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Effects of Vacuum Packaging and Chilling Storage on the Microbiological Changes of the Superheated Steam Dried Sardines

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Abstract

Fish are important sources of protein and micronutrients for combating malnutrition. Since fish are highly perishable, methods for processing and preserving them should be the main focus to increase their shelf life and maintain their quality. The present study assessed the effectiveness of the superheated steam dryer (SSD) in processing sardines, and examined the effects of packaging and storage conditions on their microbiological quality. Sardines collected from the Mafia Island were dried in SSD, air-packed and stored at room temperature (AR), air-packed and stored at chilling temperature (AC), vacuum-packed and stored at room temperature (VR), and vacuum-packed and stored at chilling temperature (VC) for 49 days. Following drying, the total viable bacterial counts (TVBC) and total yeast and mould counts (TYMC) both decreased from the initial values of 9.14 (TVBC) and 2 (TYMC) log CFU/g in the fresh samples to 0.00 log CFU/g. The AR samples had the highest microbial growths during storage (7.48 (TVBC) and 2.82 (TYMC) log CFU/g), whereas the VC samples had the lowest (1.79 (TVBC) and 1.42 (TYMC) log CFU/g). The AR treatment was rejected within 21 days, whereas the AC, VR, and VC treatments prolonged the sardines' shelf life throughout the storage time.

Keywords: Superheated steam dryer, sardine, microbial analysis, vacuum packaging, chilling storage.

Introduction

The fisheries industry in Tanzania is one of the most significant economic sectors in the nation, boosting social welfare and generating jobs that support the economy of the nation. Approximately 202,053 and more than 4.5 million Tanzanians, respectively, are employed directly and indirectly in fisheries-related activities (URT 2021). Marine sardines make about 1/3 million tons of the annual catches, according to the official statistics (SWIOFP 2012). Furthermore, this sector generates 1.71% of the GDP (URT 2021). Fish are highly perishable foods such that, if not properly processed, can deteriorate within 24 hours of being caught (Ames et al. 1991). Different methods of fish processing and preservation, including smoking, salting, and drying, are used all over the world to address this challenge. Fish processing is the technique used to give fish a longer shelf life so that they can be available year-round and reach the consumer in wholesome conditions. The most popular and least expensive technique of fish preservation in Tanzania is sun drying, but it also has the biggest post-

harvest losses, particularly during the rainy season. Fish drying using sun drying normally takes 6–8 h, but it can take up to 72 h depending on the prevailing weather conditions (Legros and Luomba 2011). Conventional drying techniques typically lead to inadequate product quality, nutritional deterioration, and uneven drying, and they consume roughly 15 to 25% of the industrial energy used in most countries (Sehrawat et al. 2016). Consumption of dried fish and shellfish infected with harmful microorganisms causes 1.5 million of gastrointestinal diseases annually characterized with diarrhoea, stomach cramps, vomiting, nausea, and fever (Todd 2014).

In order to reduce post-harvest losses and retain the quality of fish, including sardines, it is necessary to turn to attractive and sustainable solutions. Technologies like heat pump drying (HPD) and superheated steam drying (SSD) have demonstrated their potential to decrease post-harvest losses, increase availability, and preserve the nutritional qualities of fish (Sehrawat et al. 2016). Meanwhile, technological advancement has led to various preservation techniques, one of which is the hurdle technology that has been successfully involved in shelf life stabilization. Over 50 hurdles have been used to preserve various foods (Mohapatra et al. 2013). Packaging and storage temperature are among the hurdle technologies influencing the quality and safety of foods during storage. Vacuum packaging as one of the packaging technologies whereby foods are sealed within an air-free skin-tight package is of top importance concerning the quality of fish and fish products (Kaale et al. 2013). Additionally, one of the main areas of attention during the preservation of foods like sardines should be the storage temperature, which is one of the crucial factors in managing microbes. Therefore, the objectives of this work were to investigate the effectiveness of the superheated steam technique in drying sardines, and to evaluate the effects of packaging and storage conditions on the microbiological changes of sardines.

Materials and Methods

Study location

This study was conducted at Kilindoni Town, which is located in the Mafia Island at longitude 39°39'23.21"E and latitude 7°54'53.21"S (Figure 1). The Island is one of the six districts of the Pwani region, covering 972 km^2, of which 407 km^2 is dry land and 565 km^2 is water (Kweka 2017).

Fish samples collection

Fresh sardine samples were collected from local fishermen on Mafia Island at 07:00 am. The samples were packed in bags that had previously been sterilized. The bags were labeled and placed in cool boxes surrounded with ice cubes, and then transported from the Island by airplane to Dar es Salaam. The samples were delivered to the Department of Food Science and Technology at the University of Dar es Salaam Mwalimu Julius Nyerere Mlimani Campus, for laboratory analyses. On arrival at the laboratory, some of the samples were taken for microbial analyses before any treatment and the remaining samples were superheated steam dried before undergoing microbial analyses (Figure 2). Afterwards, the samples were either vacuum packed or air packed, and stored at room temperature of about 30 °C or chilled temperature of 4 °C throughout the experimental analyses (Figure 2).

Determination of moisture content

Approximately 2 g of each sample was weighed on preconditioned petri plates that were pre-heated in an oven at 105 °C for 2 h and then cooled in a desiccator for 2 h. The samples were dried in a hot air oven (Model Memmert 854) at 105 °C overnight until constant weight. The moisture content was calculated as a percentage loss in weight using the following equation:

$$Moisture\ content\ (\%) = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where: W_1 = weight of the weighing dish (g), W_2 = weight of the moist dish and sample

before drying (g), and W_3= weight of the dish and sample after drying (g).

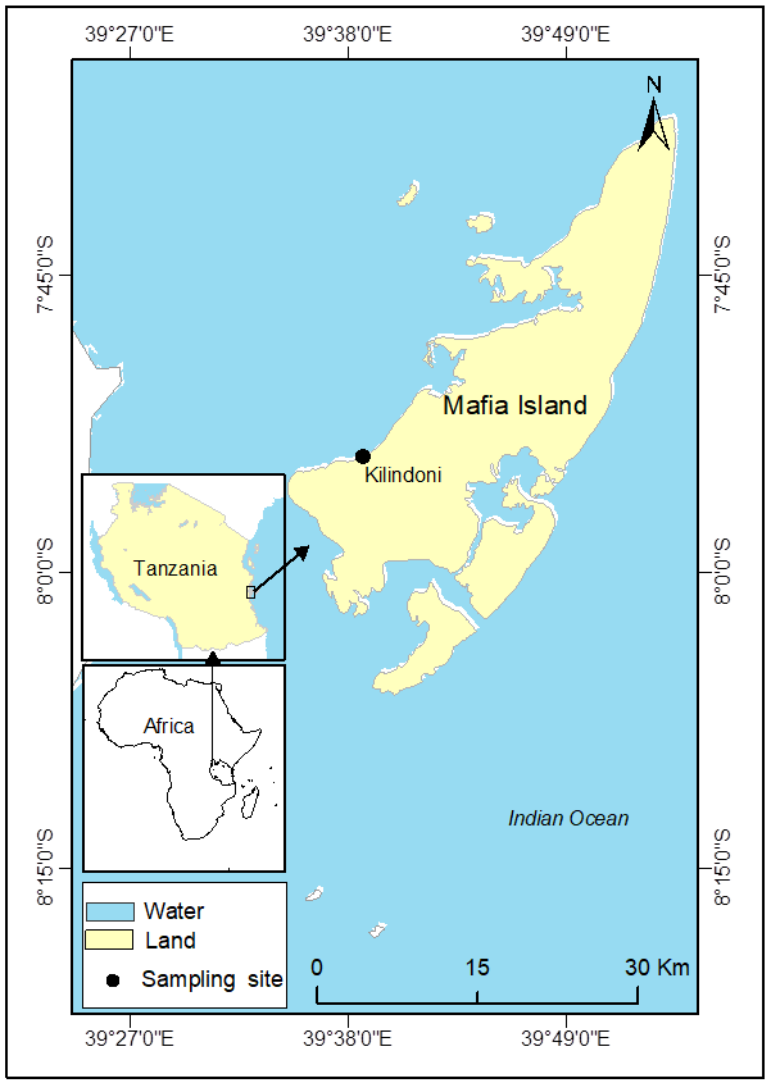

Figure 1: Map of Tanzania showing the location of the sampling site in Mafia Island (Shapefile Source: National Bureau of Statistics–Tanzania).

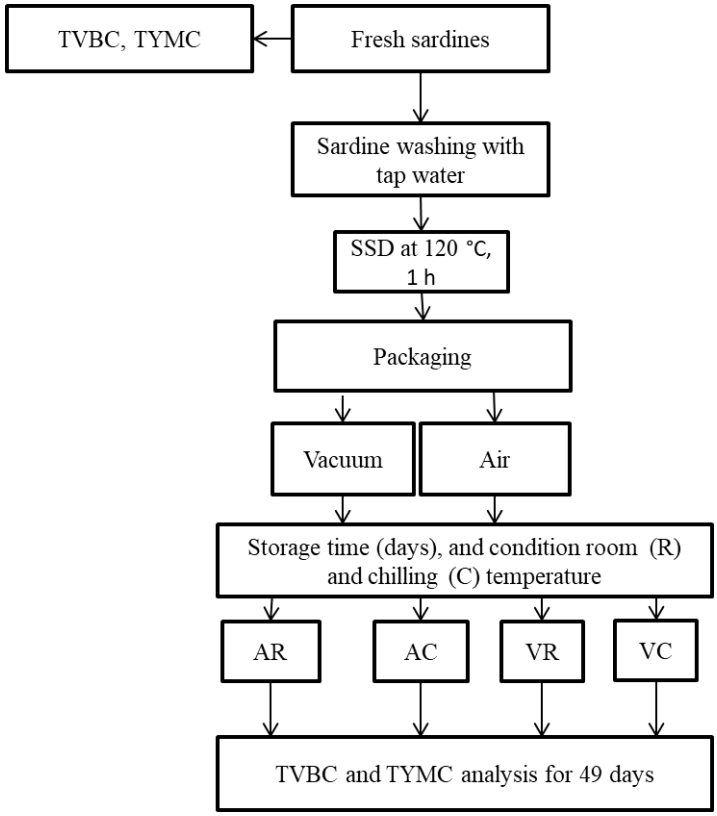

Figure 2: A flow chart showing the experimental setup for microbial analysis of the sardines.

Superheated steam drying

The Department of Food Science and Technology of the University of Dar es Salaam, Mwalimu Nyerere Campus, developed the superheated steam dryer (Figure 3). The dryer consists of a boiler, an air exchange pipe, and a drying chamber. The drying chamber is made out of a stainless-steel vessel with two partitions equipped with perforated stainless trays for holding food products. Its dimensions are 1.5 m × 1.5 m, with the capacity of holding 24 trays of 60 cm × 30 cm dimensions. Liquefied petroleum gas is used as the energy source to boil water at 100 °C under atmospheric pressure. The steam exposure to the food starts by wetting the food due to condensation but with temperature increases over 100 °C, the water in the food begins to evaporate. The water condenses on the walls of the drying chamber before being collected as condensate in the steam trap. After setting the plant ready for operation, the boiler starts by heating the air exchange pipes and simultaneously generating steam. The drained food products (sardines) shift to the steam chamber only if the boiler pressure is about 7 bars. Table 1 provides more details on how to solve specific issues that may arise when drying food using the developed superheated steam dryer.

Sardines (5 kg) were washed with clean tap water, spread out on trays, and allowed to drain for 30 minutes to remove excess surface water before superheating procedures. The trays were oiled to prevent the sardines from sticking to the drying trays during and after drying. The samples were air-dried at 100 °C for 30 min prior to being subjected to superheated steam drying to lessen the chance of the sardines breaking during the process. In the drying chamber, the sardines were superheated steam dried at 120 °C for 60 min. Following that, the sardines were moved to the conditioning room, where they remained for 3 h before packaging. The

samples were then packaged and kept in four different ways for further analysis: air-packed and stored at room temperature (AR), air-packed and stored at chilling temperature (AC), vacuum-packed and stored at room temperature (VR), and vacuum-packed and stored at chilling temperature (VC). Throughout the experiment, 11 samples from each group were used which translates to one sample from each group being used daily on days 0, 1, 2, 3, 7, 14, 21, 28, 35, 42, and 49.

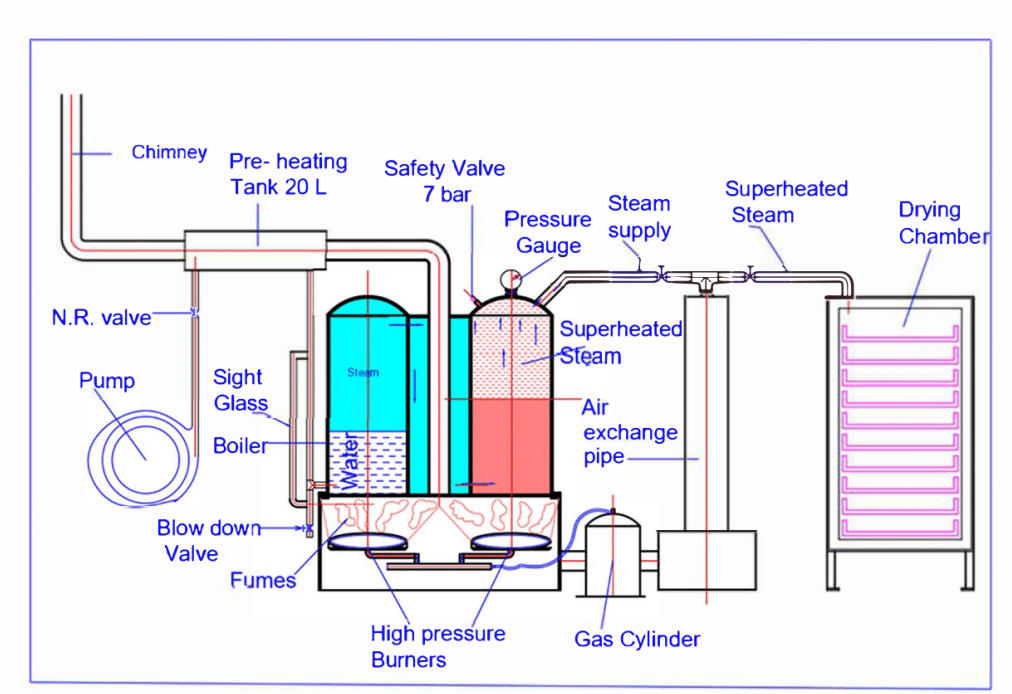

Figure 3: A schematic diagram of the low-pressure superheated steam dryer and associated units.

Table 1: Factors to consider during the superheated steam drying process

S/N	Conditions	Action taken
1	Are dryer temperature and steam pressure lower than required?	Increase gas flow.
2	Are dryer temperature and steam pressure higher than required?	Reduce gas flow.
3	Is the dryer temperature higher than required but the steam pressure less than required?	Reduce steam flow rate.
4	Is the dryer temperature lower than required but the steam pressure higher than required?	Increase steam flow rate.
5	If the temperature/pressure is constant at optimum conditions or adjusting towards optimum conditions.	No action is required.

Microbial analysis

Media preparation

Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) (Himedia, India) were used throughout the study for growing bacteria and yeasts/moulds, respectively. The culture media were prepared following the manufacturer's instructions. PCA was prepared by dissolving 23.5 g into 1000 ml of distilled water, while PDA was prepared by dissolving 39 g of PDA and 100 mg chloramphenicol (for suppressing bacterial

growth) into 1000 ml of distilled water. Thereafter, the media were autoclaved at 121 °C for 15 min before cooling.

Sample preparation and dilution

Sample preparation and dilution for microbial analyses were carried out in a sterile environment following the method described by Gutema and Hailemichael (2021). To prepare homogenate solutions, a 5 g sample, in triplicate, was aseptically homogenized for 2 min in 45 ml of 0.85% NaCl solution (saline) in the blender (BX600G Type 01, China). The resulting homogenates were then agitated on the shaker incubator (LPZ-TSI-200D, Beijing, China) at 36.5 °C and 150 rpm for 20 min to thoroughly mix the homogenate solution. 1 ml of each homogenate solution was pipetted into a clean test tube containing 9 ml of saline to achieve a 10^1 dilution. Serial dilutions were made up to 10^{-7} using the same solution.

Enumeration of the total viable bacterial counts, and the total yeasts and mould counts

Total viable bacterial counts (TVBC), and the total yeast and mould counts (TYMC) were enumerated according to the method of Gutema and Hailemichael (2021) with some modifications. 1 ml of each homogenate dilution in triplicate were poured into sterile petri plates followed by addition of 20 ml of molten media at 45 °C: PCA for TVBC or PDA with chloramphenicol for TYMC (Tournas et al. 2001). The plates were rotated clockwise-anticlockwise to thoroughly mix the inoculum (homogenate dilution) with nutrient medium, and thereafter allowed to solidify in 15 min on a biosafety cabinet (Laminar flow hood). After solidification of the inoculated medium, the petri plates were inverted and incubated at 37 ± 2 °C for 24 to 48 h in the case of bacterial enumeration, and 22 ± 2 °C for 48 to 72 h in the case of yeast/mould count. TVBC and TYMC were obtained by counting the number of colonies of bacteria, and yeasts/moulds, respectively, multiplied by the appropriate dilution factor. Microbial growth counts were expressed in the log CFU/g of the sample.

Statistical analysis

Data were analyzed in R-statistical software version 4.2.1 (The R Foundation 2022). Descriptive analyses such as means and standard deviations were analyzed and reported in Tables. One-way analysis of variance (ANOVA) was used to test for statistical differences within each treatment and within each day for both TVBC and TYMC results, a p-value < 0.05 was considered significant. Mean separation tests were done by Tukey's HSD test.

Results and Discussion

Effects of SSD technology on fresh sardines

The mean values for moisture content of the fresh and dried sardines were 72.61 and 17.22%, respectively (Table 2).

Table 2: Moisture content and microbial quality of the fresh and dried sardine samples

Sample	Moisture content (%)	TVBC (log CFU/g)	TYMC (log CFU/g)
Fresh sample	72.61 ± 0.01^a	9.14 ± 0.05^a	2.00 ± 0.00^a
Dried sample	17.22 ± 0.01^b	0.00 ± 0.00^b	0.00 ± 0.00^b

Values are expressed as mean ± Standard error (n = 3). Different statistical letters across the rows indicate statistical differences within treatments according to Tukey's HSD test.

The fresh sardines in this study had the lowest moisture content (72.61%) compared to the fresh Brazilian and Kenyan sardines reported by Saldanha et al. (2008; 82.7%) and Ochieng et al. (2015a; 75.7%), respectively. However, the moisture content level of the fresh sardines recorded in this study (72.61%) was the highest compared to the moisture content levels recorded by Basunia et al. (2011) (66.5%) and Bellagha et al. (2002)

(70%) for the fresh sardines from Oman and Tunisia, respectively. After being dried using superheated steam at 120 °C for one hour, the fresh sardines' moisture content dropped to 17.22% from the original recorded moisture content (72.61%). The study by Sablani et al. (2003) reported similar moisture contents, i.e., 16.65, 17.53, and 18.56% when dried sardines using different solar dryers. They however, dried for 200 h day and night, which is significantly longer than the time used in the present study. This implied that using superheated steam drying could potentially reduce drying time, enabling fish products to enter the market earlier.

The mean value for the total viable bacterial count (TVBC) in fresh sardines was 9.14 log CFU/g (Table 2). This value was higher than the maximum permissible limit of 7 log CFU/g recommended by ICMSF (1986). Cyprian et al. (2015) and Nguvava (2013) reported lower TVBC values for the fresh sardines, i.e., 4 and 6.08 log CFU/g, respectively. Compared to the previously mentioned research, this shows that the fresh sardines in the current work had a high amount of bacterial contamination.

On the other hand, the mean value for the TYMC in the fresh sample was 2 log CFU/g (Table 2). This value falls within the permissible limit recommended by TBS/AFDC (2017), i.e., ≤ 3 log CFU/g. According to Kombat et al. (2013), fresh round sardines in the Ghanaian cities of Tema and Accra had higher TYMC values of 2.53 and 3.15 log CFU/g, respectively. The greater microbial load (TVBC and TYMC) in the fresh sample may have resulted from on-board handling or contamination during fishing. Fish caught from contaminated water typically have high microbial loads, which are the results of the environment in which they reside (Dike-Ndudim et al. 2014). Fish can also get contaminated at many stages of transport, handling, processing, packaging, and storage, including when ice made from contaminated water is used to store fish (Kombat et al. 2013).

The TVBC was significantly ($p < 0.05$) lowered by superheated steam treatment of fresh sardines at 120 °C for 1 h (Table 2). This demonstrates the potency of superheated steam drying for the inactivation of microorganisms. According to Hamad (2012), the majority of the heat-sensitive microorganisms are destroyed when food that has been contaminated with them is heated to a temperature above 100 °C, while the heat-tolerant ones are inactivated. Nygaard and Hostmark (2008) demonstrated that a brief treatment of fish with superheated steam (120–180 °C) for 1 min results into a considerable reduction of the surface bacterial flora on dried fish products. Likewise, Sutikno et al. (2019) observed a lower bacterial count (1.77 log CFU/g) of squid processed by superheated steam at 240, 260, and 280 °C for the time duration of 3, 4, and 5 min, respectively, compared to the raw squid (3.72 log CFU/g). However, the current study recorded 0.00 log CFU/g TVBC after a 1-hour treatment with superheated steam. This might be the result of the longest drying time used in this study compared to the drying times used in the studies cited, namely Nygaard and Hostmark (2008) and Sutikno et al. (2019). Similarly, superheated steam drying significantly decreased the TYMC (Table 2).

Fish is a highly perishable food since its flesh contains 80% water; nevertheless, decreasing the moisture content to 15% prevents the formation of bacteria, extending the food's shelf life. In the current study, fresh sardines had high moisture content and were highly bacterially contaminated before drying. However, superheated steam drying decreased the moisture content and microbiological contamination, prolonging the shelf life of the product (Table 2).

Effects of packaging and storage temperatures on microbial growth during storage time

Analysis of the combined impacts of packaging and storage conditions on microbiological changes in the dried samples revealed that while growth usually accelerated with time, it lagged at 0.00 log CFU/g for the first days across all treatments (Table 3). The AR samples had the earliest microbial growths (day 2; TVBC = 1.10 log

CFU/g, TYMC = 0.33 log CFU/g), and the VC samples had the latest microbial growths (day 28; TVBC = 0.00 log CFU/g, TYMC = 1.20 log CFU/g), demonstrating the effectiveness of the VC method in controlling microbial growths. The microbial counts in the VC samples were the lowest during storage, reaching 1.79 (TVBC) and 1.42 (TYMC) log CFU/g on day 49, whereas the AR samples had the greatest counts during storage, reaching 7.48 (TVBC) and 2.82 (TYMC) log CFU/g on day 49. Generally, for all treatments, bacterial counts were shown to be greater than TYMC at a given period. From day 21 to day 49, there was a significant difference in the rate of bacterial growth (TVBC) between the treatments ($p < 0.05$). For the TYMC, significant differences were only seen between air packaging and vacuum packaging from days 28 to 49 ($p < 0.05$). All treatments in this study showed delayed microbial growth after superheated steam drying. Heat-treated foods have been threatened by the presence of highly thermo-resistant spore-forming bacteria. Spore formation has been a mechanism to survive harsh conditions by microorganisms, however, when conditions allow, they germinate to form microbes (Wells-Bennik et al. 2016).

In general, the samples that were air-packed supported more microbial growth than those that were vacuum-packed. This could be attributed to the presence of molecular oxygen in the trapped air that facilitated the growth of aerobic microorganisms (Ochieng et al. 2015b). All aerobic microorganisms require oxygen gas to oxidize their food through aerobic respiration and produce energy for driving life processes (Coles et al. 2003). Contrary to that, vacuum packaging creates anaerobic conditions by reducing oxygen tension at the same time increasing carbon dioxide concentration and lowering the pH of the food product thereby preventing the growth of aerobic microorganisms (Dordević et al. 2017). By limiting dehydration and evaporative water loss from the food's surface, vacuum packaging preserves food quality and extends the shelf life of food. On the other hand, compared to samples stored at chilling temperatures, air-packed samples stored at ambient temperature (AR) reported greater microbial counts (AC). The majority of microbes that cause food spoiling are mesophiles, meaning that they thrive at room temperature. However, in chilled storage, these microorganisms' (mesophiles') metabolic processes are slowed down by the low temperature, which has an impact on their growth and multiplication (Hamad 2012). According to reports, storing fish in cold or icy temperatures (between 0 and 2 °C) prevents microbiological growth and increases the shelf life of the fish (Jeyakumari et al. 2018). The lowest bacterial counts in the VC treatment when compared to the other treatments demonstrate the effectiveness of hurdles to the preservation of sardines, which comprised an integrated metabolic inactivation that significantly inhibited microbial development (Ochieng et al. 2015b). Similar findings were made by Ochieng et al. (2015b) and Chowdhury et al. (2020), who found that vacuum packaging combined with chilling storage (VC) significantly inhibits microbial growth more than air packaging and room temperature storage. The TVBC in the AC, VR, and VC samples remained below the acceptable limit of 7 log CFU/g (ICMSF 1986) for the duration of storage, demonstrating the efficiency of chilling storage and/or vacuum packaging. The TVBC for the AR samples started to exceed the permitted limit on day 21. Esteves et al. (2021) estimated the shelf-life of gray triggerfish fillets packed in air and vacuum after 15 days of chilling storage and found that total bacterial counts grew dramatically and consistently in the air-packed fish fillets from 2.95 log CFU/g to >10 log CFU/g. They also noted that, on day 10, the total bacterial counts in the samples that had been air-packed had exceeded the ICMSF's acceptable limit of 7 log CFU/g for meals, whereas those in the samples that had been vacuum-packed, despite rising, were within the limit (Esteves et al. 2021). Likewise, Ochieng et al. (2015b) observed that the TVBC in the solar-dried air-packed fish stored at 20 °C, and chilling temperatures

crossed the ICMSF's permissible limit on the 30^{th} and 45^{th} days, respectively, when they were respectively deemed spoilt based on sensory scores. They discovered that the TVBC for the chilled vacuum-packaged samples was within the permitted range for the duration of the examined storage time (90 days). Overall, there was no significant difference in bacterial counts with storage duration across all treatments. This might be due to the inactivation of all the microorganisms in the sardine samples as a result of heat treatment. It has been noted that the aerobic spore-forming bacteria are extremely heat resistant and known to cause food spoilage during storage (Byrer et al. 2000).

All treatments in the current study exhibited a rising trend with storage time in the TYMC; however, the trend was not statistically significant. This might be explained by the use of heat, which eliminated all yeasts and moulds that were initially present in the fresh sardines. It is documented that yeasts and moulds are less resistant to heat (killed at 85 °C for 5 min) than bacterial spores (Coles et al. 2003). This is supported by Kombat et al. (2013) who reported an increasing trend in the TYMC of the sardines from Tema and Accra, Ghana.

As compared to VR and VC (1.54 ± 0.162 and 1.42 ± 0.060 log CFU/g, respectively), the mean values of the TYMC in AR and AC were significantly higher (2.82 ± 0.007 and 2.47 ± 0.007 log CFU/g). The mean TYMC in the raised open rack dried samples as reported by Ochieng et al. (2015a) was 2.17 log CFU/g whereas that of dried samples by the conventional traditional drying method was 2.57 log CFU/g, suggesting clean and safe practices were followed during processing. The TYMC between VR and VC, on the other hand, did not show a significant difference ($p > 0.05$). Vacuum packaging limits the growth of aerobic bacteria and their detrimental effects by converting oxygen to carbon dioxide (Dordević et al. 2017). Most yeasts and moulds are aerobic and hence grow well in the presence of oxygen (Coles et al. 2003). In addition, yeasts and moulds grow slowly as compared to bacteria, and this is why most food spoilers are bacteria (Hamad 2012). Despite the fact that all treatments' TYMC increased during the storage period, none of them exceeded the maximum allowable limit of 3 log CFU/g (TBS/AFDC 2017).

Conclusions

Fish have been categorized as highly perishable foods that contain moisture above 80%. The fresh sardines were dried at 120 °C for 1 h using a superheated steam dryer in an effort to extend the shelf life of the marine sardines from Mafia Island. The superheated steam-dried sardines were examined for microbiological changes for 49 days in the appropriate packaging and storage (AR, AC, VR, and VC). It was observed that the microbial counts in all treatments decreased to 0.00 log CFU/g from the initial 9.14 TVBC and 2 TYMC log CFU/g obtained in the fresh samples. Microbial counts significantly increased during storage ($p < 0.05$) in air-packed samples compared to vacuum-packed samples. As a result, the AR samples exceeded the allowed limit on day 21, while the AC, VR, and VC samples were within the acceptable limit during the whole storage period (49 days). In this study, it was found that the use of vacuum packaging and a chilled storage method led to microbial growth control over the course of storage (49 days), hence prolonging the shelf life of dried sardines. However, this study recommends further research into the dryer's effectiveness in other food-drying applications. In addition, further research should be done into the utilization of other economically and environmentally energy sources apart from liquefied petroleum gas (LPG).

Table 3: Effects of different packaging and storage conditions on the microbiological quality of the super-heated steam-dried sardines within 49 days of storage

Storage time (days)	AR		AC		VR		VC	
	TVBC (log CFU/g)	TMC (log CFU/g)	TVBC (log CFU/g)	TMC (log CFU/g)	TVBC (log CFU/g)	TMC (log CFU/g)	TVBC (log CFU/g)	TMC (log CFU/g)
0	0.00 ± 0.000^{aE}	0.00 ± 0.000^{Ab}	0.00 ± 0.000^{Ad}	0.00 ± 0.000^{aE}	0.00 ± 0.000^{Ad}	0.00 ± 0.000^{aB}	0.00 ± 0.000^{aB}	0.00 ± 0.000^{aB}
1	0.00 ± 0.000^{aE}	0.00 ± 0.000^{aB}	0.00 ± 0.000^{Ad}	0.00 ± 0.000^{aE}	0.00 ± 0.000^{Ad}	0.00 ± 0.000^{aB}	0.00 ± 0.000^{aB}	0.00 ± 0.000^{aB}
2	1.10 ± 0.100^{aD}	0.33 ± 0.333^{aB}	0.00 ± 0.000^{Bd}	0.00 ± 0.000^{aE}	0.00 ± 0.000^{Bd}	0.00 ± 0.000^{aB}	0.00 ± 0.000^{bB}	0.00 ± 0.000^{aB}
3	2.36 ± 0.028^{aC}	0.49 ± 0.493^{aB}	0.00 ± 0.000^{Bd}	0.00 ± 0.000^{aE}	0.00 ± 0.000^{Bd}	0.00 ± 0.000^{aB}	0.00 ± 0.000^{bB}	0.00 ± 0.000^{aB}
7	3.47 ± 0.003^{aB}	2.35 ± 0.033^{aA}	2.11 ± 0.033^{Bc}	1.76 ± 0.087^{bD}	2.01 ± 0.114^{Bc}	0.00 ± 0.000^{cB}	0.00 ± 0.000^{cB}	0.00 ± 0.000^{cB}
14	3.49 ± 0.113^{aB}	2.44 ± 0.038^{aA}	3.32 ± 0.088^{Aa}	2.10 ± 0.032^{bC}	2.71 ± 0.056^{Bb}	0.00 ± 0.000^{cB}	0.00 ± 0.000^{cB}	0.00 ± 0.000^{cB}
21	7.36 ± 0.183^{aA}	2.58 ± 0.020^{aA}	5.11 ± 0.107^{Ba}	2.25 ± 0.024^{bBC}	3.15 ± 0.086^{Ca}	1.10 ± 0.100^{cA}	0.00 ± 0.000^{dB}	0.00 ± 0.000^{dB}
28	7.38 ± 0.173^{aA}	2.64 ± 0.015^{aA}	5.20 ± 0.100^{Ba}	2.29 ± 0.013^{aB}	3.16 ± 0.091^{Ca}	1.26 ± 0.140^{bA}	0.00 ± 0.000^{dB}	1.20 ± 0.100^{bA}
35	7.41 ± 0.153^{aA}	2.72 ± 0.018^{aA}	5.24 ± 0.093^{Ba}	2.35 ± 0.024^{aAB}	3.18 ± 0.093^{Ca}	1.30 ± 0.173^{bA}	0.00 ± 0.000^{dB}	1.26 ± 0.140^{bA}
42	7.45 ± 0.137^{aA}	2.75 ± 0.020^{aA}	5.25 ± 0.087^{Ba}	2.40 ± 0.009^{aAB}	3.20 ± 0.091^{Ca}	1.33 ± 0.200^{bA}	1.69 ± 0.052^{dA}	1.36 ± 0.060^{bA}
49	7.48 ± 0.129^{aA}	2.82 ± 0.007^{aA}	5.30 ± 0.084^{Ba}	2.47 ± 0.007^{aA}	3.24 ± 0.087^{Ca}	1.54 ± 0.162^{bA}	1.79 ± 0.061^{dA}	1.42 ± 0.060^{bA}

Key: AR = air-packed and stored at room temperature; AC = air-packed and stored at chilling temperature; VR = vacuum-packed and stored at room temperature; VC = vacuum-packed and stored at chilling temperature; TVBC = Total viable bacterial counts; TYMC = total yeast and mould counts. Values are expressed as mean ± Standard error (n = 3). Different statistical letters across the rows indicate statistical differences within treatments and capital letters across the column within storage time according to Tukey's HSD test.

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Conflict of interest
The authors declare no conflict of interest regarding this research work.

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