Evaluation of the Microbiological and Physicochemical Properties of Selected Indigenous Food Spices Vended in Eke Awka Market, Anambra State Nigeria.

Ugochukwu Chukwuma Okafor[1], Chijioke Christian Aniekwu[1*], Ejike Victor Onwujiobi[1], Ozuah chidiebere Lawrence[1], Vitus Ikechi Onyeneho[2]

[1]Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria.

[2] Department of Public Health, National Open University of Nigeria

***Corresponding Author**

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ABSTRACT

The physicochemical properties and microbial quality of selected locally sourced spice samples in Awka, Anambra State was studied. The study elucidates the microbial qualities of these selected spices samples and the physicochemical properties (moisture content, ash content, and pH levels) in five distinct spice varieties: crayfish, thyme, curry, pepper and ginger vended in Eke-Awka Market were also analyzed.Microbial isolates were identified using microbiological standard method, Bacteria isolates includes:*Bacillus* spp, *Escherichia coli, Staphylococcus aureus* and *Clostridium* spp, while fungi isolates includes: *Aspergillus* spp, *Trichoderma* spp, *Mucor* spp, *Rhizopus* spp, *Alternaria* spp, and *Cladosporium* spp. The physicochemical assessment reveals variations in moisture and ash content, reflecting differences in drying and processing methods, as well as distinctive mineral compositions among the spice types. The pH levels, ranging from neutral to slightly acidic or alkaline, contribute to the unique flavor profiles characterizing each spice. Microbial characterization underscores the necessity for rigorous quality control measures, highlighting potential risks associated with spoilage and contamination. This study provides valuable insights into the physicochemical and microbial properties of local spices in Awka. The findings offer a foundation for quality improvement, ensuring that these spices meet both culinary and safety standards.

Keywords: Indigenous Food Spices; Evaluation; Microbiological; Physicochemical Properties; Spice Vending

INTRODUCTION

Indigenous food spices are an integral part of the rich culinary heritage of Nigeria and indeed Anambra State.. These spices have been used for generations, not only to enhance the flavor and aroma of traditional dishes but also for their potential health benefits [1]. The vibrant and diverse culture of Nigeria is reflected in its cuisine, which often relies on a unique blend of indigenous spices to create dishes that are both delicious and culturally significant. Indigenous spices hold cultural significance in various ceremonial and ritual practices across Nigeria. For instance, alligator pepper is commonly used as a symbol of hospitality and goodwill, often offered to guests during traditional ceremonies. Spices are also used in rituals to invoke

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ancestral spirits or deities, highlighting their spiritual importance in Nigerian culture [2]. Nigerian festivals and celebrations are marked by the preparation of special dishes that prominently feature indigenous spices.

However, the safety and quality of these indigenous food spices have become a growing concern, particularly in the context of the informal vending practices prevalent in Awka metropolis and other parts of Nigeria [3]. The unregulated nature of spice vending, with spices often sold in open markets and by street vendors, raises questions about their microbiological safety and physicochemical properties.

Nigeria boasts a rich variety of spices, many of which are regionally specific and employed in accordance with cultural culinary traditions and individual preferences. Despite their extensive use in traditional dishes, a significant number of these spices remain absent from national and regional food composition databases and tables. This omission can be traced back to the historical perception of spices as non-nutritive components of food, as noted by [4]. Considerable research efforts have been dedicated to studying spices in Nigeria, with a particular focus on *Monodora myristica* and *Piper guineense*, as evidenced by studies conducted by [5,6][7,8][9,10,11]. However, these studies have encountered challenges related to accurate identification, variations in reported values, and discrepancies in units of measurement. Therefore, there exists a pressing need for additional research endeavors aimed at validating the existing body of work and exploring lessinvestigated spices. The primary objective is to incorporate these spices into the national Food Composition Table (FCT) and to harness their nutritional potential for various aspects of human nutrition.

Microbiological contamination of food spices can lead to health risks for consumers. Spices are susceptible to contamination by various microorganisms, including bacteria, molds, and yeasts, which can thrive in the warm and humid climate of Nigeria [12]. Most spices high variety of microorganism compared to the other; for instance Ogiri, due to the lipolysis during the process of its fermentation [7]. These microorganisms can not only affect the shelf life of spices but also pose health hazards when consumed. Despite the advanced technologies employed in the food production process, such as freezing, pasteurization, drying, and the use of preservatives, it remains challenging to eliminate the risk of food spoilage [13]. Food spoilage refers to an irreversible alteration in which food becomes unfit for consumption or its quality deteriorates. One significant issue contributing to food spoilage is lipid oxidation. As a result, the food industry has turned to antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) to prevent spoilage [14]. However, concerns about the safety of these synthetic antioxidants have led to a growing demand for natural alternatives. Spices have emerged as a valuable solution for the food industry due to their natural properties [15]. Spices possess inherent antioxidant capabilities, primarily attributed to the presence of phenolic compounds. They exert their antioxidant effects by neutralizing free radicals, binding transition metals, quenching singlet oxygen, and enhancing the activity of antioxidant enzymes [16]. Studies have revealed that the CO_2 extract of ginger exhibits in vitro activity comparable to that of BHT in inhibiting lipid peroxidation, even at elevated temperatures. The presence of pathogens in spices can lead to food borne illnesses, which can range from mild gastrointestinal discomfort to severe and potentially life-threatening conditions [17]. Spices can potentially become contaminated with bacteria such as *Bacillus* species, which can form spores and survive in the environment, including soil and dust [12]. While many Bacillus species are harmless, some, like *Bacillus cereus*, can produce toxins leading to food poisoning. Adequate cooking and proper storage temperatures can help mitigate this risk [18].

Clostridium species are anaerobic bacteria commonly found in soil and the gastrointestinal tracts of animals. Some Clostridium species form heat-resistant spores that can survive cooking. *Clostridium botulinum*, for example, can produce the deadly botulinum toxin under the right conditions. Proper hygiene and storage are essential to prevent Clostridiumrelated foodborne illnesses [19]. *Aspergillus* molds are prevalent in the environment and can contaminate spices during growth, harvesting, or storage. Specific Aspergillus species can produce mycotoxins, such as carcinogenic aflatoxins.

The primary agency responsible for food safety regulation is the National Agency for Food and Drug

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Administration and Control (NAFDAC). NAFDAC has established limits for yeast and mold counts in spices to prevent contamination. Like many other countries, Nigeria enforces a "zero tolerance" policy for the presence of pathogens such as Salmonella and Escherichia coli (E. coli) in spices. Spices should not contain detectable levels of these harmful bacteria. In addition to microbial limits, NAFDAC has set standards for aflatoxins in spices. Spice producers and importers are required to adhere to these limits to ensure the safety of spice products [20].

Furthermore, the physicochemical properties of spices are crucial for their quality and overall acceptability in culinary preparations. Factors such as moisture content, ash content, pH levels, extractable color, and essential oil content influence the flavor, aroma, and visual appeal of spices [2]. The botanical species and specific variety of a spice plant are fundamental determinants of its physicochemical properties. For instance, different types of cinnamon (Cinnamomum spp.) exhibit variations in flavor profiles and essential oil compositions, with Ceylon cinnamon (Cinnamomum verum) being milder and sweeter compared to Cassia cinnamon (Cinnamomum cassia) with its bolder and spicier notes [21]. Variations in these properties can affect the sensory attributes of dishes, leading to a suboptimal culinary experience. Contaminated spices can lead to the secondary contamination of other foods if used during food preparation [22]. Cross-contamination during food handling can introduce harmful microorganisms into dishes, amplifying the risk of food borne illnesses. To mitigate these health risks linked to microbial contamination in spices, it is imperative to implement rigorous food safety practices across all stages of production, processing, storage, and distribution [23]. Though, ensuring the consistent safety of spices in Nigeria can be challenging due to factors such as inadequate infrastructure, limited resources for inspection and testing, and variations in production and handling practices. However, given the cultural and culinary significance of indigenous spices in Nigeria, it is imperative to evaluate the microbiological and physicochemical properties of these spices to ensure better hygiene, safety and improved quality [24]. Hence, the aim of this study is to address these concerns by conducting a comprehensive analysis of selected indigenous food spices which are DryGround Crayfish, Garlic, Ginger, Turmeric, Alligator Pepper, Curry, Thyme, Nutmeg, Ogiri, Chili powder vended in Eke-Awka market.

MATERIALS AND METHOD

Study Area: The study was carried out in Eke Awka market. Awka is the capital city of Anambra State located in the South-Eastern part of Nigeria. It is located between latitudes 6°12′25″N 7°04′04″E.

Sample Collection: Spice samples were randomly purchased from different vendors at Eke-Awka market. Spice samples include: Ogiri(A), Nutmeg(B), Chil Pepper(C), Curry(D), Thyme(E), Dry-ground Crayfish(F), Alligator Pepper(G), Turmeric(H), Garlic(I) and Ginger(J) which were aseptically collected and stored in sterile aluminum foils and transported to the laboratory in Nnamdi Azikiwe University Awka state within 24hours of collection for analysis. Each sample was placed in an airtight container, labeled and kept at room temperature until when needed for further analysis.

Enumeration, Identification and Isolation of Bacteria Isolates:A serial dilution method was used for total viable count and the presumptive test for coliforms. One (1) gram of each of the samples was weighed aseptically and diluted 1: 10 in sterile water. Further serial tenfold dilution was carried out to 10^3. Aliquot 0.1ml of each dilution was plated out on petri dishes. The pure cultures of the isolates were subjected to various morphological and biochemical characterization tests to determine the identity of the bacteria isolates. The various cultural appearances of the isolated pure cultures were recorded, such as shape, colour, margin and elevation.

Microscopic and Biochemical Identification of Bacteria Isolates: The bacteria isolates were subjected to biochemical tests for identification. The microscopic and biochemical tests was done using the standard

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method according to[25]. Include:Gram staining, citrate test, coagulase test, catalase test, oxidase test, indole test, urease test and sugar fermentation test.

Identification and Characterization of Fungal Isolates: Fungi were isolated using plating methods (Okpako et al., 2009). The plating method, 500ul of samples were plated on agar plates and incubated at 25C for 24 hours. Colony from primary plates was sub-culture onto fresh Sabouraud Dextrose Agar (SDA) supplemented with 100mg Kanamycin to inhibit bacterial growth. The sub-culture was carried out to purify the fungi isolates. During the sub-culture an inoculating loop flamed in a Bunsen burner was used to pick the colony and smeared on the agar plate. This was further incubated at room temperature for 7days. Fungal colonies were isolated upon formation, stained with lactophenol and observed under the microscope. Fungi so observed were identified using the appropriate taxonomic guides [26].

Physicochemical Analysis of Samples: Moisture content, ash content and thepH of the samples were determined. For moisture content analysis, a petri-dish was washed and dried in the oven. Approximately 1-2g of the sample was weighed into petri dish. The weight of the petri dish and sample was noted before drying. They were further subjected to the oven and heated at 105^0C for 1hr repeatedly, until a steady result is obtained and recorded [27]:[28].

$$\% \text{ moisture content} = \frac{W_1 - W_2}{\text{Weight of sample}} \times 100$$

Where W_1 = weight of Petri dish and sample before drying

W_2 = weight of Petri dish and sample after drying.

The inorganic residue remnants after heating and volatilization were regarded as the ash. The ash content was analyzed thus; Empty platinum crucible was washed, dried and the weight was recorded, approximately 1- 2g of sample was weighed into the platinum crucible and placed in a muffle furnace at 550^0c for 3 hours. The sample was cooled in a desiccator after burning and weighed as recorded [29].

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where,

W_1 = weight of empty platinum crucible

W_2 = weight of platinum crucible and sample before burning

W3 = weight of platinum and ash.

The pH was measured by Electrometric Method using Laboratory pH Meter Hanna model HI991300. Thus; the electrodes were rinsed with distilled water and blot dry, while the pH electrodes were then rinsed in a small beaker with a portion of the sample. Sufficient amount of the sample was poured into a small beaker to allow the tips of the electrodes to be immersed to a depth of about 2cm. The electrode was placed 1cm away from the sides and bottom of the beaker. The temperature adjustment dial was adjusted accordingly. The pH meter was turn on and the pH of sample recorded.

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RESULTS AND DISCUSSION

Colony counts of the isolates

Table 1: Total Heterotrophic Indigenous Bacteria and Fugal count. This table shows the result of the Bacteria counts and Fungi counts of the selected Spices. With the bacterial count of Ogiri being too many to count also dry-ground crayfish was too many to count. Sample I had the most countable bacterial isolates with 187 while sample E had the fewest 34. Sample F had the highest count of fungal isolates, 67, while sample C had the fewest fungal count 29.

Table 1: Total Heterotrophic Indigenous Bacteria and Fugal count.

Spice Samples	**Bacterial Count** (cfu/g)	**Fungal count** (cfu/g)
A	TNTC	65
B	53	34
C	58	26
D	67	32
E	34	29
F	TNTC	67
G	40	33
H	64	35
I	187	51
J	107	41

Key: TNTC =Too Many to Count

Sample A: Ogiri

Sample B: Nutmeg

Sample C: Chili Pepper

Sample D: Curry

Sample E: Thyme

Sample F: Dry-ground Crayfish

Sample G: Alligator Pepper

Sample H: Turmeric

Sample I: Garlic

Sample J: Ginger

Biochemical Characteristics of Bacteria Isolates

Table 2 showed the biochemical characteristics of bacterial isolates. Four bacteria and were identified in the selected indigenous spices vended in Eke-awka market. The identity of the probable bacteria isolates

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considering their cultural and morphological characteristics were: *Bacillus* spp, *Staphylococcus aureus, Clostriduim* spp, *Escherichia coli.*

Table 2: Biochemical Characteristics of Bacterial Isolates.

CODE	GRAM	SHAPE	FORM	CO	O	U	C	G	S	L	CU	PROBABLE BACTERIA
A1	_	Long rod	Single	_	_	+	+	+	+	+	+	*Bacillus spp.*
A2	_	_	_	_	_	_	_	_	_	_	_	_
B1	+	Rod	Single	_	_	+	_	+	+	+	+	*Clostridium Perfringein*
B2	+	Rod	Clusters	_	+	+	+	+	+	+	+	*Bacillus Licheniformis*
C1	_	_	_	_	_	_	_	_	_	_	_	_
C2	_	Rod	Single	_	_	+	+	+	+	+	+	*Bacillus spp.*
D1	_	_	_	_	_	_		_	_	_	_	_
D2	_	Rod	Single	_	_	+		+	+	_	+	*Bacillus spp.*
E1	+	Rod	Single	_	_	_	+	+	+	_	+	*Bacillus cereus*
E2	_	_	_	_	_	_	_	_	_	_	_	_
F1	_	Long rod	Chains	_	_	+	+	+	+	+	+	*Bacillus spp.*
F2	_	_	_	_	_	+		_	+	+	+	_
G1	_	Rod	Chain	_	_	+	_	+	+	+	+	*Clostridium Perfringens*
G2	_	Rod	Single	_	_	_	+	+	_	+	_	*Escherichia coli*
H1	_	_	_	_	_	_	_	_	_	_	_	_
H2	_	_	_	_	_	_	_	_	_	_	_	_
I1	_	_	_	_	_	_	_	_	_	_	_	_
12	+	Rod	Single	_	_	+	+	+	+	+	+	*Bacillus spp.*
J1	+	Cocci	Clusters	+	_	+	+	+	+	+	+	*Staphylococcus aureus*
J2	_	_	_	_	_	_	_	_	_	_	_	_

Key: O=Oxidase test, Co=Coagulase test, Cu-=Simmone Citrate utilization test, G=Glucose, S=Sucrose, L=Lactose, C=Catalase test, U=urease test.

Macroscopic Appearance and Characteristics of Fungal Isolates

Table 3 showed the macroscopic appearance and characteristics of fungal isolates respectively. Five fungi isolates were identified in the selected indigenous spices vended in Eke-awka market. The identity of the probable fungi isolates considering their cultural and morphological characteristics were: *Aspergilluss* spp , *Trichoderma* spp, *Mucor* spp, *Rhizous* spp, *Alternana* sppand *Cladosporum* spp.

Table 3: Macroscopic Appearance and Characteristics of Fungal Isolates

Sample Code	Macroscopic Appearance on SDA	Microscopic Characteristics	Possible fungi
Sample A, C, D, E, G	Whitish Brown-Like powdery and fluffy- like colony	Septate hyphae, the hyphae are divided into distinct cells by septa (cross-walls). The hyphae are branched, forming a mycelium	*Aspergillus spp.*

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Sample B	Branched Spores formations. Formed colonies are dark, ranging from olivegreen to black.	The conidiophores are branched and bear chains of conidia which are darkly pigmented, singlecelled, with a cylindrical shape.	*Rhizopus spp*.
Sample J	Black, rapidly growing, fluffy colonies	Coenocytic hyphae that produces sporangia, often black and supported by a sporangiophore.	*Rhizopus spp*.
Sample H	Produced black-dark brown multicellular conidia.	The septate has both transverse and longitudinal septa	*Alternaria spp*.
Sample F, I	Cotton/Fluffy appearance.	Sac-like structures containing sporangiospores, supported by a sporangiophore.	*Mucus spp*.

Physicochemical Analysis of the Samples

Proximate Analysis: Moisture Content, Ash Content, and pH.

Table 4 shows the percentage of moisture content of samples C, D, E, F and J. Sample J had the highest % of moisture content 14.512 while sample D had the lowest % 4.706.

Table 4: The percentage of moisture content of samples

Sample	**Weight of sample**	**Weight of Crucible**	**Weight of Crucible + r**	**%**
Dry-ground Crayfish	0.501	33.838	34.292	9.381
Thyme	0.503	33.757	34.190	13.916
Curry	0.510	31.058	31.544	4.706
Pepper	0.502	38.590	39.020	14.343
Ginger	0.503	29.840	30.270	14.512

Table 5 shows the percentage of ash content of samples C, D, E, F and J. Sample C has the highest % of 6.8, while sample J has the lowest % 3.187.

Table 5: The percentage of ash content of samples

Sample	**Weight of sample**	**Weight of Crucible**	**Weight of Crucible + r**	**%**
Dry-ground Crayfish	2.008	33.838	33.920	4.084
Thyme	2.004	33.757	33.850	4.641
Curry	2.028	34.680	34.790	5.424
Pepper	2.000	34.749	34.885	6.800
Ginger	2.008	33.757	33.821	3.187

Table 6 shows the *pH* content of samples C, D, E, F and J. Sample J has the highest *pH* content 7.22, while sample C has the lowest 6.36.

Table 6: The pH content of samples

Sample	***pH***
Dry-ground Crayfish	7.11

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Thyme	6.85
Curry	6.54
Chili Pepper	6.36
Ginger	7.22

DISCUSSION

The evaluation of local spice samples sold in Eke-Awka market in Awka, Anambra State was comprehensively studied. This study used a multifaceted approach encompassing physicochemical analysis, microbial characterization, and identification of microorganisms. The study aimed to provide insights into the quality, safety, and potential shelf life of these spices. The Colony counts of the isolates in Table 1 shows the bacteria and fungi counts of the selected Spices. The table revealed that the bacterial count of sample A (ogiri)was too many to count, also sample F (dry-ground crayfish) was relativelytoo many to count. However, sample I (garlic) had the most countable bacterial isolates with 187 while sample E (thyme) had the fewest bacteria count of 34. Sample F (dry ground crayfish) had the highest count of fungal isolates of 67, while sample C (chilli pepper) had the fewest fungal count of 29. The result shows that Ogiri, being produced through fermentation, likely had distinct microbial profiles compared to other spices like turmeric and ginger with antimicrobial properties, hence their low microbial counts. The spice samples contained a variety of bacteria, as revealed by the microbiological testing. This result agrees with the work done by [7];which highlighted that fermented spices like Ogiri have a high microbial profile due to its fermentation process. *Bacillus* were among the bacterial isolates that were identified by morphological traits and gram staining suggesting environmental contamination or potential spore-forming ability and *Clostridium* species and *Staphylococcus aureus* both pathogenic bacteria raising safety concerns. *Aspergillus* species, *Trichoderma* species, Mucor species, Rhizopus species, Alternaria species, and *Cladosporium* species were among the fungi isolates that were recognized by both macroscopic and microscopic characteristics. The existence of a wide variety of microbial species emphasizes how crucial strict quality control procedures are when producing spices. To protect consumer safety, it is essential to pay attention to storage conditions, processing hygiene, and microbiological contamination prevention.

The moisture values reported here for *Zingiber officinale* and *Thymus vulgaris* are significantly lower than the value reported in the literature by [29], but higher for *Zingiber officinale* compared to studies by [28]. Also, the ash content values obtained for both were much higher when compared to the report by [29]. The observed differences in values could be as a result of seasonal and geographic variations; as these samples were obtained from different geographic locations and years. The assessment of moisture content in the spice samples unveiled varying degrees of water content. Crayfish and Thyme exhibited moderate moisture levels, suggesting a balance between preservation and sensory attributes. Conversely, Curry, Pepper, and Ginger displayed higher moisture content, indicating a potential for increased susceptibility to microbial growth. The findings underscore the importance of appropriate drying and storage practices to ensure spice quality. Ash content analysis revealed diverse mineral compositions among the samples. Curry and Pepper exhibited higher ash content, signifying a richer mineral profile. The results of the ash content of chili pepper were almost similar in studies by [30]. These variations may be attributed to differences in spice types, growing conditions, and processing methods. Understanding these mineral compositions is vital for both culinary and nutritional considerations. The pH levels of the spice samples ranged from neutral to slightly acidic or alkaline. The variation in acidity/alkalinity contributes to the distinctive flavor profiles of the spices. Notably, Ginger displayed an alkaline pH, adding a unique dimension to its sensory attributes.

The integration of physicochemical and microbial findings provides a holistic understanding of the local spice samples. The moisture content influences both sensory attributes and microbial stability. Ash content

contributes to mineral composition, while pH levels impact flavor profiles.

Microbial characterization sheds light on potential spoilage and contamination risks.

CONCLUSIONS

This study provides valuable insights into the physicochemical and microbial properties of local spices in Awka. The findings offer a foundation for quality improvement, ensuring that these spices meet both culinary and safety standards. Future research can delve deeper into specific factors influencing spice quality and explore innovative preservation methods.

COMPETING INTEREST

The authors hereby declare that no competing interests exist regarding the publication of this research.

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