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EFFECT OF FILLING MEDIA ON THERMAL PROCESSING PARAMETERS OF CANNED SHELLFISH FROM THE NIGER DELTA, NIGERIA

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ABSTRACT
The research evaluated the effects of different canning media on select parameters associated with the depth of effect of thermal processing on the quality and microbiological properties of canned periwinkle (Tympanostonus fuscatus), whelk (Thais callifera), oyster (Crassostrea gasar) and clam (Mercenaria mercenaria) consumed in the Niger Delta, Nigeria. The samples were processed according to the local traditional method and canned using vegetable oil, tomato sauce and brine as filling media. The products were subjected to retorting for a total processing time of 74 minutes for sterilization and cooling. The heat uptake at the slowest heating zone of the canned monitored using a temperature measuring device placed in the centre of the can. The samples were analyzed for microbial loads before and sterilization. The heat uptake data were used to compute the F value of the sterilization process while the change in the bacterial load was used in computing the D value of the contaminating microorganism isolated from the samples. The results showed that the maximum temperatures attained at the slowest heating zones and the time taken were 119.1 °C after 66 minutes, 120.3 °C after 66 minutes and 120.6 °C after 64 minutes of heating for tomato sauce, brine and vegetable oil packed samples, respectively. The F_o ranged from 9.28 min to 25.45 min. the D_{121} value for Bacillus sp isolated from the samples ranged from 12.96 min for clam in 5% brine to 39.06 min for whelk in 3% brine. Although the canning media affected the thermal processing parameters, the three canning media all delivered Fo high enough for minimum botulinum cook. Nevertheless, heat uptake by vegetable oil and brine was faster and higher, showing potential of greater microbial destruction than tomato sauce. Microbiologically, canning with vegetable oil and brine appeared the best suited canning medium for the studied shellfish species.
Keywords: **Aquatic mollusk, heat penetration, microbial log reduction, F value, D value**

I. INTRODUCTION

Agriculture has been identified as the key natural successor for growth beyond oil in Nigeria (PWC, 2016). Furthermore, aquaculture has been acknowledged as the fastest growing food production sector (FAO, 2016), with export potentials, if the vast natural resources are harnessed with appropriate technologies. Oyster (*Crassosterea graser*), periwinkle (*Tympanostonus fuscatus* and (*Tympanostomus fuscatus Var. radula*), whelk (*Buccimum undatum*) and clam (*Mercenaria mercenaria*) members of the aquatic mollusc

family (Haszpruner, 2001; Ruppet *et al.,* 2004) commonly harvested in Nigeria (Abulude *et al*., 2006; Adebayo-Tayo *et al*., 2006; Ideriah *et al*., 2006; Adebayo-Tayo and Ogunjobi, 2008). Many aquatic species produce excellent canned products, supporting human nutrition (FAO, 2005).

Shellfish are among the abundant aquatic organisms suitable for food, and are a rich source of high biological value protein (Sivasankar, 2011). They provide high quality protein with all the dietary essential amino acids for maintenance and growth of the human body (Babu *et al.,* 2011). Seafood is highly valued not only for its abundance of high-quality protein, but also for the n-3 polyunsaturated fatty acids (PUFAs), and other nutrients, such as minerals, trace elements and vitamins (FAO, 2010). These nutrients are essential for proper functioning of the body and beneficial for growth, the brain and nervous system (Hosomi *et al*., 2012). The protein quality of seafood is superior to those of meat and poultry (Arularasan *et al.,* 2009) because of the amino acid composition, including the concentration of the essential amino acids. They therefore play a significant role in human nutrition and health.

Shellfish are highly perishable due to their biological composition and their high moisture content. Under normal refrigerated storage conditions, the shelf life of shellfish is limited by enzymatic and microbiological spoilage. They begin to go bad shortly after capture unless they are subjected to processing (Ashie *et al., 1996*) in order to keep longer. More commonly among adopted processing methods for the achievement of preservation is canning (Nader *et al*., 2016; Dhinesh *et al*., 2021). Canning, reckoned among the traditional methods of processing fish, prolonging the shelf life and maintaining the nutritional content (Agwa *et al*., 2018), and shellfishes are also canned (Bratt, 2010) for similar purposes forms a convenient way of making the product available to a wider and international population (Fasogbon *et al*., 2022). According to Simpson *et al*. (2020), canned foods have a shelf life of one to four years at ordinary (room) temperatures, making them convenient, affordable, and easy to transport.

During the canning of shellfishes, the required heat treatment is such which is sufficient to destroy all heat sensitive bacteria and spores, inactivate the enzymes and cook the shellfish such that the products retain acceptability to the consumers after prolonged storage. The manner and strength of heat transfer follow the sterilization media used (Soni and Brightwell, 2022). The heat penetration gets higher and also faster based on temperature difference between the heating medium and the foods (Al-Baali and Farid, 2006). The heat transfer medium therefore affects the level of heat transfer (Simpson *et al*., 2020) and consequential level of sterilization. Several bacterial genera and species have been isolated from canned sea foods stored at different conditions, including the spoilage and pathogenic groups, which pose public health concerns (Ibe, 2008; Solomon *et al*., 2013). The different microbial species The aim of this study was therefore to monitor the microbiological quality of the canned shellfish, tailored to the shelf stability and safety of the products.

II. MATERIALS AND METHODS

A Material collection

Freshly harvested Oyster (*Crassostrea gasar*), Whelk (*Buccinum undatum*), Periwinkle (*Tympanostonus fuscatus* var) and Clams (*Mercenaria mercenaria*) were obtained from sea food market in Lagos and transported in respective ice packed containers to the Department of Fish & Fishery Product Processing Laboratory, Nigerian Institute of

Oceanography and Marine Research (NIOMR) Lagos. All chemicals and reagents that were used for the research work was obtained from the same Department and of analytical grade.

Sample preparation

For each mollusc species, a basketful ≈ 30 – 35 kg was bought. The meat samples obtained from the basket full weighed between 8.4 kg (periwinkle) and 9.6 kg (clam). The samples were prepared by using the respective traditional methods of processing the different shellfish species in Rivers State, Nigeria, as described by Kiin-Kabari and Obasi (2020), as shown in Figure 1. After the overnight soaking, the boiling and draining, the edible portions (meats) were then extracted from the shell with the aid of sterile pins in the case of the periwinkle and whelk and a sharp knife in the case of clams and oysters. The samples were frozen at – 20°C until required for use.

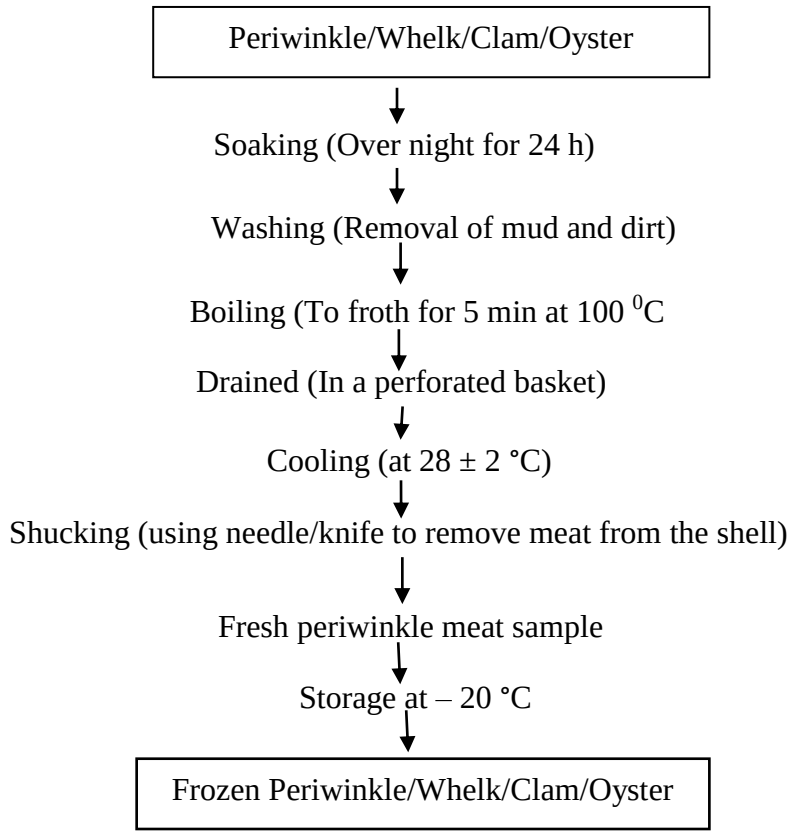

Figure 1: Traditional method of shellfish processing (Kiin-Kabari and Obasi, 2020)

B. Preparation of Filling Solutions; Canning Operation

i) Preparation of Brine Solution:

Powdered oxidized salt bought from a local supermarket was used in making the saline solution food system. Distilled water was taken from a clean rinsed beaker and the amount of water weighed to make up 5%, 3% and 2% brine solution by adding the respective weights, 5g, 3g and 2g (Onwuka, 2018) of salt and also incorporating sodium tripoly-phosphate and citric acid into the solution. The solution was boiled to expel dissolved air and was allowed to cool down to room temperature in a water bath before being added to the can.

ii) Preparation of Tomato sauce

Fresh tomatoes, pepper, onions were washed and chopped into pieces with a knife. The juice was mechanically extracted

with the aid of a Blender (Braun type: 4290 made in Germany). The extracted juice was blended followed by concentrating: frying in vegetable oil was followed with the addition of water and the tomato sauce was allowed to steam for 8 min followed by cooling.

iii) Recipe for tomato sauce preparation

Fresh tomatoes	41 kg
Onions	3 kg
Fresh pepper	3 kg
Salt	1 kg
Concentrated tomato paste	2.2 kg
Vegetable oil	2 L
Water	2 L

iv) Vegetable Oil

Refined groundnut oil (1500 mL) and 2% sodium chloride were used in preparation of this filling medium. The filling solutions was mixed with 0.1% citric acid, 0.1% sodium benzoate, and 0.1% ascorbic acid and boiled before pouring.

v) **Canning operation and storage**

The canning operation was performed by adopting the method of Cobas *et al*. (2022) with modification. The process followed the unit operations as shown in Figure 2. The frozen pre-processed samples were thawed in a refrigerator (4 °C) for 12 h. Approximately 130 g of the samples was placed in the 250 mL cans and about 100 mL of the canning media, brine, tomato sauce, refined groundnut, oil and brine was poured hot into the can. The cans were exhausted to remove the air and gases inside the filled cans using a clean stainless steel spatula and shaking the sample and swirling the can. The cans were closed by placing the lids of the cans and closing to a tight sealing using the packaging machine (VP-430 Ramon Vac Line, Barcelona, Spain). The cans containing the samples were thermally processed in an autoclave (Raypa AES-75, Barcelona, Spain) at 121 °C for 60 min before being cooled with potable water to about 40 °C. The cans were labeled and stored at room temperature for subsequent analyses. The samples were analyzed for sensory quality after canning, while the shelf stability was evaluated through the physicochemical properties and proximate composition.

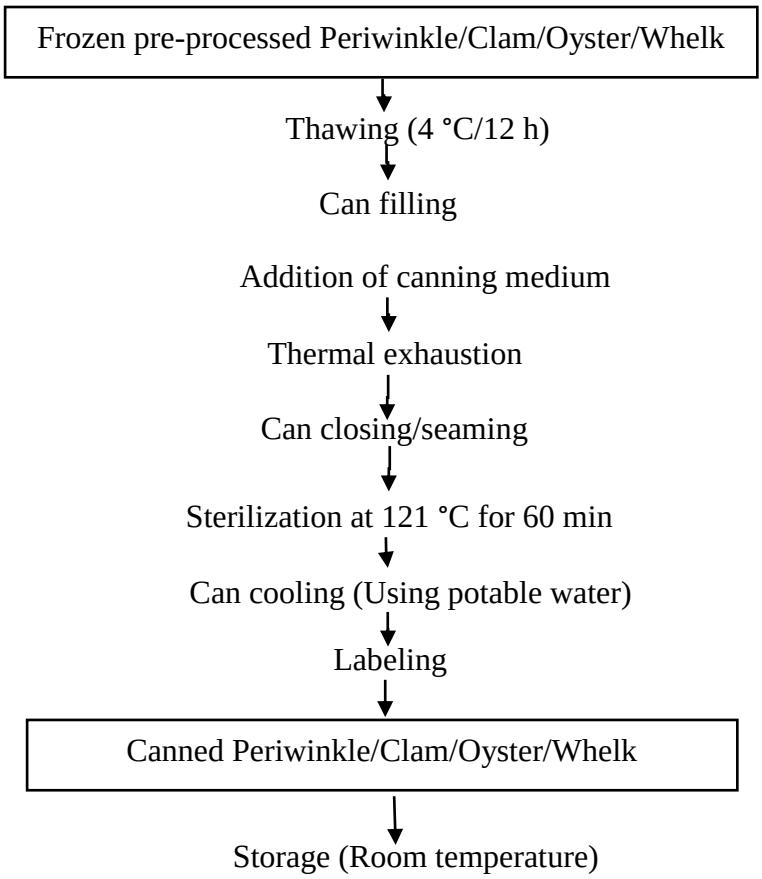

Figure 2: Canning procedure for periwinkle and clam (Cobas *et al*., 2022) modified.

C. Thermobacteriological Evaluation of Canned shellfish samples

i) Heat Penetration and F_0 of the Sterilization

The heat penetration parameter, F-value (F_0) achievable with each filling medium was obtained by following the explanation of Toledo (2007). For each filling medium, one of the cans was selected and marked "thermal uptake," a thermocouple was fixed at the centre of the can, presumed to be the coldest spot, and placed in the sterilization unit for monitoring. A can of periwinkle in 5% brine was selected for the brine-filled cans. For the cans having vegetable oil and tomato sauce as the packing media, each had a can with clam selected. Temperature at the centre of the can, showing heat uptake by the product, was taken with the thermocouple at intervals of two (2) minutes. The data were inputted in Microsoft Excel and then algorithms set for the computation of the process Lethality from the temperature data collected, per time of that temperature reading. A Time-Temperature graph was plotted and the area under the curve, which corresponded to the sum of the individual lethality values, was used in calculating the F-value of the process as:

Lethality, L at time t = $10^{(Ti-121)/z}$ …Eqn 1

Where;

T_i = Temperature at time t; z = z-value of *Clostridium botulinum* spore, assumed to be 10, and;

F value, $F_0 = \Sigma L$ (Toledo, 2007) …Eqn 2

ii) D values of the sterilized canned shellfish

The estimated D-values were determined from the microbial counts before and after the sterilization using the change in log of the microbial populations against

sterilization time by the formula below. The microbial species of interest was *Bacillus* sp., indicated by the biochemical tests.

$$D121 = \frac{Time}{(\log_a - \log_b)} \quad \text{…Eqn 3}$$

where;

a = the initial population
b = the survivors after a time interval

RESULTS

A. Heat Uptake and F values (F_0) of the Shellfish Samples Canned in Different Media

Result for heat uptake during the sterilization of canned periwinkle, whelk, oyster and clam in tomato sauce, brine and vegetable oil media is shown in Figure 3. Temperature increased from 63.1 to 119.1 °C, and declined to 101.2 °C within the heating and cooling period that totaled 74 min, for tomato sauce canning medium, with the maximum heating temperature of 119.1 °C attained after 66 min. Temperature change from the beginning to the maximum point following heat uptake during the sterilization process ranged from 109 °C to 120.3 °C and 108.4 to 120.6 °C, respectively, for the brine and vegetable oil canning media. These declined to respective 101.3 and 102.9 °C within the 0 – 74 min heating and cooling process time. For the brine medium, a maximum sterilization temperature of 120.3 °C was attained at 66 min. However, sterilization temperature of 120 °C was attained after 40 min of heating. Sterilization temperature of 120.1 °C was seen in vegetable oil medium after 34 min of heating. Maximum heat uptake of 120.6 °C was attained in the center of the vegetable oil medium after 64 min of heating. Table 1 shows the F_o of the sterilization with the different media. Cumulative F_o value of tomato sauce, brine and vegetable oil was respectively 9.13, 23.48 and 25.45, obtained within 74 minutes of heating and withdrawal of heat (cooling). Generally, Figure 3 shows that the respective initial and maximum sterilization temperatures with the different canning media were 109 °C and 120.3 °C for brine, 63.1 °C and 119.1 °C for tomato sauce and 108.4 °C and 120.5 °C for vegetable oil media.

B: D value of Canned Periwinkle and Clam in Tomato Sauce, Brine and Vegetable Oil Media

The D-values showing the estimated thermal reduction in the bacterial population are shown in Figure 4. The results ranged from 12.96 min for clam in 5% brine to 39.06 min for whelk in 3% brine. Among all the four shellfish samples studied, oyster and whelk consistently fell among samples that had higher values, while clam had the lowest value in all the canning media.

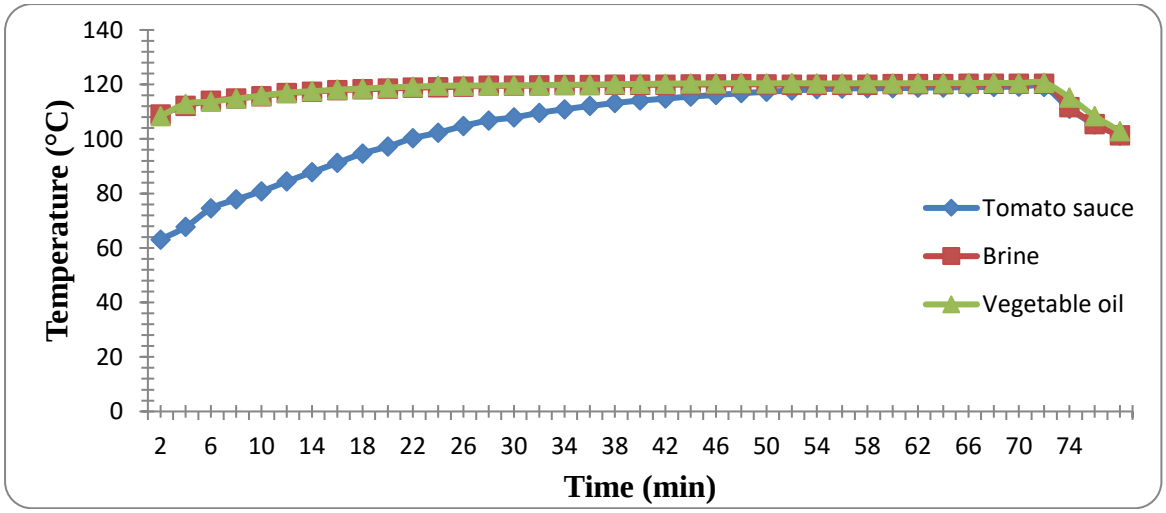

Figure 3: Heat Uptake of Canned Periwinkle, Clam, Oyster and Whelk in Tomato Sauce, Brine and Vegetable Oil Media

Table 1: Fo obtained with different media for the canning of shellfish

Canning medium	F_o (Min)
Tomato sauce	9.128
Brine (3 – 5%)	23.478
Vegetable oil	25.452

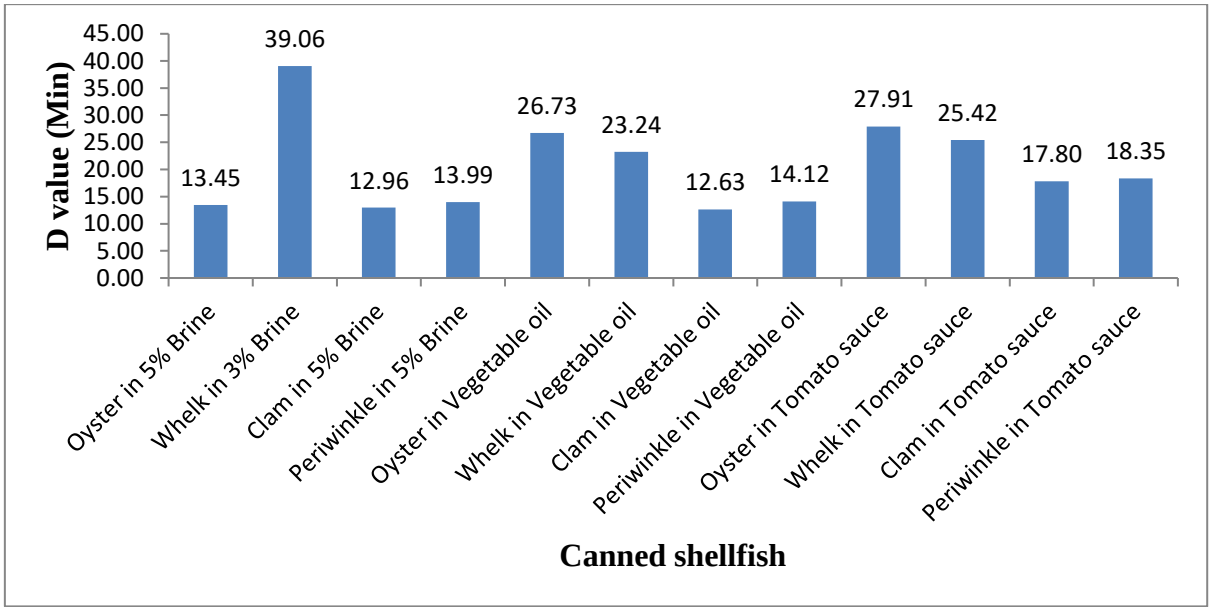

Figure 4: D_{121}-values for destruction of *Bacillus* sp. cells in periwinkle, clam, oyster and whelk canned with different filling media

DISCUSSION

Evaluation of F_o and D values of Canning Media for Shellfish samples

Sterilization value (F_o) is the thermal processing time (min), which is required at 121.1 °C to reduce the population of bacterial (*Clostridium*) spores with D-value

of $D_{121.1}$ and z-value of 10 °C to a desired reduction exponent (Cho *et al*., 1992). It is the sterilization process equivalent to time, defined as the number of equivalent minutes at T = 121.1°C delivered to a food container to make it commercially sterile and safe for consumption (Olusola, 2018). Result for heat uptake during the sterilization of canned periwinkle, oyster, whelk and clam in tomato sauce, brine and vegetable oil media is shown in Figure 3. Temperature increased from 63.1 to 101.2 °C, for a processing period of 74 min for tomato sauce filling medium. A maximum heating temperature of 119.1 °C was attained in the tomato sauce packed can at 66 min. Temperature change cycle within the 74 min total processing time showing the heat uptake and attendant temperature rise from the initial 109 °C to the maximum 120.3°C, and the return to a final 101.3 °C after a brief cooling period was observed in the brine canning medium, while sterilization of the vegetable oil packed cans gave a temperature cycle of initial 108.4 °C to a maximum 120.6 °C after 66 min, and a return to a final 108.4 °C within the total 74 min process time (heating and cooling period). For the brine medium, a maximum sterilization temperature of 120.3 °C was attained at 66 min. However, sterilization temperature of 120 °C was already attained after 40 min of heating. Sterilization temperature of 120.1 °C was achieved in vegetable oil medium after 34 min of heating. Maximum heat uptake of 120.6 °C was attained in the center of the vegetable oil medium after 64 min of heating. This was probably due to the high conductive nature of vegetable oil, with respect to heat transfer (Chibor *et al*., 2017).

Cumulative lethality giving the F_0 value of tomato sauce, brine and vegetable oil was 9.128, 23.478 and 25.452 min, respectively, obtained after the 74 minutes of heating and cooling (total process time). Sterilization values (F_0) 7.04 and 6.62 min were seen in vegetable oil and brine filling media, respectively after 26 min of heating, with temperatures at the centre of the can (slowest heating point) reading 119.7 °C and 119.5 ^{0}C, respectively. F_0 value becomes cumulatively greater with the elapsing heating time. Hence, F_0 value is estimated from the time-temperature profile during whole heating and cooling process (Cho *et al*., 1992). It is the integrated lethality during the whole sterilization process. From the result (Figure 3), the shell fish canned in tomato sauce, brine and vegetable oil filling media all attained commercial sterility with F_0 values of 9.128, 23.478 and 25.452 min, respectively. The F_0 values of a thermal process can be determined by microbiological or physical method. The former method relies on quantifying the destructive effects of heating on bacterial numbers through their enumeration before and after a thermal process (Warne, 1988), while the latter method measures the change in temperature during a thermal process at the slow heating point (SHP) of the container and relates this to the rate of thermal destruction at a reference temperature (Warne, 1988). In this study, the severity of sterilization processes was determined using only the physical method of quantifying the lethal effect of thermal processes. The target F_0-value obtained was very significant as it indicates the probability of spoilage due to under-processing (Olusola, 2018). For the attainment of microbiologically stable state, the amount of heat received by the product during thermal processing needs to be verified (Maheswara *et al*., 2011). This was determined by the temperature profile within the product during thermal processing. The recommended F_0 values for fish and fishery products range from 5 to 20 minutes (Frott and Lewis, 1994). The F_0 values obtained in this study is therefore within acceptable and recommended

values. Lower F_0 value yield microbial safe and shelf stable products without undue impairment of flavour, consistency, colour or nutrient content (Ababouch, 2000). Hence, the F_0 values of >23 – 25 min obtained in this study for brine and vegetable oil may turn out to be an overprocessing.

In smoked tuna canned in retort pouches with brine and oil as the filling media, heat penetration was faster in the brine pack compared to the oil pack (Bindu and Gopel, 2008). F_0 value of 8.79 minutes was earlier reported for canned sea food. The authors opined that the thermal process was adequate (Mallick *et al*., 2006). Safety from botulism caused by underprocessing means that the probability of *C. botulinum* spores surviving the thermal process must be sufficiently remote so as to present no significant health risk to consumers. Experience has shown that a process equivalent to twelve decimal reductions in the population of *C. botulinum* spores is sufficient for safety; this is referred to as a 12D process and assuming an initial spore load of 1 spore/g of product, it can be shown that, for such a process, the corresponding probability of *C botulinum* spore survival is 10^{-12}, or one in a trillion. This implies that for every trillion cans given a 12D process 1 and in which the initial load of *C. botulinum* spores was 1/g, there will be only one can containing a surviving spore. Such a low probability of survival is commercially acceptable, as it does not represent a significant health risk (Warne, 1988). The minimum F_0 required for canned food product is 2.52 min, to inactivate spores of *C. botulinum* (Adepoju *et al*., 2016). F_0-value (lethality) of 8.0 min was recommended for complete inactivation of all spore formers, for safe canned sea food (Ravishankar and Asok, 2015). Martin-Xavier *et al*. (2007) also recommended F_0-value of 10 min for canned sea food in vegetable oil, tomato sauce and brine filling media. The reports therefore prove that the F_0-values of 9.128, 23.478 and 25.452 min obtained in this study are adequate to guarantee commercial sterility of the canned whelk, oyster, periwinkle and clam.

The D values obtained were much higher than the D values of 0.1 – 0.25 min reported by Mohan *et al*. (2015) for two strains of *Clostridium botulinum*. The difference in the D-values could be attributed to the food systems. The tomato sauce-based samples having much higher values than those canned in brine and vegetable oil media was as should be expected. The thickness of the tomato sauce medium, giving lower rate of heat uptake by the samples resulted in the higher D values. This thus indicated that, with tomato sauce, more time would be required to destroy the microbial population to achieve a 12D sterilization of the clam and periwinkle samples. Time and temperature combination of thermal processing can depend on the food matrix, including characteristics such as moisture content and pH, and the intrinsic resistance of the bacterial species being targeted. While bacterial spore formers are known to have higher D values as compared to the non-spore forming vegetative bacterial strains, there is a significant difference in the D values among different strains of same bacterial species in various food products (Soni and Brightwell, 2022).

D Value (at 121.1°C) of Some Bacterial Spores reported by Mohan *et al*. (2015) shows that B. *stearothermophilus* has 4-5 min. *Clostridium thermosaccharolyticum* has 3-4 min; *Clostridium nigrificans* has 2-3 min; *C. botulinum* types A and B has 0.1 – 0.25 min. The D value for bacterial spores is independent of initial numbers, but it is affected by the temperature of the heating medium. The higher the temperature, the faster the rate of thermal destruction, and the lower the D value. This has been

reported as why thermal sterilization of canned fishery products relies on pressure cooking at elevated temperatures (>100 °C) rather than relying on regular cooking in steam or water, which is open to the atmosphere (Mohan *et al*., 2015).

With the faster heat transfer rate and the consequent higher heat uptake by the samples canned in vegetable oil and brine than those in tomato sauce medium, it could be expected that such could lead to an observation of no fungal species growing on samples having brine and vegetable oil as the canning media. A corresponding observation of lower bacterial counts in the brine and vegetable oil filled samples than the tomato sauce counterparts would then be adduced to explain the effect of the heat penetration on the microbial population observed on the samples in the different canning media. As fungal spores have lower thermal resistance, the destruction of the fungal spores would expectedly be more than the bacterial. This shows a more devastating effect of heat processing (including sterilization) on fungi than bacteria. High temperatures bring about inactivation of yeast cells. Yeasts are usually killed within a few minutes at temperatures over 55 °C. Unlike bacterial endospores, the ascospores or basidiospores of yeasts are only slightly more resistant to heat than the vegetative cells. The decimal reduction time (D value) at 55 °C is about 5 to 10 min, at 65 °C less than 1 min. The death rate increases tenfold when the temperature rises by 4 to 5 °C, i.e. the z-value is 4 to 5 °C. These are average values only, and the composition of the food has a substantial effect on the rate of inactivation (Deak, 2004).

CONCLUSION

The study has shown that the heat uptake by the three canning media differed and can suggest advisory consideration on the choice of appropriate medium for shellfish canning based on intended quality goals. Based on the heat uptake, maximum temperatures reached at the centres of the cans, and the F_o values obtained with the three canning media, the canning of the shellfish samples in vegetable oil, tomato sauce and 3 – 5% brine and the sterilization of the cans at 121 °C for a total processing time of 74 minutes would destroy microbial cells and enzymes and offer the products shelf life for a reasonable period before spoilage or development of safety concerns.

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