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Quantitative Microbial Risk Assessment of Ready-to-Eat Marinated Soy Cheese Sold on University Campus, Jos Nigeria

[1] Davou KV, [2] Dapiya HS, [3] Zumbes HJ, [4] Dingmun PJ, [5] Yakus SA, [6] Mathew CL, [7] Amapu TY, [8] Ibrahim HI

[1, 2, 3, 8] Department of Microbiology, University of Jos, Nigeria
[4] Department of Microbiology, Plateau State University, Nigeria
[5] Department Biology, College of Arts Science and Technology Kurgi, Plateau, Nigeria
[6] Department of Nutrition and Dietetics, College of Health Technology Zawan, Jos, Nigeria
[7] Department of Science Laboratory Technology, University of Jos, Nigeria

Corresponding Author: **Davou KV**

Abstract

Quantitative Microbial Risk Assessment (QMRA) is a scientific method used to estimate the risk of illness caused by foodborne pathogens through the consumption of contaminated food. This study determined the QMRA of ready-to-eat (RTE) marinated soy cheese sold on the University campus, Jos Nigeria. A total of 90 soy cheese samples were randomly collected from vendors, with structured questionnaires administered to consumers for consumption patterns and associated health outcomes data. A QMRA for *E. coli* and *S. aureus* was carried out using Monte Carlo simulation and the Beta-Poisson model following standard guidelines. The results revealed high total viable bacteria counts ranging from 8.16×10^2 to 8.60×10^5 CFU/g, coliforms count from 3.00×10^2 to 3.67×10^3 CFU/g, and staphylococci count from 2.67×10^2 to 3.13×10^4 CFU/g exceeding acceptable RTE food standards. Out of 186 bacterial isolates, *E. coli* (25.80%) was the most occurred followed by *S. aureus* (20.97%) while *Shigella* spp. (8.06%) were the least prevalent. Self-health risk profiling showed that 73.3% of surveyed consumers reported symptoms of illnesses including; diarrhea (56.7%), vomiting (16.7%), with nausea (13.3%) as the least clinical symptoms. Key high-risk groups identified were females, individuals aged 30–35, and frequent consumers. QMRA indicated high infection probabilities for *S. aureus* (0.99) and *E. coli* (up to 0.99 annually), reflecting a cumulative risk with repeated exposure. This high infection risk associated with ready-to-eat marinated soy cheese consumption underscores the need for improved safety practices to prevent foodborne disease outbreaks.

Keywords: Quantitative Microbial Risk Assessment (QMRA), Marinated Soy Cheese, *Escherichia Coli, Staphylococcus Aureus*, Nigeria

Introduction

Soy cheese is a soft cheese traditionally prepared by curdling hot soy milk with either a salt-based coagulant (like calcium sulfate) or an acid-based coagulant such as glucuno-D-lactose (Bagirei *et al.,* 2021) [2]. Although, soy cheese production is an eastern and southern Asia culture, its consumption has gained acceptance among diverse populations (Zinia *et al.,* 2022; Qin *et al.,* 2022) [39, 25]. Accordingly, global market size for soy cheese is projected to grow annually at CAGR of 12.50% with a volume of 37.01k kg by 2029 (Data Bridge Research, 2024) [8].

In Nigeria, the soy cheese popularly called 'awara' is commonly consumed as spicy marinated product prepared with sauce from finely chop onions and vegetables. With the rising consumption, there is a driving preparation of marinated soy cheese that is sold in open markets as a delight snack in Jos Nigeria. The marinated soy cheese is often prepared and handled not under stringent food safety and hygiene regulations, making it high-risk food harboring pathogens of great significance in the epidemiology of foodborne disease outbreaks (Saleh *et al.,* 2021; Omorodion, 2022; [23] Mengistu *et al*., 2020),. Like most ready to eat foods, it could be contaminated with pathogens such as *Escherichia coli, Salmonella*, and *Staphylococcus aureus*, major causes of foodborne illness (Bristone *et al.,* 2018; Solefack *et al.,* 2024) [3, 33]. In addition, the primary ingredients in the marination are potential source of pathogens, accountable for causing foodborne diseases outbreaks (Solefack *et al.,* 2024) [33].

Several microbiological surveys of the locally processed soy cheese reported significant contamination with coliform counts exceeded the recommended 100 CFU/g levels (Fasoyiro *et al*., 2010; Daniyan *et al.,* 2011; Bristone *et al.,* 2018) [11, 7, 3]. Foodborne pathogens such as *L. monocytogenes, E. coli* O157:H7, *S. aureus, E. aerogenes, Salmonella* sp. *Shigella* sp. *Klebsiella* sp. *Bacillus cereus*, *E. coli* and *P. aeruginosa* are dominant contaminants of the soy cheeses (Fasoyiro *et al.,* 2010; Daniyan *et al.,* 2011; Bristone *et al.,* 2018; Yakubu *et al*., 2021) [11, 7, 3, 37]. More worrisome, Odu and Akano (2016) [22] linked soy-based products to foodborne illnesses in communities of southwestern Nigeria. The species of *S. aureus* and *E. coli* are food-borne pathogen frequently associated with fatal disorders in humans (Schwendimann *et al.,* 2021) [32].

With the growing consumption of RTE marinated soy cheese, a targeted risk assessments have become crucial to control foodborne diseases outbreaks. Therefore, food safety management tools, such as Quantitative Microbial Risk Assessment (QMRA), that principally offer an evidence-based approach in estimating the risks associated with foodborne pathogens is essential (Heidinger *et al.,* 2009) [13]. The QMRA tool basically estimates public health risks related to the ingestion of pathogens in food (Codex Alimentarius Commission (CAC) 2020 [5]; Nauta, 2022 [20]; Lugue *et al*., 2024). This system conventionally utilizes four stage process to assess high risks pathogens such as of *E. coli* and *S. aureus* related to the consumption of foods (Codex Alimentarius Commission (CAC) 2020; Nauta, 2022) [5, 20]. It fundamentally appraises the level of illness to a particular pathogen in a population exposed to contaminated food (Codex Alimentarius Commission (CAC) 2020) [5]. Assessing the risks of foodborne infection in the university campus with a densely target population environment is significant for the prevention of disease outbreaks. In addition, the data on microbial risks assessment will serve as a framework for improve safety standards of RTE foods in Nigeria. This study therefore determined QMRA of 'awara' a ready-to-eat (RTE) marinated soy cheese sold on the university campus, Jos, Nigeria.

Materials and Methods

Study Area

This study was conducted at the University of Jos, located in Plateau State within Middle Belt region Nigeria. The plateau (approximately 1,200 meters above sea level) lies between latitudes 8°24′ N and 10°30′ N and longitudes 8°32′ E and 10°38′ E, covering an estimated area of 26,899 km² (Plateau State Government, 2025) [24]. Average annual temperatures range from 18°C to 22°C, with the coolest months from December to February and hottest periods occur during March and April (Wikipedia, 2023b) [36]. The rainfall is distinctly seasonal, with an annual rainfall range from about 1,317 mm to approximately 1,460 mm. The University of Jos was selected due to its significant population of marinated soy cheese vendors and a diverse student community.

Study Design

A cross-sectional study was employed to assess the bacteriological quality of ready-to-eat (RTE) marinated soy cheese. Soy cheese samples were randomly collected from campus vendors at the University of Jos over four visits between January and June 2024. Consumers were randomly selected from the student population and a structured survey questionnaire was administered to gather information on their consumption patterns and potential exposure to foodborne illnesses This was then followed by QMRA of two high risk foodborne pathogens: *E. coli* and *S. aureus* combinations following standard procedures.

Collection of Soy Cheese

A total of 90 marinated soy cheese samples were purchased from vendors in duplicate on the campus, University of Jos. Four cubes of the marinated soy cheese were collected aseptically in sterile Ziplock bags and transported immediately in ice box to the laboratory, Department of Microbiology, University of Jos for analysis.

Administration of Questionnaire

A structured survey questionnaire assessing consumption patterns and disease symptoms associated with consumption of marinated soy cheese was randomly administered to consumers at the vendors outlets.

Bacteriological Analysis

The bacteriological quality assessment of marinated soy cheese was carried out following procedures adopted by Solefack *et al.* (2024) [33]. A 25g portion of each sample was aseptically homogenized in 225 ml of sterile buffered peptone water. The resultant stock solution was then tenfold serially diluted to 10^{-5}. Afterward, the resultant aliquot was aseptically pour-plated on Plate Count Agar (PCA; Himedia M091) for total viable bacteria count, MacConkey Agar (PCA; LiofilChem, Italy) for coliform count, and Mannitol Salt Agar (MSA; LiofilChem, Italy) for staphylococci count. All the inoculated plates were incubated aerobically at 37^0C for 24 h and discrete colonies formed were then enumerated and expressed as colony forming units per gram (CFU/g) of sample.

Purification of distinct colonies was carried out by sub-culturing on sterile nutrient agar plates until pure cultures were obtained.

Characterization of Bacterial Isolates

Pure bacterial cultures were characterized based on cultural morphology and biochemical tests following conventional methods (Cheesbrough, 2006) [4]. The following biochemical tests were carried out methyl red test (MR), Voges-Proskauer Test (VP), citrate utilization test (CIT), urease test (URE), catalase test (CAT), Indole production, Nitrate reduction, Coagulase, Oxidase, Citrate, and hydrogen sulphide (H_2S) production test and fermentation of sugars as described by Cheesbrough, (2006) [4].

Microbial Risk Assessment

Microbial risk assessment of RTE marinated soy cheese was conducted in accordance with the structured framework outlined by the Codex Alimentarius Commission (2020) [5] in alignment with international food safety standards.

Hazard Identification

Model QMRA foodborne pathogens: *E. coli* and *S. aureus* were selected based on their high risk and dominant contaminants of soy cheese. Typically, *S. aureus* is responsible for staphylococcal food poisoning and predominant mastitis pathogen responsible for food-borne illnesses worldwide (Alnakip *et al*., 2014; Rajkovic *et al*., 2019) [1, 26]. *E. coli* serotypes are known to be associated with severe human diseases such as hemorrhagic colitis and hemolytic-uremic syndrome (Zengin *et al.,* 2024) [38].

Exposure Assessment

Using data generated based on weight of cheese consumed per serving and consumption frequency from the

questionnaires, dose (d) of organisms consumed per person per serving was determined.

$$CFUF = CFU/g \times W$$

Where: CFUF = the final quantity of microorganisms consumed per day or per serving, CFU/g = quantity of microorganisms per gram of cheese. W = the weight of cheese consumed per serving.

Dose-Response Assessment

The dose-response assessment was conducted to quantify the health risks associated with the consumption of QMRA pathogens using dose-response models. In this study, the bacterial load was determined by laboratory analyses. The probability of humans' infection from *S. aureus* and *E. coli* was then estimated based on model expressed as:

$$P(d)=1-e^{-rd}$$

where P(d) represents the daily probability of infection at a given dose d consumed per day, and r is the pathogen-specific infectivity rate constant for each organism. Parameters for the model included constants of 7.64×10^{-8} for *S. aureus* and 2.18×10^{-4} for *E. coli* (Cornick and Helgerson, 2004; Sabuj *et al.,* 2020) [6, 30]. Dose consumed (total exposure per day, CFUF) were input into the mathematical model to obtain the probability of infection per day. The monthly and yearly mean risk of infection was calculated as monthly = 1 - (1 - P) ^{4} and yearly = 1 - (1 - P) ^{52} where weekly risk of infection = P.

Risk Characterization

The risks characterization was determined by integrating the previous three components of QMRA to estimate the overall probability of infection resulting from exposure to microbial pathogens. An annual probability of infection was then calculated from the estimated daily probability of infection and the number of exposure days per year using a cumulative probability model,

$$P_{annaul} = 1-(1-P_{daily})^{n}$$

Where P_{annual} represents the annual probability of infection, $P_{(daily)}$ is the daily probability of infection per exposure event, and n is the number of exposure days in a year.

Data Analysis

The bacterial load is presented as mean with standard deviations and significant variations between sample area were determined using Analysis of Variance (ANOVA) with Instat version 15 software. Risk estimates were generated on a scale of 0 to 100 based on the methodology described by Ross and Sumer (2002) [28].

Results

Bacterial Load of Marinated Soy Cheese

Table 1 presents the bacterial load of marinated soy cheese sold by vendors on the University Campus, Jos. In this study, high contamination with total viable bacterial count ($8.16 \times 10^2 \pm 3.37 \times 10^2$ to $8.60 \times 10^5 \pm 1.19 \times 10^5$ CFU/g), coliform count ($3.00 \times 10^2 \pm 2.24 \times 10^2$ to $3.67 \times 10^3 \pm 1.15 \times 10^2$ CFU/g) and staphylococci count ($2.67 \times 10^{2a} \pm 1.03 \times 10^2 - 3.13 \times 10^{4a} \pm 4.70 \times 10^3$ CFU/g) was observed among the of marinated soy cheese samples. Overall, the total coliform count and staphylococci count were all above recommended standards acceptable for Ready-to-Eat foods.

Occurrence of Bacteria Contaminants of Marinated Soy Cheese

The occurrence of bacterial contaminants in marinated soy cheese sold on the university campus is presented in Table 2. Bacterial contaminants of *E. coli*, *Salmonella* sp., *Shigella* sp. *Staphylococcus aureus*, *Pseudomonas* sp., and *Staphylococcus* sp. are the most predominant. Out of 186 bacterial isolates, *E. coli* (25.80%) was the most occurred followed by *S. aureus* (20.97%) while *Shigella* spp. (8.06%) were the least prevalent.

Demographic Health Risk Profile of Consumers

The health risk exposure assessment of consumers of marinated soy cheese is presented in Table 3. Out of 90 consumers surveyed, 73.0% self-reported diarrhea (56.7%), followed by vomiting (16.7%), while both allergy and nausea (13.33%) as the least clinical symptoms.

From this study, females (75% p = 0.6813), individuals aged 30–35 (100% p = < 0.0001) and Daily/Weekly Consumers (100% p = 0.0001) are the specific high-infection risk groups associated with marinated soy cheese consumption over time. Key contributing behaviors that increased the infection risk included frequent consumption, inadequate food handling practices, and limited awareness of food safety, particularly among young adults.

Foodborne Infection Risk from Consumption of Marinated Soy Cheese

Table 4 presents the foodborne infection risk associated with consumption of marinated soy cheese sold in the university campus, Jos. The result revealed a high self-reported infection rate ranging from 71% to 100% among soy cheese consumers. Across the demographic groups, highest infection rate was observed in the 30–35 age group. Findings by sex, revealed showed that both female and male consumers experienced high infection rates with females being marginally more affected (75%) compared to males (71%).

Risk Characterization of QMRA Bacteria Pathogen Infection Linked to the Consumption of Marinated Soy Cheese

The risk of QMRA pathogens infection linked to the consumption of marinated soy cheese presented in Table 5. In this study, Monte Carlo-based risk assessment using the Beta-Poisson model confirmed high infection probabilities for both *S. aureus* and *E. coli* especially under repeated exposure scenarios. The result revealed a high infection risk from *S. aureus* in soy cheese, with a mean dose of 32,580 CFU per serving, resulting in a 0.99 infection probability across all consumption frequencies. Similarly, *E. coli* (24,435 CFU per serving) showed a rising infection risk over time, increasing from 0.32 per serving to 0.99 annually, reflecting a cumulative risk with repeated exposure.

Table 1: Mean total viable Bacteria, Total coliform and Staphylococci counts of Marinated Soy Cheese Sold in Jos Metropolis

	Total Viable Bacteria Count	Total Coliform Count	Staphylococci Count
A	$8.60 \times 10^{5a} \pm 1.19 \times 10^{5}$	$3.40 \times 10^{3a} \pm 5.72 \times 10^{2}$	$3.13 \times 10^{4a} \pm 4.70 \times 10^{3}$
B	$5.00 \times 10^{4b} \pm 1.21 \times 10^{4}$	$2.65 \times 10^{3a} \pm 4.90 \times 10^{2}$	$3.68 \times 10^{3b} \pm 1.30 \times 10^{3}$
C	$2.84 \times 10^{3c} \pm 4.21 \times 10^{2}$	$3.67 \times 10^{3a} \pm 1.15 \times 10^{2}$	$1.85 \times 10^{3c} \pm 1.39 \times 10^{3}$
D	$8.83 \times 10^{2a} \pm 2.71 \times 10^{2}$	$4.40 \times 10^{2b} \pm 3.43 \times 10^{2}$	$6.17 \times 10^{2b} \pm 5.50 \times 10^{2}$
E	$8.16 \times 10^{2a} \pm 3.37 \times 10^{2}$	$3.00 \times 10^{2a} \pm 2.24 \times 10^{2}$	$2.67 \times 10^{2a} \pm 1.03 \times 10^{2}$

Values are mean $\pm$ S.D. Mean with same superscript do not vary significantly ($P > 0.05$)

Table 2: Occurrence of Bacteria Contaminants of Marinated Soy Cheese Sold in University Campus, Jos

Bacteria Species	Number of Isolates	% Occurrence
Staphylococcus aureus	39	20.97
Escherichia coli	48	25.8
Salmonella sp.	21	11.29
Shigella sp.	15	8.06
Pseudomonas sp.	33	17.24
Staphylococcus sp.	30	16.13
Total	186	

Table 3: Demographic, Consumption Pattern and Clinical symptoms among Consumers of Marinated Soy Cheese Sold in University Campus, Jos

Parameters		Number of Consumers	% Occurrence	p-value
Age	18-23	33	36.67	< 0.0001
	24-29	54	60.00	
	30-35	3	3.33	
	>35	0	0.00	
Gender	Male	42	46.67	0.6813
	Female	48	53.33	
Frequency of Consumption	Daily	33	36.67	0.0001
	Weekly	33	36.67	
	Monthly	3	3.33	
	Yearly	21	23.33	
Quantity Consumed Per Week	Small <50 Pieces	9	10.00	0.0031
	Medium 50 - 100 Pieces	45	50.00	
	Large > 100	36	40.00	
Clinical symptoms with Consumption	Yes	66	73.30	< 0.0001
	No	24	26.67	
Associated Clinical symptoms	Allergy	12	13.33	< 0.0001
	Vomiting	15	16.67	
	Diarrhea	51	56.67	
	Nausea	12	13.33	
Frequency Clinical symptoms Per Week	Once	33	36.67	0.0003
	Twice	33	36.67	
	Thrice	6	10.27	
	Always	6	16.67	

Table 4: Foodborne Self-Reported Infection Risk Associated with Consumption of Marinated Soy Cheese Sold in University Campus, Jos

Parameters	Consumers	No. of Consumers (N)	Case Finding (D)	Risk (D/N)	Chances of Infection (%)
Sex	Male	42	30	0.71	71.00
	Female	48	36	0.75	75.00
Age	18-23	33	24	0.73	72.73
	24-29	54	39	0.72	72.22
	30-35	3	3	1.00	100.00
	>35	0	0		
Total	All	90	66	0.73	73.00

N (Number of Consumers) = Number of individuals in the demographic group, D (Case Finding) = Number of people in that group who experienced any infection risk (from the "infection Risk Associated with Consumption), Risk (D/N) = Proportion of people affected and Chances of Infection (%) = Risk × 100

Table 5: Risk of QMRA Bacteria Pathogen Infection Linked to Consumption of Marinated Soy Cheese Sold in University Campus, Jos

Bacteria Pathogen	Mean Dose of Bacteria per Serving	Risk/Serving	Mean Risk of Infection		
			Weekly	Monthly	Yearly
Staphylococcus aureus	32580	0.99	0.99	0.99	0.61
Escherichia coli	24435	0.32	0.32	0.79	0.99

Discussion

This study assessed the quantitative microbial risk assessment (QMRA) of *S. aureus* and *E. coli* infections associated with the consumption of RTE marinated soy cheese. The findings revealed that the marinated soy cheeses were contaminated at levels exceeding the acceptable microbial safety limits for RTE foods (ICMSF, 2018; Sabu *et at.,* 2019 [29]; Eze *et al*., 2020 [9]). The elevated bacterial count of the marinated soy cheese corroborated with the finding of Adimasu *et al.* (2016), who had reported these high mean viable bacteria, coliform, and staphylococci counts of 6.6 log CFU/g, 4.2 log CFU/g, and 4.3 log CFU/g, respectively. Moreover, this high bacterial load is an indicator of postprocess contamination that suggest significant poor hygiene and handling practices during production and among the vendors. Given that *S. aureus* and *E. coli* are both highly pathogenic, there is the need of strengthening food safety practices during production and sale among the unregulated producers.

A total of 73% of the consumers surveyed experienced foodborne illnesses after consumption of marinated soy cheese. This finding is similar to 70% rate self-reported among students consuming campus-sold snacks (Musa *et al*., 2018) [19]. The high pattern of foodborne disease risk is likely attributed to the increased likelihood of contamination due to the operation of informal food vendors without strict regulations within university environments. In this study, long-term consumption emerged as significant high-risk groups, substantiating the well-established correlation between frequent dietary exposure to contaminated foods and elevated foodborne illness risk. This pattern is consistent with observations in other food products, where frequent consumption significantly heightens the likelihood of infection. Specifically, high levels of *S. aureus* and *E. coli* were associated with all consumption frequencies of marinated soy cheese, with the risk being particularly elevated for individuals who consumed the product regularly or over prolonged periods. This suggest that higher likelihood of infection is reflected particularly for individuals who consume the product frequently, or for prolonged periods consistent with the observations for other food products (Lee *et al*., 2018; Liu *et al.,* 2019) [15, 16]. Moreover, this finding affirm that chronic exposure to contaminated foods exacerbates the potential for severe health outcomes.

With the annual risk of infection well above public health thresholds, a significant health risks for both infrequent and long-term consumption is eminent (FAO/WHO, 2023) [10]. This pattern of *S. aureus* and *E. coli* infections risks is a common trajectory observed for unregulated ready-to-eat foods in settings lacking adequate food safety oversight (O'Connor *et al*., 2018; Wang *et al.,* 2022) [21, 35].

Notably, this study revealed the complexity and nonlinearity between QMRA pathogen exposure and infection patterns. A higher initial risk of infection from a single weekly serving of *S. aureus* decreased with long-term consumption. This phenomenon has been attributed to "immune adaptation, a factor that influence microbial risks over time (Ho *et al.,* 2020) [14]. Moreover, at certain low-level pathogen exposures, consumers might have developed tolerance and thereby reducing the risk estimates with time (Tan *et al.,* 2019) [34]. Additionally, factors like food contamination, food types, and microbial strains influence immune adaptation, which can decrease with prolonged consumption (Rajkovic *et al*., 2019) [26]. Typically, the infection risk of *S. aureus* remained stable despite prolonged exposure, due to its resilience to survive adverse conditions (Rees *et al*., 2017) [27].

For the *E. coli* however, a cumulative repeated exposure showed significant increase in infection probabilities. Although, it has been suggested that a single exposure to *E. coli* may present substantially risk of infection (Harris and Liu, 2017) [12]. The cumulative infection risk associated with frequent consumption of contaminated food underscores the cumulative impact of pathogen ingestion over time (Odu and Akano, 2016; O'Connor *et al.,* 2018; Wang *et al*., 2022) [22, 21, 35]. Considerably, this finding also corroborates previous research that emphasized the cumulative effect of repeated *E. coli* exposure, which raises the likelihood of infection (O'Connor *et al*., 2018) [21]. The cumulative exposure to *E. coli* is a significant concern in food safety, especially for vulnerable populations, such as the elderly and immunocompromised individuals (Wang *et al.,* 2022) [35]. The study therefore, revealed that marinated soy cheese represents a significant risks food for model QMRA *E. coli* and *S. aureus* pathogens. Consistent high infection probabilities particularly with repeated exposure highlight this product as a serious public health concern. Therefore, continuous QMRA of pathogens in food consumed on university campuses and densely populated areas is essential for promotion of hygiene practices among the vendors and prevention of disease outbreaks.

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