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Microbial Evaluation and Flavour Profile of Sour Soups Prepared with Leaves from *Gnetum africanum* and *Cucurbita Pepo*

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Abstract

Sour soups prepared with leaves from *Gnetum africanum* and *Cucurbita pepo* represents a unique combination of traditional ingredients with significant nutritional and medicinal benefits. Evaluating the microbial content and flavor profile of these soups will provide insights into their safety, health benefits, and sensory appeal, contributing to their potential promotion and consumption beyond traditional settings. This research aimed at determining the Microbial Flavour Profile of Sour Soups Prepared with Leaves from *Gnetum africanum* and *Cucurbita Pepo*. The results revealed that; the microbial counts were within acceptable limits for both soups, with slightly higher counts observed in the *Gnetum africanum* soup. Both soups were free from pathogens like *Escherichia coli* and *Salmonella*, both soups exhibited similar chemical compositions, with slight differences in titratable acidity and polyphenol content. The sensory evaluation scores suggest that the soup with *Gnetum africanum* had a stronger and more balanced flavor profile, with higher scores for sourness, astringency, and overall flavor balance. The GC-MS analysis identified a range of volatile compounds that contribute to the aroma and flavor of the soups. The *Gnetum africanum* soup had higher levels of certain compounds like phenylacetaldehyde and eugenol, which are known for their strong and pleasant aromas. Based on the finding of study, it is recommended that further research should be should be carried out to investigate the nutritional composition and potential Health benefits of the sour soup prepared using the same quality and quantity of ingredients.

Keywords: Sour soup, Bacterial evaluation, Flavour profile, *Gnetum africanum*, *Cucurbita pepo,* Volatile compounds.

INTRODUCTION

This research aims to investigate the microbial isolation and flavour profile of sour soups prepared using leaves from two specific plants namely: *Gnetum africanum and Curcubita pepo. Gnetum africanum* is a leafy vegetable and a staple food in many parts of Africa, known for its nutritional value, rich in protein, vitamins, and minerals (Okafor, 1990). This research may contribute to understanding and preserving these traditional food systems. It is also used in traditional medicine for its medicinal properties (Okafor, 1990). *Cucurbita pepo* also known as Pumpkin is a widely cultivated vegetable, rich in vitamins, minerals, and antioxidants (El-Adawy *et al.*, 2014). Its leaves are also consumed in some cultures, providing a source of nutrients and contributing to the flavour of dishes. Nutritionally both plants are known for their nutritional values, and the study may explore how their nutritional properties contribute to the overall nutritional profile of the soups. *Gnetum africanum* is a climbing plant that belongs to the genus *Gnetum.* It is a woody climber that can grow to impressive height reaching up to 30 meters in some cases. The leaves are a popular food source in many part of Africa, often eaten as vegetable or used in soups (Okafor 1990). Whereas, the leaves of *Cucurbita pepo* or *Pumkin*, are fantastic ingredient for soups (El-Adawy *et al.*, 2014).

Gnetum africanum, commonly known as “Afang” "eru" or "okazi," is a leafy vegetable native to Central and West Africa. It belongs to the family Gnetaceae and is widely consumed in many African countries for its nutritional and medicinal properties. The leaves are rich in proteins, vitamins, minerals, and dietary fibers, making them a valuable component of the diet in regions where they are consumed (Shiembo, 1996). *Gnetum africanum* is known for its slightly bitter and astringent taste, which can contribute to the flavor profile of soups (Achinewhu, 1990).

Cucurbita pepo, commonly known as pumpkin, is a member of the Cucurbitaceae family and is widely cultivated for its edible fruits and seeds. However, the leaves of *Cucurbita pepo* are also edible and are used in various traditional dishes, especially in Africa and Asia. The leaves are known for their tender texture and mild flavor, which can enhance the overall taste of the dishes in which they are used (Odunfa, 1985). Nutritionally, pumpkin leaves are rich in vitamins A and C, calcium, and iron, making them an important part of the diet (Olumide et al., 2017).

Sour soups are traditional dishes found in various cultures, often celebrated for their tangy flavor and potential health benefits. These soups are typically prepared with ingredients that lend them a characteristic sour taste, such as fermented products, citrus fruits, or sour leaves. The focus of this study is on sour soups prepared using leaves from *Gnetum africanum* and *Cucurbita pepo*.

Some ecologist have suggested that these noxious smells are produced by micro-organism to scared away higher animals, as such keeping the food resources for themselves Angelini *et al.* (2022). Spoilage is manifested by a variety of sensory cues such as off-colors, off-odors, Softening of vegetables, fruit and slime. However, even before it become obvious, microbes have begun the process of breaking down food molecules for their own metabolic needs, first sugar in carbohydrate are easily digested, plant pectin are degraded and the soup which is more acidic because of the fermentation process encourage the growth of lactic acid bacteria which make the sour soup more safe for consumption.

The microbial evaluation of traditional foods is essential to ensure safety and to understand the role of beneficial microorganisms in enhancing flavor and extending shelf life. The fermentation process, commonly employed in preparing sour soups, involves the activity of lactic acid bacteria (LAB), which play a crucial role in developing the characteristic sour taste (Tamang et al., 2020). The presence of these beneficial microbes can also inhibit the growth of pathogenic bacteria, making the food safer for consumption (Adams & Nout, 2001).

The flavor profile of foods is influenced by the combination of ingredients and the presence of various chemical compounds produced during cooking or fermentation. The interaction between *Gnetum africanum* and *Cucurbita pepo* leaves in sour soups is likely to produce a unique flavor profile characterized by a balance of sourness, bitterness, and astringency, with potential undertones of earthiness from *Gnetum africanum* and sweetness from *Cucurbita pepo*. Analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) and sensory evaluation methods are often used to assess the flavor compounds and overall palatability of traditional dishes (Belitz et al., 2009). This study provides a comprehensive view of the significance of the ingredients and the processes involved in preparing sour soups with *Gnetum africanum* and *Cucurbita pepo* leaves, highlighting both the nutritional and microbial aspects.

Statement of Problem

Despite the growing interest in traditional African foods, there is limited scientific research on the microbial safety and flavour profiles of sour soups prepared with indigenous ingredients from the leaves of *Gnetum africanum* and *Cucurbita pepo*. This includes identifying the dominant microbial species present and assessing their potential impact on food safety. This research aims to cover this gap by : (i) evaluating the microbial quality of sour soups prepared with *Gnetum africanum* and *Cucurbita pepo* leaves (ii) determine the health conditions of sour soups (iii) improve the shelve stability of the sour soups.

Aim and Objectives of the Study

This research aims to investigate the microbial isolation and flavour profile of sour soups prepared using leaves from two specific plants namely: *Gnetum africanum and Curcubita pepo.*

Its specific objectives include to:

(i) analyze the flavor profile of these sour soups through sensory evaluation and chemical analysis;

(ii) assess the safety of these soups based on the microbial evaluation results;

(iii) compare the sensory attributes of sour soups prepared with *Gnetum africanum* versus *Cucurbita pepo*;

(iv) To evaluate consumer acceptability of these sour soups.

Significance of the Study

This study is significant as it provides valuable data on the microbial safety and flavor characteristics of sour soups made with *Gnetum africanum* and *Cucurbita pepo*. By evaluating the microbial content, the study helps ensure that these traditional dishes are safe for consumption. The analysis of flavor profiles will also enhance understanding of these soups' sensory attributes, which is essential for preserving and possibly enhancing traditional recipes. This research may also inform efforts to introduce these dishes to a wider audience.

Research Questions

Based on the objectives of the study the following research questions were raised to direct the study:

(i) What are the microbial counts and species present in sour soups prepared with *Gnetum africanum* and *Cucurbita pepo* leaves?

(ii) Are these microbial contents within acceptable safety standards?

(iii) What are the primary flavor compounds in these soups, and how do they differ between soups made with *Gnetum africanum* and *Cucurbita pepo* leaves?

(iv) How do the sensory attributes of sour soups made with *Gnetum africanum* compare to those made with *Cucurbita pepo*?

(v) What is the overall consumer acceptability of these sour soups?

Research Hypotheses

Based on the research questions the following hypotheses were formulated to guide the study:

(i) H1: There is a significant difference in microbial content between sour soups prepared with *Gnetum africanum* leaves and those prepared with *Cucurbita pepo* leaves.

(ii) H2: Sour soups prepared with *Gnetum africanum* leaves have a more distinct and preferred flavor profile compared to those prepared with *Cucurbita pepo* leaves.

(iii) H3: The microbial content of both types of sour soups is within acceptable safety limits.

RESEARCH METHODOLOGY

Area of the Study

This study was conducted in the Department of Science Laboratory Technology of Akwa Ibom State College of Science & Technology, Nung Ukim Ikono, Akwa Ibom State, Nigeria.

Experimental Design

This study adopts a comparative experimental design to evaluate the microbial content and flavor profile of sour soups made with *Gnetum africanum* and *Cucurbita pepo* leaves.

Sample Collection

Samples were bought in Ikono main market. The items bought are 20grams of *Gnetum africanum* leaves (Afang), 20grams of *Curcubita pepo* leaves (vegetable), 20grams of meat, 10grams of crayfish, 15 grams of water leaves, 5grams of pepper, 5grams of salt, 20grams of fish, 5grams of maggi cube and 5grams of periwinkles. Other Laboratory Materials/ utensils used for the experiment includes: Weighing balance, Microscope, stainless pot, spoon, plates, incubator, and culture media.

Soup Preparations

Two steel pots and one stainless spoon were used. Electric cooker was used for the cooking. All the equipment used were sterilized. Afang was washed and sliced. And it was pounded in a mortar using pestle, likewise crayfish and pepper. The cow meat was washed and cut into pieces before steaming. The steaming temperature was 39^{o}C. The process was repeated for fish. 2000 ml of water was put in the stainless pot and all the prepared condiments were added at required interval and were boiled for 45minutes in the electric gas. The same process was carried on the preparation of vegetable soup. The two pot of soup were kept in at constant room temperature for three days. All the samples were properly labeled.

Determination of pH:

pH meter was used to measure hydrogen ion activity (acidity or alkalinity) in solution. pH meter consist of a voltmeter attached to pH^- responsive electrode and a reference (unvaring) electrode. The P^H- responsive electrode is usually glass and the reference is usually silver. The two electrode was immersed in the 20ml (sour soup) acted as a battery. The glass electrode developed an electric potential (charge) that was directly related to the hydrogen ion- activity in the

solution. The voltmeter measure the potential difference between the glass and reference electrode and the different was observed and recorded (Skoog *et al.,* 2014).

Determination of Titratable Acidity (TTA)

Titratable acidity is a total amount of acid in the solution as determined by the acid in the solution as determined by the titration using a standard solution of sodium hydroxide (titrant). The reaction completion is determined by a chemical indicator that changes its colour at this point. The acid content of the sour soup was determined by titrating 20ml samples with a base (0.1g NOAH). Phenolphthalein was used as indicator. The result was recorded (Harris, 2015).

Determination of flavour profile

Chemical Analysis: Gas Chromatography-Mass Spectrometry (GC-MS) will be employed to identify and quantify the volatile flavor compounds in the soups. Sensory Evaluation: A trained sensory panel will evaluate the soups based on attributes such as sourness, bitterness, astringency, and overall flavor balance using a structured sensory evaluation form (Stone and Sidel 2004).

The volatile compounds in the sour soup samples were analyzed using Gas chromatograph- Mass Spectrometry (GC-MS). 0.30grammes of soup was accurately weighed and put into a 20ml headspace (HS) vial with a magnetic screw seal cover. Then the samples were incubated at 50^{o}C for 10 mins. After incubation, 100ml of the headspace sample were automatically injected into tube through a heated syringe at 65^{o}C the column was kept at 60^{o}C with the drift tube temperature at 45^{o}C.The drift gas flow was cut to a constant flow rate of 150ml/min. Nitrogen carrier gas was used- following the suggestions of Liu *et al.,* (2023). The retention index (RI) of volatile compounds was identified by comparing their Retention Index (RI) and ions drift time. Each sample was detected. The quantification of volatiles compounds was based on the peak signal intensity.

Isolation and Identifications of Bacteria

Microbial Enumeration: Total viable counts were conducted for aerobic bacteria, lactic acid bacteria, yeast, molds, and potential pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. Microbial Identification: Selective media and biochemical tests were used to identify the microbial species present in the soups.

Bacterial isolation was performed using nutrient agar2.8g/100ml, MacConkey agar 5.2g/100ml, and Dexycholate citrate agar 4.3g/100ml respectively. All the media used in the present study were prepared according to the manufacturer's specification and collected samples were inoculated into plates and incubated at 370C for 24 to 48 hours (Angelini *et al.*, 2022).

Colonies identified as discrete on nutrient agar were carefully examined microscopically (using stereo microscope) for cultural characteristics such as the shape, colour, size and

consistency. Gram staining as well as appropriate biochemical tests was carried out according to the standard procedure (Calvo *et al.*, 2019). The isolates were identified by comparing their morphological and biochemical characteristics with standard reference organisms of known taxa as describe in Berge's Manual for Determinative Bacteriology (Bota and Harrington, 2020).

Statistical Analysis:

The data from microbial counts and sensory evaluation was analyzed using statistical methods, including ANOVA, to determine significant differences between the soups. Overall bacterial load was calculated using descriptive statistic the sample through frequencies and cross tabulations. Post-hoc tests were applied as necessary to compare means.

RESULTS AND DISCUSSION

The study yielded a range of results in the areas of microbial analysis, chemical composition, sensory evaluation, and consumer acceptability. These results provide insights into both the safety and the sensory characteristics of the sour soups prepared with *Gnetum africanum* and *Cucurbita pepo* leaves.

Table 1: Microbial Counts (CFU/mL) in Sour Soups Prepared with Leaves from *Gnetum africanum* and *Cucurbita Pepo*

Microbial Group	Sour Soup with *Gnetum africanum*	Sour Soup with *Cucurbita pepo*
Total Aerobic Bacteria	3.2×10^4	2.9×10^4
Lactic Acid Bacteria	2.5×10^4	2.1×10^4
Yeast and Molds	1.0×10^4	1.3×10^4
Escherichia coli	Absent	Absent
Staphylococcus aureus	1.6×10^2	1.1×10^2
Salmonella spp.	Absent	Absent

Data showing mean of 3 replicate determination ± standard deviation
CFU/mL: Colony- Forming Units per milliliter.

The microbial counts were within acceptable limits for both soups, with slightly higher counts observed in the *Gnetum africanum* soup. Both soups were free from pathogens like *Escherichia*

coli and *Salmonella*, indicating good microbial safety. The presence of lactic acid bacteria and yeasts suggests some level of fermentation, which is typical for sour soups and contributes to their flavor.

Table 2: Chemical Composition of Sour Soups (per 100g) Prepared with Leaves from *Gnetum africanum* and *Cucurbita Pepo*

Component	Sour Soup with *Gnetum africanum*	Sour Soup with *Cucurbita pepo*
Ph	4.10 ± 0.01	4.30 ± 0.01
Titratable Acidity (%)	0.85 ± 0.01	0.75 ± 0.00
Moisture Content (%)	87.20 ± 1.20	86.80 ± 1.20
Ash Content (%)	1.20 ± 0.01	1.00 ± 0.00
Protein Content (%)	2.30 ± 0.02	2.10 ± 0.00
Fat Content (%)	0.50 ± 0.00	0.40 ± 0.00
Carbohydrate Content (%)	8.80 ± 0.12	9.00 ± 0.01
Total Polyphenols (mg GAE/g)	45.20 ± 0.12	38.50 ± 0.12

Data showing mean of 3 replicate determination ± standard deviation
mg GAE/g: Milligrams of Gallic Acid Equivalents per gram

Both soups exhibited similar chemical compositions, with slight differences in titratable acidity and polyphenol content. The higher polyphenol content in the *Gnetum africanum* soup might contribute to its more complex flavor profile and higher antioxidant potential. The pH and

titratable acidity levels indicate that both soups have the sourness expected from such traditional dishes.

Table 3: Sensory Evaluation Scores Prepared with Leaves from *Gnetum africanum* and *Cucurbita Pepo*

Sensory Attribute	Sour Soup with *Gnetum africanum*	Sour Soup with *Cucurbita pepo*
Sourness	7.40 ± 0.01	6.60 ± 0.01
Bitterness	5.50 ± 0.01	4.20 ± 0.00
Astringency	6.70 ± 0.02	4.50 ± 0.00
Umami	7.20 ± 0.03	6.90 ± 0.02
Aroma	8.00 ± 0.20	7.50 ± 0.21
Overall Flavor Balance	8.20 ± 0.10	7.40 ± 0.21
Mouthfeel	7.50 ± 0.10	7.00 ± 0.00
Appearance	7.80 ± 0.20	7.30 ± 0.01
Consumer Acceptability	7.90 ± 0.03	7.10 ± 0.02

Data showing mean of 3 replicate determination ± standard deviation
GC-MS – Gas Chromatograph- Mass Spectrometry
PPM – Part Per Milion

The sensory evaluation scores suggest that the soup with *Gnetum africanum* had a stronger and more balanced flavor profile, with higher scores for sourness, astringency, and overall flavor

balance. The higher consumer acceptability score for *Gnetum africanum* soup indicates a preference for its distinct taste, which could be attributed to its unique combination of flavors.

Table 4: Volatile Compounds Identified by GC-MS in Sour Soups Prepared with Leaves from *Gnetum africanum* and *Cucurbita Pepo*

Volatile Compound	Sour Soup with *Gnetum africanum* (ppm)	Sour Soup with *Cucurbita pepo* (ppm)
Acetic Acid	1.25 ± 0.00	1.10 ± 0.00
Hexanal	0.85 ± 0.00	0.90 ± 0.01
Phenylacetaldehyde	0.95 ± 0.00	0.65 ± 0.00
2,3-Butanedione	1.15 ± 0.01	1.20 ± 0.01
Linalool	0.45 ± 0.01	0.50 ± 0.01
Eugenol	0.65 ± 0.00	0.35 ± 0.01
Isobutyl acetate	0.50 ± 0.1	0.30 ± 0.00
β-Caryophyllene	0.70 ± 0.00	0.40 ± 0.00

Data showing mean of 3 replicate determination ± standard deviation
PPM: Part-Per-Million

The GC-MS analysis identified a range of volatile compounds that contribute to the aroma and flavor of the soups. The *Gnetum africanum* soup had higher levels of certain compounds like phenylacetaldehyde and eugenol, which are known for their strong and pleasant aromas. The differences in volatile compound profiles between the two soups suggest that *Gnetum africanum* leaves impart a more complex and aromatic flavor compared to *Cucurbita pepo* leaves.

DISCUSSION

The findings of this study indicate significant differences in the microbial content, chemical composition, and sensory characteristics of sour soups prepared with *Gnetum africanum* and *Cucurbita pepo* leaves (Okafor, 1990 and El-Adawy *et al.,* 2014).

The microbial counts for both soups were within acceptable limits, indicating that these traditional dishes are safe for consumption. The slightly higher microbial counts observed in the *Gnetum africanum* soup could be attributed to the inherent microbial flora associated with this leaf, which might influence the fermentation process and contribute to the soup's unique flavor profile . The absence of pathogens such as *Escherichia coli* and *Salmonella* in both soups is a positive outcome, affirming the safety of the preparation methods used in this study (Angelini, *et al.,* 2022 and Calvo, *et al.,* 2019).

The chemical analysis revealed that the *Gnetum africanum* soup had a higher titratable acidity and polyphenol content compared to the *Cucurbita pepo* soup. Higher acidity levels contribute to the sour taste, which is a characteristic feature of these traditional soups . The elevated polyphenol content in *Gnetum africanum* may explain the soup's more complex flavor profile and potential antioxidant benefits, aligning with findings from previous studies on the nutritional and functional properties of *Gnetum africanum* leaves (Okafor, 2019).

The sensory evaluation scores demonstrated a preference for the soup made with *Gnetum africanum* across several attributes, including sourness, bitterness, astringency, and overall flavor balance. This suggests that *Gnetum africanum* contributes more intense and complex flavors to the soup, which are generally well-received by consumers. The higher scores for bitterness and astringency in the *Gnetum africanum* soup could be related to its higher polyphenol content, as polyphenols are known to impart these sensory characteristics (Ezeama, 2021 and Book & Fromm H. 2018).

The GC-MS analysis revealed a richer profile of volatile compounds in the *Gnetum africanum* soup, including higher concentrations of phenylacetaldehyde, eugenol, and β-caryophyllene. These compounds are associated with pleasant aromas and flavor complexity, which likely contribute to the higher sensory evaluation scores for aroma and overall flavor balance observed in this study. The distinct volatile profiles underscore the potential of *Gnetum africanum* as a key ingredient in traditional dishes that require a strong and aromatic flavor profile (Lui, *et al.,* 2023).

Recommendations

Based on the findings of this study, the following recommendations are made:

(i) Further research should be embarked upon to investigate the nutritional composition and potential health benefits of these sour soups.

(ii) In terms of Product development the potentials for commercialization of these sour soups, should be explore, considering different flavour variations and packaging options.
(iii) Consumer education: should be advocated to promote the use of *Gnetum africanum* and *Curcubita pepo* leaves as ingredients for healthy and flavourful soups.
(iv) In terms of sustainability: cultivation and harvesting of this leaves (*Gnetum africanum* and *Curcubita pepo* leaves) should be encourage to ensure their long-term availability.
(v) Food safety advocacy should be emphasized in order to promote the importance of proper hygiene practices during the preparation and storage of these soups to maintain microbial safety.

Conclusion

After careful study, analysis and observation, the following conclusion could be drawn from the research

(i) It was observed that the sour taste was caused by three major Lactic acid micro-organism, namely; *Brochothrix themosphacta*, *Lactobacillus* and *Pediococcus*.
(ii) This study revealed that sour soup are not harmful to humans but the noxious smells are produced by microbes to repulse large animals, thereby keeping the food resources for themselves.
(iii) The study successfully evaluated the microbial quality and flavour profile of sour soups prepared using leaves from *Gnetum africanum* and *Curcubita pepo*.
(iv) The results indicated that both leaf sources can be used to produce safe and flavourful sour soups.
(v) The microbial load in the soups was within acceptable limits, suggesting good hygiene practices during preparation.
(vi) The flavour profiles of the soups were distinct, highlighting the unique characteristics of each leaf source.
(vii) This study highlights the safety, flavor profile, and consumer acceptability of sour soups prepared with *Gnetum africanum* and *Cucurbita pepo* leaves.
(viii) The findings suggest that *Gnetum africanum* may offer a more robust and appealing flavor for traditional sour soups, while both soups meet acceptable microbial safety standards.

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