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MICROORGANISMS IN DETERIORATING NIGERIAN MELON (*Colocynthis citrullus* L.) SOUP (EGUSI): HEAT SUSCEPTIBILITY PROFILE AND NUTRIENT DEGRADATION POTENTIAL

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Abstract

Egusi is a popular melon (*Colocynthis citrullus* L.) soup in Nigeria that deteriorates within 24 hours. The study aimed to identify microorganisms associated with spoilage of egusi soup, determine their heat susceptibility profile and nutrient degradation capabilities. Nutrient and Potato Dextrose agar were used to isolate microorganisms from egusi soup obtained from 5 restaurants and kept on the shelf for three consecutive days. The isolates were identified by standard procedure involving microscopy and biochemical tests. Egusi spoilage capabilities of the isolates were tested using losses of nutrients (lipid, carbohydrate, protein) and changes in turbidity and pH as indicators after 24 h. The isolates were also tested for survival at varying temperatures (30-80 ^{0}C). The bacteria isolated were, *Bacillus* sp., *Enterococcus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Escherichia coli and Klebsiella* sp. while the fungi were *Aspergillus niger*, *Penicillium* sp., *Microspora* sp. *and Aphanoascus* sp. Changes in pH and turbidity of egusi were minimal (pH, 6.0-6.2; turbidity reduction, 1.0-1.2%) without inter-species differences (P>0.05) while nutrient losses were 5.5-43.2% with inter-species differences for protein and lipid (P<0.05). Most isolates tolerated a temperature range of 30-50 ^{0}C with only *Bacillus* sp surviving at 80 ^{0}C. Fungal isolates were less tolerant of heat (≤40 ^{0}C). In conclusion, the isolates depleted egusi nutrients and tended to be thermophilic, thereby indicating the need for additional measures to complement 50-60 ^{0}C treatment for preservation. The findings present an insight into the strategy to be adopted to ensure microbial and shelf stability of egusi.

Keywords: Egusi, soup spoilage, microorganisms, survival temperature, nutrient degradation

Introduction

Food deterioration or spoilage is an unacceptable process that renders food products undesirable for human consumption (Bassey *et al.,* 2021) or a deviation from the food's normal state which leads to a loss in the food's inherent property and subsequently its rejection. Deterioration in food products is caused by either the enzymatic activities or the activities of microorganisms in the food. Various food products are prone to microbial spoilage which includes soups.

Egusi soup is one of Nigeria's most famous soups, and most Nigerians consume it. It is commonly known by the Yorubas as Egusi, Igbos as Egwusi and Hausas as Agusi. Egusi soup is a rich soup prepared mainly with melon seeds. Melon seed (*Colocynthis citrullus* L) is a protein-rich seed from the Cucurbitaceae plant family cultivated by many West African countries (Tahir *et al.,* 2022). Melon is rich with nutrients and is composed of 53% linoleic acid, 19% oleic acid, 35% protein,10% carbohydrate, 4% ash, 3% fiber and is rich in vitamins such as vitamin A, vitamin B1 and B2, niacin and thiamin (National Academies

of Sciences, Engineering, and Medicine, 2006; Olubi *et al.*, 2019). The condiments/flavors added during the preparation of Egusi soup may include chili pepper, onions, salt, Maggi cube, locust beans (*Parkia biglobosa*; referred to as ogriri by the Igbos, iru by Yorubas and dawadawa by Hausas). Other sources of protein (crayfish, fish) and a variety of meats (goat, cow chicken or turkey) are often added. Vegetables which may be bitter leaf (*Vernonia amygdalina*), waterleaf (*Talinum triangulare*), amaranthus green (*Amaranthus viridis*) or pumpkin leaf (*Telfairia occidentalis*), and tomatoes (*Solanum lycopersicum)* are usually included depending on preferences. Egusi soup is highly rich in nutrients, thus it serves as a suitable medium for the proliferation and growth of microorganisms.

One major challenge that besets food products is spoilage. Generally, the microbial quality of any food product is associated with the environment the food was produced, sanitary conditions, handling, packaging, and storage conditions (Jay, 2003). For example, Ejechi *et al*., (2023) reported the presence of *Salmonella* and fecal coliforms in food products including egusi soup from over 250 makeshift eateries. *Bacillus cereus, Escherichia coli, Klebsiella aerogenes, Streptococcus feacalis, Staphylococcus aureus, Pseudomonas sp., Salmonella sp,* have been isolated from deteriorating egusi soup as a result of poor hygienic, handling; and these microorganisms were found to be associated with spoilage (Ikeyi*et al.,* 2013; Zokou *et al*., 2022). Due to their ability to survive cooking temperatures, some of these microorganisms are presented as heat resistant and spore formers indicating potential risks of microbial contamination of processed food (Hryndrickx and Scheldeman, 2008).

Apart from the isolation and identification of spoilage and pathogenic microorganisms from egusi soup (Ikeyi *et al.,* 2013; Datsugwai *et al*., 2019; Zokou *et al*., 2022), research has been conducted to improve the quality and extend the shelf life of both egusi soup and egusi seed. Bankole *et al*. (2005) reported that the drying method had no significant effect on the proximate analysis of melon seed, but the oven-dried seed sensory analysis was preferred. The proximate composition and sensory properties of freeze-dried egusi soup were conducted by Omah *et al*. (2015), who reported that the sensory properties of freeze-dried soup were within acceptable limits. Furthermore, the evaluation of instant *dried* egusi soup with and without vegetables by Datsugwai *et al*. (2019) showed that the microbial load, shelf life and overall acceptability of instant egusi with vegetables were better than the instant egusi without vegetables.

However, while most of the studies on spoilage of egusi soup focus on the identity of the associated microorganisms, and sensory and nutritional properties, there is a paucity of information on the growth-limiting temperature and nutrient depletion potential of the microorganisms isolated from deteriorating egusi soup. The study was therefore designed to determine the extent individual microbial isolates can reduce the carbohydrate, lipid and protein content of egusi soup, cause pH and turbidity changes, and their heat tolerance limit. This information may prove to be useful in the formulation of preservative measures for microbial and shelf stability of egusi soup.

Materials and methods

Isolation and identification of microbial isolates in egusi soup

Freshly prepared melon soup was purchased from five “bukas (local restaurants)” in Abraka, Delta State, and set aside on the laboratory bench at room temperature (28±2

^{0}C) for 3 days. Thereafter 10g samples were collected daily and analyzed for microbial content. The samples were mixed with 90 ml of sterile physiological saline and serially diluted. A 0.1ml of each of the dilutions was used to inoculate Nutrient and Potato Dextrose agar plates for the isolation of bacteria and fungi, respectively. Nutrient agar (NA) plates were incubated at room temperature for 24h while Potato Dextrose agar (PDA) plates were for 5 days at room temperature. The bacterial isolates were identified by microscopy, visual (morphological) and biochemical characterization. Biochemical identification was based on catalase test, Triple Sugar Iron (TSI), indole test, citrate test, urease test, oxidase test, methyl red and Voges-Proskauer Test (Chesebrough, 2006). For the fungal isolates, identification was done based on macroscopic and microscopic characteristics, as described by Saxena *et al*., (2015).

Effect of temperature on the survival of isolates

PDA and NA plates were inoculated with 1 ml saline containing 10^2 fungal spores or bacterial cells, respectively and incubated at varying degrees of temperature (30, 40, 50, 60, 65, 70 and 80 ^{0}C) for 24h (bacteria) and 72 h (fungi). The plates were subsequently examined for growth and recorded.

Determination of losses of lipid, protein and carbohydrate, and changes in pH and turbidity of egusi soup

Egusi soup was prepared and sterilized in an autoclave at 121 ^{0}C for 15 minutes. Thereafter, 1 ml of standardized inoculum of each isolate was introduced into the soup and covered. The carbohydrate, protein and lipid concentrations were determined after preparation and 24 hours later. The biuret method was used for the estimation of total protein as described by Dahal, (2024), carbohydrate was by the DNSA (Dinitrosalicylic Acid) method (Miller,1959) while lipid was analyzed by the Liebermann-Burchard method (Campbell and Shawn, 2005). The pH test was determined with an electrode pH meter and turbidity by a visible spectrophotometer at a wavelength of 660nm (Hammond, 2014; Shehata *et al*., 2023).

Data analyses

ANOVA was used to analyze differences in microbial populations of egusi soup from different restaurants. It was also used for the differences in the depletion of carbohydrate, protein and lipid concentration of egusi soup by the microbial isolates.

Results

Changes in the microbial population of egusi soups

Table 1 presents the microbial load in egusi soup samples, purchased from 5 restaurants in Abraka, Delta-State. The bacterial populations were generally high both at 24 h and 48h and tended to be identical in the sample from the 5 restaurants as indicated by the absence of significant difference (Table 1). The populations became too numerous to be counted after 48h. The fungal count followed a similar trend but was countable by 72 h (Table 1).

Identity of isolates and frequency of occurrence

The identities of bacteria and fungi isolated from 'Egusi' soup can be seen in Figure 1. A total of six (6) bacteria and 4 fungi were identified. The trend of isolate detection during storage of egusi soup can also be seen in Figure 1. Almost all the isolates were repeatedly detected throughout storage except *Bacillus sp.* and *Enterococcus sp.* when all the restaurants were considered together (Figure 1) *Bacillus* was not detected on the first day but appeared after 48 and 72 hours while *Enterococcus sp* was detected at 48 hours (Figure 1). However, the presence

of each species varied with restaurant and time of isolation as some species were either not detected in some restaurants or were detected at times differently from observations in other restaurants (Figure 1). For fungal isolates, *Aspergillus niger* and *Penicillium sp.* appeared throughout the 3 days of storage while *Aphanoascus sp.* and *Microspora sp.* were detected only after 24 and 72 hours, respectively (Figure 1).

Table 1: Microbial population in deteriorating egusi soup.

Organism	Restaurants	Microbial population (log cfu/ml)		
		24h	48h	72 h
Bacteria	A	3.47	5.12	NC
	B	3.47	5.15	NC
	C	3.3	5.02	NC
	D	4.4	5.48	NC
	E	3.7	5.19	NC
Sign. diff.	>0.05	>0.05	>0.05	NA
Fungi	A	2.30	2.48	2.78
	B	2.34	2.54	2.80
	C	2.36	2.50	2.82
	D	2.30	2.49	2.78
	E	2.30	2.50	2.69
Sign. diff**.**	>0.05	>0.05	>0.05	>0.05

NC, not countable; NA, not applicable

Effect of temperature on the survival of isolates from egusi soup

Figures 2 and 3 present the effect of temperature on the survival of bacteria and fungi isolated from deteriorating egusi soup after 24h.The test showed that among the ten organisms subjected to seven test temperatures, only *Bacillus sp.* was capable of surviving at the temperature of 80 ^{0}C. All ten organisms grew at 30-40 ^{0}C and thereafter growth began to decline. Some isolates survived till 65 and 70 ^{0}C, *E. coli* and *Enterococcus* survived at 65 ^{0}C and thereafter failed to grow while *Staphylococcus* and *Streptococcus* failed to survive after 70 ^{0}C. Each species varied with restaurant and time of isolation as some species were either not detected in some restaurants or were detected at times different from observations in other restaurants (Figure 1). For fungal isolates, *Aspergillus niger* and *Penicillium sp* appeared throughout the 3 days of storage while *Aphanoascus sp* and *Microspora sp* were detected only after 24 and 72 hours, respectively (Figure 1). (Figure 2). However, with respect to fungal isolates, only *Microspora* and *A. niger* survived beyond 40 ^{0}C, but could not survive at 60 ^{0}C (Figure 3).

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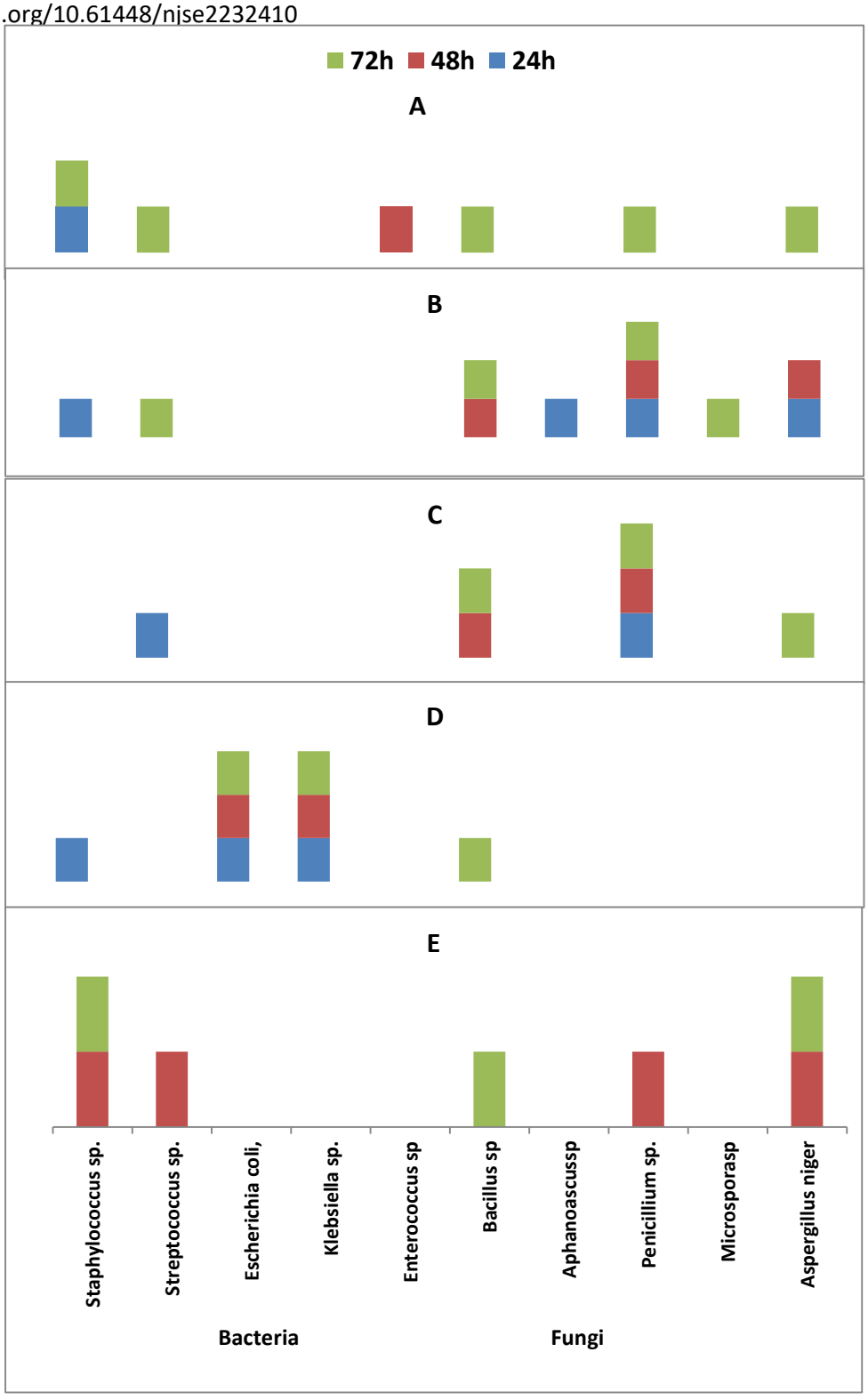

Figure 1: Detection of bacterial and fungal isolates in egusi soup at 24-72h storage : A, B, C, D, E: restaurant sources of egusi soup

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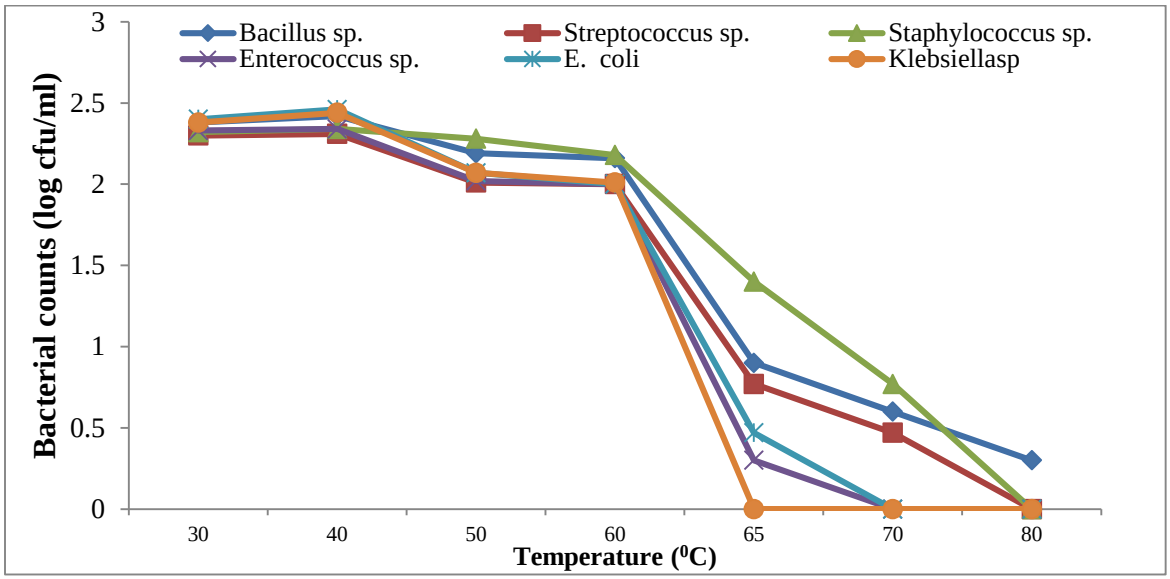

Figure 2: Effect of temperature on survival of bacteria isolated from deteriorating egusi soup

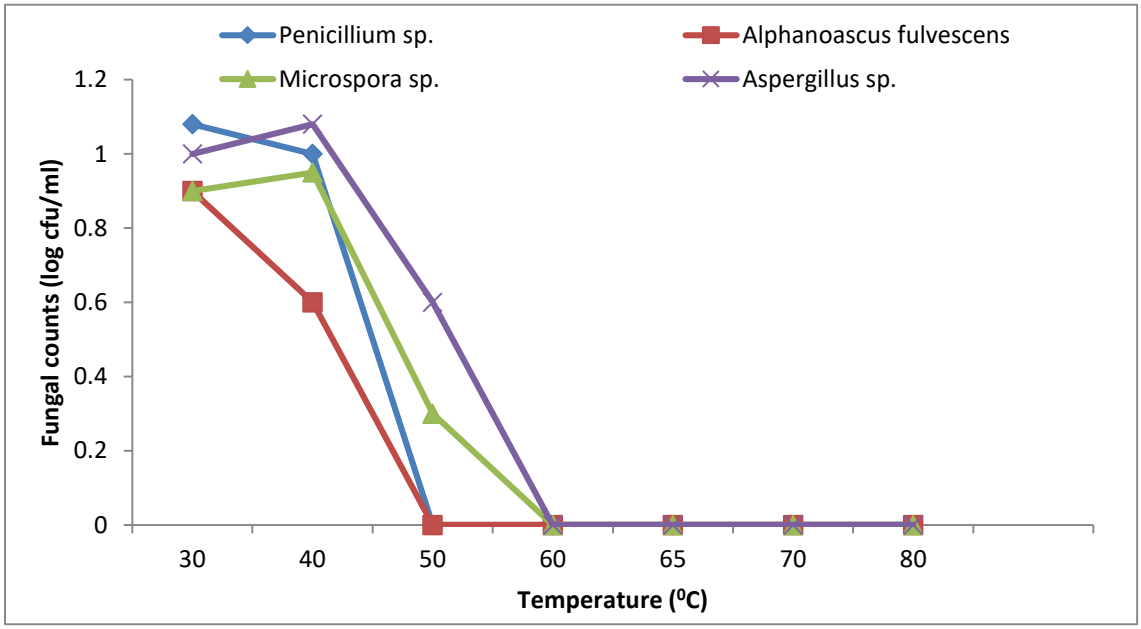

Figure 3: Effect of temperature on survival of fungi isolated from deteriorating egusi soup

Nutrient depletion in egusi soup exposed to bacterial and fungal isolates

The trends in the concentration of carbohydrate, lipid and protein contents of egusi soup after exposure to the organisms isolated from egusi soup are presented in Table 2. The losses of nutrients were generally substantial and varied with bacterial and fungal species as indicated by the result of ANOVA. Significant differences among the bacterial and fungal species occurred with nutrient depletions in all except with bacterial metabolism (Table 2). Generally, lipid was the most metabolized, followed by protein while carbohydrate was the least attacked.

Table 2: Deterioration of egusi soup by bacteria and fungi as indicated by loss of nutrients

Isolates	**Loss of nutrients after 24 h (%)**		
Bacteria	Carbohydrate	Protein	Lipid
Staphylococcus sp.	5.80 ± 0.37	11.34 ± 2.79	24.30±4.78
Streptococcus sp.	6.45 ± 0.70	17.04 ±0.72	18.7 ± 1.83
Escherichia coli,	7.07 ± 1.68	13.96 ± 4.17	32.77±4.67
Klebsiella sp.	7.77± 0.82	17.51 ± 1.74	43.2 ± 2.69
Enterococcus sp	6.59 ± 0.13	11.01 ± 3.50	15.73 ± 3.13
Bacillus sp	6.42 ± 0.58	10.32± 5.66	15.80± 2.26
Sign. diff. (*P*)	>0.05	<0.05	<0.05
Fungi			
Aphanoascus sp	6.16 ± 0.50	15.85 ± 2.68	38.93± 0.86
Penicillium sp.	8.23 ± 0.40	13.08 ± 2.83	27.17 ± 8.45
Microspora sp	7.26 ± 0.53	15.38 ± 3.90	29.07±4.88
Aspergillus niger	5.50 ± 1.38	6.85 ± 1.53	33.73 ± 2.77
Sign. diff (*P*)	<0.05	<0.05	<0.05

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Changes in the physical properties of egusi soup after exposure to isolates

The changes in pH of inoculated egusi soup in storage for 24 hours were minimal as seen in Figure 4. Except for *Enterococcus, Klebsiella* and *Penicillium* species, the difference between the species in the pH changes due to their growth activities was marginal (Figure 4). Generally, turbidity declined without marked differences between the organisms as shown in Figure 4.

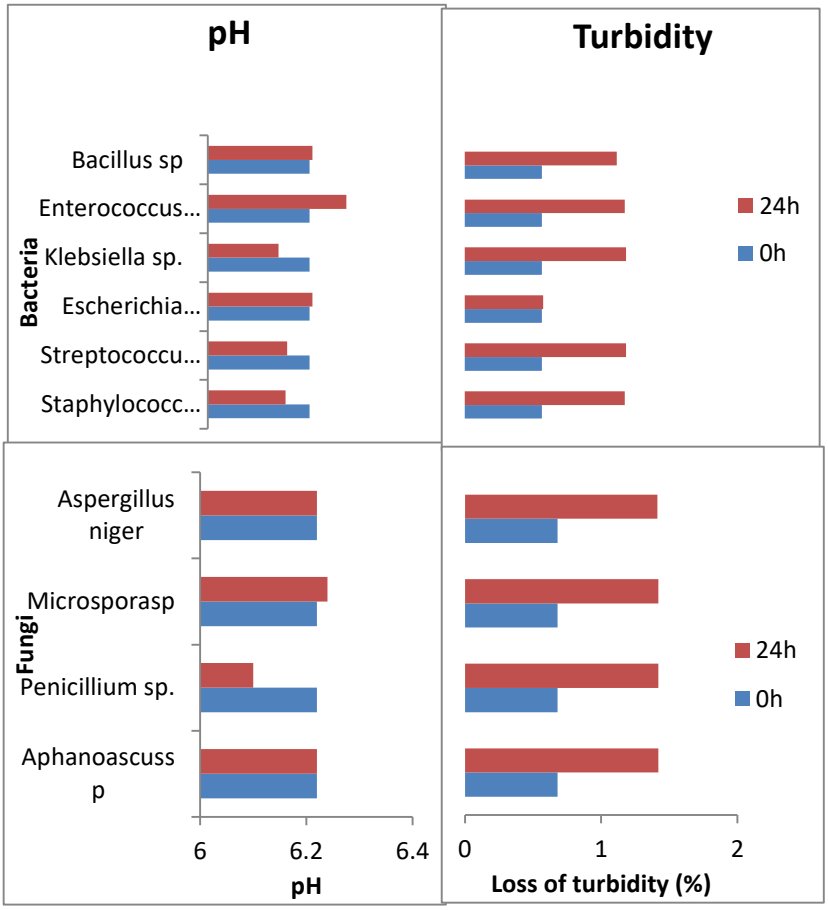

Figure 4: Changes in pH and turbidity of egusi soup after 24 h exposure to bacteria and fungi isolated from deteriorating egusi soup

Discussion

Due to its high nutrient and moisture contents, Egusi soup is highly susceptible to microbial attack. There were variations in the microbial load and species identity of the 5 samples obtained from the 5 restaurants. This could be attributed to the ingredients, sanitation, storage and hygienic conditions in which food was prepared (Ikeyi *et al.* (2013). The initial microbial population in freshly prepared egusi soup was low but increased tremendously after 24 hours of storage. The proliferation may be attributed to the presence of nutrients in the egusi soup which encouraged the growth of the microorganisms. This agrees with the report of Doyle and Beuchat (2022) that the active

multiplication and proliferation of bacteria present in soup is due to its excellence as a medium for their growth. The study showed a diverse microbial community, which is similar to isolates in the microbial profile of egusi reported by Datsugwai *et al*., (2019) in instant egusi soup.

There were changes in species with storage time. The changes and succession patterns observed here could have been driven by changes in nutrient availability and environmental conditions (Fei *et al.,* 2021). The initial colonizers utilized the available nutrients causing a change in the chemical composition, which affected the type and population of organisms that survive. In addition, the immediate environment is altered by fermentation activities resulting in the production of acid and other waste products making conditions less favorable for other bacteria, but favorable for others such as spore-forming bacteria like *Bacillus* sp which has been frequently associated with food spoilage in both egusi soup and other spoilt foods in several studies (Ossai, 2012; Ikeyi *et al*., 2013; Aminu and Ali, 2017; Ao *et al*., 2019; Eboh *et al*., 2022; Akpomie *et al.,* 2022). *Bacillus* spp are referred to, as troublesome food spoilage organisms due to their ability to survive cooking; (Turnbull, 1996; André *et al.,* 2017). This suggests that *Bacillus sp.* is the major organism responsible for the spoilage of egusi soup. The presence of *Enterococcus sp, Klebsiella* sp. and *Escherichia coli* in the soup suggests poor sanitary conditions. *Staphylococcus* sp. *and Streptococcus* sp, on the other hand, are generally part of the normal flora hence their presence in the soup might be due to contamination from food handlers or utensils (Gottfried, 2023). While *Aphanoascus* sp is less commonly reported, they are mainly isolated from soil. *Penicillium* spp. and *Aspergillus* species are known for their role in food spoilage and mycotoxin production (Pitt, 2006) and are commonly isolated from soil. *Microspora* species contribute to spoilage and indicate poor food handling practices.

The result from this analysis on how different temperatures affected the survival of bacterial and fungal isolates from deteriorating egusi soup demonstrated a distinct temperature threshold that affected the growth and survival of these organisms. *Bacillus sp.* has previously been reported to show remarkable heat tolerance (Cebrián *et al.,* 2017) which aligns with the result of this study. *Bacillus* sp. exhibited a remarkable thermal tolerance compared to other bacterial isolates, as Bacillus sp. alone withstood a temperature of 80 ^{0}C and this is associated with heat-resistant spores (Petrillo, 2020). The growth of the other bacterial isolates rapidly declined at 65 ^{0}C, conforming to the general microbial behavior where high temperatures disrupt cellular processes and denature protein (Cebrián *et al.,* 2017). In contrast, the fungal isolates showed different thermal responses when compared to bacteria. Their thermal threshold was limited to 50 ^{0}C, which was much lower than that of the bacterial isolates. Thus, this suggests that where high temperatures are involved in the cooking process, fungal isolates are not major deteriogens. Fungi are less tolerant to elevated temperatures (Abu *et al*., 2020). The result from the microbial response to different temperatures serves as leverage in the thermal processing of food and gives insight into food preservation methods in line with the organisms involved.

The ability of the isolates to metabolize carbohydrate, protein and lipid contents of egusi soup and cause changes in pH and turbidity was investigated. The concentrations of the 3 nutrients were markedly reduced to varying levels by the bacteria and fungi with losses greater in lipid as the results showed. These reductions were

not unexpected because the organisms possess the ability to metabolize them. The fluctuating reduction levels observed, suggest dynamic interactions between the soup's components, biochemical reactions and bacterial activity (active metabolic processes or degradation). The finding that lipids were the most metabolized nutrient suggests that *the organisms* likely utilized or degraded lipids more extensively compared to proteins and carbohydrates. This finding aligns with the known ability of many microorganisms to utilize lipids as a primary or significant energy source (Bustamante-Torres *et al*., 2021; Fashogbon, 2021). Proteins were also metabolized but to a lesser extent compared to lipids. Carbohydrates were the least metabolized nutrient across all tested microorganisms which seems to be an aberration because they are usually the faster source of energy. This would require further investigation. The different microorganisms showed varying levels of nutrient degradation, indicating the differences in microbial metabolism. There were minimal changes in the turbidity and pH of the soup, which could be attributed to the limited study duration (24 h). This notwithstanding, microbial metabolism was indicated by the minimal physical changes

Conclusion

The research identified a diverse range of microorganisms in egusi soup, including *Bacillus sp., Enterococcus sp., Streptococcus sp., Staphylococcus sp., Escherichia sp.,* and *Klebsiella sp*. *Aspergillus niger, Microspora sp. Aphanoascus sp* and *Penicillium sp*. with *Bacillus* as the predominant spoilage bacteria. All the organisms were capable of causing spoilage by depleting the nutritional component of the soup and altering the normal pH and turbidity of the soup. The study on the effects of temperature on the isolates in deteriorating egusi soup revealed significant insights into the thermal tolerance of these microorganisms. Notably, *Bacillus sp.* demonstrated remarkable thermal tolerance, being the sole organism capable of surviving at 80°C, indicating that some bacteria possess mechanisms to endure elevated temperatures, which could pose challenges to food quality and safety. The observation that thermophiles were among the egusi spoilage organisms suggests that heat alone cannot serve as a preservative measure for egusi soup. This insight is therefore an impetus for introducing additional preservative measures consistent with the protocol of hurdle technology if sensory properties are to be maintained. This will be the subject of another study.

Study limitation

Molecular identification was not considered necessary for the study because the organisms isolated are similar to those reported in previous studies on egusi. Instead, this study focused mainly on assessing the temperature sensitivity and nutrient degradation potential of the isolates. However, Molecular identification shall be undertaken in the follow-up to this study on preservative measures.

Conflict of interest

The authors declared no conflict of interest.

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