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MICROBIAL CHARACTERISTICS OF LAGOON CRAB (*Callinectes amnicola*) AS INFLUENCED BY PROCESSING STEPS

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ABSTRACT The Lagoon Crab (*Callinectes amnicola*) is a common palatable species consumed in Lagos State, Nigeria. This study determined the bacterial characteristics of fresh and processed crabs using microbiological standard techniques. The result revealed a Total Bacteria Counts of 6.64 x 10^5 for fresh crab while reduction in frozen and boiled samples with respective values of 5.48 x 10^4 CFU/g and 2.41 x 10^4 CFU/g. The fried crabs had the lowest Total Coliform Counts and Total Fecal Coliforms of 2.59 x 10^3 CFU/g and of 0.10 x 10^1 Cfu/g respectively. Five genera consisting both gram-positive and gram-negative bacteria were isolated from the fresh and processed crabs. The isolates were identified as *Baccillus sp.*, *Coccobacillus sp.*, *Enterobacter sp.*, *Klebsiella sp.* and *Staphylococcus aureus*. *S. aureus* had the highest prevalent rates of 25 % and 28 % in fresh and frozen crabs respectively while *Baccillus sp* and *Enterobacter sp.* had the highest prevalent rates in boiled and fried samples respectively. The inhibitory zones of antibiotic resistance and sensitivity revealed variable antibiogram patterns of the tested isolates where all the isolates were resistant to Ciprofloxacin. The comparison of the fresh and processed crabmeat showed that the processing steps have a considerable effect on the bacterial quality of fresh *C. amnicola* by reducing the distribution and abundance of the identified bacteria. However, the presence of resistant pathogens which are also human pathogens is a public health threat. Compliance with standard microbiological measures to prevent contamination by these organisms becomes very necessary.

Keywords**:** Crab, Boiling, Frozen, Frying, Lagos Lagoon.

INTRODUCTION

Although land animals provide the majority of human food protein, crabs have recently been successfully used as another source of nutrients, particularly among coastal dwellers in Southern parts of Nigeria (Moruf *et al.*, 2021). The Lagoon Crab (*Callinectes amnicola*), a popular seafood, is a cherished source of protein and minerals in human diet and the most important food organism caught in the coastal (inshore) fishery and lagoons in West Africa (Moruf and Adekoya, 2020). Its commercial fishery is rapidly growing under the increasing demand for shellfish in Nigeria. Soft shell crabs have become more popular, in part, because they are sold at the higher value, compared to hard shell crab.

Crabmeat is a highly perishable product and its microbial quality is further jeopardized by intensive handling such as backing, picking, and packing operations after cooking (Lorentzen *et al.*, 2019). Handling causes severe post-cook contamination and shortens the shelf-life of the product (Givens *et al*., 2013). Deterioration in quality of seafood is attributed to the highly sensitive proteins and fats present in aquatic organisms (Olatunde and Benjakul, 2018). The major deteriorative processes that affect the texture, color and flavor of seafood are microbial spoilage, autolysis, polymerization, deamination, decarboxylation and biochemical reactions (Tavares *et al*., 2021). Immediately after the death of crab, microorganisms and enzymes invade its flesh and react with the complex mixture of natural substances present, as fish tissues provide an ideal growth medium for bacteria (Zhuang *et al*., 2021). The rates at which microbial and autolytic spoilage occurs vary according to the species, area of catch, method of catch and, above all, processing and storage temperature (Duarte *et al*., 2020).

There is a general need in the food supply chain for rapid methods to monitor bacterial quality, and to identify hygienic and safety conditions in order to enable necessary corrective actions at the appropriate time (Hady *et al.*, 2018). In the past decades, a number of works addressing handling, processing and preservation techniques of seafood in Nigeria have been on fin fish. There seems to be a scarcity of information on the effect of processing methods on the microbial quality of indigenous crab species thus this study investigated the effect of processing methods on the microbial characteristics of *C. amnicola.*

MATERIALS AND METHODS

Sample collection and preparation

A total of 60 specimens of the gercacinid crab, *C. armatum* (length: 51.89-62.54 mm; total weight: 21.39-44.47 g) were harvested using bait traps and hand-picked from the University of
Lagos Lagoon Coast (6°31.228'N and 3°24.044'E). The specimens were kept in ice-chest before being taken to Marine Sciences Department, University of Lagos, for further analysis. The crabs were thoroughly washed, measured, de-shelled and carapace region was discarded. The samples were then separated into four groups, one group was analyzed raw (fresh); a second group frozen; third group was boiled in water while the last group was deep-fried with vegetable oil in a frying pan. Frozen was achieved by keeping samples in commercial freezer at -41^{0}C. Boiling was done in distilled water, kept boiling for about 20 minutes until the pieces were cooked and tender. Frying was achieved within 15 minutes and the temperature was about 240°C. All samples were homogenized prior to analysis.

Analytical Procedures

Ten (10) grams of each fleshy blended parts of fresh, frozen, boiled and fried *C. amnicola* were aseptically suspended into 90 mL sterile distilled water, vigorously shaken to dislodge adhered bacteria and ten-fold serial dilutions were made to obtain dilutions 10^{-1} to 10^{-3}. One (1) mL of aliquot was pour-plated in triplicate onto each plate of Nutrient Agar (NA), MacConkey Agar (MCA), Eosine Methylene Blue Agar (EMB) and the plates were aerobically incubated at 37°C for 24 hr. After incubation, colonies on plates were counted and multiplied by the dilution to obtain the Total Bacterial Counts (TBC), Total Coliform Counts (TCC) and Total Faecal Coliform Counts (TFC). The discrete colonies were sub-cultured onto freshly prepared NA plates and aerobically incubated at 37°C for 24 hr. The pure cultures of isolates were streaked onto NA slant, incubated at 37°C and stored in a refrigerator at 4 °C for characterization and identification. All isolates were gram stained and subjected to convectional biochemical tests as described by American Public Health Association (2001). Antibiotics sensitivity patterns of the bacterial isolates were determined using the disc diffusion method (Cheesbough, 2003). The discs were placed on Muller-Hinton agar plates that were seeded with the broth culture of the test organisms. The plates were inverted and left on the work bench for 30 min to allow for diffusion of antibiotics into the agar, this was followed by incubation at 37^{0}C for 48hr, after which zones of inhibition were examined and interpreted using standard charts (NCCLS, 2003).

Data analysis

The Statistical Package for Social Sciences (IBM SPSS Version 22.0) was used for data analysis. Treatments were separated using Duncan Multiple Range Test (DMRT) at 95% confidence value ($P < 0.05$).

RESULTS AND DISCUSSION

The bacteria loads of fresh and processed *C. amnicola* are presented in Table 1. The result revealed a Total Bacteria Counts (TBC) of 6.64 x 10^5 CFU g^{-1} for fresh crab while reduction in frozen and boiled samples with respective values of 5.48 x 10^4 CFU g^{-1} and 2.41 x 10^4 CFU g^{-1}. The TBC in fried samples was the lowest with 3.00 x 10^3 CFU g^{-1}. The *C. amnicola* processed by frying had the lowest Total Coliform Counts (TCU) and Total Fecal Coliforms (TFC) of 2.59 x 10^3 CFU g^{-1} and of 0.10 x 10^1 CFU g^{-1} respectively. This may be due to the high temperature involved in frying process. The acceptable microbial level for raw crabs ranges from a minimum 10^5 CFU g^{-1} to a maximum 10^6 Cfu/g as stated by International Commission of Microbiological Specification for Food (Geetha *et al*., 2016). The total bacteria count on crab rarely indicate the quality of the crab but it gives an indication of the risk of spoilage induced since contaminated organisms had different ways of effecting health conditions in consumers (Al-Sheraa *et al*., 2018)

Table 1: Bacteria loads of fresh and processed *Callinectes amnicola*
(n = 15 examined muscle tissue in each step)

Counts (Cfu g^{-1})	Fresh Crab	Frozen Crab	Boiled Crab	Fried Crab
Total Bacteria Counts	6.64 x 10^5	5.48 x 10^4	2.41 x 10^4	3.00 x 10^3
Total Coliform Counts	3.79 x 10^4	1.06 x 10^4	1.97 x 10^4	2.59 x 10^3
Total Fecal Coliforms	5.47 x 10^3	1.10 x 10^1	1.00 x 10^1	0.10 x 10^1

In this study, five genera consisting both gram-positive and gram-negative bacteria were isolated from the fresh and processed crabs. The isolates were identified as *Baccillus sp., Coccobacillus sp., Enterobacter sp., Klebsiella sp.* and *Staphylococcus aureus* using their morphological and biochernical characteristics (catalase, oxidase, indole, mannitol, citrate, spore and motility) (Table 2). This result

substantiated the report of Moruf (2022), who identified nine bacteria species (*Escherichia coli*, *Salmonella typhi*, *Shigella sp*., *Proteus vulgaris*;e, *Pseudomonas aeruginosa*, *Bacillus sp.*, *Aeromonas hydrophila* and *Vibrio cholera*) in the gut of *C. amnicola* from Epe Lagoon

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Table 2: Cellular morphology and biochemical characterization of bacteria isolated from *Callinectes amnicola*

	Gram reaction	Cell morph	Catalase	Oxidase	Indole	Mannitol	Citrate	Spore	Motility	Isolates
	+	Cocci	+	-	-	+	-	-	-	*Staphylococcus aureus*
Fresh	+	Rod	+	-	-	-	+	+	-	*Bacillus sp*
	+	Cocci	+	-	-	-		-	-	*Coccobacillus sp*
	-	Rod	+	-	-	-	+	-	+	*Enterobacter sp*
	-	Rod	+	-	-	-	+	-	-	*Klebsiella sp*
	+	Cocci	+	-	-	-		-		*Coccobacillus*
Frozen	-	Rod	+	-	-	-	+	-	+	*Enterobacter sp*
	+	Cocci	+	-	-	-	-	-	-	*S. aureus*
	+	Rod	+	-	-	-	+	+	-	*Bacillus sp*
	-	Rod	+	-	-	-	+	-	-	*Klebsiella sp*
	+	Rod	+	-	-	-	+	+	-	*B. megaterium*
	+	Rod	+	-	-	-	+	+	-	*Baccillus sp*
Boiled	+	Cocci	+	-	-	-	-	-	-	*S. aureus*
	-	Rod	+	-	-	-	+		+	*Enterobacter sp*
	-	Rod	+	-	-	-	+	-	-	*Klebsiella sp*
	+	Rod	+	-	-	-	+	+	-	*Baccillus sp*
Fried	-	Rod	+	-	-	-	+		+	*Enterobacter sp*
	-	Rod	+	-	-	-	+	-	-	*Klebsiella sp*

Based on the results from Table 3, there was an evidence for multiple contamination of the examined crabs in the fresh and all the processing forms having *Baccillus sp.*, *Coccobacillus sp.*, *Enterobacter sp.*,*Klebsiella sp.* and *Staphylococcus aureus* with different percentages. *Staphylococcus aureus* had the highest prevalent rates of 25 % and 28 % in fresh and frozen crabs respectively. The reason for this high prevalence of *S. aureus* in the samples may be attributed to the poor personal hygiene of the workers and non-hygienic practice adopted by workers as handling of crabs by persons who are harboring staphylococci in their nose, skin, or in an infected lesion. In the present study, *Baccillus sp* and *Enterobacter sp.* had the highest prevalent rates in boiled and fried samples respectively. According to Khalafalla *et al*. (2019), high contamination rate may be due to contamination from skin surface and through contaminated work surfaces and knives.

Table 3: Prevalence of isolated bacteria in fresh and processed *Callinectes amnicola* (n = 15 examined muscle tissue in each step)

	Fresh Crab		Frozen Crab		Boiled Crab		Fried Crab	
Bacteria isolated	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
Baccillus sp	13	22	11	22	9	30	6	33
Coccobacillus sp.	7	12	6	12	0	0	0	0
Enterobacter sp.	11	19	9	18	8	27	7	39
Klebsiella sp	13	22	10	20	7	23	5	28
Staphylococcus aureus	15	25	14	28	6	20	0	0
Total	59		50		30		18	

Table 4 depicts the antibiotic susceptibility pattern of the bacterial isolates from fresh and processed *C. amnicola*. The inhibitory zones of antibiotic resistance and sensitivity revealed variable antibiogram patterns of the tested isolates. Regardless of the crab form (fresh or processed), the result shows that all the

isolates were resistant to Ciprofloxacin. The gram-negative isolates (*Enterobacter sp* and *Klebsiella sp*) were susceptible to Gentamycin, Amoxacillin, Pefloxacin and Chloramphenicol while resistant to Streptomycin and Septrin. The gram-positive bacteria (*Coccobacillus sp, Bacillus sp*. and *S. aureus*) showed resistance to Gentamycin, Ampiclox, Amoxacillin and Pefloxacin while susceptible to Septrin and Erythromycin. According to Wang and Shao (2017), the high performance of antibiotics can also be due to their molecular sizes, a factor which enhances their solubility in diluents thus promoting their penetration power through cell wall into the cytoplasm of the target microorganism. The susceptibility pattern observed for the isolates in this study is comparable to those reported by Imarhiagbe *et al*. (2016) and Gufe *et al*. (2019). Bacterial resistance to antibiotics is indications of abuse and misuse of antibiotics in the environment. Bacterial groups co-habiting in a common environment may express a similar antibiotic sensitivity pattern if they share a common pool of R-factor plasmids (Laith and Najiah, 2014).

Table 4: Antibacterial susceptibility testing on the bacterial isolates from *Callinectes amnicola*

	Fresh					Frozen					Boiled				Fried		
	a	*b*	*c*	*d*	*e*	*a*	*b*	*c*	*d*	*e*	*c*	*d*	*e*	*f*	*b*	*d*	*e*
Gentamycin	R	R	R	S	S	R	R	R	S	S	R	S	S	R	R	S	S
Ampiclox	R	R	R	-	-	R	R	R	-	-	R	-	-	R	R	-	-
Amoxacillin	R	R	R	S	S	R	R	R	S	S	R	S	S	R	R	S	S
Ciprofloxacin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Streptomycin	S	S	S	R	R	S	S	S	R	R	S	R	R	S	S	R	R
Septrin	S	S	S	R	R	S	S	S	R	R	S	R	R	S	S	R	R
Erythromycin	S	S	S	-	-	S	S	S	-	-	S	-	-	S	S	-	-
Pefloxacin	R	R	R	S	S	R	R	R	S	S	R	S	S	R	R	S	S
Augmentin	-	-	-	R	R	-	-	-	R	R	-	R	R	-	-	R	R
Chloramphenicol	-	-	-	S	S	-	-	-	S	S	-	S	S	-	-	S	S

a= *Coccobacillus sp, b= Bacillus sp., c= S. aureus, d= Klebsiella sp., e= Enterobacter sp., f= B. megaterium*, - = Not applicable, R= Resistant, S=Sensitive

CONCLUSION

The comparison of the fresh and processed crabmeat showed that the processing steps (frozen, boiling and frying) have a considerable effect on the bacterial quality of fresh *C. amnicola* by reducing the distribution and abundance of the identified bacteria. However, the presence of resistant pathogens which are also human pathogens is a public health threat. Compliance with standard microbiological measures to prevent contamination by these organisms becomes very necessary.

REFERENCES

Al-Sheraa AS, Al-Khafaji KK & Al-Maleki GM 2018. Survey of pathogenic bacteria population associated with mitten crab *Eriecheir sinensis* (H. Milne Edwards, 1853) from intertidal zones in Khor Al-Zubair Canal Basra, Iraq. *International Journal of Fisheries and Aquatic Studies*, 6(5): 91-95.

American Public Health Association 2001. Drinking water quality and public health (position paper). American journal of public health 91(3): 1-499.

Cheesbrough M 2006. District Laboratory Practices in Tropical Countries. Cambridge University Press, UK. 416pp.

Duarte AM, Silva F, Pinto FR, Barroso S & Gil MM 2020. Quality assessment of chilled and frozen fish—Mini review. *Foods*, 9(12): 1739.

Geetha S1, Muddula KN, Rushinadha RK, Govinda RV & Ramesh B 2016, Microbial assessment of commercially important crabs from Visakhapatnam fishing harbour, east coast of India. *European Journal of Experimental Biology*, 6(4): 57-61.

Givens CE, Burnett KG, Burnett LE & Hollibaugh JT 2013. Microbial communities of the carapace, gut, and hemolymph of the Atlantic blue crab, *Callinectes sapidus*. *Marine biology*, 160(11): 2841-2851.

Gufe C, Canaan Hodobo T, Mbonjani B, Majonga O, Marumure J, Musari S, ... & Machakwa, J. 2019. Antimicrobial profiling of bacteria isolated from fish sold at informal market in Mufakose, Zimbabwe. *International journal of microbiology*, 1-8.

Hady AAA, Mousa M & Eissa W 2018. Quality Assurance of Some Heat Treated Meat Products. *Alexandria Journal for Veterinary Sciences*, 56(2): 131-138.

Imarhiagbe EE, Ofuya ME, Osarenotor O & Ekhaise F 2016. Antibiotic sensitivity pattern of microorganisms isolated from smoked and frozen fishes sold in Benin and Warri metropolis. *Nigerian Journal of Biotechnology*, 31: 40-45.

Jesumirhewe C & Ibeh NL 2020. Antibacterial susceptibility pattern of bacteria isolated from retail fish in Okada, Edo State Nigeria. *International Journal of Advances in Scientific Research and Engineering*, 6(4): 60-68.

Khalafalla FA, Abdel-Atty NS, Soad AN & Adel SH 2019. Potential microbiological hazards in broiler chickens during processing. *Assiut Veterinary Medical Journal*, 65: 7-15

Laith AR & Najiah M 2014. *Aeromonas hydrophila*: antimicrobial susceptibility and histopathology of isolates from diseased catfish, *Clarias gariepinus* (Burchell). *Journal of Aquaculture Research and Development*, 5(2): 1-7.

Lorentzen G, Lian F, Røhme AA, Johannessen E, Grastveit KV, Grip AE & Siikavuopio SI 2019. Effect of freezing-thawing on weight loss, melanosis, and microbial growth in mildly cooked snow crab (*Chionoecetes opilio*) clusters. *LWT*, 108: 283-288.

Moruf RO & Adekoya KO 2020. Genetic heterogeneity of portunid crab populations from three interconnecting topical lagoons. *Polish Journal of Natural Sciences*, 35(3): 301–311.

Moruf RO, Taiwo MA & Adebayo Q 2021. Nutritional and functional attributes of raw and grilled crabmeat. *Agricultural Science and Technology*, 13 (1): 83-90.

Moruf RO 2022. Isolation of gut associated bacteria from *Callinectes amnicola* (Crustacea: Portunidae) collected from two interconnecting tropical lagoons in Nigeria. *Sri Lankan Journal of Biology*, 7 (1): 31 – 39.

NCCLS 2003. Methods for disk susceptibility tests for bacteria. National Committee for Clinical Laboratory Standards, 7th ed., NCCLS Document M2-A7

Olatunde OO & Benjakul S 2018. Natural preservatives for extending the shelf-life of seafood: a revisit. *Comprehensive Reviews in Food Science and Food Safety*, *17*(6): 1595-1612.

Tavares J, Martins A, Fidalgo LG, Lima V, Amaral RA, Pinto CA & Saraiva JA 2021. Fresh fish degradation and advances in preservation using physical emerging technologies. *Foods*, 10(4): 780-786.

Wang L, Hu C & Shao L 2017. The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International journal of nanomedicine*, 12: 1227 –1249.

Zhuang S, Hong H, Zhang L & Luo Y 2021. Spoilage-related microbiota in fish and crustaceans during storage: Research progress and future trends. *Comprehensive Reviews in Food Science and Food Safety*, 20(1): 252-288.