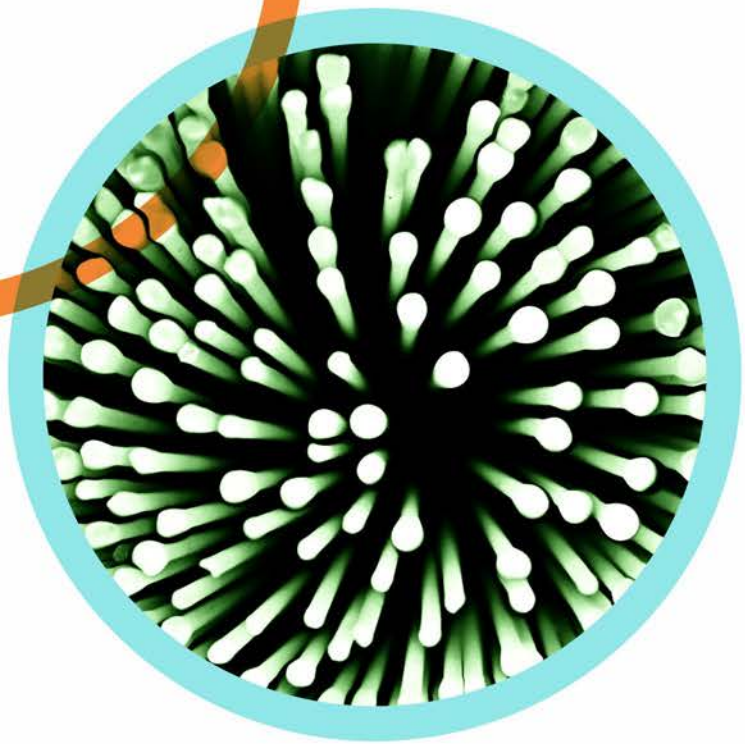

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Research Article
Assessment of Microbiological Quality of Fresh and Smoked Crabs (*Callinectes amnicola*) Fished and Sold in Southern Benin

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Abstract

Background and Objective: Crabs fished in Southern Benin are often distributed in the markets without any means of conservation or processed in very poor hygienic conditions exposing them to pathogens that can cause foodborne diseases to consumers. Therefore, there is a need to assess their safety in order to evaluate the risk for consumers. This study aimed to assess the microbial quality of fresh and smoked crabs fished and sold in Benin. **Materials and Methods:** To this end, a total of 120 fresh crabs were collected from 3 landing sites of the main water bodies and 3 retail markets and analysed using standard methods. Moreover, 30 smoked crab samples were collected from Dantokpa market and analysed. **Results:** The study revealed that all of analyzed samples had microbial loads higher than the maximum level required by Federation of Trade and Distribution for fresh crabs and the General Directorate of Food (2000) for smoked crabs. The total viable count was 7.1, 7.3 and 7.4 log CFU g^{-1} for fresh crabs from landing sites, markets and the smoked ones, respectively. Statistical analysis revealed significant difference ($p<0.01$) between microbial counts of market's fresh crabs and those from landing sites. Average fecal coliforms counts were 1.9, 2.0 and 1.4 log CFU g^{-1}, respectively with significant difference ($p<0.001$) between fresh and smoked crabs. Sulphite-reducing bacteria were 1.6 and 0.6 log CFU g^{-1} in fresh and smoked crabs, respectively. All samples were free of *Salmonella* sp. with *Staphylococcus aureus* counts satisfactory. **Conclusion:** The study demonstrates that fresh and smoked crabs sold in Southern Benin are of poor microbial quality and potentially unsafe for consumption.

Key words: Microbial quality, fresh and smoked crabs, Benin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fishing is one of the main economic activities in Benin. Continental fishing delivered about 38, 706 t to the national fish production[1] of 55, 294.29 t in 2011. The aquatic ecosystems of Benin shelter various species including fish, mollusks and crustaceans namely shrimps and crabs. These resources are intensely used by the Beninese population where fishing constitutes the first source of animal protein. This activity also contributes significantly in international trade especially for shrimps[2] and crabs[3]. Data from the fisheries directorate estimated the total number of fishers at 50.000 people and fished species to be 15. 000 t/year, for fish 4. 000 t of crabs/year and more than 3. 000 t of shrimps/year[4]. Crabs are one of the most consumed aquatic resources in Benin after fish. Two species are mainly consumed: *Callinectes amnicola* and *Cardisoma armatum*. They have interesting socioeconomic and nutritional importance with high levels of calcium, zinc, iron, sodium and other indispensable minerals[5]. However, much research has not been conducted on crabs in Benin. The only available data on this species in Benin concern its diversity and exploitation[3,6,7]. Very few studies were interested in the microbial quality of this highly consumed food while crabs are mostly fished in water bodies exposed to various microbial pollutants[8]. After fishing, crabs are often distributed in the markets without any means of conservation or processed in very poor hygienic conditions exposing them to pathogens that can cause foodborne diseases to consumers[9-14]. It is, therefore, necessary to assess the safety of these products in order to evaluate the risk for consumers. The present study was carried out to determine the microbiological quality of fresh and smoked crabs, *Callinectes amnicola* fished in Lake Nokoué, Lake Ahémé and the lagoon of Porto-Novo and their surrounding retail markets.

MATERIALS AND METHODS

Material: This study was conducted in four departments namely Atlantique, Littoral, Mono and Oueme located in southern Benin from July-September, 2016. The biological material was made of 120 fresh and 30 smoked crabs *Callinectes amnicola*. Live crabs were collected at the landing sites of Lake Ahémé, Lake Nokoué and the lagoon of Porto-Novo and in their surrounding markets (Comè, Calavi-Tokpa and Ahidaho, respectively). However, smoked crabs were obtained from Dantokpa market in Cotonou.

Methods

Sampling and transportation: Ten fresh crabs were randomly collected twice per month at each of the 6 aforementioned sampling points. The selected crabs were adults of approximately 35-95 g and kept in baskets. Five samples (of 20 individual smoked crabs performed without salting around 60-70°C for 1 h) were collected from 5 randomly selected retailers in Dantokpa market per week for 6 consecutive weeks, making a total of 30 samples. All samples were kept in sterile containers and transported to the lab with cooling elements and immediately analyzed.

Samples preparation and dilutions: Fresh whole crabs were ground using autoclaved porcelain mortar and pestle then serial dilutions were made in accordance with ISO 6887-3: 2003 standards[15]. Briefly, 25 g of ground sample was added to 225 mL of buffered peptone water in a sterile flask. Serial dilutions were thereafter made 10 times with 1 mL of the stock solution in 9 mL of peptone water.

Bacterial enumeration and detection: Bacterial counts and detections were performed following standard methods[16]. Total viable counts were determined on plate count agar (PCA OXOID CM0463) at 30°C, thermotolerant coliforms were enumerated on VRBG agar (Violet Red Bile Glucose) at 44°C, *Staphylococcus aureus*-like were counted on Baird Parker agar and sulphite-reducing bacteria on trypticase sulphite neomycin (TSN) at 37°C. For the detection of *Salmonella* sp., samples were pre-enriched in buffered peptone water (BPW OXOID CM0509), then enriched in Rappaport-Vassiliadis broth (RV OXOID CM0669) and plated on *Salmonella-Shigella* (SS) and xylose lysine decarboxylase (XLD) agars and further confirmation was done using API 20E strips. The enumeration was performed using 25 g of crab sample for mother solution and 25 g for detection of *Salmonella* sp. The obtained results were compared with the standards.

Statistical analysis: Data were analyzed using statistical analysis system[17]. Means and standard errors of microbial loads of the different flora were calculated by the Proc Means procedure. An analysis of variance one way was carried out with the procedure of generalized linear models (Proc GLM). The landing sites and the markets were considered as the sources of variations. F-test was used to determine the significance of every variation factor and the student t-test was used to compare means.

RESULTS AND DISCUSSION

Microbial quality of fresh crabs and smoked crabs: The total viable count of fresh crabs varied from 7.1-7.3 log CFU g^{-1} (Table 1) with a non-compliance rate of 100% for samples that originated from landing sites and 97% for those collected from markets. The bacterial load of the fresh crabs varied significantly between landing sites and markets ($p<0.05$) (Table 1, 4). However, no significant difference was observed within landing sites and within markets (Table 2, 3). The lagoon of Porto-Novo reported the lowest bacterial counts (7.1 log CFU g^{-1}), while Nokoué lake had the highest counts (7.2 log CFU g^{-1}). The market of Comè recorded the lowest counts (7.2 log CFU g^{-1}) and the highest bacterial counts were reported from Calavi-Tokpa market (7.3 log CFU g^{-1}). These differences observed were relatively significant from one place to another ($p<0.05$). Total viable counts of fresh crabs examined in this study were higher than the maximum limits required by FCD (2015) standards for raw crustaceans at distribution level[18]. They were also higher than the counts reported by Dègnon *et al.*[19] and Mègnon *et al.*[20], respectively for shrimps fished in Ahémé and Nokoue Lakes. Failure to respect the cold-chain, as well as the insalubrity of the markets and a deficient hygiene of the sellers are some of the risk factors.

Table 1: Microbial loads of fresh crabs in the various sampling sites

Variables	LA	MC	LN	MT	LP	MA	N	ST
TVC (10^7 CFU g^{-1})	1.69	2.01	1.95	2.20	1.43	2.56	0.1	*
FC (CFU g^{-1})	50.00	50.54	114.68	154.95	71.36	74.00	10.0	**
SRA (CFU g^{-1})	63.00	43.81	23.52	19.00	58.36	64.64	10.0	***
S. aureus (CFU g^{-1})	31.00	55.00	00.00	20.00	9.00	17.00	100.0	***
Salmonella sp. (CFU/25 g)	Abs	Abs	Abs	Abs	Abs	Abs	Abs	-

LA: Ahémé lake, MC: Market of Comè, LN: Nokoué lake, MT: Market of Calavi-Tokpa, LP: Lagoon of Porto-Novo, MA: Market of Ahidaho, TVC: Total viable count, FC: Faecal coliforms, SRA: Sulphite-reducing aerobes, CFU: Colony Forming Unit, Abs: Absence, ST: Significance test, N: Norm, *Significant at 5%, **Significant at 1%, ***Significant at 0.1%

Table 2: Microbial loads of fresh crabs sampled from the three landing sites

	Nokoué lake		Lagoon of Porto-Novo		Ahémé lake			
Variables	Mean	SE	Mean	SE	Mean	SE	N	ST
TVC (10^7 CFU g^{-1})	1.95^a	0.18	1.43^a	0.32	1.69^a	0.38	0.1	NS
FC (CFU g^{-1})	114.68^a	20.75	71.36^a	14.73	50.55^a	13.58	10.0	NS
SRA (CFU g^{-1})	23.49^b	7.07	58.36^{ab}	10.15	63.00^a	6.60	10.0	**
S. aureus (CFU g^{-1})	0.00^b	0.00	9.00^b	8.82	31.00^a	7.78	100.0	***
Salmonella sp. (CFU/25 g)	Abs	-	Abs	-	Abs	-	Abs	-

TVC: Total viable count, FC: Fecal coliforms, SRA: Sulphite-reducing aerobes, CFU: Colony forming unit, Abs: Absence, SE: Standard error, N: Norm, ST: Significance test, NS: Non-significant, **Significant at 1%, ***Significant at 0.1%. Means followed by different letters in the same row (a, ab, b) are significantly different

Table 3: Microbial loads of fresh crabs sampled from the three markets

	Calavi-Tokpa market		Ahidaho market of Porto-Novo		Market of Comè			
Variables	Mean	SE	Mean	SE	Mean	SE	N	ST
TVC (10^7 CFU g^{-1})	2.200^a	0.16	2.05^a	0.86	2.01^a	0.11	1	NS
FC (CFU g^{-1})	154.95^a	21.21	74.00^b	15.66	50.54^b	6.50	10	***
SRA (CFU g^{-1})	19.00^b	6.28	64.64^a	9.22	43.81^a	13.27	10	***
S. aureus (CFU g^{-1})	20.00^a	7.11	17.00^a	10.33	55.00^a	12.51	100	NS
Salmonella sp. (CFU/25 g)	Abs	-	Abs	-	Abs	-	Abs	-

TVC: Total viable count, FC: Fecal coliforms, SRA: Sulphite-reducing aerobes, CFU: Colony forming unit, Abs: Absence, SE: Standard error, N: Norm, ST: Significance test, NS: Non-significant, ***Significant at 0.1%. Means followed by different letters in the same row (a, b) are significantly different

Table 4: Comparative microbial loads between landing sites and markets

	Lakes		Markets			
Variables	Mean	SE	Mean	SE	N	ST
TVC (10^7 CFU g^{-1})	1.34^b	0.12	2.16^a	0.24	0.1	**
FC (CFU g^{-1})	85.52^a	11.74	108.11^a	13.91	10.0	NS
SRA (CFU g^{-1})	41.38^a	6.05	36.61^a	5.13	10.0	NS
S. aureus (CFU g^{-1})	9.50^b	3.91	28.00^a	5.31	100.0	**
Salmonella sp. (CFU/25 g)	Abs	-	Abs	-	Abs	-

TVC: Total viable count, FC: Fecal coliforms, SRA: Sulphite-reducing aerobes, CFU: Colony forming unit, Abs: Absence, SE: Standard error, N: Norm, ST: Significance test, NS: Non-significant, **Significant at 1%. Means followed by different letters in the same row (a, b) are significantly different

Table 5: Correlation between bacterial counts

Variables	TVC	FC	*S. aureus*	SRA
TVC	1	-0.357*	0.389*	-0.096^{NS}
FC		1	-0.331*	-0.168^{NS}
S. aureus			1	0.297^{NS}
SRA				1

TVC: Total viable count, FC: Fecal coliforms: SRA: Sulphite-reducing aerobes, *Significant at 5%, NS: Non-significant

Table 6: Microbial counts from smoked crab samples

Variables	Smoked crabs	DGAL (2000)	Non-compliance (%)
TVC (10^7 CFU g^{-1})	2.43	0.005	100.00
FC (CFU g^{-1})	23.90	10.00	30.00
SRA (CFU g^{-1})	4.00	2.00	46.67
S. aureus (CFU g^{-1})	4.00	10.00^2	00.00
Salmonella sp. (CFU/25 g)	Abs	Abs	00.00

TVC: Total viable count, FC: Fecal coliforms, SRA: Sulphite-reducing aerobes, CFU: Colony forming unit, Abs: Absence, DGAL: French general directorate of food

Table 7: Microbial loads of smoked and fresh crabs

Variables	Fresh crabs	Smoked crabs	ST
TVC (10^7 CFU g^{-1})	1.61	2.43	NS
FC (CFU g^{-1})	103.76	23.90	***
SRA (CFU g^{-1})	37.69	4.00	***
S. aureus (CFU g^{-1})	13.16	4.00	***
Salmonella sp. (CFU/25 g)	Abs	Abs	NS

TVC: Total viable count, FC: Fecal coliforms, SRA: Sulphite-reducing aerobes, CFU: Colony forming unit, Abs: Absence, ST: Significance test, NS: Non-significant, ***Significant at 0.1%

Regarding the fecal coliforms of these products, their count varied from 50-154.95 CFU g^{-1} of crab. The counts from landing site samples ranged between 50 and 114.68 CFU g^{-1} with an average of 85.52 CFU g^{-1}, whereas, market samples varied from 50.54-154.95 CFU g^{-1} with an average of 108.11 CFU g^{-1} (Table 4). Significant differences were observed between bacterial counts ($p<0.01$) among markets but not between landing sites ($p>0.05$). Nevertheless, fecal coliforms load in fresh crabs were lower in Ahémé Lake as compared to the lagoon of Porto-Novo and Nokoué Lake (Table 1). The occurrence of fecal coliforms in fresh crabs is due to the unsafe aquatic environment where they were fished from. Dègnon *et al.*[19] and Mègnon *et al.*[20] reported extremely high levels of fecal coliforms in Lake Ahémé (>650 CFU mL^{-1}) and Lake Nokoué (2254 CFU mL^{-1}), respectively and attributed the pollution of these waters to anthropic activities. These previous data also justify the fact that crabs from Nokoué Lake were more contaminated by fecal coliforms than those of Ahémé lake and the lagoon of Porto-Novo. Moreover, the high contamination in fecal coliforms can be explained by the increased population living around Nokoué lake[8].

The level of sulfite-reducing anaerobes bacteria varied from 23.52-63 and 19-64.64 CFU g^{-1} for fresh crabs collected from landing sites and markets, respectively (Table 2, 3). The counts were statistically lower from Nokoué Lake as compared to Ahémé and lagoon of Porto-Novo; they also varied between markets (Table 1, $p<0.001$). Sulphite-reducing anaerobes counts reported in the present study for fresh and smoked crabs were above the standard limits. The lowest counts in fresh crabs were obtained from Nokoué Lake. They were above those reported by Koussémon *et al.*[21] in fresh crabs from Ivory coast, Dègnon *et al.*[19] and Mègnon *et al.*[20] in shrimps fished respectively in Ahémé Nokoué Lakes. The counts were, however, below the counts reported by Gnakadja[22] in shrimps fished in the lagoon of Porto-Novo. The results of this study and those of the above mentioned studies were all non-complaint with the safety standards. This contamination of crabs by sulphite reducing aerobes could imply the presence of *Clostridium botulinum* and *C. perfringens* in the samples.

Staphylococcus aureus counts varied from 0 (Nokoué Lake) to 31 CFU g^{-1} (Ahémé Lake) for fresh crabs collected at the landing sites and 17-55 CFU g^{-1} for those from the markets. They differed significantly between landing sites and markets but were all in accordance with the standard limits of safety (Table 1, 2). The level of *S. aureus*-like in fresh crabs in this study is in conformity with the microbiological standards as opposed to the reports of Degnon *et al.*[19], Gnakadja[22] and Megnon *et al.*[23].

No fresh crab sample was positive for *Salmonella* sp. and such observation is in line with safety criteria. Similar situations were reported by Degnon *et al.*[19], Megnon *et al.*[20] and Degnon *et al.*[23] in shrimps. Correlation between measured variables revealed in Table 5 showed that *Staphylococcus aureus* count from the samples has a low correlation with the total viable count and sulphite-reducing anaerobes count.

Microbial quality of smoked crabs sampled from Dantokpa market: Smoked crab samples showed high non-compliance levels in total viable counts, fecal coliforms and sulphite-reducing anaerobes (100, 30 and 46.67%), respectively. However, *S. aureus* count was satisfactory regarding GDF/DGAL standards[24] and none of the samples tested positive for *Salmonella* sp. (Table 6).

Microbial loads of smoked and fresh crabs: Fresh crabs were more contaminated than smoked crabs by fecal coliforms count, sulphite-reducing anaerobes and *S. aureus* ($p<0.001$). Nevertheless, the total viable counts of smoked crabs were slightly higher than those of fresh crabs. This difference was however, not significant ($p>0.05$) (Table 7). Many factors could affect these microbial load of smoked crabs, mainly poor

hygiene during smoking, use of unsafe water for washing and the use of contaminated utensils as well as poor storage. Heat treatment application like pasteurization is necessary to kill pathogens, but crabs saleswoman sensitization could be recommended for the public health protection.

CONCLUSION

The bacteriological study of *Callinectes amnicola*, served to assess the safety risks related to the consumption of this food product. The study revealed a high level of contamination of crabs by fecal coliforms and sulphite-reducing aerobes. The study also revealed that fresh crabs collected from landing sites were less contaminated than those sampled in the markets. It is, therefore, important for fishers and sellers of these products to respect the cold-chain in order to keep the good microbial quality of the products. Consumers are also required to handle these products with good hygiene practices and cook thoroughly before eating.

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