

The Characterization of Fungi Associated with Stale Smoked Crayfish and Prawns Vended in Ilaro Town

AZEEZ A. ADENIJI¹ & OLUDAYO O. ODUWOBI^{2*}

Science Laboratory Technology Department,

The Federal Polytechnic, Ilaro, Ogun State

*Corresponding Author: dwheazy2012@gmail.com

ABSTRACT Due to their outstanding nutritional values, high protein contents and low prices, aquatic commodities have lately witnessed one of the quickest rates of development in the global agriculture sector. One meal that is used as a source of protein by persons with high and low incomes is crayfish (*Astacus leptodactylus*). Smoked dried crayfish and prawns were purchased from three different markets; Sayedero, Sabo and Orita for analyses. This research work revealed the contamination of the sampled stale smoked crayfish and prawns with fungi, with the fungal load ranges of 3.00×10^5 – 12.30×10^5 cfu/g and 6.66×10^5 – 7.33×10^5 cfu/g respectively. The fungi isolated from the stale smoked fish are *Fusarium oxysporum* (33.33 %), *Aspergillus niger* (33.33 %), *Rhizopus nigricans* (16.67%) and *Aspergillus fumigatus* (16.67 %) while the fungi isolated from the stale smoked prawns are *Fusarium oxysporum* (42.86 %), *Aspergillus niger* (28.57 %) and *Rhizopus nigricans* (28.57 %). The fungal load is highest in the smoked crayfish and prawn samples gotten from Orita market, as compared with the ones from the other two marketing sites. This study concludes that the presence of fungal contaminants might be attributable to poor or inadequate storage condition which, perhaps, was favourable for fungal proliferation. Prolonged and unsanitary storage can lead to contamination with fungi which could cause the development of food-borne fungal diseases when consumed especially in immuno-compromised individuals. Proper crayfish and prawn handling, processing and storage should be encouraged and adopted. Crayfish and prawns should not be kept unhygienically or for too long before consumption.

KEYWORDS: Contamination, Fungal Load, Fungal Proliferation, Inadequate Storage

I. INTRODUCTION

Food demand, particularly for high-protein-animal-based meals, is growing as the world's population expands (Sans and Combris, 2015; Kumar *et al.*, 2017). Due to their outstanding nutritional values, high protein contents and low prices, aquatic commodities have lately witnessed one of the quickest rates of development in the global agriculture sector (FAO, 2018). Due to its flavour, nutritional value, adaptability and speedy reproduction, the red swamp crayfish (*Procambarus clarkii*) is cultivated extensively over the world (Holdich and David, 1993; Gong *et al.*, 2008). However, as crayfish farming business has increased in size, crayfish diseases have become more widespread (Longshaw, 2011; Shen *et al.*, 2020). One meal that is used as a source of protein by persons with high and low incomes is crayfish (*Astacus leptodactylus*). It is a small freshwater shrimp that resembles a lobster. Some species dwell in freshwater rivers and streams, even as others stay in marshes, ditches and rice fields. Vitamins A, C, B6, thiamine and riboflavin are all present in exact proportions in crayfish (Willard, 2006). It is affluent in iron, zinc, copper and manganese as well as sodium, potassium and phosphorus (Orogu *et al.*, 2018). Drying, smoking, freezing, chilling and banning are all procedures used to preserve crayfish against bacterial deterioration, although, this approach is most usually done to improve the item's shell life by reducing its water content (Orogu *et al.*, 2018). When dried crayfish is exposed to insects, dust, market handling with unclean hands, its contamination risk increases. Numerous bacteria are infecting numerous crayfish species. These pathogens can represent a severe threat to public health as they are resistant to numerous medicines (Adenake *et al.*, 2016). Most of these gut bacterial species have the potential to cause gastrointestinal disease or to form the

gut flora as stable commensals (Adenalke *et al.*, 2016). Various microorganisms, including antibiotic-resistant enteric bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella* sp. can contaminate crayfish (Adenalke *et al.*, 2016).

Shrimp also is a source of animal protein in Asia and Africa. In Nigeria, where the availability of animal protein is so scarce and basically unaffordable for low-wage earners, such seafood has also replaced animal protein low in fat and calories. Shrimp contains omega-3 fatty acids, vitamin B12, zinc, iodine, phosphorus, potassium and selenium, high in iron but low in magnesium, calcium and salt, according to Olaoye, Akinemi, Odunye and Ezeri (2014). It is also low in calories. Shrimp is the world's safest source of proteins for muscles. In terms of reported illnesses per volume swallowed, it may be the least dangerous seafood product (Otwell and George, 2010). Fresh prawns must be treated further (for example, by smoking) to boost value while increasing their shelf lives. One of the typical processing processes used to eliminate or decrease post-harvest losses is smoking. Unfortunately, a lot of smoked prawns produced nowadays are only exposed to the smoke for a short amount of time, scarcely drying them (Chinivasagama *et al.*, 1996; Piggot, 2009), leaving them subject to degradation. The possibility of transferring a number of food-borne diseases via crayfish and prawn consumption to human population is now eminent due to poor handling, storage and exposure to air. The aim of this investigation is to characterize the fungi associated with stale smoked crayfish and prawns vended in Ilaro, Ogun State, Nigeria. The fungi isolated from the crayfish samples in this study are related to the study conducted by Fagbohun *et al.* (2019) on smoked dried crayfish stored for 24 weeks. They isolated *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus* sp. and other fungi species in their study.

II. MATERIALS AND METHODS

A. SAMPLE COLLECTION

Stale smoked dried crayfish and prawns were purchased from three different markets in Ilaro town, namely Sayedero market, Sabo market and Orita market. A sample each for crayfish and prawns was purchased from each market and kept in clean polythene bags. They were taken to the Microbiology Laboratory of The Federal Polytechnic, Ilaro. The samples were then milled and kept inside respective polythene bags for the mycological analyses.

B. MEDIUM PREPARATION

Potato dextrose agar (39g) was suspended in 1 liter of distilled water. The suspension was homogenized by frequent agitation for one minute to completely dissolve the medium. It was sterilized by autoclaving at 121°C for 15 minutes. The molten suspension was cooled to 45°C and then dispensed into sterile Petri plates.

C. ISOLATION OF FUNGI

The milled stale crayfish and prawns were presented for mycological analysis. Each sample (1g) was introduced into 9.0ml of distilled water and then serially diluted to dilution 10^{-5} to give a five serial ten-fold dilution. Dilutions 10^{-2} , 10^{-4} and 10^{-5} were inoculated on PDA plates by pour-plate technique and incubated at room temperature for 5 days. After incubation, the organisms were sub-cultured on freshly-prepared PDA agar to get pure cultures (Ibrahim & Rahma, 2009). The fungal load was determined and recorded.

D. MICROSCOPIC IDENTIFICATION

Portions of fungal growth from the sub-cultures were transferred onto clean glass slides to prepare thin smears. 2-3 drops of lactophenol-cotton-blue stain were dropped on the slides and cover slips were carefully applied to avoid the formation of air bubbles. After 5 minutes, the slides were microscopically observed under low-power objective lens for identification and the results were compared with a Fungi atlas.

III. RESULTS AND DISCUSSION

This study focused on the isolation and identification of fungi associated with stale smoked crayfish and prawns gotten from different markets in Ilaro. Varied fungi were isolated from the stale smoked crayfish and prawns purchased from the different locations. Table 1 presents the fungal load of the fungi isolated from the stale smoked crayfish and prawns. The mean fungal load of the stale smoked crayfish samples ranges from 3.00×10^5 cfu/g – 12.30×10^5 cfu/g. The crayfish samples gotten from Sabo market have the highest level of fungal contamination with a fungal load of 12.30×10^5 cfu/g, followed by Orita markets with a fungal load of 7.00×10^5 cfu/g and Sayedero markets with the least fungal load of 3.00×10^5 cfu/g. The mean fungal load of the stale smoked prawn samples ranges between 6.66×10^5 cfu/g and 7.33×10^5 cfu/g. The prawn samples gotten from Orita market have the higher level of fungal contamination with a fungal load of 7.33×10^5 cfu/g than Sayedero markets with a fungal load of 6.66×10^5 cfu/g which is the same with Sabo market's with a fungal load of 6.66×10^5 cfu/g too. The fungal load ranges reported are higher than the result of Olaoye *et al.* (2014) that recorded a range of 8.00×10^2 cfu/g – 1.68×10^4 cfu/g in their study.

Table 1: Fungal Load of Stale Smoked Crayfish and Prawns

Location	Fungal Load			
	10^{-5}	10^{-5}	10^{-5}	Mean Fungal load
OC	8	8	5	7.00
OP	10	8	4	7.33
SC	5	3	1	3.00
SP	10	6	4	6.66
SaC	20	13	4	12.30
SaP	10	8	2	6.66

Keys: OC = Orita Crayfish, OP = Orita Prawn, SC = Sayedero Crayfish, SP = Sayedero Prawn, SaC = Sabo Crayfish, SaP = Sabo Prawn

Table 1 shows the fungal load of stale smoked crayfish and prawns purchased from the three different markets in Ilaro, in triplicates. Crayfish samples obtained from Sabo market have the highest fungal load (12.30×10^5 cfu/g), followed by Orita market samples and then Sayedero market samples. Prawn samples obtained from Orita market have a higher fungal load than Sayedero market samples and Sabo samples with the same value. Table 2 presents the morphology and microscopy of the isolated fungal species. A total of 4 different fungal species were isolated from the crayfish and prawn samples gotten from Orita market, Sayedero market and Sabo market which are *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus fumigatus* and *Rhizopus nigricans*.

Table 2: The Fungal Isolates from the Stale Smoked Crayfish and Prawns

S/N	Morphology	Microscopy	Probable Organism
OC1	Black mould characterized by dark spores	Colonies are jet-black, globular or spherical, profuse conidia, conidia are biserial	<i>Aspergillus niger</i>
OC2	Green mould with velvety-powdery growth	Septate hyphae, short conidiospore, flask-shaped vesicles, uniseriate phialides and columnar conidia	<i>Aspergillus fumigatus</i>
OP1	Whitish to brownish cotton colony	Branching mycelia with hyphae and unbranching sporangiophores	<i>Rhizopus nigricans</i>
OP2	White mould	Multicellular distinctive sickle-shaped macroconidia, aseptate, floccose with sparse mycelia	<i>Fusarium oxysporum</i>

OP3	Black mould characterized by dark spores	Colonies are jet-black, globular or spherical, profuse conidia, conidia are biseriate	<i>Aspergillus niger</i>
SC1	White mould	Multicellular distinctive sickle-shaped macroconidia, aseptate, floccose with sparse mycelia	<i>Fusarium oxysporum</i>
SC2	Whitish to brownish cotton colony	Branching mycelia with hyphae and unbranching sporangiophores	<i>Rhizopus nigricans</i>
SP1	Surface is granular in texture with whitish colouration that rapidly turned black at the centre	Colonies are jet-black, globular or spherical, profuse conidia, conidia are biseriate	<i>Aspergillus niger</i>
SP2	White mould	Multicellular distinctive sickle-shaped macroconidia, aseptate, floccose with sparse mycelia	<i>Fusarium oxysporum</i>
SaC1	White mould	Multicellular distinctive sickle-shaped macroconidia, aseptate, floccose with sparse mycelia	<i>Fusarium oxysporum</i>
SaC2	Surface is granular in texture with whitish colouration that rapidly turned black at the centre	Colonies are jet-black, globular or spherical, profuse conidia, conidia are biseriate	<i>Aspergillus niger</i>
SaP1	Whitish to brownish cotton colony	Branching mycelia with hyphae and unbranching sporangiophores	<i>Rhizopus nigricans</i>
SaP2	White mould	Multicellular distinctive sickle-shaped macroconidia, aseptate, floccose with sparse mycelia	<i>Fusarium oxysporum</i>

Keys: OC = Orita Crayfish, OP = Orita Prawn, SC = Sayedero Crayfish, SP = Sayedero Prawn, SaC = Sabo Crayfish, SaP = Sabo Prawn

Table 3 shows the fungal isolates gotten from the crayfish are dominated by *Fusarium oxysporum* and *Aspergillus niger* with 33.33 % each, followed by *Rhizopus nigricans* and *Aspergillus fumigatus* with 16.67 % each too. This is closely related to the work of Bassey and Asamudo (2020) that also isolated *Aspergillus niger* in their study. Table 4 shows the fungal isolates gotten from the stale smoked prawn and they were also dominated by *Fusarium oxysporum* with a prevalence of 42.86 %, followed by *Aspergillus niger* and *Rhizopus nigricans* with 28.57 % prevalence each. This is contrary to the result of Olaoye *et al.* (2014) that isolated *Aspergillus niger*, *Rhizopus* sp., *Penicillium* sp., *Aspergillus oryzae*, *Aspergillus flavus*, *Mucor* sp. and *Candida krusei* in their work. They recorded *Aspergillus niger* as the most prevalent fungus (31.03 %) and *Rhizopus* sp. as the least prevalent fungus (6.90 %).

Table 3: The Frequency of Occurrence of the Fungal Isolates from the Stale Smoked Crayfish

Fungal Isolates	No. of Occurrence	Frequency of Occurrence (%)
<i>Fusarium oxysporum</i>	2	33.33
<i>Aspergillus niger</i>	2	33.33
<i>Rhizopus nigricans</i>	1	16.67
<i>Aspergillus fumigatus</i>	1	16.67
Total	6	100

Table 4: The Frequency of Occurrence of the Fungal Isolates from the Stale Smoked Prawns

Fungal Isolates	No. of Occurrence	Frequency of Occurrence (%)
<i>Fusarium oxysporum</i>	3	42.86
<i>Aspergillus niger</i>	2	28.57
<i>Rhizopus nigricans</i>	2	28.57
Total	7	100

Prolonged storage can stimulate the contamination by fungi which in turn could cause the development of food-borne fungal diseases when consumed by immuno-compromised individuals. The presence of these fungi in the crayfish and prawn samples might be due to contamination during handling and storage by the vendors. The contamination might have been due to the condition of storage which might have favoured the growth of the fungi thus isolated.

IV. CONCLUSION

In conclusion, this research work revealed the contamination of stale smoked crayfish and prawns with fungal contaminants. The fungal load was highest in the smoked crayfish and prawn samples gotten from Orita market. The study also concludes that the presence of the fungal contaminants might be attributable to the storage condition which possibly favoured the fungal proliferation. Proper crayfish and prawn handling, processing and storage should be encouraged and adopted. Crayfish and prawns should not be kept unhygienically or for too long before consumption.

REFERENCES

- [1] I. N. Bassey, and N. U. Asamudo, "Comparative studies of fungi associated with sea foods in different wetlands of Akwa Ibom State," *Journal of Aquatic Sciences*, 34(1), 41-47, 2019.
- [2] H. N. Chinivasagam, H. A. Bermner, S. J. Thrower, and S. M. Nottingham, "Spoilage pattern of 5 species of Australian Prawn," *Journal of Af. Food Prod tech*, 5(1), 25 -50, 1996.
- [3] E. D. Fagbohun, A. O. Durojaiye, and O. A. Popoola, "Mycoflora, Proximate Composition and Mineral Analysis during the Storage of Smoked Dried Crayfish (*Penaeus natialis* Shrimps)", *Asian Journal of Fisheries and Aquatic Research*, 4(1), 1-8, 2019.
- [4] Food and Agriculture Organization (FAO), "The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals," Rome, 2018.
- [5] S. Gong, J. L. and R. Sun, "Studies on the reproductive biology of *Procambarus kirschner*. *Freshw. Fish.*", 06, 23-25, 2008.
- [6] D. M. Holdich, and M. David, "A review of astaciculture: freshwater crayfish farming. *Aquat. Living Resour.*", 6, 307-317, 1993. doi: 10.1051/alr:1993032.
- [7] S. Ibrahim, M. A. Rahma, "Isolation and Identification of Fungi associated with date Fruits (*Phoenix dactylifera* Linn.) sold at Bayero University, Kano, Nigeria," *Bayero Journal of Pure and Applied Sciences*, 2(2), 127 - 130, 2009.
- [8] P. Kumar, M. K. Chatli, N. Mehta, P. Singh, O. P. Malav, and A. K. Verma, "Meat analogues: health promising sustainable meat substitutes," *Crit. Rev. Food Sci. Nutr.*, 57, 923-932, 2017. doi: 10.1080/10408398.2014.939739.
- [9] M. Longshaw, "Diseases of crayfish: a review," *J. Invertebr. Pathol.*, 106, 54-70, 2011. doi: 10.1016/j.jip.2010.09.013.
- [10] O. J. Olaoye, A. A. Akinoyemi, O. A. Oduneye, and G. N. Ezeri, "Micro-organisms associated with smoked prawn (*Macrobranchium* spp.) in selected market locations in Abeokuta Metropolis of Ogun State, Nigeria," *Journal of Agricultural Science and Environment*, 14, 109-117, 2014.
- [11] J. O. Orogu, A. E. Aphair, E. I. Oghonyon, "Bacteriological analysis of dried crayfish and stockfish in Ozoro Market," *Indo American Journal of Pharmaceutical Sciences*, 5(6), 5234- 5239, 2018.
- [12] W. S. Otwell, and J. F. George, "A HACCP Program for Raw, Cultured Penaeid Shrimp. Florida Sea Grant College Program," *E/TP-1 P.*, 218, 2010.
- [13] G. Shen, X. Zhang, J. Gong, Y. Wang, P. Huang, Y. Shui, "Transcriptomic analysis of *Procambarus clarkii* affected by "Black May" disease," *Sci. Rep.*, 10, 21225, 2020. doi: 10.1038/s41598-020-78191-8.
- [14] H. H. Willard, "*Instrumental Method of Analysis*," Van Nostrand Co.: New York, 2006, pp. 208.