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Original Article

Microbial quality and physicochemical analysis of some selected soybean cheese sold at different locations in Ilorin, Kwara State

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ABSTRACT

The microbiological and physicochemical quality of street vended fried soyabean cheese products from three different locations was assessed. Standard pour plate technique was used to examine the microbial load of the soyabean cheese. The pH ranged from 4.9 to 5.3. The bacterial count, total coliform and faecal coliform of the soyabean cheese samples ranged from 1.0 x10^3 and 3.0 x 10^5 CFU/g, 6.0 x 10 to 1.0 x 10^5 CFU/g, and 2.0 x 10^1 to 4 x 10^3 CFU/g respectively. The fungal count of the soyabean cheese samples ranged from 7.0 x 10^5 CFU/g, to 3.0 x 10^{-3}. The highest fungal counts were recorded from samples from Al-Hikmah with 7.0 x 10^5 and the lowest fungal counts was recorded from the samples used as control recorded 3.0 x 10^{-3}. Eight tentative bacterial species were isolated and identified

as (tentative) *Bacillus subtilis, Escherichia coli Micrococcus sp., Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter aerogenes and Klebsiella pneumoniae*. Similarly, four fungal species were isolated and identified as *Mucor racemosus, Aspergillus fumigatus, Saccharomyces cerevisiae, and Rhizopus stolonifera.* The results suggest that the soyabean cheese samples were contaminated with pathogenic microorganisms and could cause health hazard to the consumers. There is the need to improve personal hygiene and environmental sanitation during and after production of the soyabean cheese.

Keywords: Microbial Quality, Physicochemical Analysis. , Soybean Cheese Kwara State

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INTRODUCTION

Soybean cheese, widely known as *wara soya* in Nigeria, is a plant-based cheese substitute derived from soy milk. This dairy alternative is rich in proteins, essential amino acids, and isoflavones, making it a preferred choice for individuals with lactose intolerance, vegetarians, and those seeking an affordable protein source [1]. In Nigeria, soybean cheese is traditionally produced by coagulating soy milk using acidic agents such as lemon juice, vinegar, or calcium sulfate. However, its production is largely unregulated, often involving unhygienic processing and handling methods, which can lead to microbial contamination [2]. Various studies indicate that microbial load in soybean cheese varies significantly across different locations due to differences in processing methods, handling, and storage conditions [3]. Microbial contamination in soybean cheese poses significant public health concerns, as it can introduce harmful pathogens such as *Escherichia coli, Salmonella*, and *Staphylococcus aureus*, leading to foodborne diseases [4]. Therefore, this study aims to evaluate the microbial quality and physicochemical properties of soybean cheese sold at different locations in Ilorin, Kwara State, to assess its safety, nutritional value, and potential health risks. Understanding these factors will help improve food safety standards and promote better handling practices among local vendors.

Soybean cheese is primarily composed of proteins, fats, carbohydrates, and bioactive compounds such as isoflavones, which contribute to its nutritional benefits [2]. The production process involves soaking and grinding soybeans, extracting soy milk, and coagulating it using an acidic agent like lemon juice or calcium sulfate.

The resulting curds are then pressed to remove excess moisture and molded into desired shapes [2]. The physicochemical properties of soybean cheese, such as moisture content, pH, texture, and protein composition, are influenced by the type of coagulant used and processing conditions. Research has shown that the nutritional quality of soybean cheese can be enhanced by fortifying it with plant-based proteins such as tiger nut or coconut milk [5]. However, due to its high moisture content, soybean cheese is highly perishable, requiring proper storage conditions to prevent microbial growth. Studies indicate that traditional processing methods often lack proper hygiene standards, leading to contamination with spoilage and pathogenic microorganisms [6]. Improving production techniques, such as heat treatment and controlled fermentation, can enhance the microbial safety and shelf life of soybean cheese, making it a viable alternative to dairy-based cheese in regions with limited access to refrigeration.

The microbial quality of soybean cheese is a critical food safety concern due to the risk of contamination during processing, storage, and retailing. Several studies have reported the presence of bacterial pathogens such as *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Salmonella*, and *Listeria monocytogenes* in locally retailed soybean cheese [7]. These microorganisms can originate from contaminated water, unclean processing equipment, or improper handling by vendors. In Ilorin, microbial contamination levels in soybean cheese vary depending on the location, with higher bacterial loads observed in open markets compared to supermarket-sourced products [8]. Poor hygiene during preparation, exposure to environmental contaminants, and lack of refrigeration contribute to the high microbial load in soybean cheese. The high moisture content and neutral pH of soybean cheese provide a suitable medium for microbial growth, increasing the risk of spoilage and foodborne illnesses [9]. To mitigate these risks, vendors should adopt strict hygiene practices, including proper handwashing, equipment sterilization, and appropriate storage methods. The implementation of microbial testing protocols and regulatory standards can further improve the safety of soybean cheese, ensuring that it meets acceptable public health requirements before reaching consumers.

Physicochemical properties play a crucial role in determining the quality, nutritional composition, and shelf life of soybean cheese. Key

parameters such as moisture content, pH, protein level, fat composition, and ash content influence the cheese's texture, taste, and stability [10]. The moisture content of soybean cheese typically ranges between 50% and 70%, making it prone to microbial spoilage if not properly stored. pH levels in soybean cheese range from 4.5 to 6.0, affecting both its microbial stability and sensory characteristics [10]. Higher acidity levels can help prevent microbial growth but may alter the taste and texture of the cheese. Research indicates that the protein content of soybean cheese is significantly influenced by the type of coagulant used, with calcium sulfate yielding higher protein retention compared to lemon juice or vinegar [6]. Additionally, the fat content of soybean cheese is relatively low compared to dairy cheese, making it a healthier alternative for individuals seeking reduced-fat diets [11]. Understanding these physicochemical properties is essential for optimizing soybean cheese production, ensuring consistency in quality, and developing improved preservation techniques to enhance its shelf life and consumer acceptability.

Soybean cheese is a widely consumed plant-based protein source in Nigeria but concerns over its microbial quality and physicochemical properties necessitate scientific evaluation. The justification for this study lies in the need to assess the safety and nutritional value of soybean cheese sold in Ilorin, Kwara State, where inconsistent hygiene practices and storage conditions may contribute to microbial contamination. Understanding these factors is crucial for improving food safety standards, reducing the risk of foodborne illnesses, and ensuring consumer health. The significance of this study extends to both public health and the food industry, as findings will provide data to guide regulatory policies, improve production techniques, and educate vendors on best practices for handling and storage. Additionally, this research supports the growing demand for plant-based protein alternatives by promoting safer, higher-quality soybean cheese.

MATERIALS AND METHODS

Test tubes, test tube rack, conical flask, measuring cylinder, foil paper, masking tape, slide, cover slip, microscope, micro pipette, Pasteur pipette, spatula, inoculation loop, universal container, petri dishes, autoclave, cotton wool, colony counting machine, electric weighing balance, mortar and pestle, potato dextrose agar, nutrient agar, eosin methylene blue agar, macconkey agar, foil paper.

Sample collection, preparation and Sterilization

Three samples of already processed soybean cheese (fried) from different locations and vendors; Mandate market (North and South) and Al-Hikmah mosque area. The soybean cheese samples were collected into sterile containers and transported immediately to the laboratory for analysis. Information such as time of purchase, container used by vendor, date of purchase of samples were recorded.

Hot air oven was used to sterilize all glass wares namely; conical flask, beakers, pipettes, test tubes, and petri dishes at 180^0C for an hour. Disinfectant such as Dettol was used to disinfect the work bench, ceramic mortar and pestle and wire loop which was flamed before and after every use.

Physicochemical Analysis

Determination of pH

The pH values were determined using the pH meter (Crison basic 20, Barcelona). One milliliter (1 ml) of the diluted sample was weighed and poured into a sterile beaker containing distilled water. The calibrated pH meter was inserted into the beaker and readings were recorded.

Determination of Temperature

The temperature of the soybean cheese samples was taken immediately at the point of purchase or collection by using a thermometer.

Determination of Moisture Content

One gram of each macerated sample was weighed into a crucible of known weight and then dried in an oven at temperature of 170°C, after 1 hour the samples were taken out of the oven and allowed to cool down. The weight of the crucible was subtracted from the weight of the dried sample leaving only the weight of the dried sample (AOAC, 2006). The percentage moisture content was then calculated thus:

$$\text{Moisture (\%)} = \frac{(W1-W2)}{W1} \times 100$$

Where: W1 = weight (g) of sample before drying

W2 = weight (g) of sample after drying

Preparation of Media

The following media was used and were all prepaid according to the manufacturer instruction Potato Dextrose Agar, Nutrient Agar (NA), MacConkey Agar (MA), Eosin methylene blue Agar (EMB) and Mannitol salt agar (MSA) [12].

Serial dilution preparation

Serial dilution was prepared by introducing 1gram of soybean cheese with a sterile blade. A test tube rack was arranged with sterile test tubes containing 9ml of distilled water. A tenfold serial dilution was carried out by homogenizing 1 gram of the sample into test tubes and was labelled as 10^{-1}. It was mixed thoroughly and 1ml was taken again from the 10^{-1} dilution tube and transferred into the next test tube (labelled 10^{-2}). Each test tube was shaken vigorously before each transfer as described by Parumasivam et al. [13].

Inoculation and Incubation

Inoculation was done using the pour plate method. One milliliter from the dilution 10^{-2} was taken using sterile pipette and then introduced into sterile petri dishes, these were done in triplicate of each one of the three samples of soybean cheese. The prepared media were poured into the petri dishes containing 1ml of diluted culture. The plates swirled to mix properly. All plates were allowed to solidify on a bench before incubation. The plates were incubated at 350^0C for 24 hours. Similarly yeast and mould were incubated at room temperature for 7 days and were observed daily to allow them to grow for easy identification.

Viable cell count

Colony counting machine was used for counting the total bacterial aerobic plate count of the plates

Gram Staining Technique

Gram staining reaction has the wide application that is capable of distinguishing virtually all gram positive and gram-negative bacteria. Smear of each isolate was made on the slide and heat fixed. Primary stain (crystal violet) was applied for 45 seconds and washed with gentle running water. Lugol's iodine was also added for 45 seconds and was decolorized with acetone-alcohol and washed with clean water. The slides were counter stained with 30 percent safranin for 30 seconds and washed. It was then air dried and examined at under oil immersion lens of the microscope used [14].

Motility test

Motility was performed using agar with concentration of 0.2-0.5 percent (W/V) was inoculated with the test organism. A stab of each inoculum was made at the centre of each tube. The tube was incubated at 35^0C for 24 hours. The temperature was reduced for Pseudomonas. A diffused growth at place of

inoculation is considered as positive and restricted growth is considered as negative [14].

Catalase test

Catalase test used to determine whether or not a microorganism produces catalase enzyme. A loop full of the culture was placed on a clean grease free slide. The culture was emulsify with a loop full of freshly prepared 3% hydrogen peroxide (H_2O_2) on the slide and the reaction was observe immediately for catalase positive or negative organism [14].

Coagulase test

Coagulase test is particularly employed to differentiate pathogenic Staphylococcus aureus from the non-pathogenic species. Coagulase test was done by slide method using culture from solid media. A clean grease free slide was divided into two using a grease pencil. A drop of normal saline (0.85%) was placed on each of the portions and 18-hour culture of the tests organism was emulsified in little quantity on each of the drops of normal saline until a uniform suspension was obtained. A drop of rabbit plasma was added to one of the suspensions and stirred for about 5 seconds for the presence of Coagulase positive or negative organisms [14].

Oxidase test

Oxidase test was carried out using petri dish method. A drop of 1% aqueous solution of the reagent was placed on the portion of the culture plate containing the test organism. The reaction was observed between 10 seconds [14].

Urease test

Urease test was carried out to determine if a microorganism produces the enzyme urease. Urease broth was prepared according to direction from the manufacturers and 5 ml potion was dispensed into clean test tubes to obtain a slope of I inch built. It was sterilized at 121 0C for 15minutes before the test tube was kept in a slanting position to set. The slope surface was inoculated by streaking with a loop full of the peptone water broth culture. It was incubated at 35oC for 24 hours before the reaction was observed. Pink colour of the media indicated Urease positive [14].

Indole test

Peptone water was prepared by adding 10 g tryptone to 100 ml distilled water. Exactly 0.5 g of sodium was added to the solution and the pH was adjusted to final pH of 7.2. Then, 5 ml was dispensed into sterile test tubes and were covered loosely and autoclaved at 121oC. It was allowed to cool to 30oC and was inoculated and incubated at 35oC for 48 hours. After that, 0.5 ml of kovac's

reagent was added and was gently shaken. It was allowed to stand for 10 minutes. The reaction was observed for red positive colour [14].

Voges-proskauer test

Glucose-phosphate was prepared and sterilized as the growth medium and allowed to cool to at room temperature for 24 hours. Then 1 ml of 40% potassium hydroxide and 3 drops of alpha-napthol was added. The tube was shaken well and allowed to stand for 5 minutes before observation [14].

Citrate utilization test

This test was carried out using Simon's citrate agar method. The media was prepared and dispensed into clean test tubes. It was sterilized at 121oC for 15 minutes and slopes of about 1 inch were made. The test tubes were kept in a slanted position to set and the surfaces of slopes were inoculated by streaking with a loop full of the peptone water broth culture. They were incubated for 48 hours and observed for citrate utilization. [14]

Methyl red test

The glucose phosphate broth was prepared according to manufactures instructions. The medium was, dispense in 9 ml amount into clean test tubes and was loosely tightened and sterilized at 121 OC for 15 minutes. It was allowed to cool to about 30oC then the test organism was inoculated in duplicates. It was incubated for 24 hours and a few drops of methyl red reagent wad added into broth culture and observed [14].

Colony Morphology

The morphological structures of the colony such as shape, color, optical characteristics, surface texture, type of edge and consistency when probed with inoculating loop were observed and recorded.

Enumeration of Fungi

After incubating at room temperature for 5-7 days, plates were carefully observed and all discrete colonies including pin head size were counted with the aid of colony counter. Potato dextrose agar was used for fungi enumeration. The numbers was expressed as colony forming unit per milliliter (cfu/ml) and colony forming unit per gram (cfu/g).

Pure culture

Agar plates were inoculated by streaking using inoculums taken from the primary plates of the fungal isolates earlier carried out at room temperature for fungi.

Characterization and identification of fungal isolates

The pure isolated fungi were identified using cultural and morphological features according to the documented keys in fungal

identification. The isolates were also identified by comparing their characteristics with those of known taxa, and also used standard book for identification of fungi.

Stock culture

Representative colonies on the potato dextrose agar were picked from a sector of plates and further sub-cultured by streaking on a fresh media until pure cultures were obtained. The purified isolates were stored as stock culture on sabouraud dextrose agar slants respectively at 4^0C until required for identification.

Staining of fungal hyphae (cotton blue)

A drop of cotton blue-in-lactophenol was dropped on a clean glass slide (grease free). With the aid of mounted needle a small piece of mycelium free of medium picked and transferred to stain on slide. The mycelium was spread very well on slide with the aid of two mounted needles, cover dlip was gently lowered, taking care to avoid air bubbles. Excess liquid was wiped off putting the slide between two filter papers and applying gentle pressure around cover slip. The slide was first examined under low power and then under high power microscope [14].

RESULTS

The physicochemical properties of soybean cheese samples varied slightly among locations. The pH values ranged from 4.9 (AA) to 5.3 (AN and AS), indicating a slightly acidic nature.

Table 1: Physicochemical Analysis of Selected Soybean Cheese

Sample codes	Ph	Moisture (%) Contents
AN	5.3	13.94
AS	5.3	14.22
AA	4.9	14.90

Key: AN= Awara north; AS= Awara south; AA= Awara Alhikmah

The total fungal count among soybean cheese samples varied signi ficantly, indicating differences in fungal contamination levels. AA (Awara Al-Hikmah) recorded the highest fungal count (7.0×10^5 CFU/mL), suggesting that this sample had the most fungal exposure or ideal conditions for fungal proliferation.

Table 2: Total Fungal Count of Selected Soybean Cheese Samples

Sample codes	FUNGAL COUNT (CFU/ML)
AA	7.0×10^5
AS	4.0×10^3
AN	3.0×10^1
AS2	3.9×10^1
C	3.5×10^{-3}

Key: AN= Awara north; AS= Awara south; AA= Awara Alhikmah

The total bacterial count (TBC) varied significantly among the soybean cheese samples, with AN (Awara North) showing the highest bacterial load (3.0×10^5 CFU/mL).

Table 3: Total Bacterial Count of Selected Soybean Cheese Samples

Sample Codes	Bacterial Count (CFU/ML)	Total coliform count (CFU/ML)	Fecal coliform count (CFU/ML)
AN	1.0×10^5	6×10	1×10^3
AS	11×10^3	10×10	2×10^3
AA	7.0×10^3	2×10^3	4×10^3
C	1.0×10^3	1.0×10^5	2.0×10^1
AN 2	3.0×10^5	1×10^3	3×10^3
AN 3	2.0×10^5	4×10^3	1×10^3

Key: AN= Awara north; AS= Awara south; AA= Awara Alhikmah

The fungal isolates from the soybean cheese samples exhibited distinct colonial morphologies, with variations in texture, elevation, shape, and pigmentation.

Microscopic examination of fungal isolates revealed structural differences among species, aiding in identification

Table 4: Colonial Morphology of Fungal Isolates

SAMPLES CODES	TEXTURE	ELEVATION	SHAPE	PIGMENTATION
AN	Woolly	Raised	Circular	Black
AS	Woolly	Raised	Circular	Black
AA	Smooth	Raised	Irregular	Whitish
C	No growth	No growth	No growth	No growth

Key: AN= Awara north, AS= Awara south, AA= Awara Alhikmah, C = Control

Table 1: The colonial morphology of bacterial isolates from selected soybean cheese samples

SAMPLES CODES	TEXTURE	ELEVATION	SHAPE	PIGMENTATION
AN	Shiny	Flat	Circular	Yellow
AS	Shiny	Convex	Circular	Creamy
AA	Shiny	Flat	Circular	Whitish
C	Shiny	Convex	Circular	Creamy

Key: AN= Awara north; AS= Awara south; AA= Awara Alhikmah; C = Control

The bacterial isolates showed variations in texture, elevation, shape, and pigmentation, which are important in microbial identification.

Table 6: Microscopic Characteristics of Fungal Isolates

ISOLATE CODES	MICROSCOPIC CHARACTERISTICS	PROBABLE IDENTITY
AS	Hypha profusely branched, septate and hyaline. Flat and smooth conidia are borne in chains at the tip of the sterigmata.	*Aspergillus niger*
AN	Isolates were globose to sub-globose, the cells were seriate, the metulae enclosed the vesicles surface and emitted in all directions, the conidia were globose, thin walled, slightly roughed.	*Aspergillus flavus*
AA	Spheroidal to oval ellipsoidal occuring singly in pairs.	*Saccharomyces cerevisiae*
C	Spheroidal to oval ellipsoidal occuring singly in pairs.	*Saccharomyces cerevisiae*
AS2	The hypha are thick and non-septate, columella is round. The sporangiophore departing laterally from mycellium, sporangia filled with spores	*Mucor sp*

Key: AN= Awara north; AS= Awara south; AA= Awara Alhikmah; C = Control

Table 7: Biochemical characteristics of bacterial isolates from selected soybean cheese samples

Bacterial Isolates	Shape	Cell Arrangemen	Gram Staining	Catalase	Motility	Citrate	Coagulase	Oxidase	Indole	Glucose	Sucrose	Lactose	Fructose	Maltose	Methyl red	Voges Proskaur	Nitrate Rreduction	Urease	OF	Spore Formation	H_2S	Probable identity
1	Cocci	Pairs	+	+	-	+	+	-	-	A	AG	AG	AG	AG	-	+	-	+	Fe	-	+	*Staphylococcus aureus*
2	Rod	Single	_	+	+	+	-	-	-	A	AG	AG	AG	A	-	+	+	-	Fe	-	+	*Enterobacter Aerogenes*
3	Rod	Single	-	+	+	_	-	-	+	AG	AG	AG	AG	AG	+	-	+	-	Fe	-	+	*Escherichia coli*
4	Rod	Singles	-	+	-	+	-	-	-	AG	A	AG	AG	A	-	-	+	+	Fe	-	-	*Klebsiella pneumoniae*
5	Rod	Single	-	+	+	+	-	+	-	AG	A	AG	AG	A	-	-	+	+	Fe	-	+	*Bacillus subtilis*
6	Rod	Pairs	+	+	+	+	-	-	-	AG	AG	A	AG	AG	-	+	+	-	Fe	+	-	*Pseudomonas aeruginosa*
7	Cocci	Clusters	+	+	-	+	+	+	-	A	AG	A	AG	A	+	+	-	+	Fe	-	-	*Micrococcus sp*

+= positive, -= negative, OF= oxidation-fermentation, A=acid only, AG= acid and gas

Table 2: Distribution of the fungal and bacterial isolates from the selected soybean cheese samples

TENTATIVE ORGANISMS	AA	AS	AN	C
Staphylococcus aureus	+	+	+	-
Enterobacter Aerogenes	+	+	+	-
Escherichia Coli	+	-	-	+
Klebsiella Pneumoniae	-	+	+	-
Bacillus Subtilis	+	+	+	-
Pseudomonas aeruginosa	-	+	+	-
Micrococcus sp	-	+	+	-
Aspergillus niger	+	+	+	+
Aspergillus flavus	+	+	+	-
Saccharomyces cerevisiae	+	+	+	-
Mucor sp	+	+	+	+

KEY: + = PRESENT, - = ABSENT

The frequency and percentage occurrence of microbial isolates provide **a comprehensive overview of the dominant bacterial and fungal species in soybean cheese**. ***Escherichia coli*** **exhibited the highest occurrence rate (25%)**, with presence only in the **control sample (C), indicating potential contamination from external sources** such as **water, utensils, or human handling**. Other bacterial isolates, including ***Staphylococcus aureus***, ***Enterobacter aerogenes***, Klebsiella ***pneumoniae***, ***Bacillus subtilis***, ***Pseudomonas aeruginosa***, and ***Micrococcus*** **sp.**, each had a **7.5% occurrence rate**, suggesting **diverse contamination sources, including environmental exposure and cross-contamination during processing**. Among the fungal isolates, ***Aspergillus niger***, ***Aspergillus flavus***, ***Saccharomyces*** **cerevisiae, and** ***Mucor*** **sp.** each had a **7.5% occurrence rate**, reinforcing the **high risk of fungal contamination in soybean cheese**. The **consistent presence of Aspergillus spp. is particularly concerning due to their ability to produce mycotoxins, which can pose serious food safety risks**. These findings highlight the **need for stringent microbial monitoring,**

effective preservation techniques, and enhanced hygiene practices to ensure the microbial safety and quality of soybean cheese sold in different locations.

Table 3: Frequency and percentage occurrence of fungal and bacterial isolates from the selected soybean cheese samples

TENTATIVE ORGANISM	AA	AN	AS	C	% OCCURENCE
Staphylococcus aureus	1	1	1	0	7.5
Enterobacter Aerogenes	1	1	1	0	7.5
Escherichia Coli	0	0	0	1	25
Klebsiella Pneumoniae	1	1	1	0	7.5
Bacillus Subtilis	1	1	1	1	7.5
Pseudomonas aeruginosa	1	1	1	1	7.5
Micrococcus sp	1	1	1	1	7.5
Aspergillus niger	1	1	1	1	7.5
Aspergillus flavus	1	1	1	1	7.5
Saccharomyces cerevisiae	1	1	1	1	7.5
Mucor sp	1	1	1	1	7.5

DISCUSSION

Physicochemical Analysis of Selected Soybean Cheese

The pH and moisture content of soybean cheese significantly influence microbial stability, texture, and shelf life. The observed pH values (4.9–5.3) indicate that soybean cheese is slightly acidic, which can inhibit bacterial growth but may favor fungal proliferation [15]. The AA sample (4.9) had the lowest pH, suggesting higher fermentation activity or microbial

metabolism, while AN and AS (5.3) exhibited lower acidity, possibly due to differences in processing methods or ingredient composition [16].

Moisture content is a critical determinant of spoilage, as higher water activity facilitates bacterial and fungal growth. The AA sample had the highest moisture content (14.90%), indicating a higher susceptibility to microbial spoilage, while AN had the lowest (13.94%), which may contribute to extended shelf stability [17]. Studies have shown that higher moisture levels in soy-based cheese products correlate with shorter shelf life due to increased microbial activity [18].

To enhance product safety and longevity, soybean cheese producers should consider moisture reduction techniques, controlled fermentation, and improved packaging. Employing modified atmospheric packaging (MAP) and natural antimicrobials could also help preserve quality while maintaining microbial safety. The findings highlight the importance of physicochemical properties in determining soybean cheese's microbial stability and consumer acceptability.

Total Fungal Count of Selected Soybean Cheese Samples

Fungal contamination in soybean cheese is a significant food safety concern, as molds can produce mycotoxins that pose serious health risks [19]. The fungal load varied significantly among the samples, with AA (7.0×10^5 CFU/mL) having the highest fungal count, likely due to higher moisture content and prolonged exposure to airborne contaminants. The lower counts in AS (4.0×10^3 CFU/mL) and AN (3.0×10^1 CFU/mL) suggest better processing hygiene and reduced exposure to fungal spores, As reported by Madsen et al., [20].

Aspergillus species, commonly found in contaminated cheese, are known to produce aflatoxins, which are potent carcinogens affecting liver function, as also repoted by Navale *et al.*, [21]. Studies indicate that fungal growth in soybean-based products is influenced by humidity, temperature, and post-processing storage conditions [21].

To mitigate fungal contamination, manufacturers should implement proper storage techniques, such as refrigeration and controlled humidity, and use natural antifungal preservatives. Regular microbial testing can help identify contamination risks early, ensuring safer soybean cheese production.

Total Bacterial Count of Selected Soybean Cheese Samples

The total bacterial count (TBC) in soybean cheese is a key indicator of hygiene, processing conditions, and shelf stability. AN (3.0×10^5 CFU/mL) had the highest bacterial load, indicating potential contamination during processing or storage. The fecal coliform count in AA (4×10^3 CFU/mL) raises public health concerns, as it suggests possible fecal contamination from water or handling IRESA, [22].

Escherichia coli, found in some samples, is a strong indicator of inadequate sanitation, while the presence of Bacillus subtilis and Pseudomonas aeruginosa suggests spore-forming bacteria that may survive pasteurization. Bacterial contamination in cheese is often due to poor hygiene, improper storage, or contaminated raw materials [23].

Implementing good manufacturing practices (GMP), improved sanitation protocols, and microbial testing before distribution are essential to reduce contamination risks and ensure consumer safety.

Colonial Morphology of Fungal Isolates

The morphological characteristics of fungal isolates provide insight into potential spoilage organisms in soybean cheese. AN and AS exhibited woolly, black fungal colonies, indicating Aspergillus species, known for producing toxic aflatoxins [15]. AA displayed smooth, whitish colonies, likely Saccharomyces cerevisiae, a yeast involved in fermentation or spoilage [16].

Fungal contamination in dairy alternatives such as soybean cheese is often associated with improper hygiene, storage conditions, and airborne spores [18]. The control sample (C) exhibited no fungal growth, reinforcing that contamination results from handling or environmental exposure [17].

Strict storage guidelines, antifungal treatments, and aseptic handling measures can reduce fungal proliferation, improving product quality and shelf life.

Colonial Morphology of Bacterial Isolates

Bacterial morphology helps in identifying potential pathogens and spoilage organisms. AN exhibited flat, yellow colonies, indicative of Staphylococcus aureus, a pathogen commonly linked to foodborne illnesses [15]. AS had convex, creamy colonies, likely Enterobacter aerogenes, a coliform associated with fecal contamination [16]. AA displayed whitish colonies,

suggesting Escherichia coli, a serious foodborne pathogen [18].

The findings highlight the importance of hygiene in soybean cheese production, as contamination could arise from unclean water, utensils, or handling practices [17]. Proper sanitation protocols and microbial surveillance are necessary to reduce risks.

Microscopic Characteristics of Fungal Isolates

Microscopic examination confirmed the presence of Aspergillus niger, Aspergillus flavus, Saccharomyces cerevisiae, and Mucor sp., all of which are common spoilage organisms [16]. Aspergillus species pose serious health risks due to their ability to produce aflatoxins, which have been linked to liver cancer and immunosuppression [17]. Saccharomyces cerevisiae was present in some samples, possibly due to fermentation or contamination during storage [18].

To prevent fungal contamination, manufacturers should implement better storage conditions, lower moisture content, and antifungal treatments [15].

Biochemical Characteristics of Bacterial Isolates from Selected Soybean Cheese Samples

The biochemical characteristics of the bacterial isolates revealed the presence of diverse microorganisms associated with both pathogenicity and food spoilage. The identification of Staphylococcus aureus, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa, and Micrococcus sp. indicates significant microbial contamination in soybean cheese samples. Staphylococcus aureus, a Gram-positive bacterium, was found to be catalase- and coagulase-positive, characteristics that contribute to its virulence and resistance to immune responses [15]. The presence of E. coli in some samples is concerning, as it is an indicator of fecal contamination and poor hygiene during processing and handling [16].

The detection of Pseudomonas aeruginosa suggests potential spoilage risks, as this bacterium is known for its high adaptability and resistance to antibiotics, making it a frequent contaminant in moist food products [17]. Additionally, Bacillus subtilis, a spore-forming bacterium, was detected, which may indicate contamination from soil, air, or processing equipment. The oxidase and catalase activity of Micrococcus sp. suggests environmental exposure, possibly from airborne contamination or handling [18].

These findings reinforce the importance of strict hygiene protocols in soybean cheese production, including the use of sterilized equipment, proper refrigeration, and improved sanitation practices. Failure to address microbial contamination could lead to foodborne illnesses and reduced product shelf life, which may negatively impact consumer health and economic viability.

Distribution of Fungal and Bacterial Isolates from Selected Soybean Cheese Samples

The distribution of bacterial and fungal isolates among soybean cheese samples revealed a high prevalence of contamination, with significant variations across different locations. Staphylococcus aureus, Enterobacter aerogenes, and Bacillus subtilis were present in all tested samples, except the control, suggesting that contamination occurs primarily due to handling and environmental exposure [16]. The detection of Escherichia coli in AA and C samples is a major concern, as E. coli is an indicator of fecal contamination and potential public health risks [17].

Fungal isolates, including Aspergillus niger, Aspergillus flavus, Saccharomyces cerevisiae, and Mucor sp., were detected across all soybean cheese samples. The presence of Aspergillus species raises concerns about aflatoxin production, which can lead to chronic toxicity, liver damage, and carcinogenic effects [15]. Saccharomyces cerevisiae, commonly associated with fermentation, was present in multiple samples, indicating possible yeast contamination or fermentation during storage.

The absence of major contaminants in the control sample highlights the importance of proper storage, processing, and handling practices in mitigating microbial risks. Strict quality control measures, including heat treatment, improved packaging, and microbial testing, are necessary to prevent fungal and bacterial contamination.

Frequency and Percentage Occurrence of Fungal and Bacterial Isolates from Selected Soybean Cheese Samples

The occurrence and frequency analysis of bacterial and fungal isolates in soybean cheese samples provides insight into the dominant microbial contaminants and potential food safety risks. Escherichia coli had the highest occurrence rate (25%), suggesting a high probability of fecal contamination, which may result from unhygienic handling or contaminated water sources [16].

Staphylococcus aureus, Enterobacter aerogenes, Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa, and Micrococcus sp. each had a 7.5% occurrence rate, indicating diverse contamination sources, including processing environments, equipment, and personnel hygiene [15].

The consistent presence of Aspergillus niger, Aspergillus flavus, and Mucor sp. across all samples suggests a high risk of fungal contamination, particularly due to high moisture content and storage conditions [21]. The dominance of Aspergillus species is particularly concerning, as they are known for producing mycotoxins, which can pose severe health risks.

To mitigate contamination, manufacturers should implement hygienic handling practices, quality assurance protocols, and adequate storage conditions. The findings reinforce the need for strict microbial control strategies, including proper refrigeration, disinfection of equipment, and training of food handlers to minimize contamination risks.

CONCLUSION

This study highlights the microbial quality and physicochemical characteristics of soybean cheese sold in different locations in Ilorin, Kwara State. The pH and moisture content varied among the samples, influencing their susceptibility to microbial contamination. The high fungal and bacterial loads detected in some samples indicate poor hygiene practices, inadequate storage conditions, and possible fecal contamination. The presence of Escherichia coli, Staphylococcus aureus, and Aspergillus species raises significant public health concerns, as these microorganisms are known to cause foodborne illnesses and toxin production. The detection of Bacillus subtilis and Pseudomonas aeruginosa further suggests possible contamination from environmental sources or processing equipment. Proper hygiene, microbial testing, and effective storage techniques are essential to minimize contamination risks and ensure consumer safety. Overall, the study underscores the need for strict microbial surveillance, improved handling practices, and awareness among producers and consumers to enhance the safety and shelf life of soybean cheese.

REFERENCES

1. Arise, A., Ahmed El-Imam, A., & Solola, F. (2022). *Effect of different pre-treatment methods on the quality, sensory properties and*

storability of Bambara groundnut spread. Journal of Culinary Science. Taylor & Francis

2. Odebisi-Omokanye, M. B., Zakariyah, R. F., & Jimoh, F. A. (2016). *Microbiological and physiochemical assessment of street vended fried soybean cheese sold within Ilorin metropolis.* Journal of Science and Technology.
3. Olaniran, A. F., Taiwo, E. A., & Osemwegie, O. (2023). *Application and acceptability of microbiomes in the production process of Nigerian indigenous foods.* PMC. Link
4. Uduku, N. D., Chinwe, I. F., Happiness, O., Immaculate, N. M., Favor, N. O., Peter, I. U., ... & Iroha, I. R. (2023). Antibiotics Susceptibility Profile of Biofilm-Forming Bacteria Isolated from Soybean Milk Drinks Sold in Abakaliki Metropolis, Nigeria.
5. Balogun, M. A., Oyeyinka, S. A., & Kolawole, F. L. (2019). *Chemical composition and sensory properties of soy-tiger nut cheese.* Ceylon Journal of Science. PDF
6. Malomo, S. A., & Arise, A. K. (2023). *Food chemistry advances: In vivo anti-diabetic activity, physicochemical and sensory properties of kunu enriched with African walnut.* ScienceDirect. Link
7. Olea-Rodríguez, M. D. L. Á., Chombo-Morales, P., Nuño, K., Vázquez-Paulino, O., Villagran-de La Mora, Z., Garay-Martínez, L. E., ... & Torres-Vitela, M. R. (2021). Microbiological characteristics and behavior of Staphylococcus aureus, Salmonella spp., Listeria monocytogenes and staphylococcal toxin during making and maturing cotija cheese. Applied Sciences, 11(17), 8154.
8. Borode, O. F. (2017). *The effect of water and ethanol extracts of ginger and garlic on the nutritional quality and physico-chemical properties of stored soymilk.* Academia.edu. PDF
9. Abdulkareem, L. O. (2018). *Quality, sensory and storage attributes of cheese produced from cow milk supplemented with tigernut and coconut milk.* Academia.edu. PDF
10. Bansal, V., & Veena, N. (2024). Understanding the role of pH in cheese manufacturing: General aspects of cheese quality and safety. Journal of food science and technology, 61(1), 16-26.
11. Ray, A., Dev, M., & Das, M. (2024). A concise review on current trend of replacing fat

in the development of non-dairy cheese analogues. *Food Science and Biotechnology*, 1-8. a. *Advance in Research*, *24*(6), 168-176.

12. Elbing, K., & Brent, R. (2002). Media preparation and bacteriological tools. *Current Protocols in Molecular Biology*, *59*(1), 1-1.
13. Parumasivam, T., Riazi, M., & Mohamad, S. (2024). Microbiology for Pharmacy: A Practical Guide. Penerbit USM.
14. Mansur, M. M., Aliyu, R., Mustapha, S. B., Maryam, B. M., Habibu, A., Harris, G. N., & Mohammed, A. O. (2024). Assessment of bacterial contamination on surgical instruments commonly used in Dutse General Hospital, Jigawa State, Nigeria. Dutse Journal of Pure and Applied Sciences, 10(4c), 296-304.
15. **Muzamail, S., & Javid, H. S. (2023).** Physicochemical changes in zinc-fortified soy cheese: A two-month storage study for enhanced production and shelf life optimization in the dairy industry. *ResearchGate*. Link
16. **Iammarino, M., Bevilacqua, A., & Speranza, B. (2024).** Novel chemical, microbiological, and physical approaches in food safety control, volume II. *Frontiers in Nutrition*. DOI: 10.3389/fnut.2024.1513864
17. **Bodhankar, H., Nagda, P., & Dhanmeher, B. (2023).** Development and nutritional evaluation of vegan authentic cheese balls; a rose-flavored rasgulla. *SSRN Electronic Journal*. Link
18. **Oupathumpanont, O., Wisansakkul, S., & Jindaprasert, A. (2024).** Texture and nutritional value development of plant-based analog cheese products. *SSRN Electronic Journal*. Link
19. Tian, F., Woo, S. Y., Lee, S. Y., Park, S. B., Im, J. H., & Chun, H. S. (2022). Mycotoxins in soybean-based foods fermented with filamentous fungi: Occurrence and preventive strategies. *Comprehensive Reviews in Food Science and Food Safety*, *21*(6), 5131-5152.
20. Madsen, A. M., Frederiksen, M. W., Jacobsen, M. H., & Tendal, K. (2020). Towards a risk evaluation of workers' exposure to handborne and airborne microbial species as exemplified with waste collection workers. *Environmental Research*, *183*, 109177.
21. Navale, V., Vamkudoth, K. R., Ajmera, S., & Dhuri, V. (2021). Aspergillus derived

mycotoxins in food and the environment: Prevalence, detection, and toxicity. *Toxicology reports*, *8*, 1008-1030.

22. IRESA, T. T. (2021). Assessment of pathogenic bacteria and hygienic practice of vendors from a traditional fermented milk/yoghurt among yoghurt vended house in Negele Arsi town, Oromia, Ethiopia.
23. Elegbeleye, J. A. (2020). *Characterisation and Spoilage Potential of Bacillus subtilis Complex Group Isolated from Milk Processing Environment* (Doctoral dissertation, University of Pretoria).
24.