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Mycoflora, Proteolytic Potential and Quality Implication of Dried Crayfish at Uyo Urban Market, Uyo Nigeria.

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Abstract

Smoke dried crayfish sold by heaping open in an urban market in Uyo, Akwa Ibom State of Nigeria was procured and taken to microbiology laboratory for selected microbial quality tests. Total bacterial count ranged from 4.2 x 10^2 to 4.4 x 10^2 cfu/g. Coliform count ranged from 2.0x10^2 to 2.8 x 10^2 cfu/g. Other organisms *Bacillus subtilis, Bacillus polymyxra*, *Staphylococcus aureus* and *Penicillium chrysogenum* exhibited relatively higher proteolytic potential. The study revealed that smoke drying which is expected to preserve crayfish at post harvest, may not be effective in controlling proteolysis elaborated by these organisms under the existing handling method. Awareness on better post harvest handling of smoke dried crayfish among the rural retail traders in the markets for quality and safety is advocated.

Key Words: Smoke-dried crayfish, microflora, proteolytic – potential.

Introduction

Crayfish (*Procambarus clarkii*) is a crustacean that forms greater proportion of shellfish, abundant in the fresh waters of the Delta region of Nigeria. Abundance is not limited to the Tropical waters but extend to Europe (Guner & Harlioglu, 2010). Artificial rearing of crayfish is not popular compared to fish but production level in Nigeria is conservatively estimated at about 12,000mt per annum. Bulk of the activities including processing are handled by rural fishermen.

Crayfish is economically valuable in many riverine countries particularly in countries where fish production account for more than 75% of the total value of their commodity trade. Many Nigerian riverine Delta region women source their livelihood from marketing of smoke-dried crayfish. The commodity is processed and packaged in woven polythene or hessian bags or woven baskets and transported in dugout wooden boats from processing centres in creeks to onshore markets.

Crayfish is classified as animal polypeptide consisting about 36 – 45% protein (Ibironke et al 2014). Like most sea foods, it contributes immensely in the nutrition of consumers. The protein is relatively cheaper (Abou-Zaid & Mohammed 2014) than other animal protein and possess high nutritional value. The nutritional benefits of crayfish were reported by earlier workers (Ibironke et al 2012, Nahid & Fayza 2009, Swahu 2004). Crayfish is used to a large extent in local food preparation in Nigeria. Reports (Ibironke et al 2014) showed that crayfish has found use in complementary food formulations.

Like most marine products, it is susceptible to microbial damage which may create quality and safety problems. Mould on crayfish deposit substance that have anti-vitamin effect (Ladoke 1991). Preservation is either by salting, freezing, canning, sun-drying or smoke-drying. Sun and smoke-drying are common preservation method

because of their relative lower costs. The use of smoke in local fish preservation was reported (Eyo, 2000) earlier. The implication of poor postharvest handling of crayfish has also been reported (Kumolu-Johnson et al. 2010). Smoke drying is done to partially cook, remove water, obtain brown colour, improve organoleptic flavour and control microbial and enzymic actions that may cause spoilage. Preservation effects of smoke derived from the antioxidant and antimicrobial properties of its phenolic compound have been reported (Shehu et al. 2013), Abou-zaid & Mohammed, 2014).

There is gap of knowledge in the impact of traditional smoke drying and handling of crayfish in Nigeria (Akintola et al. 2013). Crayfish has potential for export to market in countries with high population of people from the producing countries. Such cross-country trade demand high consideration for quality and safety. It is an important flavour ingredient in many Nigerian Local preparations. In local markets, crayfish is retailed open as small heaps on tables to attract consumers. Information on duration of effectiveness of smoke drying on crayfish quality is scarce. The essence of processing is to stop bacterial action (Omomo and Kareem 2014) and retain quality.

The study therefore determines the organisms that may prevail and cause spoilage including their proteolytic potential on smoke dried crayfish during retail handling in Uyo markets, Akwa Ibom State, Nigeria. The information will be useful in post harvest handling for quality and safety of consumers.

2. Materials and Method

Smoke-dried crayfish (5kg) was purchased randomly from 10 crayfish retailers in Uyo urban market, packaged in plastic container with tight fitting lid and transported to the microbiology laboratory for analysis.

2.1 Microbiological analysis

A representative sample portion was taken and milled (Master Chef Model B919A) for microbiological tests. Milled crayfish (1g) was homogenized in 9.0ml of sterile peptone water. The samples were serially diluted until 10^{-5} dilution was obtained. Isolation and identification of infecting organisms were done according to the methods of Ogbulie et al. (2005; ICMMSF (1978). Nutrient agar was used for culturing non-fastidious bacteria while McConkey agar was used for isolation and differentiation of enteric bacteria. Soboraud agar was used for isolation of fungi (Klich, 2002).

Total viable count was determined in accordance with (Odom et al. 2012). Colony forming units (cfu/g) were enumerated by pour plating 1.0ml of 10^{-5} diluent incubated at 37^{o}C for 48h. Total count for fungi isolates were done in triplicates. Pure cultures of bacteria and fungi isolates were obtained on nutrient agar and Sabouraud dextrose agar respectively.

2.2 Characterization and identification of isolates.

For bacteria, cultural characteristics were examined microscopically while physiological and biochemical tests followed for further confirmation of organisms. Tests done were gram stain, catalase test, coagulase test, oxidase test, motility test, spore stain, fermentation test, methyl red-voge, Proskaur test, methyl Red test, Voges

Proskaur, indole test and citrate tests. Isolated fungi were identified by staining with cotton blue lacto phenol and visualized under X40, (Klich 2002).

2.3 Determination of proteolytic activities of Isolate

Geletin (0.4%) was added as protein source to the nutrient agar containing protease produced by the microorganisms. The inoculated plates were incubated for 7 days. Saturated solution of ammonium sulphate was poured over the agar surface to enhance visibility of the zones of proteolysis (SAB 1951).

3. Result and Discussion

The prevalence of microbial isolates in crayfish is presented in Table 1. The following bacteria species *Escherichia coli, Staphylococcus aureus, Bacillus subtillis, Bacillus polymyxa, Micrococcus iuteus and Proteus vulgaris* were isolated from the smoke dried crayfish. Fungal isolates detected were *Candida albicans, Penicillium chrysogenium, Fusarium moniliform, Aspergillus flavus, Aspergillus candidus* and *Cephalosporium* species. Earlier workers (Wu et al. 1981; Wantanabe 1971) reported the presence of some of these organism on crayfish.

The morphological and biochemical characteristics of bacteria isolated from smoke dried crayfish are presented in Table 2. Total bacterial count ranged from 4.2 x 10^2 to 4.4 x 10^2 cfu/g while coliform count ranged from 2.0 x 10^2 to 2.8 x 10^2 cfu/g (Table 3). The low level total count may be associated with the antagonistic effect of smoke constituents during drying which subsisted during handling. Shehu et al. (2013) reported the preservative effect of smoke during drying of fish. Although smoke concentration was not determined, report (Eyo 1979) showed that levels of smoke deposit on crayfish up to 450mg/kg inhibited growth of bacteria. The concentration of smoke on dried crayfish is hardly determined under traditional process.

The incidence of some of these organisms *E. coli, Proteus sp, Bacillus sp* and *Staphylococcus* species on smoke-dried crayfish in the market may be due to poor handling which predisposes the commodity to contamination. Poor handling further exposes the commodity to insect infestation and elaboration of moulds which in turn reduces the quality of the commodity.

The proteolytic potential of the isolated organisms are shown in Table 4. Some of the organisms (*B. subtilis, B. polymyxra, Staphylococcus aureus and Penicillium chrysogenum)* exhibited relative higher proteolytic potential indicating ability to produce enzymes for spoilage of crayfish during handling. Bacillus sp was reported (Ouoba et al. 2003) to cause proteolysis in fermenting seeds. The presence of these organisms in crayfish may cause both physical and chemical quality defects with time of exposure. Some of the defects include discoloration, odour deterioration and poor flavour in food preparations. The commodity consequently looses market appeal and rejection by consumers.

4. Conclusion

Preservation of crayfish through traditional smoke-drying appears not adequate in protecting the commodity from spoilage organisms and proteolys during retail handling as practiced in the market. The handling method of the produce in the local markets overtime may encourage build-up of spoilage organisms and thus reduce the quality desired by consumers.

5. Recommendation

Creation of awareness on better and safe handling method for smoke-dried crayfish among retailers in rural and urban markets in Nigeria is highly desirable. The programme will help to reduce possible losses that can be encured by retailers and ensure that consumers get value for money spent on the purchase.

Table 1: The Prevalence of the Microbial Isolates in the Crayfish Sample

Bacterial Isolates	Plate Number										
	1	2	3	4	5	6	7	8	9	10	Total
Bacillus subtilis	+	-	+	+	-	-	-	+	+	-	5
Baallus polymyxa	+	+	+	-	-	-	-	-	-	-	3
Escherichia coli	+	+	+	-	+	-	-	+	-	+	6
Micrococcus luteus	+	-	+	+	+	-	-	+	-	-	5
Proteus vulgaris	-	+	-	-	+	+	+	+	-	-	5
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	10

Bacterial Isolates	Plate Number										
	1	2	3	4	5	6	7	8	9	10	Total
Aspergillus candidus	+	-	-	+	+	-	-	+	+	-	5
Aspergillus flavus	+	+	-	-	+	+	-	-	-	-	4
Aspergillus flavipes	+	+	+	+	-	-	+	-	+	-	6
Candida albicans	+	-	-	+	+	+	-	-	+	-	5
Cephalosporium Sp	+	+	-	-	-	+	+	-	+	+	6
Fusarium moniliforme	+	-	+	-	+	-	+	+	-	-	5
Penicillium expansum	+	-	+	+	-	+	-	-	-	-	4
Penicillum Chrysogenium	+	+	-	+	+	-	-	-	+	+	6

Table 2: Morphological and Biochemical Characteristics of Bacterial Isolated from Crayfish

Cultural characteristics	Isolates	Shade	Grain stain	Catalase	Spore stair	Indole test	Coagulate	Urease	Oxidase	M.R	V.R	Motility	Glucose	Sucrose	Fructose	Lactose	Mannitoe	Probable organisms
Large, watery rose pink colony	A	Rods in chains	-	+	+	+	-	-	-	+	-	+	AG	AG	A	AG	A	Eschenchia coli
Golden yellow with smooth raised colony	B	Cossi in clusters	+	+	-	-	-	+	-	+	+	-	A	A	A	A	A	Staphylococcus
Dry flat, irregular colony preading	C	Long rods in chains	+	+	+	-	ND	+	+	+	-	-	A	-	+	-	+	Bacillus subtilis
	D	Short rods in chains	+	+	+	-	+	-	-	+	-	-	A	A	A	A	A	Bacillus polymyxa
Smooth smooth white, raised colony	E	Cocci in pairs	+	+	-	-	-	ND	ND	+	-	-	-	A	A	-	-	Micrococcus luteus
Whitish smooth Colony	F	Rods	-	+	-	+	-	+	+	+		+	AG	A	-	-	-	Proteus vulgaris

Keys:

+	=	Positive
-	=	Negative
A	=	Acid production
AG	=	Acid and Gas production
ND	=	Not determined

Table 3: Microbial Counts of Crayfish Sample

Sample	Total anaerobic bacteria count (cfu/g)	Total coliform count (cfu/g)	Total fungal count (cfu/g)
1.	4.4×10^2	2.8×10^2	1.9×10^2
2.	4.2×10^2	2.0×10^2	2.1×10^2
3.	2.9×10^2	1.4×10^2	1.4×10^2

Table 4: The Proteolytic Potential of Microorganisms Isolated From the Crayfish Samples

S/N	Bacterial isolates	Proteolytic potential
1.	*Bacillus subtilis*	+++
2.	*Bacillus polymyxa*	+++
3.	*Escherichia coli*	++
4.	*Micrococcus lufeus*	++
5.	*Proteus vulgaris*	++
6.	*Staphylococcus aureus*	+++

	Fungal Isolates	
1.	Aspergillus candidus	++
2.	Aspergillus flarus	++
3.	Aspergillus flaripes	++
4.	Candida albicans	+
5.	Cephalosporium sp	-
6.	Fusarium moniliforme	-
7.	Penicillium expansium	++
8.	Penicillium chrysogerium	+++

Keys: +++ = High proteolytic potential
++ = Moderate proteolytic potential
+ = Low protelytic potential
- = No proteolytic potential

References

Abou-Zaid, A. M. & Mohammed, A.S.E (2014). "Production and Quality Evaluation of Nutrition, of High Quality Biscuits and Potato Puree Tablets Supplemented with Crayfish (*Procombarus clarkia*) Protein Products". *Journal of Applied Science Research* 10(7) 43-53.

Adams, A.J. &Tobias, W.J (1999). "Red Mangrove Prop-root Habit as Fin Fish Nursery Area: A Case Study of Salt Rivers Bay, St. Croix, U.S.V.I", *Proceeding of the 40th Gulf and Caribbean Fisheries Institute*. 46, 22 - 46.

Cann, D. C. (1976). "Bacteriology of Shell Fish with Reference to International Trade". *Proceeding of Conference on the Handling, Processing and Marketing of Tropical Fish, London*, 377-397.

Cheikyula, T. J & Awobade, H. O. (2014). "Microbial Flora and Nutrient Content of Market Bought Smoked African Crayfish (Clarias gariepinus) from Jos, Nigeria", *Food Science and Quality Management* (Online) 32, 34 – 40.

Eyo, A. A. (2000). "Fish Processing Technology in the Tropics", *University of Ibadan Press, 165 - 168.*

Ibironke, S. I., Joseph, B. F & Morakinyo, M. I (2014). "Nutritional Quality of Animal Polypeptide (Crayfish) Formulated into Contemporary Foods". *American Journal of Food & Nutritional*. 2 (3) 39 – 42.

Ibironke S. I. Fashakin, J.B & Badmus O.A (2013). "Nutritional Evaluation of Contemporary Food Developed from Plant and Animal Protein sources". *Nutrition of Food Science* 42 (2) 111 – 120.

Klich, M. A. (2002). "Identification of Common Aspergillus". *Central Voo Schimmal Cultures*. Utrecht 1st edition, The Neterland, 1 - 113.

Kumolu - Johnson, C. A., Aledetohun, N. F & Ndimele, P. E. (2010). "The Effect of Smoking on Nutritional Qualities and Shell-life of Clarias Gariepinus (BURCHELL 1882)", *African Journal of Biotechnology*. Vol. 9 (1), 073 - 076.

Nahid, F. Z & Fay Za, E (2009). "Study on Chemical Quality and Nutritional Value of Fresh water Crayfish (Procambarus Clarkii)". *Journal of the Arabian Aquaculture Society* 4 (1) 1 – 18.

Omomo, D. E; Sunday, R. M & Kareem, I. (2014). "Effect of Smoke Drying Techniques on the Proximate and Mineral Composition of *Macrobranchium Vellenhovenic* African River Prawn)", *International Journal of Biological, Biomdecular, Agricultural, Food and Biotechnological Engineering*. 8, (2) 189 - 192.

Ouobia, L.I.I Rechinger, K. B; Barkholt, V.; Diawara B.; Traore, A. S. & Jakobsen, M. (2003). "Degradation of Protein during the Fermentation of Africa Locust Bean (*Parkia biglobosa*) by Strains of *Bacillas Subtillis* and *Bacillus Pumuilus* for Production of Soumbala". *Journal of Appl Microbiology* 94, 396 - 402.

Philips, S. and Wallbridge, A. (1976). "The Mycoflora Associated with Dry Salted Tropical Fish". Proceedings of the Conference on the Handling, Processing and Marketing of Tropical Fish, 353-356.

Saliu J. K (2008). "Effect of Smoking and Frozen Storage on the Nutrient Composition of Some African Fish". *Adv. Nat. Appl*. Sc 2 (i): 16 - 20.

Shehu, L. A, Ayodele, B., Abubakar, B., Oluwafemi, D. O. & Omolola Bello, B. (2013). *Pol. J. Food Nutrisci*. 63 (4) 227 - 237.

Swahu, J. O (2004). "The Cultural History of crayfish". *Bull Fr Pech Piscic* 372 – 373; 243 – 251.

Utku, G & Muzafter M. H (2010). "Status of Fresh Water Crayfish Distribution in Trace Regions of Turkey". *Reviews in Fisheries Science*. 18 (1 - 1), 1 - 6.