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Microbial Isolates from the Roasted Larva of the Palm Weevil (*Rhynchophorus phoenicis* [F]) from Edo and Delta States of Nigeria

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Abstract: The microbiological assessment of prepared edible larvae of *Rhynchophorus phoenicis* (F) sampled from four different locations of Benin – Sapele Express way in Edo and Delta states was carried out at the Microbiology Research laboratory of the Benson Idahosa University, Benin City, Nigeria. From the assessment, five bacterial genera and three fungal genera were consistently isolated and identified coupled with their percentage frequency of occurrence. Bacteria species isolated were *Staphylococcus aureus* (100%), *Baccilus cereus* (30%), *Escherichia coli* (20%), *Enterococcus faecalis* (45%) and *Pseudomononas aeruginosa* (35%), while the fungal isolates include *Aspergillus niger* (63%), *Mucor species* (21%) and *Rhizopus species* (16%). The mean viable count for bacteria was 6.2 X 10^6 cfu/g and fungi ranged from 6.5 X 10^4 and 1.2 X 10^5 cfu/g. The results indicated high level of contamination by the identified species thus posing serious threat to public health. Improved preparatory practices, cheap as well as easy packaging techniques are solicited for the delicacy not to be a vehicle for transmitting these contaminants which could serve as disease pathogens.

Key words: Roasted Larva, *Rhynchophorus phoenicis*, Microbial Isolates, Nigeria

INTRODUCTION

The larval of the palm weevil (*Rhynchophorus phoenicis*) is cherished as food among many communities in Nigeria and around the world. The larva is commonly called edible worm or maggot and can be seen hawked along major roads and markets in Edo and Delta States. The larva, popularly known as "Edible worm" is a delicacy in many parts of Nigeria and other countries in Africa where it is found. It is known by various names by the different ethnic groups in Nigeria who strongly believe it to have high nutritive value as well as certain pharmaceutical potentials especially its proteineous content (Ande, A.T. 1991). The mode of preparing it for eating differs from one geographical locality to another.

Evaluation of the nutritive value of this larva has been reported. The larvae are nutritious, containing easily digestible proteins, fats, carbohydrates, minerals and vitamins (Meyer-Rochow, V.B., 1978). The larva of this insect (*Rhynchophorous phoenicis*) is able to make up for our dietary imbalance as they form real sources of food for man and other animals (Fasoranti, J.O. 1997).

Larvae of the palm weevil are able to thrive under conditions, provided by the palm whose trunks are rich in vitamins and other nutrients. The nutritional requirements of insects are much the some as those of mammals; water, inorganic ions and essential amino acids. Vitamins of the B group are needed. Those of A and D are not essential and the insect can synthesize ascorbic acid (Vitamin C). They cannot and do not synthesize adequate qualities of cholesterol. Thus, cholesterol can be defined as a vitamin for insects (Barker, T.W. and Worgan, J.T. 1981). The larvae are rich in nutrients and usually have yeast associated with their digestive tract.

Foods are not only of nutritional value to those who consume them but often are ideal culture media for microbial growth. These foods can serve as a vehicle for disease transmission. The entire sequence of food handling; from the producer to the final consumer, micro-organisms can affect food quality and human health and contamination by disease – causing microorganisms can occur at any point in the food handling sequence

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(Prescott, L.M., *et al.,* 1999).

Microbial growth in foods involves successional changes, with intrinsic and extrinsic factors. The intrinsic factors or food related factors include pH, moisture content, oxidation – reduction potential, water activity, physical structure of the food and available nutrients. Extrinsic or environmental factors include temperature, relative humidity, gases present (carbon dioxide, oxygen), and the type and number of microorganism present in the food (Prescott, L.M., *et al.,* 1999).

As a general rule, the type and number of microorganisms present in a finished (prepared) food is influenced greatly by the environment from where the food was originally obtained, the microbial quality of the food in its raw or unprocessed state, the sanitary conditions under which the food is handled and processed (Jay, M.J., 1978). Others include the adequacy of subsequent handling, packaging and storage condition of the product. Examination of a wide range of aerial portions of the oil palm revealed the source of yeast to be the xylem stream since they are consistently seen at the tapping sites (Faparusi, S.I. 1973). The microorganisms commonly associated with insect larvae vary and are related to the microorganisms associated with the palm and sap environments (Hartley, C.W.S., 1977). Identification of the source of microorganisms revealed the back of the male inflorescence since the organisms found on the scrapings of it were also found on the wine except *Pseudomonas* species which were assumed to have been found in the environment of wine unsuitable (Okafor, N., 1978). He reported that lipolytic moulds such as *Aspergillus, Penicillium, Fusarium, Trichoderma, Cunninghamela* species could be isolated from Nigerian palm fruits and sap, but isolated microorganisms from palm fruits of western Nigeria were found to be dominated by *Rhizopus* and *Mucor* species while *Aspergillus* species predominated isolates from mid-western and eastern Nigeria (Kuku, F.O. and Adeniji, M.O. 1976). Other microorganisms isolated were *Micrococcus*, *Mycobacterium*, *Pseudomonas, Bacillus, Leuconostoc, Streotococcus, Lactobacillus, Acetobacter, Corynebacterium, Pediococcus* and *Zynomonas* species (11. Bassir, O. 1962; Okafor, N. 1978).

This present work seeks to assess the microbiological qualities of processed edible worms in the Benin – Sapele axes of Edo – Delta States of Nigeria as most consumers do not process the worms further before consumption.

MATERIALS AND METHODS

Study Area and Larva collection:

The study was carried out in Benin City. Benin City is a cosmopolitan city mostly populated by the Edo indigenes and people from other parts of the world. These maggots are mainly sold by hawkers along the ever busy Benin-Sapele express way without proper form of packaging. Samples from this axis were purchased in batches at interval of six days and their microbial load compared. The batches are represented in Table 1.

Sample Processing:

Pieces of the edible worm samples were transferred into peptone water and incubated at room temperature for two hours.

Isolation and Identification of Micro-organisms:

Subcultures were then made into Macon key agar, blood agar, salmonella-shigella agar and potato dextrose agar. The agar plates were re-incubated at 37^0C for 24 hours.

Plate Reading:

The organisms were identified on the basis of their colonial and morphological features i.e. size, elevation, and color.

Gram Staining:

Colonies of the suspected isolates were gram-stained. Gram positive organisms were recorded as those retaining their primary color which is crystal violet while the gram negative organisms were recorded as those retaining their secondary color which is neutral red.

Oxidase Test:

A smear of the test organism was made on a Whiteman filter paper soaked with oxidase reagent. Oxidase production was indicated by a change of color on the smear to deep purple within five seconds.

Indole Test:

To an overnight broth culture of the organism, few drops of kovac's reagent were added. Indole production was indicated by the formation of a red-pink ring at the surface of the broth.

Citrate Test:

The test organism was cultured into a citrate medium and incubated overnight at 37^0C.

Sugar Fermentation Test:

The test organism was cultured into sugar media (glucose, sucrose, mannitol and doxytol). Sugar fermentation is indicated by change of colour from blue to yellow.

Catalase Test:

A colony of the test organism was emulsified into a drop of hydrogen peroxide (H_20_2). Catalase production was indicated by effervescence.

Coagulase Test:

A colony of the test organism was emulsified into a drop of plasma on a clean glass, slide coagulate production was indicated by formation of clumps.

Motility Test:

This was carried out in a semi-solid medium. About 15ml of the motility agar was dispensed into McCartney bottles, autoclaved and allowed to gel. Using an alcohol sterilized and flamed straight needle, each isolate was inoculated by stabbing to half of its depth and incubated at room temperature for about 48hrs. Growth away from the inoculation was recorded as evidence of motility.

RESULTS AND DISCUSSIONS

The results obtained from this study showed that a wide range of micro-organisms is consistently associated with roasted edible larvae which corroborate with the findings of earlier worker (Fasoranti, J.O. 1997). The results of this investigation revealed micro-organisms from eight different microbial genera. Five of the isolates were found to belong to the bacterial group while the other three were of the fungal group.

Based on cultural, morphological and biochemical characteristics, the bacterial isolates are presented in Table 3. The bacterial isolates consistent were *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Enterococcus feacalis* and *Pseudomonas aeruginosa*.

Table 1: Maggot sampling points

Sampling sites	Symbol
Sapele Roundabout	Batch 1
Expressway 1	Batch 2
Main town	Batch 3
Expressway 2	Batch 4

Table 3: Cultural, Morphological Characteristics of Bacteria Isolate

	Cultural				
characteristics	B1	B2	B3	B4	B5
Surface	Smooth	Rough	Irregular	Smooth	Smooth
Growth	Diffuse	Diffuse	Diffuse	Diffuse	Diffuse
Shape	Circular	Irregular	Circular	Circular	Circular
Margin	Entire	Filamentous	Entire	Entire	Entire
Elevation	Convex	Low convex	Convex	Convex	Convex
Size of Colony	Pin-Point	Spreading	0.5 – 1mm	Regular	Pin-Point
Colour	Yellow/Whitish	Creamy	Grayish	Creamy	Whitish
Optical Character	Opaque	Opaque	Translucent	Opaque	Opaque

Table 3: Continued.

Morphological					
Gram Stain	+	+	-	-	+
Shape	Cocci	Rods	Rods	Rods	Cocci
Cell arrangmenet	Cluster	Single pair	Single Pair	Single Pair	Chain like
Spore Formation	-	+	-	-	-
Motility	-	+	+	+	-
biochemical & sugar fermentation test					
Catalase	+	+	+	+	-
Oxidase	-	+	+	-	NA
Coagulase	+	-	-	NA	NA
Citrate utilization	-	+	+	-	NA
Indole	-	-	-	+	-
Methyl Red	+	-	-	-	NA
Glucose	A	A	-	+	A
Lactose	A	A	-	+	+
Mannitol	A	-	-	+	A
	S. aureus	*B cereus*	*P. aeruginosa*	*E. coli*	*Enterocucus feacalis*

key
A – Acid
AG – Acid & Gas
NA – Not Applicable
+ = Positive
- = Negative

The work also revealed that *Pseudomonas aeruginosa, micrococcus spp, Proteus spp, Aspergillus niger, Penicillum oxalicum* and *Fusarium oxysporium* are normal flora of the larvae and this is in agreement with previous findings (Kuku, F.O. and Adeniji, M.O. 1976). They are present in the substratum and are saprophytic in soil, water, sewage and air. These microorganisms are likely to have a good start in reaching and colonizing exposed larvae. Therefore they are expected to play an important role in spoilage of larvae and may be pathogenic when given necessary conditions, such as factors that enhance their excessive multiplication.

Table 4 shows the isolated fungi based on their cultural and morphological characteristics. The isolated and identified fungi are *Aspergillus niger, Rhizopus* species and *Mucor* Species. Fungal isolates consistent in this work concurred with previously published research which asserted that *Aspergillus niger, Mucor species* and *Rhizopus* species are fungi predominant in the mid-western and eastern Nigeria (Okafor, N. 1978; Kuku, F.O. and Adeniji, M.O. 1976).

The viability count results presented in Table 5 for total viable Bacterial counts and Table 6 for total viable fungal count on PDA indicate that as dilution factor correlate positively with microbial population.

Table 3: Cultural and Morphological features of fungal isolates

Isolate	Colour	Surface	Hyphal type	Spore Forming
Aspergillus niger	Brownish	Fluffy	Septate	Conidiophoroes
Rhizopus	Brownish	Cottony	Non-septate	Clamydiophores
Mucor	Whitish	Cottony	Non-septate	Clamydiophores

Table 4: Total Viable Aerobic Bacterial Count

Batch 1		Batch 2		Batch 3	
Dilutions	Bacterial (CFU)	Dilutions	CFU	Dilutions	CFU
10^{-1}	TNTC	10^{-1}	TNTC	10^{-1}	TNTC
10^{-2}	TNTC	10^{-2}	TNTC	10^{-2}	TNTC
10^{-3}	8.3×10^{3}	10^{-3}	0.2×10^{3}	10^{-3}	9.9×10^{3}
10^{-4}	7.7×10^{4}	10^{-4}	8.8×10^{3}	10^{-4}	8.0×10^{4}
10^{-5}	6.9×10^{5}	10^{-5}	7.4×10^{5}	10^{-5}	7.0×10^{5}
10^{-6}	5.8×10^{6}	10^{-6}	6.6×10^{6}	10^{-6}	6.5×10^{6}
10^{-7}	4.1×10^{7}	10^{-7}	5.4×10^{7}	10^{-7}	5.9×10^{7}
10^{-8}	2.7×10^{8}	10^{-8}	3.9×10^{8}	10^{-8}	5.0×10^{8}
10^{-9}	1.9×10^{9}	10^{-9}	1.8×10^{9}	10^{-9}	3.9×10^{9}

The presented frequencies of occurrence of isolates in Figures 1 and 2 reveals that *Staphylococcus aureus* occurred in all the specimens investigated while *Bacillus cereus* had the lowest occurrence. On fungal isolates, *Aspergillus niger* was most frequent while *Mucor species* was least as presented in figure 2.

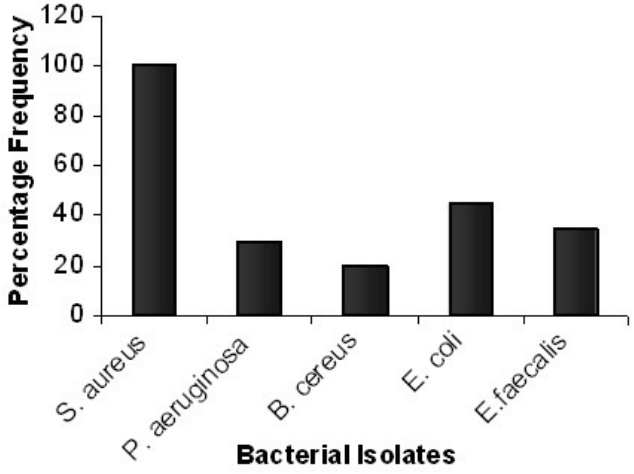

Fig. 1: Percentage Frequency of Bacterial Isolated.

The presence of *Enterococcus feacalis* may indicate faecal contamination probably through handlers who are most of the time along the roads and use neighbouring bushes as their toilet and do not wash up properly after use.

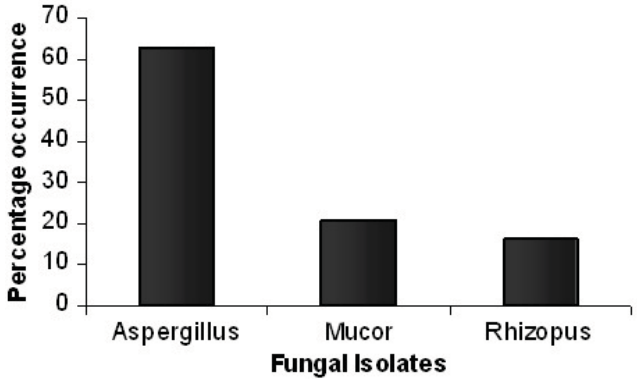

Fig. 2: Percentage Frequency of occurrenence of Fungal Isolates.

Table 5: Total Fungal Count on PDA:

Batches	Dilutions	Fungal count
1	10^{-3}	120 X 10^{-3}
2	10^{-3}	80 X 10^{-3}
3	10^{-3}	65 X 10^{-3}

The presence of *Bacillus cereus* is not unexpected since it is present in large numbers in the soil and is able to survive long periods in the environment due to the production of spores. Its presence in food is of great concern since it causes food poisoning by production of an enterotoxin on ingestion of the spores (Jawetz, J.L. and Adeberg, E.A. 1984).

Although the micro organisms isolated are part of the normal flora of the larvae, palm and palm sap, the quantity in which they occur is of public importance. Of the isolates, only *Staphylococcus aureus* can be said to be a non-floral microbe of the larvae, palm and palm sap. This is an indication that the preparatory methods, handling and sale have loopholes for which the delicacy is contaminated.

Also of significant is the relative density in which the different isolates occurred. An organism that is a normal flora may become virulent if the factor of population is enhanced. The result of the present work showed that populations were greatly enhanced as could be shown from the serial dilution results. This again would lead to increased food poisoning and also enhancement of infectious diseases as pathogens can easily be transmitted by merely eating such unwholesome foods.

This calls for public health awareness, improved methodology and adequacy in collection of larvae and better sanitary handling of larvae during retail. The chief purpose of microbiological standards is to give assurance among others so that the food being consumed will not be an avenue for spread of infectious disease or food poisoning (Ikene, 1990). It is hoped therefore that, food packaging experts should seek to advance improved packaging techniques, which would be cheap and easy to explore by local industries to increase the acceptability of the delicacy.

REFERENCES

Ande, A.T. 1991. Some aspects of the biology of insects (Lepidoptra: Saturnidae, Coleoptera: curculionidae). Nigeria Journal of Entom., 24: 106-129.

Meyer-Rochow, V.B., 1978. The Diverse Uses of Insects in Diverse Traditional Societies. *Ethnomed*., 31: 287-308.

Fasoranti, J.O., 1997. The Value of African Insects as Human Food Supplements. Jour. Sci. and Tech., 10(2): 1-5.

Barker, T.W. and J.T. Worgan, 1981. The utilisation of palm oil processing effluents as substrate for microbial protein production by the fungus Aspergillus oryzae. Eur. J. Appl. Microbio. & amp: Biotechn., 11(4) :234-240.

Prescott, L.M., J.P. Hartley and D.A. Klein, 1999. *Microbiology*. McGraw-Hill Publishing, New York, 96.

Jay, M.J., 1978. Modern Food Microbiology 2nd Ed. van Nostrand Reinhold Pub. Co. Berkhire., pp: 479.

Faparusi, S.I., 1973. Origin of the Initial Microflora of Palm Wine from Oil Palm Trees (*Elaesis guineensis*). J. Appl. Bact., 36: 559-565.

Hartley, C.W.S., 1977. Diseases and Pests of the Oil Palm. *The Oil Palm*. 2nd ed. Longman, London., pp: 607.

Okafor, N., 1978. Microbiological and Biochemistry of Oil Palm Wine. Adv. Appl. Microbial., 24: 237-256.

Kuku, F.O. and M.O. Adeniji, 1976. The Effect of Moulds on Quality of Nigerian Palm Kernel. International Biodeteriorration Bulletin., 12: 37-41.

Bassir, O., 1962. Observations on the Fermentation of Palm Wine. West African Journal Biol. Appl Chem., 6: 20-25.

Jawetz, J.L. and E.A. Adeberg, 1984. Review of Medical Microbiology. Large Medical Publications., pp: 153-216.

Ikene, A.I., 1990. Meat Science and Technology a Comprehensive Approach. (AF) African FEB Publishers Limited, Ibadan., pp: 267.