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Effects of Preservatives on the Bacteriological, Chemical and Sensory Qualities of Mangrove Oyster (*Crassostrea gasar*)

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Authors' contributions

This work was carried out in collaboration between both authors. Author BJOE designed the study and supervised the work. Author LOA wrote the first draft of the manuscript and managed the literature searches and analysis of data. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This work was undertaken to investigate the quality changes in mangrove oysters (*Crassostrea gasar*) exposed to various preservative treatments (PTs) including sodium benzoate (NaB), sodium chloride (NaCl), potassium aluminum sulphate (PAS) and green lime juice filtrate (LJF) during ambient temperature storage (30±2ºC) to enhance the shelf-life.
Study Design: Oyster samples were subjected to various preservative treatments to enhance the shelf-life and the bacteriological, chemical and sensory qualities determined and the data obtained were analyzed.
Place and Duration of Study: Department of Microbiology (Ofirima Complex), University of Port Harcourt, Port Harcourt and Department of Science Laboratory Technology, Rivers State Polytechnic, Bori, Nigeria during the dry and rainy seasons between June, 2008 and May, 2009.
Methodology: Freshly harvested oysters (200) were steamed for 5 min and manually shucked. The oyster meat samples were then subjected to four PTs as follows: 0.1% (w/v) NaB, 1.0% (w/v)

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NaCl, 1.0% (w/v) PAS, 10% (v/v) LJF while the control samples were subjected to sterilized distilled water and analyzed for 3days.

Results: Bacterial flora isolated varied; with control samples showing nine bacterial genera which included *Bacillus* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus* spp., *Vibrio* spp., *Proteus* spp., *Micrococcus* spp., *Lactobacillus* spp. and *Corynebacterium* spp. but fewer (five) bacterial genera were isolated from PAS-preserved oysters. The bacterial population of control and preservative-treated samples increased with storage time but minimal increase occurred in PAS-preserved samples. The pH of the samples differed with treatment but the control and NaB-preserved samples had the highest (4.72- 5.03) while PAS- and NaCl-preserved samples showed the lowest (3.20 -4.05).

The sensory attributes of all samples decreased significantly ($p<0.05$) and became unacceptable after one day but PAS-preserved samples remained highly acceptable throughout the storage.

Conclusion: Of all the samples, the PAS-preserved samples presented the best bacteriological and organoleptic qualities during the storage. Thus, the PAS-preservative treatment is highly recommended for shelf-life extension of oysters.

Keywords: Oysters; bacterial profiles; preservative treatments; shelf-life.

1. INTRODUCTION

Oyster is a popular shellfish that is highly valued worldwide [1,2]. As filter-feeders, they bioaccumulate, retain and concentrate different pathogens such as bacteria, viruses and protozoa [3,4,5]. Oysters are excellent sources of protein and consumed raw or lightly cooked in some parts of the world leading to the transmission of pathogenic microorganisms [6,7].

The diseases caused by these pathogens range from mild gastroenteritis to life-threatening syndromes [5,8,9]. Similarly, a wide range of microorganisms including pathogens have been isolated from oyster meats [10,11]. Therefore, post-harvest and/or processing treatments that inhibit the presence of these pathogens prior to consumption of oyster meats are most desirable. Traditionally, steaming/cooking, smoking and sun-drying are the common post-harvest preservation methods which have remarkably extended the shelf-life of oyster meats [11,12].

Use of chemical and phytochemical agents that will prevent biodeterioration of oyster meats without adversely affecting the organoleptic properties is of continuous research interest. Consequently, sodium benzoate being one of the Generally Recognized as Safe (GRAS) preservatives has been evaluated as an antimicrobial additive in the food industry [13]. Similarly, antibacterial activity of lime juice on clinical bacterial isolates has earlier been reported [14,15]. In addition, sodium chloride is often added to food products for various purposes such as decrease in water activity, reduction in microbial load and enhancement of functional properties leading to extended shelf-life [16]. It has been reported also, that PAS (alum) has antimicrobial activity and has been used in treatment of foods [17,18] as well as in domestic and industrial water purification [19-21]. However, there is little or no information on the preservative potential of PAS, NaB, NaCl and LJF on seafoods particularly oysters. Therefore, the objectives of this study were to evaluate the preservative effects of PAS (alum), NaB, NaCl and LJF on the bacterial profiles, organoleptic qualities and shelf-life of oyster meats during storage for 3 days at ambient temperature ($30\pm2^{o}C$).

2. MATERIALS AND METHODS

2.1 Collection of Oyster Samples

The oysters (*Crassostrea gasar*) were harvested from Gbolokiri creek (average temperature 24ºC and 30ºC for rainy and dry season respectively; salinity of 15ppt and 18.5ppt for rainy and dry season respectively) of the New Calabar River in Obio/Akpor Local Government Area, Port Harcourt, Rivers State, Nigeria. They were transported in polyethylene bags to the laboratory in less than 3h after harvest for analyses.

2.1.2 Collection of green lime fruits and chemicals

Green lime fruits (*Citrus aurantifolia*) were purchased from mile 3, market, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

Sodium benzoate (NaB) was obtained from M&B Laboratory Chemicals Ltd, England while sodium chloride (NaCl) was obtained from East Anglia Chemicals, England and potassium aluminum sulphate (PAS) obtained from Vickers Laboratories, Ltd, England. These chemicals were of analytical grade.

2.2 Preparation of Oyster Meat Samples

Mangrove oysters (200) were steamed at 100°C for 5min [10,22]) and manually shucked as traditionally practiced. The oyster meat samples were subjected to preservative treatments (details in 2.2.2) and evaluated for bacteriological, chemical, proximate composition and sensory quality attributes [23]. The samples (treated or untreated) were then subjected to ambient temperature (30±2ºC) storage and representative portions taken for analyses every 24h for 3 days.

2.2.1 Preparation of lime juice filtrate (LJF)

Lime fruits were washed with sterilized distilled water and then cut with a pre-sterilized knife and the juice squeezed out into a sterile 500mL conical flask. The juice was filtered with cheesecloth to remove the seeds and other debris [14,15].

2.2.2 Preservative treatment of samples and storage at ambient temperature

Shucked samples were divided into five (5) subsamples with each consisting of 150g. Four (4) of the subsamples were dipped into 300mL of sterile solutions of 0.1% (w/v) NaB, 1.0% (w/v) PAS, 1.0% (w/v) NaCl and 10.0% (v/v) of LJF contained in 500mL capacity sterile conical flasks respectively while the remaining subsample (i.e. control) was dipped into 300mL of sterilized distilled water in 500mL sterile conical flask [10,24]. Following these treatments, the flasks were then sealed with aluminum foil before ambient temperature storage.

2.3 Determination of pH of Samples with or Without Preservative Treatments

A 10g portion of oyster meat sample was homogenized in 20mL sterile distilled water (1:2 ratio) using Moulinex electric blender (France). The pH of homogenate or slurry was determined with a calibrated digital pH-meter (LABTECH, India.) as described previously [10,24].

2.4 Proximate Composition and Trimethylamine (TMA) of Samples with or Without PAS

The proximate composition of oyster meat samples with or without potassium aluminum sulphate (PAS) were determined for percentage total available carbohydrate, moisture content, crude fibre, ash, crude fat and crude protein as described in AOAC [25]. TMA contents were determined using the methods described by Osborne and Vogt [26] and Malle and Poumeyrol [27]. Control samples were analysed only on days 0 and 1 due to obvious spoilage while analysis of PAS-preserved samples was carried out on days 0 and day 3.

2.5 Microbiological Analysis

Aerobic plate count (APC) was determined by blending 25g of shucked oyster samples in 225mL 0.1N alkaline peptone water to obtain a 10^{-1} homogenate. Further serial dilutions were made from the homogenate and 0.1 portions were spread-plated in duplicate on plate count agar (Scharlau Chemie S.A. Spain) supplemented with 1.0% NaCl [28]. Coliforms including *Escherichia coli* were determined on pre-poured, surface-dried MacConkey agar (Oxoid Ltd., UK) while *Vibrio* counts (VCs) were determined on surface-dried thiosulphate-citrate-bile-salt-sucrose agar (Lab M Ltd, UK) using spread-plate method and plates were incubated at 37ºC for 24h respectively.

Representative colonies (30-300) were enumerated as colony forming units (CFUs) and identification of bacterial isolates was carried out based on cultural, morphological and biochemical characteristics [29,30].

2.6 Sensory Evaluation of the Samples with or Without Preservatives

Shucked, preserved or unpreserved samples were evaluated for sensory attributes (visual appearance, aroma and firmness) at intervals of 24h for 72h by 10- member panelists consisting of students and members of staff familiar with oyster sensory qualities. The samples were evaluated using the hedonic scale of 1-9 where 1= dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5= neither like nor dislike, 6= like slightly, 7= like moderately, 8= like very much and 9= like extremely [31].

2.7 Statistical Analysis

The analyses were carried out in duplicates on two different occasions. ANOVA used was based on software of SPSS version 15 for Windows and the significance of the mean differences determined at p<0.05 (SPSS Inc. 2007)

3. RESULTS

3.1 Changes in pH Values of Samples Following Preservative Treatments

Significant (p<0.05) differences were observed in the pH values of the samples with or without preservatives during ambient temperature storage (Table 1). The pH values of the control and sodium benzoate samples were significantly (p<0.05) higher than those of PAS and NaCl-preserved samples during the storage (Table 1).

3.2 Changes in Aerobic Plate Counts and Other Bacterial Groups as Affected by Preservative Treatments during Ambient Temperature Storage

All the samples except control did not show any bacterial growth on day 0 but thereafter, the aerobic plate counts (APCs) increased drastically in both control and preservative- treated samples resulting in 1.60 $x10^8$ cfu g^{-1} for control sample and 1.65 $x10^7$ cfu g^{-1} for NaB- and 1.25 $x10^7$ cfu g^{-1} for NaCl- preserved samples respectively on day 2 (Table 2). In contrast, PAS-preserved samples showed only 5.00 $x10^4$ cfu g^{-1} on day 2 with growth of *Vibrio* spp. and *E.coli* being undetectable for NaB, PAS and LJF-preserved samples on day 2 (Table 2). Overall, microbial and organoleptic changes induced spoilage in the samples except PAS-preserved sample on day 3 hence the discontinuation of their bacteriological analysis on day 3 (Table 2). The most heterogeneous bacterial genera (10) occurred in raw (unsteamed), control (9) and NaCl-preserved (8) samples with the least occrrence (5) being observed in PAS-treated samples (Table 3).

The sensory scores for the various attributes of the oyster meat samples treated with or without preservatives are shown in (Table 4). The samples were highly rated (acceptable) on day 0 due to their freshness. Thereafter, all the samples were rated low and unacceptable except PAS-preserved sample. The samples treated with NaB, LJF and NaCl were unacceptable after day 1 due to obvious physical and bacterial induced deterioration whereas the PAS-treated sample which retained high level of acceptability was subjected to further bacteriological analysis on days 1 to 3 due to its extended shelf-life.

(Table 5) shows the percentage changes in proximate composition of oyster meat samples treated with or without preservatives. The proximate composition (PC) values for crude protein, fat, crude fibre and carbohydrate in the PAS-treated samples were comparable with those of the control on day 0 but decreased in all the PC parameters except in the ash and moisture contents on day 3 (Table 5). After day 1, all the other samples became bacteriologically and organoleptically unacceptable except the PAS-preserved sample. Higher TMA values were observed in control samples on days 0 and 1 compared to those of PAS-treated sample on days 0 and 3 respectively but control samples were not analysed on day 3 due to obvious spoilage (Table 5).

4. DISCUSSION

The significant differences observed in the pH values of the samples during storage indicate the influence of the preservatives and the related microbial activities. For example, the hydrolysis of PAS in water/moist foods results in formation of sulphuric acid [32] which must have decreased the pH of the PAS-preserved samples. In addition, the decomposition of glycogen to lactic acid in seafoods [2,33] might have also contributed to the reduced pH values. Furthermore, the wide range of bacterial flora (Table 2) and the microbial dynamics involving fermentative and proteolytic activities may be attributed to the differential pH changes (Table 1) and associated “souring” of oyster samples [10,34].

Preservative effects of antimicrobial agents are critical for the shelf-life of foods due to their inhibitory properties. The occurrence of low microbial populations on day 0 demonstrates the influence of preservative treatments on microorganisms such that, some of the microorganisms (*Acinetobacter* and *E. coli*) were eliminated immediately after treatment (Table 2) suggesting their susceptibility to preservatives in food ecosystems [35,36] while the detection of *Staphylococcus aureus*, *Bacillus* spp, *Streptococcus* sp and *Vibrio* spp is indicative of their relative heat tolerance and resistance to some preservatives [37-40]. Thus, the significant increases in the bacterial populations after day 1 may be attributed to waning preservative effects

and resultant microbial recovery. This phenomenon may be ascribed to several factors such as bacterial types, microbial population dynamics and concentration of preservatives in the food ecosystem as previously reported [23,37,39]. Additionally, the non-detectability of *Vibrio* species in all the preservative-treated samples (Table 3) demonstrates the high sensitivity of these microorganisms to preservative treatments [41]. Similarly, the significant (p<0.05) decrease in sensory quality attributes of the samples (except PAS-preserved samples) clearly underscores PAS treatment as the most beneficial of the preservatives (Table 4).

Table 1. Changes in pH values of oyster meat slurry with or without preservative-treatments following storage at ambient temperature

	pH changes during storage (days)			
Samples	**0**	**1**	**2**	**3**
Control	5.50±0.12^{c}	4.72±0.06^{d}	5.00±0.06^{c}	5.03±0.02^{c}
NaB	5.32±0.04^{c}	4.72±0.06^{d}	4.75±0.09^{c}	4.78±0.03^{b}
PAS	4.10±0.06^{a}	3.20±0.04^{a}	3.71±0.06^{a}	4.00±0.06^{a}
NaCl	4.15±0.04^{a}	3.90±0.06^{b}	3.92±0.07^{a}	4.05±0.03^{a}
LJF	4.50±0.09^{b}	4.33±0.25^{c}	4.39±0.02^{b}	4.45±0.06^{b}

Key: Control = untreated oysters but others were treated with the following preservatives: NaB = Sodium benzoate, PAS = Potassium aluminum sulphate, NaCl = Sodium chloride, LJF = Lime juice filtrate, Each value represents mean ± standard deviation (SD) of four determinations, Values in columns at the respective time intervals having different letters are significantly (p < 0 .05) different

Table 2. Bacterial loads (cfu g^{-1}) of oyster meat samples with or without preservative-treatments following storage at ambient temperature

	Duration of storage (days)			
	0	**1**	**2**	**3**
Control				
APC	3.0x10^{3}	1.55x10^{6}	1.60x10^{8}	ND
CC	2.6x10^{2}	1.15x10^{5}	2.40x10^{6}	ND
VC	1.0x10^{1}	5.60x10^{3}	1.20x10^{3}	ND
EC	NGD	NGD	NGD	ND
NaB				
APC	ND	8.30x10^{6}	1.65x10^{7}	ND
CC	ND	1.77x10^{5}	1.63x10^{5}	ND
VC	ND	NGD	NGD	ND
EC	ND	NGD	NGD	ND
PAS				
APC	ND	5.30x10^{3}	5.00x10^{4}	2.30x10^{5}
CC	ND	6.00x10^{2}	3.40x10^{2}	2.50x10^{3}
VC	ND	NGD	NGD	NGD
EC	ND	NGD	NGD	NGD
NaCl				
APC	ND	2.50x10^{5}	1.25x10^{7}	ND
CC	ND	1.50X10^{4}	2.50x10^{5}	ND
VC	ND	1.25x10^{3}	3.20x10^{4}	ND
EC	ND	NGD	NGD	NGD
LJF				
APC	ND	1.35x10^{5}	4.30x10^{6}	ND
CC	ND	7.50x10^{4}	5.50x10^{5}	ND
VC	ND	NGD	NGD	ND
EC	ND	NGD	NGD	ND

ND = Not determined; NGD = No growth detected, APC = Aerobic plate count; CC = Coliform count; VC = Vibrio count; EC = Escherichia coli count, N/B: ND = Under day 0 column, day of preservative-treatments while under day 3 was due to obvious spoilage of samples, Each value represents mean of four determinations

Table 3. Genera of bacteria isolated from oyster meat samples with or without preservative-treatments following storage at ambient temperature

	Genera of bacteria isolated
Raw (unsteamed)	*Bacillus, Staphylococcus, Pseudomonas, Vibrio, Proteus, Micrococcus, Escherichia, Lactobacillus, Acinetobacter, Corynebacterium.*
Control	*Bacillus, Streptococcus, Pseudomonas, Staphylococcus, Lactobacillus, Proteus, Corynebacterium, Vibrio, Micrococcus*
NaB	*Bacillus, Streptococcus, Pseudomonas, Lactobacillus, Staphylococcus, Proteus, Micrococcus.*
PAS	*Bacillus, Pseudomonas, Staphylococcus, Proteus, Lactobacillus*
NaCl	*Bacillus, Streptococcus, Pseudomonas, Lactobacillus, Staphylococcus, Proteus, Vibrio, Micrococcus*
LJF	*Bacillus, Streptococcus, Staphylococcus, Proteus, Lactobacillus, Pseudomonas, Micrococcus*

Preservative treatments of processed oyster samples included: NaB = Sodium benzoate; PAS = Potassium aluminum sulphate (alum); NaCl = Sodium chloride; LJF = Lime juice filtrate

Table 4. Changes in sensory attributes of oyster meat samples with or without preservative treatments following storage at ambient temperature

		Preservative treatments				
Duration (days)	**Attributes**	**Control**	**NaB**	**PAS**	**NaCl**	**LJF**
0	App	7.40±0.80^{b}	7.60±0.66^{b}	8.40±0.49^{c}	7.80±0.75^{b}	7.70±0.64^{b}
	Aro	8.00±0.00^{b}	8.00±0.00^{b}	8.40±0.49^{c}	7.60±0.92^{a}	8.10±0.30^{b}
	Fir	7.80±0.75^{b}	7.80±0.75^{b}	8.20±0.60^{b}	8.00±0.63^{b}	8.00±0.63^{b}
1	App	4.40±1.62^{c}	4.70±2.05^{c}	7.30±1.42^{f}	3.60±2.42^{b}	6.90±1.37^{e}
	Aro	2.90±0.83^{a}	5.30±2.10^{e}	8.30±0.64^{f}	4.00±2.76^{c}	5.40±1.28^{e}
	Fir	2.60±1.36^{b}	4.40±1.62^{c}	8.10±0.70^{f}	6.40±2.33^{e}	5.90±1.64^{d}
2	App	2.30±0.68^{b}	2.50±0.81^{b}	8.30±0.64^{e}	3.20±1.89^{c}	4.80±2.09^{d}
	Aro	1.80±0.75^{a}	3.50±1.02^{c}	6.90±1.92^{e}	2.80±1.78^{b}	3.80±1.94^{c}
	Fir	2.00±1.73^{b}	2.00±0.63^{b}	7.00±1.34^{e}	3.60±2.91^{c}	5.20±2.32^{d}
3	App	1.70±0.46^{a}	1.90±0.30^{a}	7.30±0.90^{d}	3.00±2.00^{b}	5.00±1.00^{c}
	Aro	1.30±064^{a}	1.90±0.83^{b}	6.90±1.92^{e}	2.50±1.36^{c}	4.90±1.14^{d}
	Fir	2.40±0.92^{c}	1.10±0.30^{a}	7.00±1.34^{e}	2.70±1.42^{c}	3.90±1.45^{d}

Key: App = Appearance; Aro = Aroma; Fir = Firmness. Preservative-treatments included NaB = Sodium, benzoate,PAS = Potassium aluminum sulphate, NaCl = Sodium chloride. LJF = Lime juice flitrate, Higher values represent better sensory quality (higher acceptability), Each value represents mean±standard deviation (SD) of four (4) determinations, Values in rows for the respective time intervals with the same letters are not significantly ($p<0.05$) different

Table 5. Changes in proximate composition and trimethylamine of oyster meat samples preserved with or without PAS-treatment following storage at ambient temperature

Composition	**Samples and duration (days)**			
	Control Day 0	**PAS-treated Day 0**	**Control Day 1**	**PAS- treated Day 3**
	(%)	**(%)**		**(%)**
Crude protein	62.72±0.40	62.75±0.35	ND	60.80±0.10
Fat	10.32±0.07	10.41±0.19	ND	10.24±0.05
Crude fibre	3.02±0.07	3.30±0.10	ND	2.51±0.09
Carbohydrate	8.74±0.05	8.92±0.07	ND	6.60±0.10
Ash	4.00±0.04	4.35±0.15	ND	4.45±0.05
Moisture	12.20±0.15	10.27±0.13	ND	15.51±0.09
TMA (MgN/100g)	6.30±1.05	5.85±0.16	13.75±0.25	13.65±0.70

ND = Not determined (due to obvious spoilage), Values represent mean±standard deviation (SD) of four determinations of samples on dry weight basis, Control sample was not analysed on day 3 due to obvious spoilage whereas PAS-treated sample was analyzed on day 3 due to enhanced shelf-life

Several factors influence the quality and shelf-life of fresh and processed seafoods and these include the storage conditions, proximate composition and microbial profile [42,43,44]. The marginal changes in proximate composition (including the low moisture content) of PAS-preserved samples (Table 5) may be attributed to dehydration and astringency induced by PAS-treatment as earlier reported [17].

Total Volatile Nitrogen (TVN) was not measured because it serves as a comparable quality indicator to TMA in seafoods [24] and the maximum value of 35mg/100g flesh seafood is stipulated by the EC guidelines. In contrast, 10-15mg TMA/100g of seafood is typical range for spoilage detection [24]. Thus, the negligible adverse changes coupled with the relatively low TMA values (13.65mgN/100g) for PAS-preserved samples (Table 5) are indicative of PAS being the most effective of all the preservatives used. Furthermore, TMA values of fishery products have been attributed to bacterial and endogenous proteolytic enzymatic actions associated with spoilage of oysters [45,46]. Therefore, the occurrence of 13.65mgN/100g TMA on day 3 for PAS-treated sample which coincided with the APCs of $\log_{10}$ 5.7 clearly corroborates these two parameters as useful indices of spoilage of bivalve mollusks as earlier reported [47,48]. Evidently, based on the European Council Directive 93/493 EEC [49] of critical value of 10^5 cfu g^{-1} APCs in cooked shellfish, only the PAS-preserved samples are therefore considered safe on day 2. Conclusively, of all the treatments, PAS was the most effective, both microbiologically and organoleptically and this was followed by lime juice filtrate treatment. These are therefore highly recommended for use for the shelf-life extension of oysters.

5. CONCLUSION

The preservative treatments resulted in differential bacterial profiles in the samples with most diverse genera (9) occurring in control samples as compared with five bacterial genera in PAS-preserved oysters. Lowest bacterial population also occurred in PAS- treated samples. Overall, the best quality attributes including shelf life were observed in the PAS-preserved samples during the storage.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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