THE EFFICACY OF GARLIC AND TURMERIC IN EXTENDING THE SHELF LIFE OF SUN-DRIED MARINE SARDINES (*Stolephoruscommersonnii*)

Abstract

Increasing the shelf life of sun-dried marine sardines is critical to maintaining their quality and cutting down on food waste. The use of spices is common in different localities, however, there is a pressing need to optimize the use of spices for enhanced quality and shelf life of seafood. This study aimed to optimize the use of spices during the storage of sundried marine sardines. Design expert software was used for this purpose. Three levels of two independent factors were used to obtain different combinationsGarlic (0-3%) and Turmeric (0-3%). A linear equation was developed to indicatethe relationship between variables (independent and dependent variables). The bacterial and fungal counts (cfu/ml) werereduced with increased concentrations of garlic, and turmeric,singly and in their combinations. The optimum reduction of bacteria (0cfu/ml) and fungi (cfu/ml) was obtained withthe combination of garlic and turmeric at 1.5% and 3% respectively. The bacterial count (0 cfu/ml) was observed in sun-dried sardines treated with garlic and turmeric at 1.5%, 3%, or its interactions respectively, whereas the highest bacterial count (360cfu/ml) was observed in control samples followed by??. Further, the lowest fungal count (0 cfu/ml) was observed in sun-dried sardines treated with garlic and turmeric at 3% concentration only or its interactions whereas the highest fungal count (68 cfu/ml) was found in the control samples. Analysis of variance indicated that interactive effects significantly ($p \leq 0.05$) affected bacterial and fungal count growth.Further results indicate that about 55% of the isolated samples were not colonized with bacteria while 25% and 20% of the isolated samples were contaminated with *E.coli* and *S. aureus* respectively. About 63% of the isolated samples showed no fungalgrowth, while 20% and 17% of the isolated samples were contaminated with *Mucor* and *Aspergillus flavus* respectively. Generally, optimizing the use of spices such as garlic improves quality and extends the shelf life of sun-dried marine sardines, and hence recommended for seafood preservation.

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Keywords: Marine sardines, Sun-dried marine sardines, fish preservation, food preservation, garlic, turmeric, spices.

Introduction

Background Information

Marine sardine is widely distributed small pelagic fish around the world. These fish play a significant role in the food and nutritional security of many low-income households in developing countries as they provide all the components of a wholesome and nutritionally ideal food supply for the growing populations (Isaacs, 2016). According to the Food and Agriculture Organization (FAO), small pelagic fish such as sardines are the most consumed fish in Sub-Saharan Africa (FAO, 2018). Moxness-Reksten et al., (2020) revealed that small pelagic fish such as Sardinellaspp and horse mackerel account for 50% of the important fish for domestic food supply in the SSA. Countries such as Zambia, the Democratic Republic of Congo, Burundi, and Malawi import a substantial number of sardines from Tanzania and are mostly preferred fish for the majority of low-income families (URT, 2022)

Further, During Sun drying, the moisture content and water activity of fish are reduced (Majumdar *et al,* 2023), which essentially may stop or delaythe growth of microbes (Alp and Bulantekin, 2021). However, sun-dried sardines are accompanied byseveral challenges such as rancidity (Alex and Eagappan, 2017), and the loss of sensory and nutritional value due to moisture absorption from the surroundings (Alex, 2016). Further, according to Patterson et al., (2018), fish that are sun-dried frequently produce low-quality products because the fish dry slowly, and are vulnerable to contamination and infestation. Despite the wide use of fish drying method in fish preservation, the technique is confronted with low microbial quality of stored fish.

Spices and herbs are natural preservatives that may be used to enhance the microbial quality of sun-dried sardines. Spices contain various bioactive compounds that have antimicrobial, antioxidant, and anti-inflammatory properties, which may inhibit the growth of spoilage bacteria and delay lipid oxidation in fish. Adding spices to dried fish is a common practice aimed at

lowering its water activity (aw) (Fitri*et al.*, 2022), improving product quality and thereby reducing microbial proliferation. According to Alex and Eagappan, (2017), the bioactive compounds found in spices are very essential for reducing microbial growth in sun-dried fish. Studies by Kumar *et al.* (2019), and Nair *et al.*, (2016) discovered that applying a blend of spices to sun-dried sardines (Sardinellalongiceps) enhanced the fish's microbiological quality and prolonging its shelf life in contrast to sun-drying the fish without any spices. Compared to the control sardines, the spice-treated sardines exhibited a reduced bacterial load, a higher phenolic content, and stronger antioxidant activity (Ndife*et al.*,2018)

Further, spices can help to lower the microbial load of the dried fish (Karam *et al.*,2021). However, not every spice will work well with sun-dried sardines. Certain herbs and spices may have negative interactions with other ingredients or environmental elements, or they may change the fish's flavor, color, or texture (Guneri*et al.*,2021). Due to this fact, it is critical to choose the right herbs and spices for sun-drying sardines based on their availability, affordability, chemical makeup, and antibacterial action. Thus, to utilize, apply, and quantify the technique of spices for preserving sun-dried marine sardines in various contexts, more research is of paramount importance. This study will specifically evaluate the effects of spices (garlic and turmeric) in reducing microbial load in sun-dried sardines, assess the storage time in microbial load, and examine the shelf life of fish stored with garlic and turmeric.

Materials and Methods

Materials

Selected organic spice powders (turmeric and garlic) were bought from Sustainable Agriculture Tanzania (SAT)organic shop in KilakalaMorogoro and transported at ambient temperature to the Department of Food Sciences and Agro Processing, SUA, Morogoro for the preparation of spices water extract.

Sample collection

Sardine samples were purchased from fishermen in the three landing sites of Tanga, Dar es Salaam, and Kilwa regions. These were chosen for the assessment of potential variations in microbial contamination of the sardines. About 27 kg of fresh sardines were randomly collected from fishermen early morning at landing sites. They were subdivided into 3 groups of a nearly equal weight of 1kg each resulting in a total of 27 samples. Analysis wascarried out in duplicate. Sampleswere stored in ice-insulated boxes and immediately transported to the SUA microbiology laboratory for analysis

Formulation of spices extract for mixing with marine sardine

The choice of Garlic and turmeric extracts were selected to show antimicrobial activities and determine optimal concentrations to inhibit microbial growth on sun-dried marine.Garlic contains compounds like allicin, which have been shown to exhibit antibacterial and antifungal properties (Hayat *et al*.,2016), while turmeric contains curcumin, which has strong antimicrobial effects (Chen *et al.*,2018). Preliminary experiments were carried out using various concentrations of Garlic and Turmeric extracts, to determine the minimum and maximum concentration for inhibiting bacterial and fungal growth. Preliminary experiments identified 1 to 3%. spice concentration hadnotable effectiveness in reducing microbial growth The design software was then used to develop a matrix with different combinations of turmeric and garlic to create several formulations (Anteneh *et al*, 2023). The developed matrix with different combinations of garlic and turmeric was taken to the laboratory for further experiments on microbial growth.

Randomized order	Run	Salt%	Garlic%	Turmeric%	Microbial response (cfu/ml)
10	1	2	1.5	1.5	
11	2	2	1.5	1.5	
4	3	2	3	3	
12	4	2	1.5	1.5	
9	5	2	1.5	1.5	
13	6	2	1.5	1.5	
1	7	2	0	0	
5	8	2	0	1.5	
2	9	2	3	0	
8	10	2	1.5	3	
6	11	2	3	1.5	
7	12	2	1.5	0	
3	13	2	0	3	

Table 1: Formulations of spice cocktails for mixing with marine sardines

Solution preparation

To prepare spice formulations, a mixture with different ratios of turmeric and garlic powder (Table 1)was passed through a 250um sieve was and mixed with 1litre of boiling water with continuous stirring (Kim *et al* 2011) The mixture was boiled for 15 min and subsequently cooled to 0–5°C in a refrigerator. This was followed by sieving to remove the particles. This solution was used for 1 kg of sardines (Kim *et al.*, 2011).

Sardines' preparation

Sardines were blanched with 10% sodium chloride for 15 minutes to improve their texture. They were then soaked inspices water extract (1:2 w/w) for 40 min (Alex, 2016). The mixture of spices' water extract of turmeric and garlic was0-3g/l according to design expert software. Sardines without spices were used as a control. Subsequently, he spicedmarine sardines that weresolar-dried.

Drying of marine sardines

The spicedmarine sardines were subjected to controlledsolar drying in a small room environmentenclosed by corrugated iron sheets on the sides and a roof overhead.Theywere exposed to indirect sunlight to prevent any external contamination. They weredried for three days and then stored in Zip Lock polyethylene bags at room temperature for 8 weeks.

Microbiological Laboratory Analysis

Spiceddried marinesardine samples were analysed for Total Viable Count (TVC), and fungi. Isolation and identification of *Escherichia coli* and *Staphylococcus aureus* was also done. The preparation of analytical reagents (diluents) and media was according to the manufacturer's instruction(s) and the specific test method requirements.

Determination of CFU counts

Bacteria identification

The standard pour plate technique method was applied for quantitative CFU (Colony Forming Unit) counts determination of respective groups of microorganisms in 10g of marine sardines. Preparation of homogenized samples of marine sardines were prepared based on a decimal dilution, For Total Bacterial Count (TBC), molten nutrient agar was used. The molten agar was poured into petri dishes and left to solidify. One milliliter of a sample in serial dilution (by performing 6 serial dilutions where 1 ml of the sample is transferred into 9 ml of sterile diluent, mixed thoroughly, and then 1 ml from this mixture is transferred into another 9 ml of sterile diluent; this process is repeated to achieve the desired dilution) was then inoculated in nutrient agar plates. These plates were incubated in an oven at 37°C for 24 hrs, as described in ISO 4833-1:2013.For mould count, molten potato dextrose agar at a temperature of 40-45°C was used. In the same way, the agar was poured into petri dishes and allowed to solidify. One milliliter of the sample in serial dilution was then inoculated in duplicate onto the potato dextrose agar (PDA) plates. These plates were aerobically incubated, with the lid positioned uppermost in an upright position, in the incubator at 25°C ± 1°C for 5 days, as described in ISO 21527-2:2008.

Morphology

Assessment of bacteria colony morphology characteristics on solid agar plates was used as the first stage for identification, where *E. coli* was identified using Mac Conkey plate agar and *Staphylococcus spp* using Mannitol Salt Agar.

Gram staining technique

The standard Gram staining procedure was used to classify bacteria as gram-positive and gram-negative based on chemical and physical properties as developed by Hans Christian Gram in 1884.

Isolation of bacteria colonies

A pure colony of *Staphylococcus* spp and *E. coli* was isolated from the petri dish containing the bacteria by using a sterile wire loop. These colonies were sub-cultured on Mannital Salt Agar and Mac Conkeyplate agar.for *Staphylococcus spp* and *E. coli* respectively.

Biochemical test: Conformation test of *Staphylococcus aureus*

The presence of *S. aureus* was determined using the spread plate technique by using Mannitol salt agar as described by ISO 6888-3:and presumptive colonies were confirmed by the Coagulase test, as described in ISO 6888:1983.

Biochemical test: Conformation test of *E. coli*

The presence of *E. coli* was determined using the pour plate technique as per ISO 7251-1:2005 and presumptive colonies were confirmed as described in ISO 166499- 1: 2001, and triple sugar iron as described in ISO 6579 -1:2017

Data Analysis

Statistical data analysis of the laboratory results was carried out using design expert software version 13. Analysis of variance (ANOVA) was used to test the significant effect of the spices formulations on bacterial and fungal counts

Results and Discussion.

The effects of garlic and turmeric on bacterial growth

Table 2 shows microbial growth in spiced sardines after storage for two months.Bacterial countranged from0 to360 cfu/ml) in sundried marine sardines treated with garlic and turmeric at different ratios (Table 2).A study by Kombat *et al.* (2017) and Gul and Bakht, (2015) reported

bacterial growth of 0-255 cfu/ml and 0-320 cfu/ml respectively when treated with garlic and turmeric spices. However, the earlier author did not examinethe interactive effects of the two spices indicating that while garlic and turmeric individually show antibacterial properties, their combined effect could be more potent in inhibiting bacterial growth.The low bacterial count (0cfu/ml) was recorded in sundried marine sardines treated with garlic and/or turmeric while the bacterial count was high(360cfu/ml) in control samples. Similar findings were reported in the study by Arulkumar *et al.,* (2017), and Tairu*et al.,* (2017) whereby low bacterial count was observed in fish treated with turmeric and garlic spices respectively. The highmicrobial load in control samples might be due to low antibacterial activity, as it was treated with neither of the spices (Bhatwalkar*et al.,* 2021). On the other hand, the low bacterial count in sundried sardines treated with garlic and turmeric extracts might be attributable to the high antibacterial activity found in the garlic and turmeric spices (Nwinee *et al.,* 2022, Alex, 2016).

Table 1: Bacterial and fungal count (cfu/ml) of sun-driedsardines spiced with variouscombinations of garlic and turmeric concentrations

Randomized order	Run	Garlic%	Turmeric%	Bacterial count (cfu/ml)	Fungal count (cfu/ml)
10	1	1.5	1.5	0	3.3
11	2	1.5	1.5	0	3.5
4	3	3	3	0	0
12	4	1.5	1.5	0	3.3
9	5	1.5	1.5	0	3.2
13	6	1.5	1.5	0	3.3
1	7	0	0	360	68
5	8	0	1.5	0	3.3
2	9	3	0	0	0
8	10	1.5	3	5.3	2.5
6	11	3	1.5	13.2	8.7
7	12	1.5	0	13	1.7
3	13	0	3	18.8	3.7

Analysis of variance (ANOVA) in design expert software indicates that the model (eq 1) was significant ($p<0.05$) while all factors (garlic and turmeric) had a non-significant linear effect ($p>0.05$) on the bacterial count (Table 3). However, the interactive effect of garlic and turmeric was significant ($p<0.05$).The effect of garlic and turmeric might be due to the synergetic effects of allicin and curcumin and their high bioavailability in the two spices.According to Delgado *et al.,* (2021) combination of the active compounds from garlic and turmeric and their bioavailability enhances the synergetic effects of these compounds towards inhibition of microbial growth and proliferation Thelack of fit was not significant ($p>0.05$) indicating adequacy fit of the actual data in the model and suitability of the model in predicting patterns of variation and therefore can be useful for navigating the design.

Table 2:ANOVA Results for the Linear Model (Equation 1) Analyzing Bacterial Growth

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	71670.12	3	23890.04	4.7	0.0306	Significant
A-Garlic	22277.23	1	22277.23	4.39	0.0657	
B-Turmeric	20288.54	1	20288.54	4	0.0767	
AB	29104.36	1	29104.36	5.73	0.0403	
Residual	45704.95	9	5078.33			
Lack of Fit	45704.95	5	9140.99			
Pure Error	0	4	0			
Cor Total	1.17E+05	12				

Interactive effect showed a positive linear relationship with bacterial count this implies that when both garlic and turmeric are present together, their combined effect leads to a decrease in bacterial count, whereas garlic and turmeric showed a negative relationship with the bacterial countmeaning that decreasing the concentration of either garlic or turmeric tends to increase the bacterial count, resulting in a model graph in a curvilinear form.

Garlic and turmeric showed a negative relationship indicating that the decrease in garlic and

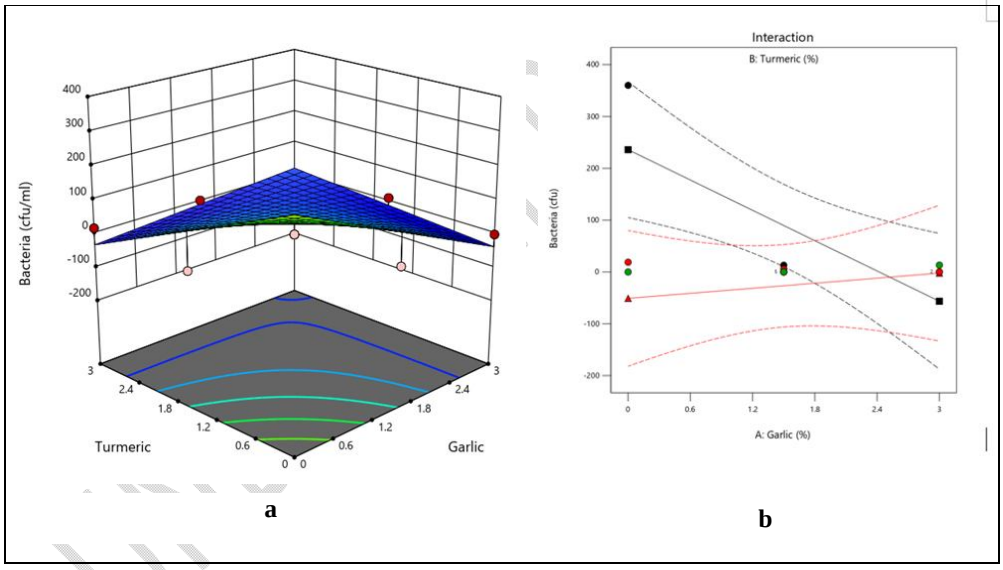
$$Y=+31.56-60.93\times A-58.15\times B+85.30AB \quad \text{eq 1}$$

Comment [F5]: Why didn't you write the whole equation?

Whereas Y is bacterial count, A is garlic, and B is turmeric.

Furthermore, the effects of garlic and turmeric spiceextracts were depicted in the 3D graphs (Figure 1a-c), whereas the darker red regions present the optimum values. The graphs show that bacterial count decreased linearly with increased concentrations of independent variables up to the optimal values (Figure 1a). Increased concentrations of garlic and turmeric reduced (inhibited) the growth of bacteria to the optimal values as explained in the model (eq 1).

Desirability values ranging from 1 to 0.1 represent the ideal case, where higher values indicate a closer position to the desired outcome (Majaliwa *et al.*,2019). A desirability value of 1, as observed in Figure 1c, implies a positive result, suggesting that the experimental conditions led to an outcome closely matching the desired objectives. This high desirability indicates that the observed reduction in bacterial load is likely attributable to the antibacterial properties of garlic and turmeric.The significant reduction in bacterial count, tied with the high desirability value, underscores the statistical strength of the data and supports the implication that garlic and turmeric are effective in inhibiting bacterial growth. This finding raises the possibility of utilizing garlic and turmeric in combination, as natural preservatives for seafood.The optimal bacterial counts were achieved at concentrations of 1.5% and 3% for garlic and turmeric respectively.


Bacteria (cfu/ml)
400
300
200
100
0
-100
-200
Turmeric
Garlic
3
2.4
1.8
1.2
0.6
0
Interaction
B: Turmeric (%)
Bacteria (cfu)
A: Garlic (%)

a **b**

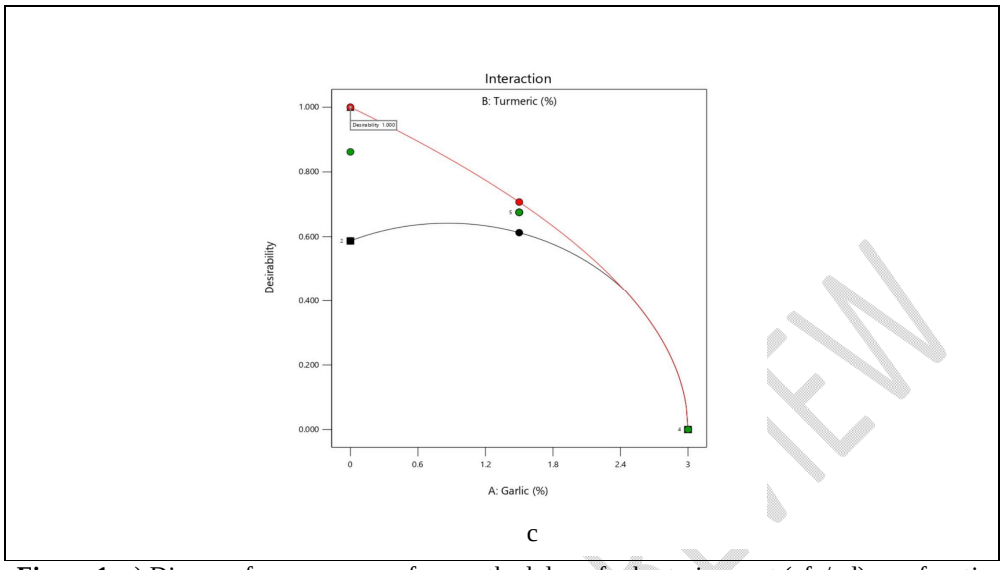


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Figure 1: a) Diagram for response surface methodology for bacteria count (cfu/ml) as a function of turmeric and garlic spices, b) interaction effects of garlic and turmeric, c) optimization of turmeric and garlic spices interactive effects on bacteria growth

Susceptibility of bacteria isolates to sun-dried marine sardines treated with spices

Further, Figure 2 shows that no bacterial growth appeared in 55% of the isolated samples, whereas 25% and 20% of the isolated samples were contaminated by *E. coli*and *Staphylococcus aureus* respectively. The presence of *E.coli* and *S. aureus* underscores deviation from hygienic practices or poor storage conditions during handling or processing (de Jesus-Rodrigues *et al.*, 2016). Fecal contamination is a major source of *E. coli* and when ingested in substantial quantities can cause food-borne illnesses (Bintsis, 2017).

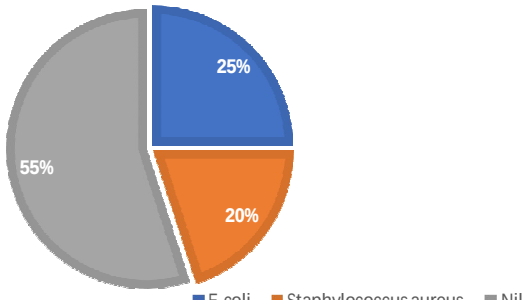

Figure 2: Bacterial load in sun dried marine sardines treated with different spice formulations
NB: Nill indicates no microbial growth

The results in Figure 2 indicate that most of the samples didn't provide favorable conditions for the growth of these bacteria. (Suleiman *et al*., 2023) had similar observations. It is possible that the antibacterial activity found in the spice formulations inhibited the growth of these spoilage bacteria (Liu *et al.,* 2017). Garlic is rich in organosulfur compounds such as allicin, vinyldithins, ajoene, ally sulfides, and allicin (Ribeiro *et al.,* 2021; Bhatwalkar*et al.,* 2021) while turmeric is rich in curcumin, which are important bioactive compounds with bactericidal properties. For instance,the sulfur compounds found in garlic can inhibit biofilm formation and remove the resistance factor of bacteria, thereby reducing its multiplication (Jikah and Edo, 2023). Curcumin compounds are effective bactericidal against gram-positive and gram-negative bacteria (Dai *et al.,* 2022). According to Adamczak *et al*., (2020), bacterial gene expression and DNA replication can both be inhibited with curcumin. The bacterial cell membrane can be disrupted with curcumin which also interferes with microbes' metabolic pathways and ATP synthesis inhibition (Gupta *et al*., 2012). These mechanisms contribute to the preservation of fish. Generally, the use of spices in the preservation of sun-dried sardines showed low bacterial contamination incidence, however, hygienic procedures should be adhered to during handling and processing of sardines.

Furthermore, the common bacteria colonies observed were medium to large with sharp borders, round and convex in shape with creamy to golden color, and some with zones of clear beta-hemolysis on Mannitol Salt Agar. Such colonies were suspected to be of *Staphylococcus spp*. *E. coli* appeared lactose fermenters, smooth colonies and medium in size, flat pink reddish colonies on Mac Conkey plate agar.*Staphylococcus aureus* was gram-positive, cocci in shape, grape-like in clusters. Therefore *E. coli* was gram-negative, with rods in the shape of single.

The Effectsof Garlicand Turmericon FungalGrowth

In this study, fungal count (cfu/ml) ranged from 0-68 cfu/ml (Table 2). The minimum fungal count was recorded in sundried marine sardines treated with garlic and/or turmeric while the fungal count was high in control samples. The study by Magawata and Shina, (2013), and Tairu*et al*., (2017) reported similar findings with low-recorded fungal count in fish treated with spices such as turmeric and garlic. The low fungal count in sundried sardines treated with garlic and turmeric extracts might be attributable to the high antifungal activity found in the garlic and turmeric spices. (Tairu*et al*, 2017). In this study, the antifungal activity of garlic and turmeric against different fungal strains was high at both 1.5% and 3% concentrations. However, the high fungal count in control samples might be due to low antifungal activity as the control sample was not treated with either of the spices (Prakash *et al.*, 2011; Kortei *et al*., 2022). This underscores the effectiveness of these spice formulations in mitigating fungal contamination during storage. This observation highlighted the importance of using spice extracts in preventing fungal contamination in dried sun-dried sardines. Thus, the consistently low CFU/ml in dried sun-dried marine sardines suggests that the spice formulations can be useful in controlling the microbiological quality of dried sardines throughout storage.

Analysis of variance (ANOVA) indicates that the model (eq 2) was significant ($p<0.05$) while all factors (garlic and turmeric) had a non-significant linear effect ($p>0.05$) on the fungal count (Table 4). However, the interactive effect of garlic and turmeric was found to be significant ($p<0.05$). The presence of allicin in garlic spice (Hayat *et al*., 2016) and curcumin in turmeric (Chen *et al*., 2018) and their synergistic effects which are important in controlling fungal proliferation, might have contributed to this observation. The lack of fit was not significant ($p>0.05$) indicating adequacy fit of the actual data in the model and suitability of the model in predicting patterns of variation and therefore can be useful for navigating the design space.

Table 3: Results for Analysis of variance (ANOVA) for the linear model (eq 1)

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Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2438.28	3	812.76	4.84	0.0284	Significant
A-A	732.62	1	732.62	4.37	0.0662	
B-B	672.04	1	672.04	4	0.0764	

AB	1033.62	1	1033.62	6.16	0.0349
Curvature	0	0			
Residual	1510.27	9	167.81		
Lack of Fit	1510.22	5	302.04		
Pure Error	0.048	4	0.012		
Cor Total	3948.55	12			

The interactive effect showed a positive linear relationship with fungal countsuggesting that as the combined action of garlic and turmeric increases, there is a corresponding decrease in fungal count. whereas garlic and turmeric showed a negative relationship with the fungal count this means that as the concentrations of garlic and turmeric decrease, the fungal count tends to increase. However, when plotting the relationship between the concentrations of garlic and turmeric and fungal count, a curvilinear form emerged.

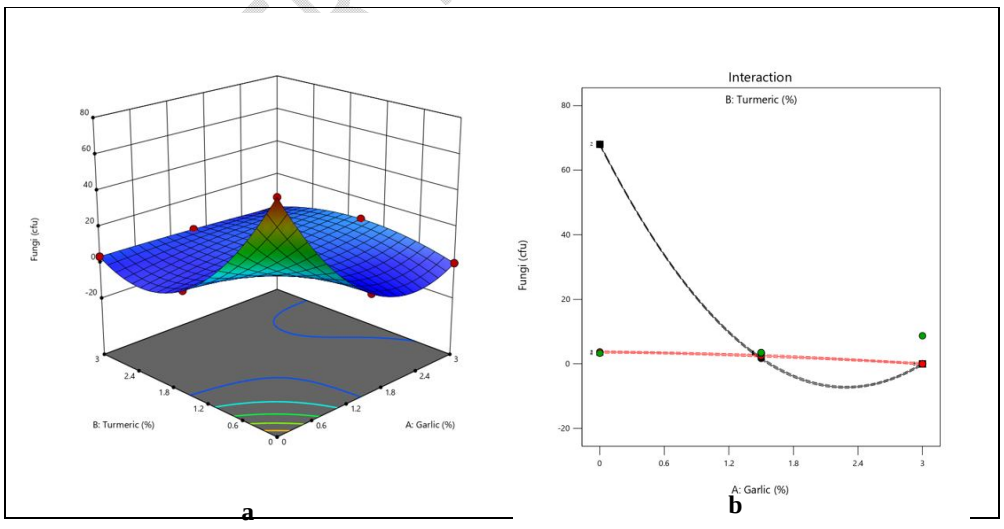
$Y=+8.04-11.05A-10.08B+16.07AB$……..eq 2

Whereas Y is fungal count, A is garlic, and B is turmeric.

Furthermore, the effects of garlic and turmeric spice extracts were presented in the 3D graphs (Figure 3a-c), whereas the darker red regions indicate the optimum values. As depicted in the graphs, fungal count decreased linearly with increased concentrations of independent variables up to the optimal values (Figure 3a). At low concentrations of the garlic and turmeric extracts, the fungal count was higher, whereas the increase in the concentrations of garlic or turmeric contributed to the reduced fungal count. This can be explained by the negative coefficients in the model (eq 2). The results in Figure 3b further suggest that an increase in garlic concentration with increased turmeric concentration led to a slight increase in the microbial count which is also explained in the model (eq 2).

Conversely, Figure 3c results showed a desirability score of 1, which is extremely ideal.The optimal value was 1.5% and 3%. The results suggest that both turmeric and garlic showed remarkable efficacy in reducing fungal populations. The statistical strength of the results is emphasized by their high desirability, which implies that the antifungal properties of turmeric and garlic are the cause of the observed fungal load reductions. Further, the significant reduction

in the fungal count raises the possibility that garlic and turmeric could be used together to preserve seafood.



a **b**

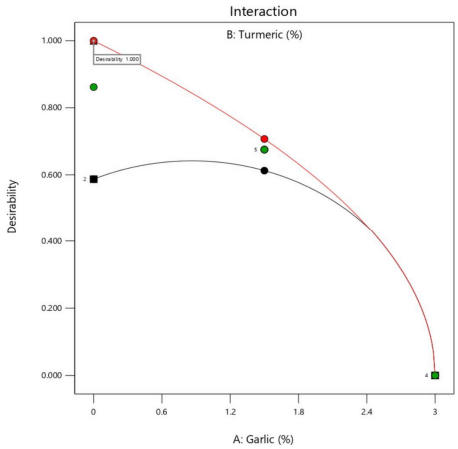


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Figure 3: a) Diagrams for response surface methodology for fungal count (cfu/ml) as a function of turmeric and garlic spices, b) interaction effects of garlic and turmeric, c) optimization of turmeric and garlic spices interactive effects on fungal growth

Susceptibility of fungi isolates to sun-dried marine sardines treated with spices

Further, Figure 4 reveals that about 63% of the isolated samples treated with spice formulations showed no bacterial growth, while 20% and 17% of the isolated samples were contaminated with *Mucor* and *Aspergillus flavus fungi* respectively. The presence of *Mucor* and *Aspergillus flavus* in the isolated samples can be attributed to moisture and temperature during processing and storage. Fungus such as Aspergillus flavus produce mycotoxins which may endanger the consumers and cause serious illnesses (Odeyemi *et al.*, 2020). Moreover, spoilage of food products such as sardines as a result of fungus contributes to the major health concern of foodborne illnesses (Benedict *et al.*, 2016). The study by Deng *et al.*, (2021) isolated the three dominant fungal genera in dried preserved fish which include *fusarium*, *aspergillus*, and

penicillium. Daramola *et al.,* (2023) reported similar findings where seven fungal microbes were isolated and found to be

prevalent in dried fish. *Aspergillus flavus, Mucorsp, Alternaria, Aspergillus niger, Rhizopussp, Aspergillus fumigatus, and Candida sp* were the most prevalent isolates (Daramola *et al.,* 2023).

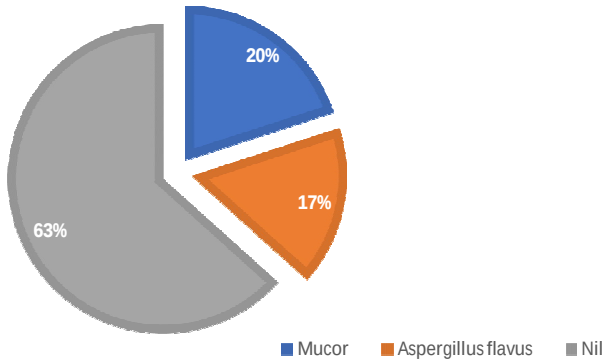

Figure 4: Fungal load in sun dried marine sardines treated with different spice formulations NB: Nill indicates no microbial growth

Results in Figure 4indicate that most of the isolated samples didn't provide optimal growth conditions for fungi. This can be due to the presence of the bioactive compounds in garlic and turmeric spices which pose antifungal properties. Garlic poses an allicin bioactive compound (Hayat *et al.,* 2016) while turmeric poses curcumenol, curdione, curzerene, germacrone, curcumin, β-elemene, germacrone, isocurcumenol, and curcumol which are an important antifungal compound (Chen *et al.,* 2018). The growth of different fungal species isinhibited with curcumin and allicin through cell membrane disruption, interference with respective cell division, cell wall synthesis, and inhibiting fungal metabolism (Yang *et al.,* 2023; Martins *et al.,* 2009). Ergosterol, an important component of fungal cellsmay decrease with the presence of curcumin as a result of lowering ergosterol production (Nath *et al.,* 2021) and eventually leadto fungal cell deaths (Rodrigues, 2018). Thus, many studies have reported that turmeric and garlic

are good candidatesfor antifungal activity and have proven inhibitory effects against fungal growth and colonization (Oza *et al.,* 2021; Li *et al.,* 2022). However, the results from this study implythat despite the effectiveness of turmeric and garlic in preventing the growth, survival, and proliferation of fungus, most resistant strains may continue to survive in the preserved fish. This underscoresthe need for further investigation of the most effective spices and/or combination of spices to be used for fish preservation.

Effect of Storage Time on Microbial Load

Figure 5 shows the effectsof storage time onbacterialandfungal growth and multiplication. No significant variation was observed in the microbial load during storage was observed ($p>0.05$). Figure 3 indicated a slight difference in the bacterial load, with a higher mean CFU/ml in the second month compared to the first month. The slight increase in microbial load over time indicates bacteria adaptation to the storage conditions and an increase in the optimal growth conditions, which favor bacterial growth and multiplication. During storage, moisture from the surroundings could be absorbed thus creating favorable conditions for growth and multiplication of microbes (Hakeem *et al.,* 2023). Similar findings by Dutta *et al.* (2018) observed increased bacterial load in stored smoked fish in later stages of storage. Under favorable conditions, the growth and replication of bacteria takeplace rapidly and their number may increase two times every 20 minutes (Vollum *et al.,* 2014). Additionally, it is possible that certain bacterial species present initially developed resistant strains, contributing to the observed increase in bacterial load over time (Hawkey *et al.,*2008)

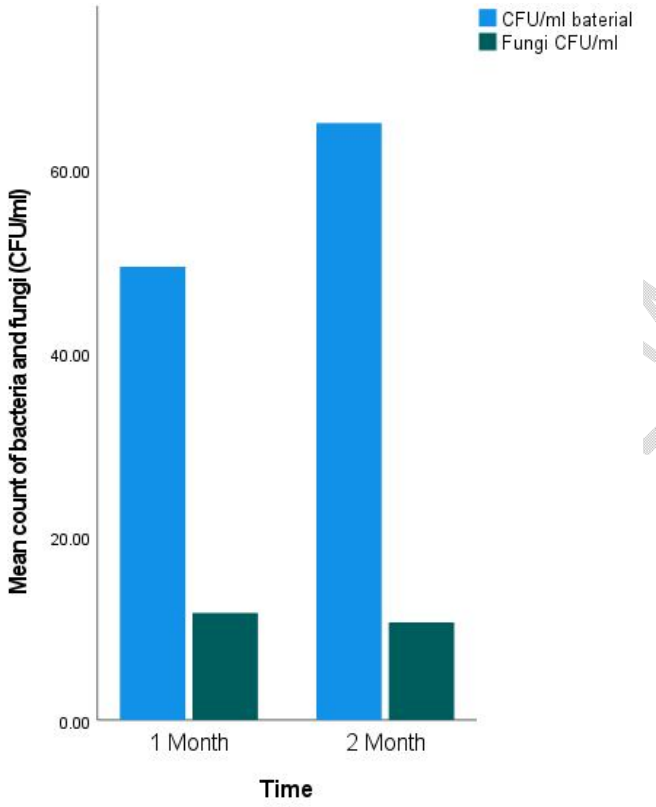

Figure 5: Overall mean microbial load of spiced solar-driedsardines during two monthsof storage time

The fungal load was consistent throughout the storage duration (Figure 5) indicating the stability of the fungal population throughout the storage duration. The presence of inhibitory factors such as bioactive compounds (allicin and curcumin) of garlic and turmeric or antagonistic interactions among fungal species could have contributed to the observed stability in fungal load over time (Paris *et al.,*2020). The results are consistent with the findings by Adu-Gyamfi et *al.,* (2006) who reported consistent fungal load following the addition of ginger and pepper in dried fish for all twelve months of storage. Prolonged storage and high temperature and humidity conditions are some of the factors that accelerate fungal development (Marijan *et al.,*2019).

In this study, we observed that the microbial population on sun-dried marine sardines treated with turmeric and garlic extract met the standard set (cfu/g) by the Tanzania Bureau of Standards

(TBS) except for E. coli, where the maximum limit is 0. However, we found that 25% of E. coli was present in the samples. Despite this, the total viable count, Staphylococcus aureus, and fungi were all below the maximum limits set at 10^5, 10^3, and 10^3 respectively. This indicates that the spices used in sun-dried marine sardines demonstrated effectiveness in antimicrobial activity.

Implications of Turmeric and Garlic Spices to Sardines Shelf-Life Extension

The antimicrobial and antioxidantproperties of spices such as turmeric and garlic are effective in sardines' shelf-life extension (Güneri, 2021). This hasbeen evidenced with low or zero fungal and bacterial load (CFU/ml) in the sardines treated with formulated spices (garlic, turmeric) at 1.5% and 3% in all two months of storage. The use of spices such as garlic and turmeric hasbeen recognized as animportant harmless method of preserving food resources (Fitri*et al.,* 2022). Turmeric extracts are effective in slowing down microbial proliferation and hence extend the shelf life of fish (Arulkumar *et al.,* 2017).

Garlic and turmeric have antibacterial properties that help to slow down the rate of spoiling, extending the shelf life of preserved fish without sacrificing its quality or safety (Teshome *et al.*, 2022). Producers will find this longer storage duration convenient, as it allowsthem tostockpile larger quantities of goods without the fear of rapid spoilageand lower food waste. This has real-world applications that result in lower costs, more effective supply chains, and higher producer profitability (Herbon, 2023). Furthermore, customers gain access to better-quality fish products for extended periods, which raises overall satisfaction and value (Soro *et al.,* 2021).

Consumers often prefer natural preservatives in the process of obtaining healthy food products (Güneri, 2021). The use of spices such as turmeric and garlic ensures the shelf-life extension of seafood with little or no microbes at all which helps to keep seafood free from poisoning and intoxication (Mei *et al.,* 2019). Through natural preservatives, consumers are guaranteed seafood free from toxins and foodborne illness (Pinto *et al.,* 2023). However, despite the usefulness of spices in extending the shelf life of seafood products, exceptional instances may occur where the microbial load can be high in fish (Fitri*et al.,* 2022) due to the resistance of some strains of bacteria and fungi to the bioactive compounds of spices such as turmeric and garlic. Thus, further studies need to be done to explore the potential usefulness of spices to some resistant strains of bacteria

Conclusion

Comment [F7]: Sensory analysis is necessary for such a study.

Generally, this study found that the mixture of garlic and turmeric extract has beneficial effects in controlling dried sardines' microbial quality. The antimicrobial activity of the garlic and turmeric at 1.5% and 3% was found to be the most effective combination. Sun-dried sardines treated with spices formulations at different levels of combinations showed low bacterial and fungal count (CFU/ml) compared to control. Further studies could explore the specific antimicrobial compounds present in these formulations and assess their safety. This strategy helps to raise the bar for seafood microbial safety and quality requirements in addition to satisfying consumer demand for natural, sustainable preservation techniques.

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