Tropical Journal of Applied Natural Sciences

Vol. 4 Issue 1 (2026)

Tropical Journal of Applied Natural Science

ISSN: 2449-2043

https://tjansonline.org/view-paper.php?id=72;

**Volume/Issue:** Volume 4, Issue 1 **Published:** March 17, 2026

# EVALUATION OF MICROBIAL QUALITY OF SEASONINGS PREPARED USING *ARACHIS HYPOGEAE* AND *LACTOBACILLUS PARACASEI*

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## ABSTRACT

Consumption of unwholesome food has being the major threat to the public health as shown in high rate of morbidity and mortality. Microbial contamination introduces potent toxins that are highly debilitating to visceral organs and overall metabolic processes in the body. This study evaluated microbial quality of seasonings prepared using *Arachis hypogeae* and *Lactobacillus paracasei*. Groundnut seeds (*Arachis hypogeae*) and dried tilapia fish were purchased at Eke Awka, Awka South L.G.A. Anambra State. The natural seasonings were prepared using solid state fermentation process while the fermenter was *Lactobacillus paracasei* obtained from Zaharm Analytical and Research Laboratory, Amawbia, Anambra State. The microbial quality of the prepared seasonings was examined using standard microbiological technique. The result revealed total aerobic heterotrophic bacterial count (THABC) in all the batches of which Batch A was highest ($4.0X10^2$ cfu/g) while the lowest was Batch E ($3.1X10^2$ cfu/g). However, the microbial counts were within the standard stipulated by the International Standard Organization (ISO). Furthermore, Total *Salmonella count*; Total coliform count; Total Faecal coliform count; Total *Shigella* count; Total *Staphylococcus aureus* count; Total yeast count; Total mould count; Total *Vibrio* count, and Total *Listeria monocytogenes* counts yielded no growth after 48 h incubation at 37°C. Therefore, the seasonings are wholesome for human consumption due to satisfactory microbial quality observed.

**KEY WORDS**: Seasoning; *Lactobacillus paracasei*; *Arachis hypogeae*; Microbial Quality

## 1.0 INTRODUCTION

*Arachis hypogeae* is a legume commonly known as groundnut or peanut (Akram et al., 2018). The seed is globally consumed due to the presence of vital nutrients that the body needs such as protein, carbohydrate, fibre, calories etc. (Akram et al., 2018). Groundnut is affordable due to adaptability to most soil and climatic conditions, though there are regions that are well known as cultivators of groundnut (Akhtar et al., 2014). Groundnut is consumed in different forms such as cooking, frying, raw consumption, salting among others (Arya et al., 2016)

It is worthy to note that several people dislike consuming groundnut in any of the forms outlined above due to lack of the desired satisfaction (Adedokun et al., 2013). For instance, some consumers have complained of emergence of pimples in the facial region, due to excessive oil it contains while some have also complained of stomach upset after consumption (Akram et al., 2018).. This clearly indicates that there should be alternatives form of preparing groundnut in order to optimize its nutritious composition and enhance its consumption (Eniodiok et al., 2017).

Research has revealed that certain bacterial species are capable of fermenting groundnut seed into a form that is more palatable to taste bud (Yang et al., 2016; Chukwu et al., 2017; Chukwu et al., 2018b; Chude et al., 2020). Fermentation of groundnut changes its original form, due to the activity of fermenting bacteria such as *Lactobacillus* species, *Bacillus* species among others (Akram et al., 2018). The product of microbial fermentation of groundnut can be used as a condiment or seasoning, for food preparation such as stew, porridge, and other local dishes (Adedokun et al., 2013).

It has been established that any product that is meant to be consumed must undergo microbial quality assessment to ensuring that it is pathogen free (Yang et al., 2016a; Chukwu et al., 2017; Chukwu et al., 2018b, Chude *et al*., 2020). This is essential because introduction of contaminated food into the system could pose threat to the structure and function of the body. Different pathogenic bacteria and fungi have been isolated and characterized in environmental samples and ready to eat food products sold to the public, and the effect has been revealed on the increase

in hospital cases on daily bases (Akram et al., 2018; Iheukwumere et al., 2018), due to food poisoning and food intoxication such as *Escherichia coli, Salmonella* species, *Listeria monocytogenes, Shigella* species, *Klebsiella* species among others (Chude et al., 2020)..

Several researchers have studied the microbial quality of ready to eat food products such as Yang et al. (2016a); Chukwu et al. (2017); Chukwu et al. (2018b) and Chude et al. (2020) but few studies are available on evaluation of microbial quality of seasoning prepared using *Arachis hypogeae* and *Lactobacillus* species. Hence, the aim of this study is to evaluate microbial quality of seasonings prepared using *Arachis hypogeae* and *Lactobacillus* species.

## 2.0 MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out at Amawbia, Awka South L.G.A. in Anambra State, Nigeria. Amawbia is 325m above sea level and lies between latitude 06$^{o}$11.434'N – 06'11.643N and longitudes 07$^{o}$03.649'E – 07$^{o}$03.691'E. It falls within the humid tropical climate belt of Nigeria. There are two seasons which are well marked in this region where the maximum average rainfall is experienced during July and August. The mean annual rainfall is in range of 1500-2500 mm. Amawbia has a mean annual maximum temperature of 32.9$^{o}$C, mean annual minimum temperature of 23.4$^{o}$C, while the soil monthly mean temperature is 30$^{o}$C. The major anthropological activities are farming and hunting. Amawbia is situated along Enugu-Onitsha express road. It is about 35 km from Onitsha and it has boundaries with Awka on the west, Nawfia, Umuokpu on the south, and on the North, Enugu Agidi.

### 2.2 Sample Collection

Groundnut seeds were purchased at Eke Awka, Awka South L.G.A. Anambra State. The seeds were put in a sterile polyethylene bag, and appropriate label was placed on top of the bag for easy identification before transporting to the laboratory for analysis. Similarly, dried fish samples (Tilapia) were purchased at Eke Awka Market and put into polyethylene bag with appropriate label for easy identification before transporting to the laboratory for analysis. The *Lactobacillus paracasei* used as fermenter was obtained at Zaharm Analytical and Research Laboratory, Amawbia, Anambra State, Nigeria.

### 2.3 Preparation of *Arachis hypogea* Seeds

Five hundred grams (500 g) of the groundnut seeds were weighed using an analytical weighing balance (JJ224BC). The seeds were sundried for 72 h to facilitate removal of the outer layers. The seeds were ground using an electric blender (SC-1589) which had been disinfected using 70% ethanol. The dried fish samples were descaled and deboned using a sterile kitchen knife before blending using the same electric blender mentioned above. The blended groundnut seeds and dried fish were put in a sterile container and covered tightly.

Plate 1: Seeds of *Arachis hypogeae*

## 2.4 Formulation of the Seasoning

### 2.4.1 Solid state fermentation of *Arachis hypogea*

The powdered form of the seed was stored in a sterile rubber container prior to fermentation. Similarly, the dried tilapia fish which had been deboned and descaled was blended using the same electric blender and was stored in a sterile rubber container prior to fermentation. Fermentation was carried out using the method described in a study published by Chude et al. (2020). The starter culture (*Lactobacillus paracasei*) was washed using sterile water and normal saline (0.85% NaCl) in a centrifuge (80-1) five times in order to remove odour. The cells were obtained in a sediment form and normal saline was added to obtain a liquid preparation which was used for solid state fermentation. The fermentation was done using sterile beakers (250 mL). Twenty grams (20 g) of the powdered groundnut were weighed out using an analytical weighing balance (JJ224BC) and put into the fermenting beakers. Likewise, the same quantity of the powdered groundnut was added into the beakers. Then, 5 mL of the *Lactobacillus paracasei* was measured using a sterile syringe and put into the beakers. Additional 10 mL of sterile water was added into the beakers. The content of the beakers was thoroughly mixed using a sterile glass rod. Then, the preparation was allowed to ferment at 30±2°C for 96 h.

After fermentation process, other excipients such as salt, vitamins, magnesium, potassium, and calcium were added in minute quantity (0.1 g per 100 g of the seasoning) and the product was thoroughly mixed together using a sterile spoon. The product was dried using an electric oven at 80°C for 7 days**.** After drying, water activity of the fermented samples was determined, then, it was ground into powder, and stored in a sterile screw capped container for subsequent analysis. Packaging of the product was done using a manual moulder into colourful shape and wrapped using a sterile coloured aluminum foil.

## 2.5 Microbial Quality of the Prepared Seasoning

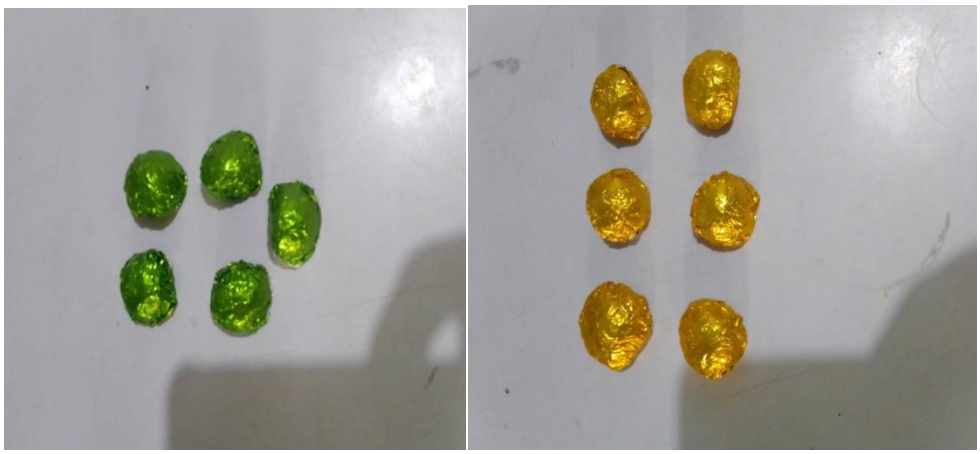
The seasoning was prepared in $10^{-1}$ dilution by weighing 1.0 g of the sample into 20 mL test tube (Pyrex). This was followed by addition of 4 mL of peptone water which had been sterilized, and it was well mixed, and then filled up to 10 mL using the diluent. The same method was used in all the samples prior to culture on nutrient agar (BIOTECH),

MacConkey agar (BIOTECH), Eosin methylene Blue agar (BIOTECH), Deoxycholate Citrate agar (BIOTECH), Mannitol salt agar (BIOTECH), Thiosulfate – Citrate Bile salts sucrose agar (TCBS/BIOTECH), and Brain Heart Infusion agar using pour plate methods for total heterotrophic aerobic bacterial counts (THABC), total coliform counts (TCC), total faecal coliform counts (TFC), total *Salmonella* counts (TSC), total *Shigella* counts (TSHC), total *Staphylococcus aureus* counts (TSAC), total *Vibrio* counts (TVC) and total *Listeria* monocytogenes count (TLMC), respectively. The total yeast counts (TYC) and total mold counts (TMC) were evaluated by culturing 0.1 mL of the samples on Sabourand Dextrose agar (SDA/BIOTECH) using spread plate method. The THABC and TYC were enumerated after 24 h at growth temperature of 35±2$^{o}$C, TCC, TSC, TSHC, TSAC, TLCC, and TVS were enumerated after 48 h at growth temperature of 35±2$^{0}$C, TFCC was enumerated after 48 h at growth temperature of 44.5$^{o}$C and TMC was enumerated after 7 days at room temperature (30±2$^{o}$C), as describe in the study published by Iheukwumere et al. (2018).

## 3.0 RESULTS

### 3.1 Evaluation of Microbial Quality of the Seasoning

The microbial quality of the seasoning is presented in Table 1. The result revealed that the five batches of the seasoning recorded similar microbial quality but total aerobic heterotrophic bacterial count (THABC) yielded growth in all the batches though batch A recorded the highest bacterial count of 4.0X10$^{2}$ followed by batch B (3.8X10$^{2}$), batch C (3.6X10$^{2}$ ), and batch D (3.2X10$^{2}$) while the lowest was batch E, which recorded a bacterial count of 3.1X10$^{2}$ but all the bacterial growth was within the standard stipulated by the International Standard Organization (ISO). Meanwhile, TSC= total *Salmonella count*; TCC = Total coliform count; TFCC = Total Faecal coliform count; TSC= *Shigella* count; TSAC= Total *Staphylococcus aureus* count; TYC= Total yeast count; TMC= Total mould count; TVC = *Vibrio* count, and TLMC= Total *Listeria monocytogenes* count were zero in all the batches, which met the standard stipulated by the International Standard Organization (ISO).



A B

Plate 1: Seasonings prepared using peanut (A: AHS; B: AHCS)

Table 4: Microbial quality of the seasoning

| Parameter | Seasoning | | | | | ISO Standard (CFU/ml) |
|---|---|---|---|---|---|---|
| | Batch A | Batch B | Batch C | Batch D | Batch E | |
| TSC(CFU/g) | 0 | 0 | 0 | 0 | 0 | 0 |
| THABC (CFU/g) | $4.0X10^2$ | $3.8X10^2$ | $3.6X10^2$ | $3.2X10^2$ | $3.1X10^2$ | <106 |
| TCC(CFU/g) | 0 | 0 | 0 | 0 | 0 | <100 |
| TFCC(CFU/g) | 0 | 0 | 0 | 0 | 0 | 0 |
| TSAC(CFU/g) | 0 | 0 | 0 | 0 | 0 | <100 |
| TSC(CFU/g) | 0 | 0 | 0 | 0 | 0 | 0 |
| TVC(CFU/g) | 0 | 0 | 0 | 0 | 0 | 0 |
| TYC(CFU/g) | 0 | 0 | 0 | 0 | 0 | <100 |
| TMC(CFU/g) | 0 | 0 | 0 | 0 | 0 | <100 |
| TLMC | 0 | 0 | 0 | 0 | 0 | 0 |

TSC= *Salmonella count*; THABC = Total heterotrophic aerobic bacteria count; TCC = Total coliform count; TFCC = Total Faecal coliform count; TSC= *Shigella* count; TSAC= Total *Staphylococcus aureus* count; TYC= Total yeast count; TMC= Total mould count; TVC = *Vibrio* count, TLMC= Total *Listeria monocytogenes* count.

## 4.0 DISCUSION AND CONCLUSION

Generally, consumption of food is a basic requirement for growth and metabolism, but it is worthy to note that quality food is highly essential for longevity, optimum anatomy and physiology of the body. Also, food consumption is enhanced when the food is prepared using ingredients that improve taste, and adopting good method of food preparation. Individuals in the society are eager to optimizing ingredients in order to enhance the taste of food without considering the health impact of the ingredients after consumption. There are numerous food enhancers in the market such as spices and condiments that have been processed for food preparation and affordability has led to high patronage, especially the low income earners that do not afford meat and fish. These artificial food sweeteners are the major challenges to healthful living, and there is an urgent need for an alternative food sweetener of natural origin, which can restore certain damages that had been sustained due to consumption of unwholesome foods.

The absence of faecal coliform such as *Escherichia coli* and other pathogenic bacteria such as *Staphylococcus aureus* , *Salmonella* species, *Shigella* species, and *Listeria monocytogenes* indicates that the seasonings were prepared in a sterile environment, and are therefore wholesome for consumption as stipulated by the International Standard Organization (ISO). Similar observation was reported by several researchers (Yang et al., 2016a; Chukwu et al., 2018b; Chude et al., 2020; Sandya et al., 2021; Chawafambira et al., 2022). However, this observation does not corroborate to the study conducted by Zabat et al. (2018) who evaluated the final product of sauerkraut and discovered the presence of *Enterobacter* and *Pseudomonas* bacteria in minute microbial load, which they attributed to contamination from the handlers. Meanwhile, the presence of total heterotrophic aerobic bacterial count in the present study was in accordance to the stipulation of ISO, and they are not sources of threat to the final consumers. Similar conclusions were drawn by several researchers (Yang et al., 2016a; Chukwu et al., 2017; Chukwu et al., 2019; Chude et al., 2020; Sandya et al., 2021; Chawafambira et al., 2022) who evaluated microbial quality of their condiments.

**Conclusion**

This study has revealed that seasonings produced using *Arachis hypogeae* and *Lacticaseibacillus paracasei* strain in solid state fermentation are microbiologically safe for public consumption due to the conformity to the standard as stipulated by the International Standard Organization (ISO). Therefore, it is recommended to the public for use in food preparation. However, there is need for toxicity profile and nutritive composition analyses of the seasonings for further authentication.

**Acknowledgement**

I sincerely acknowledge the effort of my Supervisor; Dr. I.H. Iheukwumere (Associate Professor of Medical Microbiology) who ensured that this study was completed at his own personal Laboratory (Zaharm Analytical and Research Laboratory, Amawbia). Furthermore, the effort of my second Supervisor; Dr. B.O. Uba is highly commendable because he provided candid advice that led to completion of this study. Remain blessed.

**Conflict of Interest**

The authors declared that there was no conflict of interest in the study.

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