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Research Article

Assessment of Microbial Characteristics of Processed Palm Weevil "*Rhynchophorus phoenicis*" Larvae Sold in some Market Areas in Bayelsa State, Nigeria

Enetimi Idah Seiyaboh[1], Sylvester Chibueze Izah[2]

[1]Department of Biological Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.
[2]Department of Microbiology, Faculty of Science, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria.
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I N F O

Corresponding Author:
Sylvester Chibueze Izah, Department of Microbiology, Faculty of Science, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria.
E-mail Id:
chivestizah@gmail.com
Orcid Id:
https://orcid.org/0000-0001-5526-006X
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A B S T R A C T

Problem Statement: The larvae of palm weevil (*Rhynchophorus phoenicis*) is commonly processed and consumed as food in the Niger Delta region of Nigeria especially in the rural areas.

Aims and Objective: This study investigated the microbial characteristics of processed larvae of *Rhynchophorus phoenicis* sold in some market areas in Yenagoa metropolis, Bayelsa State, Nigeria.

Methods: Triplicate samples of processed *Rhynchophorus phoenicis* larvae were obtained from vendors in Opolo and Tombia market areas in Yenagoa metropolis, Nigeria. The samples were analyzed following standard microbiological procedures.

Result: Results showed that the total heterotrophic bacteria, *Enterobacteriaceae* bacteria and total fungi counts ranged from 0.85-8.6 x 10^5 cfu/g, 1.18-8.73 x 10^2 cfu/g and 1.52-7.97 x 10^3 cfu/g, respectively. There was statistical deviation ($P<0.05$) among the *Rhynchophorus phoenicis* samples. The microbial isolates found in the samples were *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus, Proteus* and *Micrococcus* species (bacteria), *Aspergillus niger, Aspergillus flavus, Penicillium* and *Rhizopus* species (fungi). The similarity in interaction of the microbial diversity between samples from each vendor ranged from 57.14-82.35% for Sorenson qualitative index and 0.40-0.70 (which is equivalent to 40-70%) for Jaccard index. The similarity is above a critical level of significance of 0.5 or 50% except for samples from vendor A - C using Jaccard index.

Conclusion: Most of the microorganisms isolated especially the fungi could produce toxins and are pathogenic, hence, there is a need to frequently monitor the processed *Rhynchophorus phoenicis* larvae sold in the study area to ascertain its acceptability with respect to microbial quality.

Keywords: Contaminants, Food, Microorganisms, Public Health, *Rhynchophorus phoenicis*

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Introduction

The practice of entomophagy especially the edible ones have a long history in the nutrition of human.[1] Some insects are intentionally consumed. In such a case, they are prepared as a delicacy. This practice is predominantly found in some Africa, Latin America and Asia region of the world, where about 2.5 billion people eat insects as part of their diets.[2] In Africa, the practise of entomophagy is common in Côte d'Ivoire.[2] Nigeria, Ghana, Kenya, Cameroon among others.[3] Most of the insects that are consumed in West Africa are termites, locusts, lepidopteran caterpillars, beetles,[4] crickets, bees, weevils etc. In the Niger Delta region of Nigeria about 20 edible insects belonging to 6 orders; Isoptera, Orthoptera, Coleoptera, Lepidoptera, Hemiptera, Diptera and Coleoptera with the common species being *Macrotermes* and *Zonocerus* species, *Rhinoceros oryctes, Brachytrypes membranaceus, Rhynchophorus phoenicis, Heteroligus meles, Sitophilus oryzae*, *Callosobruchus maculatus*, *Dermestes maculatus*, *Daraba (Sceloides) laisalis*, *Gonimbrasia Belina*, *Apis mellifera*, *Musca domestica*, uncertain species of Cotton stainer, aphids and locust have been reported in literature.[5,6] Among these insects, some of them are consumed unintentionally especially when they occurs in grains such as maize, beans and rice.

The larvae of some insects are used as food in some part of Nigeria. Some of the insects whose larvae are popularly consumed as food in the Niger Delta include palm weevil, *Rhynchophorus phoenicis* which belong to the Curculionidae family[3] and *Oryctes owariensis*,[7] a beetle that belongs to the subfamily Dynastinae (family: Scarabaeidae). These two insects are popularly known as Bayalsa Suya in Bayelsa state, Nigeria.[3,7] There are different methods of preparing edible insects for consumption. These include frying, drying, cooking, roasting and raw form.[3] Of these, frying is the most acceptable form with acceptability level of 92-100% in the Niger Delta.[3]

The larvae of *Rhynchophorus* species are mainly found in decaying Coconut, Raphia and Oil palm truck and they are prepared and consumed by the inhabitants of the area. This may be associated with its protein and other proximate composition content. *Rhynchophorus phoenicis* is exceedingly cherished in many tropical cultures especially in southern Nigeria.[8] They are sold in some market areas in some state capitals in Nigeria. For instance, they are processed, staked and sold in some markets within the Yenagoa metropolis, Nigeria.

Rhynchophorus phoenicis is commonly known as palm weevil.[4,8-11] The proximate composition, minerals, vitamins, amino acids, fatty acid profiles of *Rhynchophorus phoenicis*,[1,8,11] traditional consumption rate[3] have been documented in literature. Bayelsa state is among the areas in the central Niger Delta that the consumption of *Rhynchophorus phoenicis* larvae is high among the rural communities. The picking, processing and marketing of larvae of *Rhynchophorus phoenicis* are a source of livelihood to several families to the indigenous inhabitants of the area.

Microorganisms in ready to eat food is a serious concern to Food microbiologist and public health experts. This is because food is one the major route of contracting foodborne diseases. Some of the microbial pathogens are highly detrimental to the human body. Studies on the microbial quality of the processed *Rhynchophorus phoenicis* have been reported around Okija and Oba Junctions, Anambra State[9] and along Onitsha-Owerri expressway, southeastern Nigeria.[10] But information about the microbial quality of processed larvae ready for consumption in Bayelsa State is scanty in literature. Therefore, this study aimed at assessing the microbial characteristics of processed *Rhynchophorus phoenicis* larvae sold in some market areas in Bayelsa State, Nigeria.

Materials and Methods

Field Sampling

The samples of fried *Rhynchophorus phoenicis* larvae were obtained from five vendors in triplicate in Edepie roundabout (Sample A, B, and C) and Opolo markets (Sample D and E) in Bayelsa State, Nigeria. The samples were collected in the month of August 2017. The samples were packaged in Ziploc bag and microbiological examination was carried out in <24 hours after purchasing the samples.

Sample Preparation

The samples were macerated using a blender. Approximately 20g of the sample was blended in 180 ml of sterile peptone water.[13] Before re-use, the blender was washed with sterile water.

Enumeration of Bacterial and Fungal Counts

Salmonella-Shigella Agar, Nutrient Agar, MacConkey agar and Potato Dextrose Agar were used to enumerate the density of Salmonella-Shigella counts, total heterotrophic bacteria, bacteria of the *Enterobacteriaceae* family and total fungi counts, respectively. The microbial population was determined in line with pour plate protocol previously described by authors.[13,14] About 1.0ml of the serially diluted samples was aseptically plated in prepared Salmonella-Shigella Agar, Nutrient Agar, MacConkey agar and Potatoes dextrose Agar, and then incubated at 37ºC for 24-48 hours (for all bacteria related organisms) and 30ºC for 4 days for fungi. At the end of the experiment, the colonies that emerged were counted and expressed as colony-forming units per gram of the samples. The resultant isolates were isolated into the pure culture before identification.

Identification of the Microbial Isolates

The bacteria isolates were characterized following the

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biochemical test previously described authors.[14,15] The characteristics displayed by the bacteria isolates were compared with those of known taxa.[15,16] The bacteria isolates were also streaked in Kligler iron agar and the characteristics displayed were compared with the identification guide.[15]

Total fungi isolates were identified based on the macroscopic/colonial and microscopic characteristics previously presented by authors.[13,14,17-20] Lactophenol cotton blue stain method previously described by authors[13,14] was used for the identification of the fungi isolates.

Statistical Analysis

SPSS software version 20 was used to carry out the statistical analysis. The data obtained were expressed as Mean±standard deviation. Significant deviation was established using one-way analysis of variance at P=0.05, and means were separated using Duncan statistics. Jaccard index and Sorenson qualitative index[21] was used to determine the microbial diversity similarity between samples from the different vendors, and the critical level of significance is 50% or 0.5 for similarity.

Result

Table 1, shows the microbial density of *Rhynchophorus phoenicis* larvae sold in Bayelsa state, Nigeria. The total heterotrophic bacteria count ranged from 0.85-8.6 x 10^5 cfu/g. Statistically, there was deviation among the samples from different vendors. However, Duncan multiple test statistics showed that there is no significant difference ($p>0.05$) between vendor B and D, and between vendor A and E. The *Enterobacteriaceae* bacteria counts ranged from 1.18-8.73 x 10^2 cfu/g, being significantly different ($p<0.05$) across the samples from the various vendors. Furthermore, mean separation revealed that there is no significant deviation between vendor A and E, and between vendor C and D. The total fungi counts ranged from 1.52-7.97 x 10^3 cfu/g, being significantly different ($p<0.05$) among the vendors. The multiple comparisons showed no significant deviation between vendor A and B and between vendor C and E. Samonella and Shigella counts was not detected in the samples.

Table 2, shows the microbial isolates found in the processed *Rhynchophorus phoenicis* larvae sold in Bayelsa state, Nigeria. The isolates identified in the processed *Rhynchophorus phoenicis* larvae was *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus, Proteus* and *Micrococcus* species (bacteria), *Aspergillus niger, Aspergillus flavus, Penicillium* and *Rhizopus* species (fungi).

Table 1.Microbial density of Rhynchophorus phoenicis larvae sold in some market areas in Yenagoa metropolis, Bayelsa state, Nigeria

Vendors	Total heterotrophic bacteria counts, x 10^5 cfu/g	*Enterobacteriaceae* bacteria counts, x 10^2 cfu/g	Total fungi counts, x 10^3 cfu/g	Salmonella-Shigella counts, cfu/g
A	1.96±0.45b	1.55±0.45a	6.53±1.58c	0.00±0.00
B	0.94±0.11a	8.73±1.29c	7.97±1.47c	0.00±0.00
C	8.60±0.70c	5.07±0.95b	1.52±0.48a	0.00±0.00
D	0.85±0.13a	4.80±0.66b	4.10±0.70b	0.00±0.00
E	2.09±0.19b	1.18±0.03a	1.79±0.30a	0.00±0.00

Data are expressed as mean±standard deviation (n=3); Dissimilar letters (a, b and c) along the column indicate significant alteration according to Duncan statistics.

Table 2.Microbial isolates found in Rhynchophorus phoenicis larvae sold in Bayelsa state, Nigeria

Microorganisms	A	B	C	D	E
Pseudomonas aerugionsa	+	+	+	+	+
Escherichia coli	+	+	+	+	+
Bacillus species	-	-	+	+	-
Staphylococcus aureus	+	+	+	+	+
Proteus species	-	+	+	-	-
Micrococcus species	-	+	+	-	-
Penicillium species	+	+	-	-	+
Aspergillus niger	+	+	+	-	-
Aspergillus flavus	+	+	-	+	-
Rhizopus species	-	+	+	+	+

Note: The isolates were present in at least one of the triplicate samples.

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Table 3.Similarity index of microbial diversity found in the processed Rhynchophorus phoenicis larvae according to each of the vendor

Pair-wise of the vendors	Sorenson qualitative index	Jaccard index (data converted to a percentage)
A – B	80.00	0.67 (67%)
A- C	57.14	0.40 (40%)
A – D	66.67	0.50 (50%)
A – E	72.73	0.57 (57%)
B – C	82.35	0.70 (70%)
B – D	66.67	0.50 (50%)
B – D	71.43	0.56 (56%)
C – D	71.43	0.56 (56%)
C – E	61.54	0.44 (44%)
D – E	72.73	0.57 (57%)

The similarity of the microbial isolates between each of the vendor based on Sorenson qualitative and Jaccard index are presented in Table 3. The similarity interaction between each vendor with regard to the microbial diversity ranged from 57.14-82.35% for Sorenson qualitative index and 0.40-0.70 (which is equivalent to 40-70%) for Jaccard index.

Discussion

The significant variation observed may be associated with variation in handling and hygiene practices by the processors/handlers during processing, packaging and marketing. The microbial population was within tolerable limits (10^4-10^5) for total aerobic bacteria, while the *Enterobacteriaceae* family bacteria counts and total fungi counts were within the acceptable limit of ≤10^3 as specified by the International Commission on Microbiological Specification for Food.[22] Furthermore, the values reported in this study has some similarity with the works of other authors on palm weevils. For instance, a study on microbial characteristics of palm weevil larvae sold at Okija and Oba Junctions, Anambra State had mean value of 6.24x10^4 cfu/g for total viable bacteria counts and 4.1x10^4 cfu/g total viable fungi counts [9]. In a related study, mean total viable bacteria and fungi counts of 1.72 x 10^6 cfu/g and 4.3 x10^2 cfu/g, respectively were reported in roasted *Rhyncophorus phoenicis* sold along Onitsha-Owerri expressway, Nigeria.[10] The slight variation observed could be due to the difference in handling and packaging as well as other anthropogenic activities peculiar to the environment that it is being sold.

From Table 2, *Pseudomonas aerugionsa, Escherichia coli* and *Staphylococcus aureus* were the predominant isolates occurring in at least a sample obtained from each of the vendors. Some of the isolates are of public health importance. The microbial isolates found in this study had some similarity with the ones previously reported in palm weevil (*Rhyncophorus phoenicis*). For instance, *Staphylococcus aureus, Escherichia coli* and *Salmonella* species have been reported in a fresh *Rhynchophorus phoenicis* larva obtained from Mgbo, Oba in Idemili Local Government Area, and *Bacillus subtilis, Pseudomonas aeruginosa* and *Proteus vulgaris* (bacteria), and *Cladosporium* species and *Aspergillus flavus* (fungi) in roasted *Rhynchophorus phoenicis* obtained along Onitsha-Owerri expressway all in Anambra State, Nigeria.[10] In a related study, *Escherichia coli, Staphylococcus aureus,* and *Bacillus* species (bacteria), and *Aspergillus niger, Rhizopus* and *Mucor* species were reported in palm weevil (*Rhyncophorus phoenicis*) sold at Okija and Oba Junctions, Anambra State, Nigeria.[9] Authors have attributed the microbial contamination to ready to eat food to dust during hawking in a busy highway.[9] Some of the isolates are of health concern. Among the isolates with highest occurrence rate in the processed *Rhynchophorus phoenicis* larvae are *Pseudomonas aeruginosa* (which could lead to folliculitis, ecthyma gangrenosum, ventilator-associated pneumonia, bacteremia),[23,24] *Staphylococcus aureus* (which produces toxins that could aggravate the sternness of food poisoning, septic shock, and toxic shock syndrome)[23] and *Escherichia coli* (which could lead to diarrheal illness, urinary tract infections, sepsis, wound infections, dysentery etc).[23,25]

Some of the isolates found in this study have been reported in ready to eat food sold such as meat-pie,[26] sliced fruits,[12] Kunu drink,[27] Zobo drink,[28] garri,[29] smoked fish,[23] suya[30] in some locations in Bayelsa state, Nigeria. Some of them especially fungi are known to produce mycotoxins that could be detrimental to humans. For instance, most species of *Aspergillus* could produce aflatoxins, ochratoxins and sterigmatocystine.[23,31] Specifically, *Aspergillus flavus* produces aflatoxin that contaminates cereals.[32] *Penicillium* species are also known to produced mycotoxins. Authors

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have reported that aflatoxins could have toxigenic, immunotoxigenic, and mutagenic effects on biodiversity including humans.[23,32-35] Generally, the health condition associated with exposure to the isolates identified in this study have been widely reported in literatures.[12,23,27,36]

Apart from the microbial diversity from vendor A-C using Jaccard index in Table 3, the similarity is above the critical level of significance at 0.5 or 50% for similarity. This is an indication the microbial contaminants in the processed *Rhynchophorus phoenicis* larvae sold in the study area are significantly similar. This is because the higher the joint occurrence of the microbial isolates the higher similarity.

Conclusion

The larvae of *Rhyncophorus phoenicis* is one of the species of the class insecta that is consumed as food. This study assessed the microbial characteristics of roasted larvae of *Rhyncophorus phoenicis* sold in some market areas (Opolo and Etegewe junction) in Yenagoa metropolis, Bayelsa state, Nigeria. The study found that there is a statistical deviation in the microbial population of the samples from each of the vendors. The microbial population is within the tolerable level for ready to eat food as specified by the International Commission on Microbiological Specification for Food. Some of the microbial diversity is of public health importance. Hence, there is a need to improve on the handling, preservation, packaging and marketing strategies to advert the potential health concerns associated with the consumption processed *Rhyncophorus phoenicis* larvae in the study area.

Conflict of Interest: None

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