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Research Article

Preservation of Periwinkle (*Tympanotonus fuscatus*) with Potassium Sorbate and Natural Agent at Ambient Temperature

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ABSTRACT

The aim of this study was to evaluate the microbiological and preservative effect of potassium sorbate and natural agent (potassium aluminium sulphate) also known as alum, on periwinkle during ambient temperature storage. Periwinkle meat was shucked and prepared aseptically and preservatives; potassium sorbate 0.1% (w/v), potassium aluminium sulphate 1% (w/v) and combination of both preservatives were separately applied on the sample. The samples were stored at ambient temperature and microbial analysis was carried out for 2 days. The total heterotrophic bacterial count of the control sample from the initial time to day 2 had the highest value of 5.1 $\pm 0.0\times10^{2}$ to $2.24\pm0.1\times10^{7}$ CFU/g while the total count of alum treatment was the least $3.4\pm0.01\times10^{2}$ to $8.7\pm0.03\times10^{3}$ CFU/g. The highest count of total coliform for control sample was $6.3\pm0.01\times10^{4}$ to $1.92\pm0.01\times10^{6}$ (initial time to day 2) while the least count of total coliform with the treatment with Alum was $2.1\pm0.1\times10^{2}$ to $1.92\pm0.01\times10^{3}$ CFU/g. The count of *Salmonella* and *Shigella* for the treatment with Alum had the least value of $1.04\pm0.02\times10^{3}$ CFU/g while the control sample had the highest value of $8.4\pm0.02\times10^{4}$ to $1.53\pm0.01\times10^{5}$ CFU/g. The least count of *E. coli* ($3.6\pm0.1\times10^{3}$ CFU/g) was in the sample treated with Alum preservative while the control sample had the highest count of $9.4\pm0.01\times10^{3}$ to $1.24\pm0.1\times10^{5}$ CFU/g from the initial time to day 2. The fungal count of the samples was recorded only on day 2 (no growth on the initial time and day 1) and the Alum preservative had the least value of $3.3\pm0.02\times10^{2}$ CFU/g and the control had the highest count, $7.2\pm0.01\times10^{4}$ CFU/g. The bacteria isolated and identified through biochemical test were *Escherichia coli*, and species of *Micrococcus, Staphylococcus, Enterobacter, Pseudomonas, Salmonella, Shigella, Proteus, Citrobacter, Alcaligenes* and *Bacillus*. While fungi were *Candida*, *Aspergillus niger* and *Penicillium* sp. Some bacteria identified molecularly with ascension numbers were *Alcaligenes faecalis*, MN833516, *Pseudomonas* aeruginosa, MN985328, *Escherichia coli*, MN396566 and *Bacillus niacin*, JF496375. From the study, it can be recommended that Potassium aluminium sulphate (Alum) could be applied in the preservation of shucked periwinkle meat to improve the shelf-life and microbial safety as it produced better attribute compared to other preservative treatments.

Keywords: Periwinkle (*Tympanotonus fuscatus*), Potassium sorbate, Potassium aluminum sulphate, preservation.

Introduction

Periwinkle (*Tympanotonus fuscatus*) is one of the most consumed proteinous seafood consumed in the riverine area of south-south region of Nigeria and other parts of West Africa. They are univalve invertebrates belonging to the phylum Mollusca. The shellfish is predominantly of three genera, *Tympanotonus*, *Pachymelania* and *Merceneria* (Oghenemowho and Ahaotu, 2021; Ekop *et al.*, 2021). In Nigeria, their major habitat is in lagoons, estuaries, and mangrove swamps and are basically of two genera in Nigeria in the species of *Tympanotonus fuscatus* and *Pachymelania aurita* usually harvested by hand picking (Ekop *et al.*, 2021). The high content of iodine necessitates the use of periwinkle meat in treatment of endemic goiter and other ailments. The high content of calcium in periwinkle, 41.98mg/100g, suggests that its consumption can increase the body's calcium and help in the blood clotting process (Ekop *et al*., 2021).

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As a result of the habitat of the shellfish, and periwinkle in polluted bodies of water, there is tendency for the contamination of the seafood which translates into the ready to eat product. Hence may result in significant health hazard as microbes such as, *Vibrio, Bacillus, Escherichia coli,* and *Micrococcus,* that are known to be flora of polluted water body are responsible for diseases associated with seafood when consumed without adequate preparation and their microbial load results in diseases such as cholera, Camphylobacteriosis, gastroenteritis, Shigellosis, Salmonellosis, typhoid fever, Poliomyelitis, Brucellosis, Amoebiasis and (Adebayo-tayo *et al*., 2006; Ngozi *et al*., 2020).

Preservatives are natural or synthetic substances that are added to fruits, vegetables, prepared food items, cosmetics and pharmaceuticals in order to increase their shelf life and maintain their quality and safety by inhibiting, retarding or arresting their microbial contamination, fermentation, acidification, and decomposition (Amand *et al*., 2013).

The best method to process periwinkles before consumption differs among the populace because of different reasons. Some people believe that periwinkles should be thoroughly washed; its pointed end cut off and then cooked with its shell because of its perceived medical and nutritive value while some other people believe that the shell should be removed and the meat washed thoroughly before cooking (Omenwa *et al.*, 2011)

Periwinkle like other foods is subjected to food preservation methods such as roasting and drying which reduces the microbial load of the food product and extends the product shelf life. However, the product can be re-contaminated by pathogenic microorganisms in the environment due to poor handling (Akintola *et al.*, 2013; Oghenemowho and Ahaotu, 2021).

This study hypothesized that the shelf-life shucked periwinkle can be extended by some preservatives by preventing the growth of spoilage microorganisms.

The study objectives were to determine the preservative effect of potassium sorbate and aluminum sulphate (alum) on the microbial quality and shelf-life of shucked periwinkle.

Materials and Methods

Preparation and Preservative Treatment of Periwinkle Meat Samples and Storage

In the preparation of the periwinkle, the periwinkles were steamed at 100℃ for 5mins and manually shucked as traditionally practiced. The periwinkle meat samples were subjected to preservative treatments of Potassium Aluminum sulphate (Alum), Potassium sorbate and combination of Potassium sorbate and Alum. Untreated samples served as control. Shucked samples were divided into four (4) subsamples with each consisting of 150g of the shucked periwinkle sample. Three of the subsamples were dipped into 300mL of sterile solutions containing 1% (w/v) Potassium sulphate (alum), 300ml of distilled water containing 0.1% (w/v) Potassium sorbate and 300ml of distilled water containing a combination of 0.5% (w/v) Alum with 0.05% of Potassium sorbate contained in 500mL capacity sterile conical flasks respectively. The remaining subsample (i.e., control) was dipped into 300mL of sterilized distilled water in 500mL sterile conical flask. Following these treatments, samples were immediately collected from each treated and control batch for analysis after which the other samples were each placed in separate sterile flasks and were then sealed with aluminum foil before storage at an ambient temperature of 30±2ºC. Thereafter, representative portions were aseptically taken for analyses every 24h/daily for 2 days and the samples were evaluated for difference in the microbial population and diversity.

Microbial Analysis of the Periwinkle Samples

The bacteria and fungi in the samples were analysed by weighing 25g of the samples into 225ml of 1% sterile peptone water (diluent). The measured samples were blended aseptically and homogenized in the diluent. Serial dilution was carried out aseptically using sterile pipette. After dilution, aliquot (0.1ml) of the diluted samples were cultured on different media, MacConkey, Nutrient Agar (NA), Potato Dextrose Agar (PDA), Eosin Methylene Blue (EMB), Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS) and *Salmonella Shigella* Agar (SSA), using sterile hockey stick. The cultured plates were incubated aerobically at 35ºC (for MacConkey, NA and SSA) for 24hours and at 28-30ºC (for PDA) for 48hours.

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Enumeration and Purification of the Isolates

After culture incubation, the Total Heterotrophic count of the bacteria (THB) and total fungi (THF), Total coliform Count, Total count of *Vibrio* were determined by counting the colonial growth on the cultured plates and the colony forming unit (CFU/g) were calculated.

The different isolates of the cultures were purified by streaking the bacterial isolates on the freshly prepared nutrient agar plates based on their different cultural morphological features and incubated at 37ºC for 24 hours to have a pure culture of the isolates.

The fungi isolates were subcultured on freshly prepared PDA plates and incubated at 30ºC for 48-72 hours.

Characterization of the bacterial isolates

Motility test and Gram's staining of the bacterial isolates were carried out using the method described by Cheesbrough (2002). Also carried out were biochemical tests which include catalase, citrate, indole, methyl red, Voges-Proskauer, oxidase, hydrogen sulphide production and sugar fermentation. The Bergey's Manual of Determinative Bacteriology was used as a guide for identification of the bacteria (Holt *et al*., 1994).

Characterization of the fungal isolates

Morphological and microscopic characteristics of fungi isolated from the samples is a guide towards identification of the fungi. Type of mycelium and pigmentation of the sporulating structures were noted.

After lactophenol cotton blue staining of the fungal isolates, they were examined microscopically. Identification of the fungal isolates was based on cultural characteristics, morphology of the cells, spores and hyphae.

Statistical analysis

The analyses were carried out in duplicates on two different occasions. The data obtained during the study was analyzed statistically using SPSS version 22 for analysis of variance (ANOVA) of the data to determine the significance of the mean differences at $p<0.05$.

Results

The count of Total Heterotrophic Bacteria (THB) in the different treatment samples over the time of storage is represented graphically in Figure 1. The total heterotrophic bacterial count of the treatment with Alum increased minimally from $3.4\pm0.01\times10^2$ CFU/g at the initial time to $1.32\pm0.01\times10^3$ CFU/g at the day 1 of storage to $8.7\pm0.03\times10^3$ CFU/g after day 2 of storage compared to the treatment with combination of Potassium sorbate and Alum which had increased count of heterotrophic bacteria from the initial time to the day 2 of monitoring. The count of heterotrophic bacteria in combination treatment increased from $3.1\pm0.02\times10^2$ CFU/g at the initial time to $2.6\pm0.2\times10^4$ CFU/g at the day 1 of storage and to $9.3\pm0.0\times10^4$ CFU/g at the day 2 of storage.

The treatment of the periwinkle with Potassium sorbate in comparison with other treatments showed a slightly higher increased in heterotrophic bacteria count over the time of storage. The THB count of treatment with Potassium sorbate increased from $4.3\pm0.1\times10^2$ CFU/g at initial time to $6.4\pm0.01\times10^4$ CFU/g at day 1 and increased to $2.8\pm0.01\times10^5$ CFU/g. An exponential increase in the count of heterotrophic bacteria was recorded in the control samples from the initial time to the day 2 of storage. The count in the control sample increased from $5.1\pm0.0\times10^2$ CFU/g at the initial time to $9.8\pm0.02\times10^5$ CFU/g on day 1 to $2.24\pm0.1\times10^7$ CFU/g on day 2 of storage. There was significant difference ($p<0.05$) in the Total Heterotrophic Bacteria counts among the treatments in relation to the time of observation.

The count of coliform in the treatment samples is presented in Figure 2. All the treatment samples recorded no growth of coliform at the initial time of the analysis. From the day 1 (24hour) of the storage, to the day 2 (48hour), the control treatment sample recorded the highest increased count of coliform compared to other treatment samples increasing from $6.3\pm0.01\times10^4$ CFU/g to $1.92\pm0.01\times10^6$ CFU/g.

The treatment sample with Potassium sorbate recorded coliform count ranged from $3.3\pm0.02\times10^3$ CFU/g on the day 1 (24 hour) to $9.4\pm0.0\times10^3$ CFU/g after 48hours of storage. The treatment with combination of Alum and Potassium sorbate recorded coliform count of $3.0\pm0.01\times10^2$ CFU/g on the day 1 (24 hour) of storage and $2.4\pm0.03\times10^3$ CFU/g was recorded on the day 2 (48 hour) of the storage.

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The treatment sample preserved with only Alum produced the least count of coliform from the day 1 (24hour) up till day 2 (48 hour) during the study recording increased count from $2.1\pm0.1\times10^2$ CFU/g to $1.92\pm0.01\times10^3$ CFU/g of the stored sample. There was significant difference ($p<0.05$) in the Total counts of coliform among the treatments in relation to the time of observation.

The count of *Escherichia coli* on EMB medium is presented graphically in Figure 3. All the treatment samples recorded no count of *Escherichia coli* on EMB medium at the initial time during the study. The control sample recorded more increased count of *E. coli* from the day 1 (24 hour) to the day 2 (48hour) during the study followed by the treatment with Potassium sorbate only and the combination treatment with Potassium sorbate and alum. Control treatment sample recorded count of $9.4\pm0.01\times10^3$ CFU/g on the day 1 (24our) and $1.24\pm0.0\times10^5$ CFU/g on the day 2 (48hour) of the study. The treatment with Potassium sorbate recorded count of $8.2\pm0.03\times10^3$ CFU/g on the day 1 (24hour) and $1.88\pm0.1\times10^4$ CFU/g on the day 2(48 hour). The combination treatment recorded count of $1.1\pm0.02\times10^2$ CFU/g on the day day 1(24hour) and $6.0\pm0.02\times10^3$ CFU/g on the day 2. For the treatment of the sample with Alum, there was no growth of *E. coli* at the initial time and day 1 however, the count of $3.6\pm0.01\times10^3$ CFU/g was recorded on the day 2 (48 hours) of the study. There was significant difference ($p<0.05$) in the counts of *Escherichia coli* among the treatments in relation to the time of observation.

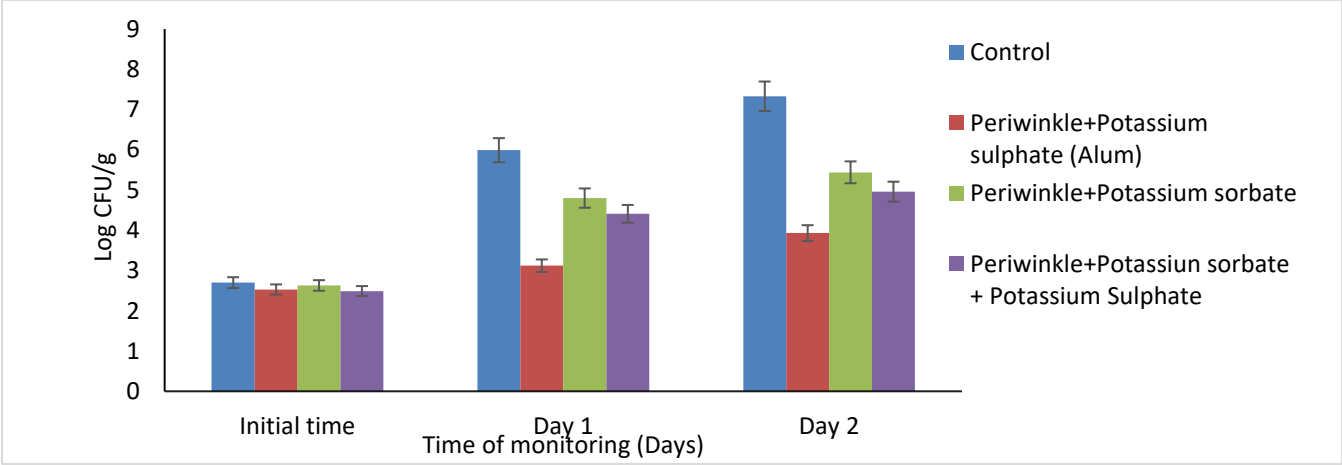

Fig. 1: Total Heterotrophic bacteria count of the periwinkle samples

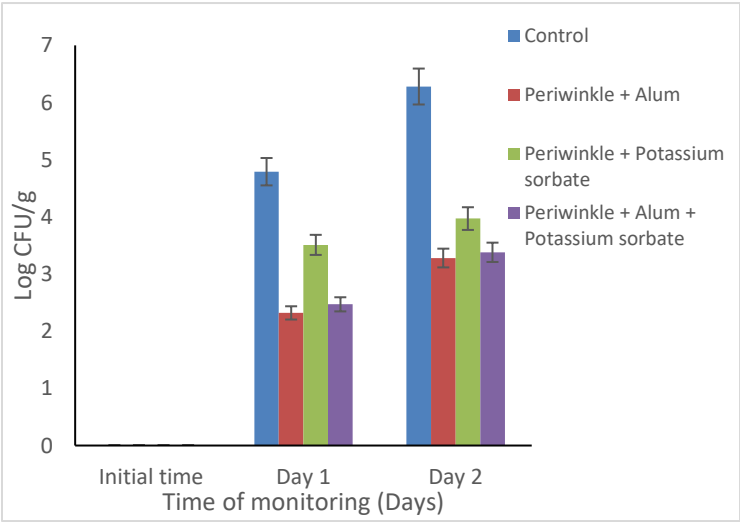

Fig. 2: Total coliform count of the periwinkle

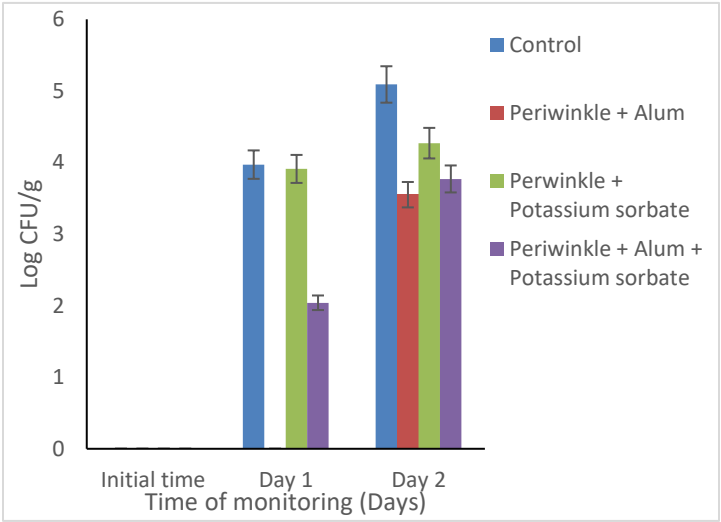

Fig. 3: Count of *E. coli* of the treated periwinkle

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The count of *Salmonella* and *Shigella* in the treatment samples during the storage period is represented graphically in Figure 4. No growth of *Salmonella* and *Shigella* was recorded at the initial time for all the treatment samples. The control sample recorded more increased count of *Salmonella* and *Shigella* from the day 1 (24hour) to the day 2 (48hour) during the study followed by the treatment with Potassium sorbate and the combination treatment with Potassium sorbate and alum.

There was a slight increase in the count of *Salmonella* and *Shigella* count from $8.4\pm0.02\times10^4$ CFU/g (on the day 1) to $1.53\pm0.01\times10^5$ CFU/g (on day 2). The count in the Potassium sorbate treatment increased from $4.4\pm0.1\times10^3$ CFU/g on the day 1 (24hour) to $1.36\pm0.1\times10^4$ CFU/g on the day 2 (48hour). The count of *Salmonella* and *Shigella* in the combination treatment was recorded on the day 1 (24hour) as $8.5\pm0.02\times10^2$ CFU/g to $8.3\pm0.2\times10^3$ CFU/g on the day 2 (48hour). No obvious count of *Salmonella* and *Shigella* was recorded at the initial time and day 1, nevertheless, on the day 2 (48hour), a count of $1.04\pm0.02\times10^3$ CFU/g was recorded on the day 2 of the treatment sample with Alum.

The Total Fungal count of the treatment samples is presented graphically in Figure 5. There was no count of fungal recorded in all the treatment samples at the initial time and day 1 however, fungal growth was observed on the day 2 (48 hour). On the day 2, the control sample recorded the highest count of $7.2\pm0.01\times10^4$ CFU/g followed by the treatment with Potassium sorbate with $5.7\pm0.03\times10^3$ CFU/g followed by the combination treatment with the count of $2.1\pm0.1\times10^3$ CFU/g and the least count of fungi was recorded in the treatment with Alum with the count of $3.3\pm0.02\times10^2$ CFU/g. There was significant difference ($p<0.05$) in the Total Fungal counts among the treatments in relation to the time of observation.

Table 1 shows the different bacterial isolate identified through biochemical test, macroscopy and microscopy. The bacteria identified were *Micrococcus* sp, *Staphylococcus* sp, *Enterobacter* sp, *Pseudomonas* sp, *Escherichia coli*, *Salmonella* sp, *Shigella* sp, *Proteus* sp, *Citrobacter* sp, *Alcaligenes* sp and *Bacillus* sp.

The fungal isolates identified through macroscopy and microscopy were *Candida*, *Aspergillus niger* and *Penicillium* sp (as shown in Table 2)**.**

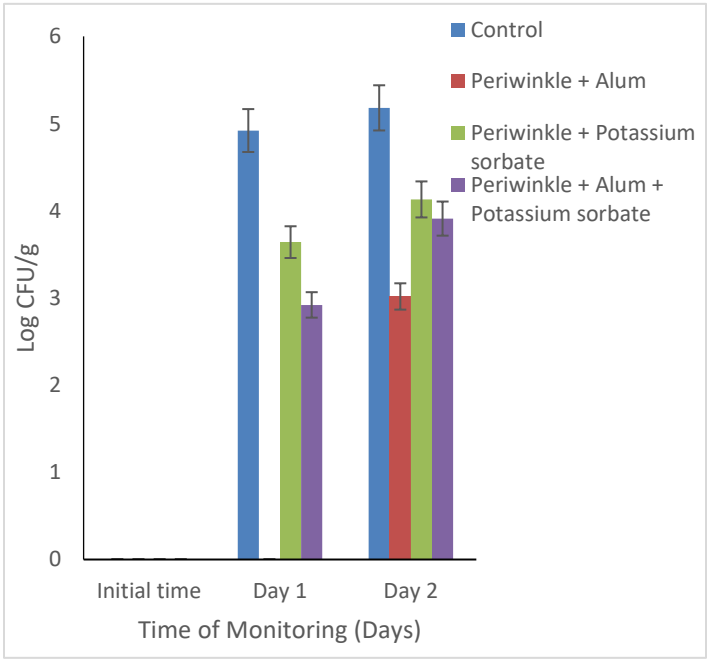

Fig. 4: Total count of *Salmonella* and *Shigella* of samples

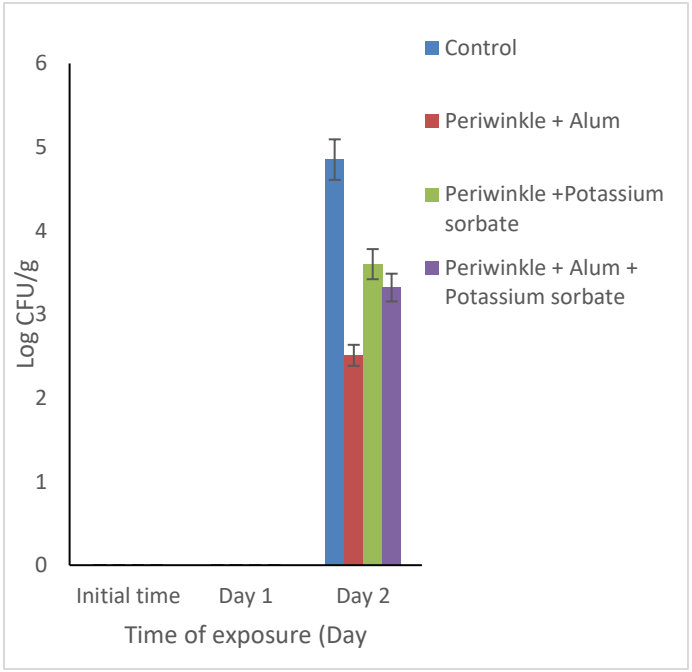

Fig 5: Total Fungal count of periwinkle

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Table 1: Biochemical test results of the bacterial isolates of periwinkle

Isolates code on medium	Grams reactio	Gram's shape	Oxidase	Catalas e	Citrate	Indole	MR	VP	Glucose	Sucrose	Lactose	Maltose	Motility	Probable organism
NA1	+	Rod	+	+	-	-	-	+	A	A	-	-	+	*Bacillus* sp
NA2	+	Rod	+	-	-	-	+	+	A	A	A	-	+	*Bacillus* sp
NA3	+	Cocci	+	+	-	-	-	-	A	A	-	A	-	*Micrococcus* sp
NA4	+	Cocci	-	+	-	-	-	+	A	A	-	-	-	*Staphylococcus* sp
NA5	+	Rod	-	+	+	-	-	-	A	A	-	A//G	+	*Bacillus* sp
NA6	+	Cocci	-	+	-	-	+	+	A	A	-	-	-	*Staphylococcus* sp
NA7	-	Rod	+	+	-	-	-	-	A	A	-	A	-	*Pseudomonas* sp
NA8	+	Rod	+	+	-	-	+	+	-	-	A	-	+	*Alcaligenes*sp
NA9	+	Rod	+	+	+	-	+	-	A	A	-	-	-	*Bacillus* sp
NA10	+	Rod	+	+	-	-	--	-	A	A	-	-	-	*Bacillus* sp
MAC1	-	Rod	+	+	+	-	-	-	-	-	-	+	-	*Pseudomonas* sp
MAC2	-	Rod	+	+	-	+	-	-	A	A	-	-	+	*Escherichia coli*
MAC3	-	Rod	-	+	+	-	-	+	A	A	-	+	+	*Enterobacter* sp
MAC4	-	Rod	-	+	-	-	-	-	AG	AG	-	A	+	*Enterobacter* sp
MAC5	-	Rod	-	+	+	-	-	-	AG	AG	-	-	-	*Proteus* sp
MAC6	-	Rod	-	+	+	+	-	-	AG	AG	AG	AG	+	*Escherichia coli*
EMB1	-	Rod	-	+	+	+	-	-	AG	AG	AG	AG	+	*Escherichia coli*
EMB2	-	Rod	-	+	+	+	-	-	AG	AG	AG	AG	+	*Escherichia coli*
EMB3	-	Rod	-	+	+	+	-	-	AG	AG	-	AG	+	*Escherichia coli*
EMB4	-	Rod	-	+	-	-	-	-	AG	AG	AG	A	-	*Citrobacter* sp
SSA1	-	Rod	-	+	+	-	-	-	AG	AG	-	A	+	*Salmonella* sp
SSA2	-	Rod	+	+	+	-	-	-	AG	AG	AG	A	+	*Salmonella* sp
SSA2	-	Rod	+	+	-	+	-	-	AG	-	-	A	-	*Shigella* sp

Note: NA = Nutrient Agar, MAC = MacConkey Agar, EMB = Eosin Methylene Blue, SSA = *Salmonella Shigella Agar*

Table 2: Microscopic and Macroscopic Characterization of the Fungal Isolates of periwinkle

S/N	Isolates	Macroscopy	Microscopy	Probable Fungus
1.	THF1	Greenish velvety surface with white, rough reverse side.	Septate hyphae with simple conidiosphores. The phialiades end having brush-like clusters.	*Penicillium* sp
2.	THF2	Black-brownish, powdery surfaced mycelia with cracked reverse	Septate hyphae with conidia arranged with conidia like a mop-head.	*Aspergillus niger*
3.	THF3	White-creamy colonies	Oval shaped large cells	*Candida albicans*

Note: THF = Total Heterotrophic Fungi

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Discussion

The total heterotrophic bacterial count showed that there was minimal increase in the population of heterotrophic bacteria in the treatment of the periwinkle meat samples with Alum from day 0 to day 2 which was lower in comparison to the increase in population of total heterotrophic bacteria in the treatment of the periwinkle meat samples with Alum+Potassium sorbate which also was lower than the population of total heterotrophic bacteria in the treatment with Potasssium sorbate only which was significantly ($p<0.05$) lower than the control sample setup. The low increase in the growth of total heterotrophic bacteria in the samples treated with Alum and Potassium sorbate during the monitoring is in consonant with the report of Efiuvwevwere and Amadi (2015) in which treatment with preservative, alum reduced mesotrophic bacteria count in comparison with the control sample. The reduced microbial population recorded in the samples on the day 0 may be as a result of the freshness of the samples and the less microbial presence after preparation which is susceptible to microbial increase over time of storage as shown in the control samples. Increase in the population of heterotrophic bacteria in the control sample can be attributed to the microbial flora which could be from the environment of their habitat harvested from (Oghenemowho and Ahaotu, 2021).

According to Obire *et al*. (2017), the bacteria flora of fresh molluscan shellfish which include periwinkle is largely dependent on the environment where they were harvested as well as handlers of the product and not the periwinkle. The presence of enteric microorganisms in freshly harvested periwinkle is a strong indication that aquatic environment where they were harvested is polluted (Adebayo-tayo *et al.*, 2006). Seafood such as periwinkle are highly perishable. In other words, they have a short shelf life. This could be attributed to the chemical effects of atmospheric oxygen and activities of aerobic microorganisms' time (Oghenemowho and Ahaotu, 2021).

The International Commission on Microbiological Specifications for Food (ICMSF) recommends that total plate count (TPC) of shellfish should not exceed 5 log10CFU/g (Adebayo-tayo *et al.*, 2006). According to Amadi *et al*. (2014), a standard threshold of 10^4 CFU with respect to total fungal count of food should not be exceeded for it to be considered safe for human consumption. Considering the limits set by both standards, the treatment with alum and the combination treatment of alum and potassium sorbate for the period of two days are safe for human consumption.

No growth of coliform, *Shigella*, *Salmonella*, *Escherichia coli* and fungi was observed at the initial time of the analysis and on the day 1 and day 2, growth of the coliform, *Shigella* and *Salmonella* were recorded however low population of the organisms was observed in the sample treated with the preservative. This is in line with the study of Efiuvwevwere and Amadi (2015) in which lower microbial growth of enteric bacteria and coliforms was observed in the sample treated with preservative. The presence of the coliform, *Shigella* and *Salmonella* at the later days of the storage is an indication of the potential of the presence of other pathogenic microorganism when periwinkle meats are stored over time (Oghenemowho and Ahaotu, 2021). The growth of heterotrophic fungi was recorded on the day 2 for all the samples and this can be attributed to the reduction in the pH of the medium as observed in the study in relation to the increase in the days and thus might have necessitated the growth of fungi as fungi grow better in acidity condition. The fungi, *Aspergillus niger*, *Candida albicans* and *Pencillium* sp isolated are in consonant with those isolated in the study of Ngozi *et al*. (2020) which among others, isolated similar fungi from dried periwinkle.

Among the treatments, Alum exhibited more preservative ability especially in the limitation of microbial growth population followed by the treatment with the combination of Alum and Potassium (PS+AL) followed by treatment of the periwinkle sample with Potassium sorbate however, showed less inhibition of microbial growth in the periwinkle meat sample. Hence, Alum treatment (only) could reduce the condition of microbial spoilage of periwinkle meat compare to Potassium sorbate and the combination. The hydrolysis of Potassium aluminum sulphate (alum) in moist/water foods results in the formation of sulphuric acid which results in the decrease of pH of the alum-preserved food samples (Efiuvwevwere and Amadi, 2015). The potency of alum as antibacterial agent had been visibly demonstrated over the years through the myriads of its beneficial activities and relevance in a broad spectrum of human research and development (Atah *et al*., 2022).

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The relative inhibitory effect of potassium sorbate in combination with alum as observed in this study can be attributed to their synergistic effect it has with other chemical preservative. This is in line with the study of Stanojevic *et al*., (2009) reported relative microbial inhibition by a combination of potassium sorbate with sodium nitrate.

The molecularly identified organisms were shown to be *Alcaligenes faecalis, Pseudomonas aeruginosa, Escherichia coli and Bacillus niacini*. The organisms identified through biochemical test, *Escherichia* coli, *Bacillus niacini, Proteus, Shigella* sp, *Salmonella* sp, *Alcaligenes faecalis, Pseudomonas aeruginosa, Citrobacter* sp, *Enterobacter* sp, *Proteus* sp, *Staphylococcus* sp were similar to other studies of periwinkle meat samples which can be attributed to the habitat in which periwinkle dwell or harvested from (Nwiyi and Okonkwo, 2013; Oghenemowho and Ahaotu, 2021). *Escherichia coli* is part of the intestinal flora of humans and vertebrates. In humans, some species of *Escherichia* are associated with infantile diarrhea and newborn meningitis. It has been reported that some species of *Enterobacter* are responsible for septicemia and neonatal meningitis (Adebayo-tayo *et al*., 2006).

The production of enterotoxins is associated with some strains of *Staphylococci* and *Bacillus* which poses a serious threat to consumers of food containing large population of these organisms (Ngozi *et al*., 2020). According to Obire *et al*., (2017) toxin production by *Staphylococcus aureus* which is a mesophilic organism occurs when the population of the bacterium exceed 10^6 CFU/g in an appropriate temperature. *Pseudomonas aeruginosa* is a common opportunistic pathogen ubiquitous in nature. It is present in some blood infections, burns, and wounds. Since the aquatic environment where periwinkle is harvested determines its bacteria flora, the presence of *Pseudomonas* sp. in periwinkle could be traced to individuals bathing inside the water with open wounds or other infections (Omenwa *et al*., 2011). Isolation of *Salmonella* species from the shellfish samples can be attributed to possible chronic carriers, from feaces to other persons by the oral-feacal route, which may be water-borne, food borne or by contact with hands and other formites (Ngozi *et al*., 2020). The increase in the bacterial populations after day 1 may be attributed to waning preservative effects and resultant microbial recovery.

This may be attributed to several factors like bacterial types, microbial population dynamics and concentration of preservatives in the food ecosystem as previously reported. Furthermore, the non-detection of *Vibrio* species in all the preservative-treated samples and the control sample throughout the study may demonstrate the elimination of the microbial species during preparation. This is contrary to the study of Efiuvewevwere and Amadi (2015) in the study of preservative treatment of oysters in which *Vibrio* count was recorded in the control sample.

Conclusion

From this study, among the preservative treatments, the treatment of the periwinkle with Alum was the most effective microbiologically and this was followed by the treatment with Potassium sorbate. The study revealed that, *Escherichia coli, Bacillus niacini, Proteus, Shigella* sp, *Salmonella* sp, *Alcaligenes faecalis, Pseudomonas aeruginosa, Citrobacter* sp, *Enterobacter* sp, *Proteus* sp, *Staphylococcus* sp and fungi, *Aspergillus niger, Candida albicans* and *Penicillium* sp were identified as isolated microorganisms from the storage of shucked periwinkle meat (at ambient temperature) whose growth population is relative to preservatives used in this study, Potassium Aluminum sulphate (Alum) produced better preservative results compared to Potassium sorbate and the combination of Alum and Potassium sorbate.

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References

Adebayo-tayo, B. C., Onilude, A. A., Ogunjobi, A. A. and Adejoye, D. O. (2006). Bacteriological and proximate analysis of periwinkles from two different creeks in Nigeria. *World Applied Sciences Journal*. *1(2)*: 87-91.

Akintola, S. L. B., Bakare, A., Osowo, O. D. and Bello, O. B. (2013). Effect of hot smoking and sundrying processing on nutritional composition of giant tiger shrimp (Panaeus monodon, Fabricius, 1798). *Polish Journal of Food and Nutritional Science*. *63*: 227-237.

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Amadi, J. E., Onyejekwe P. C., Ozokonkwo C. O. and Adebola M. O. (2014). Isolation and identification of moulds associated with four selected snacks sold in Nnamdi Azikiwe University, Awka and its environs. *Asian Journal of Agriculture and Food Science*. *2(2)*: 145-150.

Amand, S. P. and Santi, N. (2013). Artificial Preservatives and their Harmful Effects; Looking towards Nature for Safer Alternatives. *International Journal of Pharmaceutical Sciences and Research*. *4(7)*: 2496-2501.

Atah, S. N., Amadi, L. O. and Olufunmilayo, J. O. (2022). Enhanced Antibacterial Potential of *Xylopia aetiopia* Extract in Consortium with Alum on Some Bacteria Isolated from *Achatina achatina* (Land snail*). International Journal of Current Microbiology and Applied Sciences*. *11(11)*: 92-102.

Cheesbrough M. (Eds) (2002). Biochemical tests to identify bacteria. In: *Laboratory practice in tropical countries, Cambridge edn*. 36-70.

Ekop, I. E., Simonyan, K. and Onwuka, U. N. (2021). Effect of Processing factors and conditions on the cracking efficiency of *Tympanotonus fuscatus* and *Parachymelania aurita* periwinkles, Response surface approach. *Journal of Agriculture and Food Research*. *3*: 1-7.

Efiuvewevwere, B. J. O. and Amadi, L. O. (2015). Effect of Preservatives on the Bacteriological, Chemical and Sensory Qualities of Mangrove Oyster (*Crassatrea gasar*). *British Journal of Applied Science and Technology*. *5(1)*: 76-78.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Stanley, J.T. and William, S.T. (1994) *Bergey's Manual of Determinative Bacteriology. Williams and Wilikins, Baltimore*, 786-788.

Ngozi, C. O., Theodora, O. and Obhioze, A. A. (2020). Microbiological Assessment of Roasted Dried Periwinkle (*Tympanotonus fuscatus*) sold in Yenegoa, Bayelsa. *International Journal of Applied Biology*. *4(2)*: 37-45.

Nwiyi, P. and Okonkwo, C. (2013). Pathogenic microorganisms isolated from periwinkles in creeks South-South of Nigeria. *Online Journal of Animal and Feed Research*. *3(4)*: 186-188.

Obire, O., Nwosu, O. R. and Wemedo, S. A. (2017). An evaluation of the bacteriological quality of some molluscan shellfish preserved with different drying methods. *Current Studies in Comparative Education, Science and Technology*. *4(1)*: 240-253.

Oghenemowho, E. and Ahaotu, I. (2021). Microbiological Assessment and Shelf-life of Periwinkle Preserved by smoking, polythene and vacuum-packing. *International Journal of Research Studies in Microbiology and Biotechnology*. *1*: 9-24.

Ogunbenle, O., and Omowole, C. (2012). Use of seashells and marine resources. *Journal of Environmental Science*. *11*: 27–34.

Omenwa, V. C., Ansa, E. J., Agokei, O. E., Uka, A. and George, O. S. (2011). Microbiological Quality of Raw and Processed Farm-Reared Periwinkles from Brackish water earthen pond, Buguma, Nigeria. *African Journal of food Agriculture, Nutrition and Development*. *11(2)*: 4623-4628.

Stanojecvic, D., Comic, L., Stefanovic, O. and Solujic-Sukdolak, S. (2009). Antimicrobial Effect of Sodium Benzoate, Sodium nitrite and Potassium sorbate and their synergistic action in vitrol *Bulgarian Journal of Agricultural Science*. *15(4)*: 307-31.

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