MICROBIOLOGICAL QUALITY OF RAW AND ROASTED AFRICAN PALM WEEVIL (*RHYNCHOPHORUS PHOENICIS*) CONSUMED IN THE SOUTH EASTERN NIGERIA

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ABSTRACT

The level of microbial contamination of African palm weevil (Rhynchophorus phoenicis) was carried out to assess the health implications of consumption of the larva in raw and roasted forms. Raw Rhynchophorus phoenicis larva collected from rotting palm at Mgbo, Oba in Idemili Local Government Area and roasted Rhynchophorus phoenicis purchased along Onitsha-Owerri expressway all in Anambra State, Nigeria were used for the study. Streak method was used in the assessment of the microbial load in the raw Rhynchophorus phoenicis whereby fluid from intestinal content was inoculated to Nutrient and MacConkey agar and incubated at 37°C for 48 hours, while those on Sabaroud agar were incubated at room temperature for five days. The roasted ones were milled before plate count method was applied. In this method one tenth milliliter (0.1ml) of the 4th part of diluents produced after serial dilution to the concentration of 10^{-6} was aseptically inoculated into MacConkey agar (3 plates), Nutrient agar (3 plates) and Sabaroud agar (3 plates) respectively. The result showed three species of bacteria: Staphylococcus aureus, Escherichia coli and Salmonella spp. in the live APW and three species of bacteria: Bacillus subtilis, Pseudomonas aeruginosa and Proteus vulgaris as well as two species of fungi: Cladosporum spp. and Aspergillus flavus in the roasted Rhynchophorus . Total bacterial count in the roasted Rhynchophorus phoenicis was 1.72 x 10^6 CFU/g while Total fungal count was 4.3 x10^2 CFU/g. Rhynchophorus phoenicis though reported to be highly nutritious in terms of amino acid profile and presence of unsaturated fatty acid can be a source of food poison if not properly handled in sanitary manner during collection, processing and post processing period.

Keywords: African palm weevil, *Rhynchophorus phoenicis, Staphylococcus aureus, Escherichia coli, Salmonella* spp., *Bacillus subtilis, Pseudomonas aeruginosa, Proteus vulgaris, Cladosporum* spp., *Aspergillus flavus*

INTRODUCTION

The concept of food safety has evolved in the developed worlds over the past few years owing to food related diseases, but most of the regulations to ensure food safety are yet to be adopted in many African countries including Nigeria.

Control over the occurrence of potential hazard in the food supply is a *sine qua non* in increasing the consumer confidence in the safety of food. A food material adjudged to be a delicacy can be a pot of poison if it contains disease causing pathogens. It becomes imperative to assess microbiological qualities of food relished by humans to ensure that the

nutritive quality not devalued by its microbial content.

The African palm weevil (APW) – *Rhynchophorus phoenicis* Fabricius 1801 is highly cherished in many parts of the country to the extent that it is called by various descriptive names in many parts of the country; Akwa Ibom State – *Nten,* Edo State (*Bini*) – *Orhu*, Edo State (*Eshan*) – *Okhin*, Delta State (*Itsekiri*) – *Ikolo*, Delta (*Urhobo*) – *Edon*, Delta (*Isoko*) – *Odo*, Oyo State (*Oyo*) – *Awon*, Osun State (*Ilesha*) – *Ekuku*, Anambra State (most parts) – *Akpa ngwo*, Anambra State (Ihiala LGA) – *Nza*, Abia State – *Eruru ngwo*, Benue State (*Idoma*) – *Eko-ali* (Ekpo and Onigbinde, 2005).

Several reports in literature expressed the nutritional and medicinal value of *Rhynchophorus phoenicis* larvae (Ekpo and Onigbinde, 2004; Ekpo and Onigbinde, 2005; Banjo *et al.,* 2006; Edijala *et al.,* 2009; Nzikou *et al*., 2010; Womeni *et al*., 2012; Ebenebe and Okpoko, 2014). Ebenebe and Okpoko (2014) reported that *R. phoenicis* compares favourably with other meat proteins as it contains 21.1% crude protein, 65.2 % lipid and 5.2% ash. Banjo *et al.* (2006) reported crude protein content of 28.42%. Amino acid profile of *R. phoenicis* larvae reported by Womeni *et al.* (2012) showed that the larva contained all essential amino acids (EAA); lysine, valine, leucine, isoleucine, phenylalanine, threonine and methionine. According to them EAA like lysine and threonine normally deficient in grain and cereals were in high concentration in the larvae but low when compared to reference value for humans (FAO/WHO, 1991), while the other with the exception of tyrosine and phenylalanine were in high concentrations. The presence of essential fatty acids linoleic and linolenic acids further elucidate the fact *R. phoenicis* is a highly nutritious food material. Igwe *et al.* (2011) also reported appreciable amounts of Vitamin B3 (Niacin) and B1 (Thiamine) in addition to Vitamin A and C in the termite (*Macrotermes nigeriensis*). Apart from the nutritional value, Ekpo and Onigbinde (2004) reported the medicinal value of *R. phoenicis* on the basis of the high iodine value of the larval oil, which they stated is an index of the degree of un-saturation of the larval oil and its usefulness in the prevention of arteriosclerosis and other heart related diseases.

APW larval oil was also reported to have high pharmaceutical potentials based on the specific gravity and refractive index. According to Ekpo and Onigbinde (2004), *R. phoenicis* larval oil has specific gravity and refractive index lower than arachis oil, linseed oil and olive oil, meaning that the larval oil more may be valuable in the pharmaceutical industries.

However the food value of APW can only be appreciated if the hazard analysis in the process of its collection, processing and preparation underscores the larva as a safe food for human nutrition. Several reports have indicated that food borne pathogens are responsible for death of human and animals in several food related diseases (ICSMF, 1986; Siame *et al.,* 1996; Voetsch *et al.,* 2004; Yu *et al.,* 2005; EFSA, 2008, Majawicz *et al.,* 2010; Hald *et al.,* 2012; Rafal *et al.,* 2012). It is therefore important to assess the microbiological quality of the APW in two forms (raw and roasted) in which it is consumed in Nigeria. Estimation of microbial numbers in food is frequently used in the assessment of microbiological quality of food or to validate the presumptive "safety" of foods. This procedure requires that samples are taken of the food, microbiological tests or analyses are performed and the results evaluated to ascertain the health risks of the food.

MATERIALS AND METHODS

Raw *Rhynchophorus phoenicis:* Live *R. phoenicis* larva used in the study were collected from rotting raffia palm at Mgbo (swampy land with a stretch of river and small tributaries running through its surrounding), Oba in Idemili Local Government of Anambra State. The larva were collected together with the frass (chewed up palm pith) from three different rotting raffia palm and they were placed in a well aerated plastic bucket before being transported to the Entomological Unit of the Department of Zoology for identification using the key of Giblin-Davis *et al.* (2013). The samples were later taken to Microbiological Laboratory for microbiological analysis by Dr. I. F. Okonkwo a

microbiologist in Department of Agricultural Engineering and Bioresources. Only streak method was applied for the live larva. Nine larvae were randomly picked from the samples from each of the three different rotting palms. Each of the larva was cut open with lancets and the fluids from the intestinal contents aseptically inoculated into nine prepared plates, three containing MacConkey agar, three Nutrient agar and three Sabaroud agar respectively using wire loop. The plates containing Nutrient agar and the inoculums, as well as those with MacConkey agar and inoculums were incubated at 37^{o}C for 48 hours while the remaining three plates with Sabaroud agar and inoculums were incubated at room temperature for five days.

Roasted *R. phoenicis:* The roasted *R. phoenicis* were purchased from stationed sellers along Oba – Nnewi new road around Oba junction in Anambra State, Nigeria. Extraneous materials including pepper and other condiments used in the preparation were removed from the sample and they were spread out to sundry to some extent to make it crispy before milling. Twenty grammes of the milled larva was measured out and mixed with 180 ml sterile distilled water. One milliliter portion was aseptically transferred into 9ml distilled water and serial dilution continued until concentration of 10^{-6} was obtained in accordance with Braide and Nwaoguikpe (2011). One tenth milliliter (0.1ml) of the 4th part was then aseptically inoculated into the MacConkey agar (3 plates), Nutrient agar (3 plates) and Sabaroud agar (3 plates), respectively. Inoculums were spread evenly over the surface of the agar plates using a sterile spreader (Braide and Nwaoguikpe, 2011). The nutrient and MacConkey agar plates (three for each) were incubated at 37^{o}C for 48 hours while the Sabaroud agar plates were incubated at room temperature for seven days.

Microbial Counting: On establishment of colonies in the plates containing roasted APW, the number of colonies was counted using an electronic counter. The mean count for each triplicate plate was obtained multiplied with the dilution factor to obtain the total viable cells per unit weight of the sample expressed as the colony forming unit per gram (CFU/g) of the sample (Cheesebrough, 2000).

Characterization of Bacterial Isolates: Colonies of bacterial isolates were subjected to colonial characterization using the methods described by Pelczar *et al.* (1993) and Cheesebrough (2000). Further characterization was based on microscopic and biochemical methods (Pelczar *et al.,* 1993; Cheesebrough, 2000). Cultures of fungi isolated only in the roasted *Rhynchophorus* larva were identified on the basis of macro and micromorphology, reverse and surface colouration of colonies in accordance with McCance (1990) and Abbey (2007).

RESULTS

The result obtained from the microbiological analysis of the raw/live *R. phoenicis* larva showed only bacterial contamination including *Staphylococus aureus, Escherichia coli* and *Salmonella* species. No fungus was detected. The colonial characteristics of the isolates are presented in Table 1.

Table 1: Colonial characteristic of bacteria isolated in the fresh *Rhynchophorus phoenicis* larva

Colony	Agar Plate	Colonial morphology
A	Nutrient agar	Circular, moderately large, convex, shiny, light yellow colonies
B	Nutrient agar	Circular, small, raised, smooth, shiny white colonies
C	MacConkey agar	Smooth colourless colonies

The microscopic and biochemical characterization of the bacteria isolated are presented in Tables 2 and 3, respectively. Microbiological analysis of the roasted *R. phoenicis* larva on the other hand showed both bacterial and fungal contamination. Bacteria species isolated included *Bacillus subtilis, Pseudomonas aeruginosa* and *Proteus vulgaris*, while the two species of fungi isolated were *Cladosporum sp. and Aspergillus flavus.* Total bacterial count was 1.72 x10^{6} CFU/g and Total fungal count 4.3 x 10^{2} CFU/g respectively.

Table 2: Microscopic characteristics of bacterial isolates in fresh *Rhynchophorus phoenicis* larva

Colony	Mot	Gram rxn	Flagella
A	-	+	-
B	+	-	+
C	+	-	+
	Cell morphology		**Most probable identity**
A	Oval cells in clusters		*Staphylococcus aureus*
B	Short rods		*Escherichia coli*
C	Short rods		*Salmonella* spp.

Table 3: Biochemical characterization of bacterial isolates in fresh *Rhynchophorus phoenicis* larva

Colony	Catalase	Oxidase	Coagulase	H_2S
A	+	-	+	-
B	-	-	-	-
C	+	-	-	+
	Urease	**Indole**	**Citrate**	**ID of Isolate**
A	-	-	+	*Staphylococcus aureus*
B	-	+	-	*Escherichia coli*
C	-	-	+	*Salmonella* spp.

Table 4: Total heterotrophic counts and colonial characteristic of bacteria isolated in the roasted *Rhynchophorus phoenicis* larva

Colony	Agar Plate	Colonial morphology	MPI
D	Nutrient agar	Large but short, wavy, rough, dull white, single rods	*Bacillus* spp.
E	Nutrient /MacConkey agar	Small, short, single rods, bluish green on nutrient agar, pale yellowish on MacConkey agar	*Pseudomonas* spp.
F	Nutrient /MacConkey agar	Circular, Smooth cream coloured raised	*Proteus* spp.

Total heterotrophic Count 1.72 X 10^6CFU/g MPI = Most Probable Identity

The total heterotrophic counts and colonial characteristic of bacteria isolated in the roasted *Rhynchophorus phoenicis* larva indicated the presence of *Bacillus* spp. *Pseudomonas* spp. and *Proteus* spp. on Nutrient and MacConkey agar (Table 4).

Microscopic characteristics of bacterial isolates in roasted *Rhynchophorus phoenicis* larva showed the presence of oval cells in clusters of *Bacillus* spp. and short rods of *Pseudomonas* spp. and *Proteus* spp., respectively (Table 5). The biochemical characteristics of bacterial isolates in roasted *Rhynchophorus phoenicis* larva indicated the catalse and H_2S in all the bacterial isolates, citrate in *Bacillus subtilis* and *Pseudomonas aeruginosa* and urease and indole only in *Proteus vulgaris* (Table 6). The microscopic characteristic of fungal isolates in the roasted *Rhynchophorus phoenicis* Larva indicated the presence of *Cladosporum* spp. and *Aspergillus flavus* (Table 7).

DISCUSSION

The result obtained from the microbiological analysis of live and roasted African Palm weevil (APW) *Rhynchophorus phoenicis* indicates the presence of three species of bacteria; *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. in the live APW and three species of bacteria; *Bacillus subtilis, Pseudomonas aeruginosa* and *Proteus vulgaris* as well as two species of fungi; *Cladosporum* spp. and *Aspergillus flavus* in the roasted *Rhynchophorus*. Total bacterial count in the roasted APW was 1.72 x 10^6 CFU/g while Total fungal count was 4.3 x10^2 CFU/g.

Staphylococcus aureus in ready to eat food is usually as a result of human contamination through improper handling during preparation. *Salmonella* presence in food is a useful indicator of poor hygiene and post processing contamination of food. Although the total viable count was not obtained in this study, ICMFS (1986) noted that a level greater than 104 per gram in ready to eat foods is unacceptable as it is an indication that contamination has occurred. Absence of *Salmonella* in the roasted APW may be due to the effect of heat treatment. *Salmonella* is one of the most important food borne zoonotic pathogens with significant health and economic impacts in humans and animals (Voetsch *et al.,* 2004). Majawicz *et al.* (2010) reported that non typhoid *Salmonella* is the leading cause of food borne

illness, estimated to be implicated in 93.8 million cases of gastroenteritis globally. *Salmonella* can be eliminated by heat treatment depending on treatment time, temperature and extent of moisture content during cooking as considerable resistance to heat is observed in dry materials particularly if the material is wrapped by lipids as in the APW (EFSA, 2008).

Table 5: Microscopic characteristics of bacterial isolates in roasted *Rhynchophorus phoenicis* larva

Colony	Mot	Gram rxn	Flagella
D	+	+	+
E	+	-	+
F	+	-	+
	Cell morphology	**Most probable identity**	
D	Oval cells in clusters	*Bacillus subtilis*	
E	Short rods	*Pseudomonas aeruginosa*	
F	Short rods	*Proteus vulgaris*	

Mot = Motility, Gram rxn = Gram reaction

Table 6: Biochemical characteristics of bacterial isolates in roasted *Rhynchophorus phoenicis* larva

Colony	Catalase	Oxidase	Coagulase	H_2S
D	+	-	-	+
E	+	+	-	+
F	+	-	-	+
	Urease	**Indole**	**Citrate**	**ID of Isolate**
D	-	-	+	*Bacillus subtilis*
E	-	-	+	*Pseudomonas aeruginosa*
F	+	+	-	*Proteus vulgaris*

Table 7: Microscopic characteristic of fungal isolates in the roasted *Rhynchophorus phoenicis* larva

Colony	Colonial morphology	Most Probable
G	Conidia borne of short branched clusters with varying sizes and shapes. Conidiophores had no swelling.	*Cladosporum* spp.
H	Un-branched conidiophores, Swelling at the apex, Conidia were borne on short chain of sterigma	*Aspergillus flavus*

Total heterotrophic Count 4.3 X 10^2CFU/g

Escherichia coli presence in the live APW is also undesirable as many young people and some communities that use the APW for medicinal purposes eat it raw, uncooked and unprocessed in any form. ICSMF (1986) reported that *E. coli* is not supposed to be detected in foods, only levels less than 3 per gram is given satisfactory category. *Escherichia coli* infection is associated with eating improperly cooked or undercooked food. *Escherichia coli* infection can cause disease such as urinary tract infection, bacteraemia and meningitis.

Bacillus subtilis observed in roasted APW is also associated with food borne disease. *Bacillus subtilis* is an endophore that predominates in the soil (Braide and Nwaoguikpe, 2011). ICMFS (1986) showed that *B. cereus* in cooked food is as a result of inadequate temperature controls. Levels greater than or equal to 10^4CFU/g is considered potentially hazardous.

The presence of *Pseudomonas aeruginosa* in roasted APW is also undesirable, though Kruick (2013) noted that *P. aeruginosa* is a common environmental organism that poses no health risk to healthy people, he also indicated that most severe infection occurs in people who are already hospitalized with another disease condition. Besides, the presence of *P. aeruginosa* will reduce the nutritional and eating quality of the weevil as Nester *et al.* (1998) reported that *P. aeruginosa* produce protease and lipases that can catalyze reactions leading to degradation of proteins and fats, thus an undesirable flavor of the food product.

Proteus vulgaris are pathogens that degrade food with high protein content. Braide and Nwaoguikpe (2011) noted that they do not give rise to food borne diseases but they lower the nutritive value of foods. The total viable count of 1.72 x 10^6CFU/g suggests contamination and health risk to consumers.

The observation of two species of fungi in the roasted APW; *Cladosporum* spp. *and Aspergillus flavus,* is not out of place because the weevils are sold uncovered in an open place and the unsold larva are warmed for sale in subsequent days. *Cladosporum* is a cosmopolitan organism with its spores found in the air, water and soil. They cause deterioration and spoilage of foods. Rafal *et al.* (2012)

showed that *Cladosporum* spp. dominate 80% of spores in the air in various parts of Europe. *Cladosporum* spp. can cause allergic reactions in human which sometimes lead to asthma.

Aspergillus flavus are well known producers of aflatoxin whose primary target is the liver and they are potent carcinogens, mutagens and teratogens, thus they are acutely toxic to man and animals (Siame *et al.* 1996). Yu *et al.* (2005) reported that *Aspergillus flavus* are the cause of invasive and non invasive aspergillosis in humans, animals and insects, and it causes allergic reactions in man.

Rhynchophorus phoenicis though rich with proteins, essential unsaturated fats, vitamins and minerals could pose health risks for consumers in the forms in which it is presently consumed, and this may negate the plans for the promotion of entomophagy in this part of the world. The use of spices like garlic (*Allium sativum*) and Negro pepper (*Xylopia aethiopica*) which have anti microbial properties in the preparation of roasted APW though useful in controlling bacterial contamination, other hygienic procedures like washing before roasting, salting and use of preservatives that will control fungal growth, as well as adequate packaging to prevent exposure to fungal spores must be adopted to make the roasted APW fit for human consumption.

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