria in Soured Soups:** Five milliliters (5 ml) of each soured soup sample was homogenized and 1 ml from the stock was used for serial dilutions. The pour-plate method and

streaking techniques were used to isolate the bacteria present. One milliliter (1 ml) of the dilutions  $10^4$ ,  $10^3$ ,  $10^2$ , and  $10^1$ , were pipetted into nutrient agar plates, Sorbitol-MacConkey agar, blood agar, TCBS agar (Thiosulfate-Citrate-Bile Salts-Sucrose), Mueller-Hinton agar and Salmonella-Shigella agar. The isolates obtained were carried out using colonial morphology using oil immersion lens ( $\times 100$ ). The plates were incubated in triplicates aerobically and anaerobically at  $37^\circ$  for 24 hours. Later, discrete colonies were sub-cultured on fresh medium for the development of pure isolates, which were stored on nutrient Agar slants for biochemical test.

### 3.1 Testing Some Soured Soups for Contamination with *Bacillus Cereus*, *Clostridium Botulinum*, and *Staphylococcus Aureus* Toxins

- **Procurement of Test Kits:** The lateral flow assay device (Duopath cereus Enterotoxin immunoassay) and Biothreat Alert test- strip were purchased from Merck, White House Station, New Jersey, USA while capture antibodies against SEA to SEE (Staphylococcal Enterotoxin A-E), were obtained from R-Biopharm GmbH, Darmstadt, Germany.
- **Preparation of Activated Charcoal Solution:** The activated charcoal was bought from the Conference Headquarters of the Seventh- Day Adventist Church in Rumuokwuta and transported to the laboratory for preparation of solution. About forty grams (40g) of the Enzyme Immunoassay (EIA) Kit which utilizes five monovalent capture antibodies against SEA of SEE (Staphylococcal Enterotoxin A-E), was used. The soured soup sample was diluted with phosphate-buffered saline and adjusted to pH 7.4. The buffered sample was transferred to reaction tubes and sealed with Silicon stoppers to avoid enclosure of air. The enterotoxin assay will be performed by the methods recommended by the manufacturer of the kit and results will be recorded visually after 15 minutes. One hundred and fifty microliters (150  $\mu$ l) of sample were added into each of the immunoassay wells and allowed to stand for 20 minutes after which results is read as positive or negative. A positive result indicates two red lines while one red line indicates a negative result. activated charcoal was soaked in three hundred millilitre (300ml) of each solvent namely cold water, hot water and ethanol. Each conical flask was covered with cotton wool wrapped with aluminium foil and were left in a rotary shaker for two days. The solutions were filtered using sterile filter paper (Whatman No 1). The filtrates then serve as the solutions [19].
- **Detection of *Bacillus Cereus* Toxin in Soured Soups:** The lateral flow assay device (Duopalh® cereus Enterotoxin Immunoassay) was used for the detection of *Bacillus cereus* toxin. Ten milliliters (10 ml) of sample buffer (provided) was mixed with the soured soup samples and homogenized and centrifuged at 7000 rpm for 30 minutes. The supernatant was passed through 3.45 $\mu$ m membrane filter. One hundred and fifty microliters (150 $\mu$ l) of each of the filtered sample was added to the immunoassay port, following the manufacturer's instructions. The results were read as positive if red line was visible after 20 minutes incubation at room temperature. Tests were considered valid only when control lines were visible. Two red lines indicate positive while one red line indicates negative results.
- **Detection of *Clostridium Botulinum* Toxin in Soured Soups:** Biothreat Alert test strip for the detection of BONT/A/B was used. Fifty millimeters (50 ml) of soured soup sample was mixed with 10 ml of sample buffer homogenized with a bench top stomacher (Seward, Cincinnati, OH) to make a homogenous suspension. The food-buffer mixture was centrifuged at 7,000  $\times$  g for 30 mm. at  $4^\circ\text{C}$  to remove solid particles, and then filtered through a membrane filter. Five hundred microliters (500 $\mu$ l) of soured soup sample supernatant were thoroughly mixed with 500 $\mu$ l of sample buffer in a glass test tube and used for the experiment. Each test device was removed from a protective pouch and placed on a flat surface. 150 $\mu$ l of the sample was placed into the round sample sort according to the manufacturer's instructions and results were recorded visually after 20 minutes. The appearance of red lines indicates positive (one of the red lines is the control line) while only one red line indicates negative results.
- **Detection of *Staphylococcus Aureus* Toxin in Soured Soups:** Enzyme Immunoassay (EIA) Kit which utilizes five monovalent capture antibodies against SEA of SEE (Staphylococcal Enterotoxin A-E), was used. The soured soup sample was diluted with phosphate-buffered saline and adjusted to pH 7.4. The buffered sample was transferred to reaction tubes and sealed with Silicon stoppers to avoid enclosure

of air. The enterotoxin assay will be performed by the methods recommended by the manufacturer of the kit and results will be recorded visually after 15 minutes. One hundred and fifty microliters (150 µl) of sample were added into each of the immunoassay wells and allowed to stand for 20 minutes after which results is read as positive or negative. A positive result indicates two red lines while one red line indicates a negative result.

**Table 1.** Isolation of Bacteria in Soured Soups

Soup Samples	Bacteria Associated
Okra	Lactobacillus sp, Bifidobacterium sp, Streptococcus sp, Pediococcus, sp Leuconostoc sp, Campylobacter jejuni, Listeria monocytogenes, Salmonella sp.
Banga	Lactobacillus sp, Clostridium botulinum, Bifidobacterium sp, Streptococcus sp, Pediococcus, sp Leuconostoc sp, Escherichia coli, Vibrio parahaemolyticus, Campylobacter jejuni.
Native	Lactobacillus sp, Bifidobacterium sp, Streptococcus sp, Pediococcus, sp Leuconostoc sp, Staphylococcus aureus, Shigella sp, Salmonella sp, Clostridium perfringens, Yersinia enterocolitica, Bacillus cereus.

- **Absorbing Effect of Activated Charcoal Solution on Clostridium Botulinum:** Five millilitre (5ml) of activated charcoal were added into each soured soup (10ml) sample and the mixture was transferred into a pot placed on fire and heated to a temperature of 1000 C. Later, analysis was done to determine the toxin content. 150µl of the sample was placed into the round sample sort (lateral flow assay device) according to the manufacturer's instructions and results were recorded visually after 20 minutes. The appearance of red lines indicates positive (one of the red lines is the control line) while only one red line indicates negative results.
- **Absorbing Effect of Activated Charcoal Solution on Staphylococcus Aureus Toxin:** Five millilitres (5ml) of activated charcoal solution were added into each soured soup (10ml) sample and the mixture was transferred into a pot placed on fire and heated to a temperature of 100<sup>0</sup> C. Later, analysis was done to determine the toxin content. 150µl of the sample was placed into the round sample sort (Enzyme Immunoassay (EIA) Kit) according to the manufacturer's instructions and results were recorded line) while only one red line indicates negative results [20].

#### 4. RESULTS AND DISCUSSION

Table 1 shows that Lactobacillus sp, Bifidobacterium sp, Streptococcus sp, Pediococcus, sp Leuconostoc sp Bacillus cereus, Clostridium perfringens, Campylobacter jejuni, Vibrio parahaemolyticus, Yersinia enterocolitica, Salmonella sp, Shigella sp, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, and Clostridium botulinum were associated with the soured soups.

**Table 2.** Bacteria Toxin Detected in Soured Soups

Soured Soup	Toxin		
	Clostridium Botulinum	Bacillus Cereus	Staphylococcus Aureus
Okra	-	-	-
Banga	+	-	-
Native	-	+	+

Absence of toxin; -

Presence of toxin; +

Table 2 shows that Staphylococcus aureus enterotoxin and Bacillus cereus toxin were detected in the native soured soup, Clostridium botulinum was observed in the banga soured soup while none was detected in the okra soured soup. This signals a potential health risk upon consumption. The presence of these toxins in the Native and Banga soured soups highlights the importance of stringent food safety

measures during preparation, storage, and handling and stresses the need for thorough cooking practices, proper storage conditions, and careful monitoring to prevent bacterial contamination and toxin production in food items. The varying toxin profiles across different soured soup samples suggest that not all soured soups carry the same level of risk. The absence of detectable toxins in the Okra soured soup implies a lower risk compared to the Native and Banga soured soups.

**Table 3.** Effect of Activated Charcoal Solution on Bacteria Toxins in Soured Soups

Soured Soups	Effect of Activated Charcoal on Bacteria Toxins		
	<i>Clostridium Botulinum</i>	<i>Bacillus Cereus</i>	<i>Staphylococcus Aureus</i>
Banga	-		
Native		-	-

Positive; +

Negative; -

**Table 3** shows the effectiveness of activated charcoal solution in absorbing (removing) the bacteria toxins in soured soups. The results suggest that activated charcoal solution shows promise in absorbing or removing bacteria toxins from soured soups, signifying a potential method for detoxification or reduction of toxin levels in contaminated soups. This could have implications for the food industry, potentially leading to the development of protocols or procedures for the treatment of contaminated soup products and reducing economic losses due to spoilage.

**Table 1** showed that *Lactobacillus* sp, *Bifidobacterium* sp, *Streptococcus* sp, *Pediococcus* sp, *Leuconostoc* sp *Bacillus cereus*, *Clostridium perfringens*, *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, *Salmonella* sp, *Shigella* sp, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Clostridium botulinum* were associated with the soured soups. This agrees with the work of, who reported that these organisms are major food spoilage microorganisms. The study also agrees with the findings of [1] Whose works isolated these organisms from various cooked food items.

**Table 2** shows that *Staphylococcus aureus* enterotoxin and *Bacillus cereus* toxin were detected in the native soured soup. *Clostridium botulinum* was observed in the banga soup, while none was detected in the okra soup. This is in line with the findings of [21], [11], [9] which noted the presence of *B. cereus* toxins in rice dishes. Also, it agrees with the findings of [8], which analyzed vanilla sauce and detected *B. cereus* toxins. Also, [1], confirmed *B. cereus cereulide* in foods and found that cereulide production occurred in rice dishes and other starchy foods. *B. cereus* bacteria is mostly found in rice, rice products, grains, oriental dishes, spice meats, vegetable dishes, soups, and sauces. This bacterium also grows well in a variety of cooked foods, such as meats, poultry, sauces, puddings, soups, rice, potatoes, and vegetables. *Bacillus cereus* is a spore-former that grows in the presence of oxygen; the spores survive boiling for several hours and remain viable in cooked foods and at suitable temperatures; and it grows, multiplies, and produces toxins.

Moreover, detecting *B. cereus* toxins in soured, especially egusi soup, may be due to the presence of its heat-resistant spores from the ingredients (melon seed or vegetable) used in the preparation of the soup, which, when the soup cools down, germinate, multiply, and produce toxin. Inadequate temperature management occasionally plays a part in *B. cereus* food poisoning; the bacterium multiplies in food that has been precooked and then not heated up enough or else not adequately cooled down beforehand. Also, *B. cereus* can generate spores that can survive high heat and are still capable of generating viable cells at low temperatures; these then often secrete toxins that are heat-stable. Although this is contradictory to the findings that *B. cereus* diarrhoeal enterotoxins are unstable; heat or temperatures of >55°C for 20 minutes will denature the toxin, while that of Emetic toxin cereulide is highly resistant to heat and to all normal food processing and preparation temperatures. This agrees with the findings of [9], who detected *S. aureus* in many cooked foods, and those of [21], who noted that toxins from *S. aureus* are the most frequent cause of food-borne diseases in many countries. Other workers [15], [2], [3], also reported the presence of enterotoxigenic *S. uteritis* and its enterotoxin in raw milk and cheese. *Staphylococcus aureus* cells grow on many food items, under aerobic or microaerophilic conditions at 37°C and are resistant to dry heat. The toxins are produced easily in foods that require hand preparation, such as vegetable salad, potato salad,

sandwich spreads, cooked meat sauce, and soup. Contamination may be introduced into foods by direct contact with human hand or arm lesions and by coughing or sneezing and that *Staphylococcus aureus* food poisoning is the most widespread and frequent cause of outbreaks of foodborne diseases in many countries and occurs after eating foods containing enterotoxins. Symptoms include nausea, vomiting, abdominal pain, diarrhoea, headache, and a drop in blood pressure and in Nigeria and some other developing countries, this disease is usually under-reported due to a lack of notification to the surveillance health authority.

Table 3 indicates the effectiveness of the activated charcoal solution in removing the bacteria and toxins present in the soured soup samples. This occurrence is due to the fact that activated charcoal has the ability to absorb poisons. This disagrees with the findings of [9] who noted that onion and red oil cannot neutralize bacterial toxins; also, [10] who noted that heating soured soup does not denature the toxins in it. It disagrees with the findings of, who noted that Staphylococcal Enterotoxins (SEs) are heat-stable and resist high temperatures and environmental conditions of drying and freezing, and that usual cooking procedures, pasteurization, and drying do not inactivate these toxins. It also does not correspond with the studies of [12], in which *B. cereus* toxin remains active after treatment with other plant extracts. However, the findings of [20] showed that diarrhoeal toxin of *B. cereus* can be inactivated by heating with other treatments for 5 minutes at 56°C [22].

## 5. CONCLUSION

*B. cereus*, *C. botulinum*, and *S. aureus* toxins were detected in the soured soups. Activated charcoal has the potential of absorbing (removing) the bacterial poison produced by *C. botulinum*, *S. aureus* and *B. cereus*, implying that activated charcoal has absorbing (removing) effect on the toxins produced in the soured soups by the bacteria. It is, therefore, safe to consume soured soups when it has been treated with activated charcoal. The use of activated charcoal in the treatment of soured soups as a way of repairing the soup for consumption is a reality.

### Recommendations

Based on the findings of this study, the researcher hereby submits the following recommendations:

- Soured soups of any kind should not be consumed because it contains bacterial toxins, which are harmful to harm the body.
- The use of activated charcoal in communities should be encouraged as treatment for soured soups as it removes bacterial toxins from the soups.

### Acknowledgments

The authors have no specific acknowledgments to make for this research.

### Funding Information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

### Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Thankyou Saturday Okpabi	✓	✓	✓	✓		✓		✓	✓	✓	✓		✓	✓
Sopakiriba Lawson	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓
Woke Gift		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	
K. H. Ogbonda	✓	✓	✓		✓	✓	✓	✓		✓	✓	✓	✓	

C : Conceptualization

M : Methodology

So : Software

Va : Validation

I : Investigation

R : Resources

D : Data Curation

O : Writing - Original Draft

Vi : Visualization

Su : Supervision

P : Project administration

Fu : Funding acquisition

Fo : Formal analysis

E : Writing - Review & Editing

### Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

### Informed Consent

All participants were informed about the purpose of the study and their voluntary consent was obtained prior to data collection.

### Ethical Approval

Not Applicable.

### Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.




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**How to Cite:** Thankyou Saturday Okpabi, Sopakiriba Lawson, Woke Gift, K. H. Ogbonda. (2024). Detoxification of bacterial toxins in soured soups using activated charcoal, *International Journal of Agriculture and Animal Production (IJAAP)*, 4(2), 58.67. <https://doi.org/10.55529/ijaap.45.9.18>

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