MICROFLORA OF THE SHELLS OF READY-TO-EAT CONOPHOR *(Tetracarpidium conophorum)* NUTS ON RETAIL SALE IN OWERRI

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Abstract: A study was undertaken to determine the bacterial population and diversity of the shells of ready-to-eat conophor *(Tetracarpidium conophorum*) nuts retailed in Owerri, Imo State, Nigeria. Fifty samples (5 nuts per sample) of ready-to-eat conophor nuts were purchased from 50 retailers in Ekeonunwo market (EM), Okigwe road motor park (ORMP), Arugo motor park (AMP), New market (NM) and Relief market (RM) and analyzed for total coliform, total aerobic heterotrophic bacteria (TAHB), and the presence of indicator and pathogenic bacteria using standard plate count methods. It was discovered that samples from RM and AMP accounted for the highest and lowest TAHB values of 6.30 and 4.20 logio cfu/g, respectively while total coliform counts in the samples ranged from 1.04 logio cfu/g for RM to 4.18 logio cfu/g for EM. Bacterial species associated with the samples were identified as *Salmonella* sp., *Shigella* sp., *Enterobacter* sp., *Klebsiella* sp., *Bacillus* sp., *Staphylococcus aureus, Lactobacillus* sp., *Escherichia coli*, Diphtheroids and Actinomycetes. The predominant bacterial isolates were *Staphylococcus aureus* and *Lactobacillus* sp. with the later being more predominant. Samples from AMP and ORMP showed *E. coli* contamination. *Salmonella* sp. *and Shigella* sp. were found in samples from AMP, EM and RM. *Enterobacter* sp. *and Klebsiella* sp. were confirmed in samples from NM, RM and EM. The presence of indicator of feacal contamination and pathogenic bacteria in the shells of ready-to-eat conophor nuts poses potential risks to the consuming public. The results emphasized the need for appropriate cooking of the nuts to ensure decontamination, maintenance of good hygienic practices during preservation, selling and handling prior to consumption of conophor nuts.

Key words: Bacterial population and diversity, conophor nut shells, Owerri

INTRODUCTION

Tetracarpidium conophorum, which belongs to the family of Euphorbiaceae is also known as conophor nut (Enujiugha, 2003) and popularly referred to as African walnut by consumers in Nigeria. This perennial climbing shrub, often grown in the moist forest zones of sub-Sahara Africa (Oladiji *et al.*, 2009), is chiefly grown for its kernels (Enujiugha, 2003), which are cooked and consumed as snacks (Oke, 1995) because of their nutritive value (Oke and Fafunso, 1975; Ogunsua and Adebona, 1983; Nwokolo, 1987).

During the rainy season (from the months of April to September) of every year, many inhabitants in Southern Nigeria purchase cooked conophor nuts from hawkers and eat them as snack on transit often without washing their hands before eating or as take-away for relations at home. Most consumers of conophor nuts are not interested in

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Nigerian Journal of Microbiology 2015, 29: 2964-2971
Published online at www.nsmjournal.org

cooking the nuts in their homes. Cooking without dehulling the shells is the only known traditional method of preparing conophor nuts as food. Shells of ready-to-eat conophor nuts are often wet, dark brown-black, unsightly and in no way sterile. To reach the edible kernel, the hard shell is usually cracked with the teeth before separating it from the kernel. It is rather impossible to reach the kernel of conophor nut without transferring some microorganisms from shell to the kernel. Pathogenic bacteria were implicated in twelve types of edible nuts including groundnut and walnut which were unhygienically prepared with or without their shells and retailed without packaging in Diyarbakir, Turkey (Vural and Erkan, 2008). Also, decades earlier, independent researchers have reported the presence of *Escherichia coli* and other bacteria of public health risks in edible nuts (Kokal, 1965; Hall, 1971; Beuchat, 1973).

Conophor nut has been basically studied with respect to the nutritive value of the nuts (Oke and Fafunso, 1975; Adebona *et al.*, 1988; Akpuaka and Nwankwo, 2000; Enujiugha, 2003; Edem *et al.*, 2009). There is dearth of reports on the microbial quality of ready-to-eat conophor nuts.

Therefore, the objective of this study was to determine the load and identity of the bacteria present in the shells of ready-to-eat conophor nuts retailed in Owerri, Imo State, Nigeria. It also underscored the likely public health hazards associated with the method of removing the nut shells before consumption of the kernel.

MATERIALS AND METHODS

Sample collection

In August 2010, 250 ready-to-eat (cooked) conophor nuts with intact shells were purchased from five different centers (Arugo motor park (AMP), New market (NM), Ekeonunwo market (EM), Okigwe road motor park (ORMP) and Relief market (RM)) in Owerri, Imo State, South-Eastern, Nigeria. In each centre, 10 samples (made up of 5 nuts per sample) were randomly collected from 10 different retailers, who retailed conophor nuts in open wheel barrows, or big trays. The nuts were not packaged and the retailers did not wear protective coverings. Samples were put in sterile plastic bags labeled and taken to the laboratory within 30 min for analysis.

Isolation of bacteria

Ten samples (50 nuts with intact shells) from each sampling centre were gathered together and 50 g portion of this composite sample was aseptically weighed into 450 ml sterile deionized water. This was allowed to stand for 10 min, vigorously shaken and serially diluted (10-fold) using the same diluent. Dilutions were plated out in duplicate on nutrient agar (Antec) and MacConkey agar (Oxoid) for total aerobic heterotrophic bacteria (TAHB) and total coliform counts, respectively, using the spread plate method of Miles and Misra (Collins *et al.*, 2004). Inoculated nutrient agar and MacConkey agar plates were incubated aerobically at room temperature (30 ± 2°C) and 37°C, respectively for 18–24 h. For lactobacilli count, dilutions were plated on de-Man Rogosa and Sharpe (MRS) agar (Lab M) using pour plate method (Collins *et al.*, 2004) and incubated for 3 days at 35°C. Isolated

microorganisms were purified by repeated streaking using the medium and incubation conditions of the original isolation, stored on agar slants at 4°C before being characterized.

Characterization and identification of bacterial isolates

Analyses of the bacterial isolates for colony morphology, cultural appearance, cell micromorphology together with biochemical characterization tests for the identification of the isolates were all carried out according to the methods detailed by Collins *et al.* (2004). The tests carried out include Gram staining, spore staining, motility, production of catalase, oxidase and urease; citrate utilization, oxidative/fermentative (O/F) utilization of glucose. Others include utilization of sugars; indole production; hydrolysis of casein, starch and tween 80; methyl red and Voges Proskaur test; gelatine liquefaction; coagulase production; haemolysis; nitrate reduction; gas production at 44°C; decarboxylation of amino acids; phenylalanine deaminase. Identification of bacteria followed the scheme of Holt (1982).

Pink to red colonies without precipitate from the MacConkey agar plates were streaked onto Eosine methylene blue (EMB) agar (Oxiod) and incubated at 35°C for 24 h. Isolates which appeared on EMB as mucoid, pink, confluent colonies with gray-brown centre in transmitted light were identified as *Enterobacter* and *Klebsiella* and confirmed based on differential test results.

To detect *Salmonella* and *Shigella* species, colourless, translucent colonies from the MacConkey agar plates were streaked onto EMB agar and then *Salmonella Shigella* (SS) agar (Oxoid). Emergent colourless, transparent colonies on EMB agar, which appeared red and dark centred, were selected as species of *Salmonella* whereas those that appeared red and translucent without black centre, were selected as species of *Shigella.*

RESULTS

Total count of bacteria in the Shells

Bacterial count in shells of ready-to-eat conophor nuts from five different centres in Owerri are detailed in Table 1. The range of total aerobic heterotrophic bacteria (TAHB) counts is (log_{10} cfu/g): 4.20–6.30 as recorded in samples from Arugo motor park (AMP) and Relief market (RM), respectively. The range of the total coliform counts obtained from the five centres is (log_{10} cfu/g): 1.04–4.18 for Relief market (RM) and Ekeonunwo market (EM), respectively. On the other hand, the lowest and highest values of lactobacilli counts were recorded by samples from Arugo motor park (AMP) and Relief market (RM), respectively.

Diversity of bacteria in the Shells

Bacteria associated with the samples were identified as *Staphylococcus aureus, Salmonella* sp., *Shigella* sp., *Lactobacillus* sp., *Bacillus* sp., *Escherichia coli, Enterobacter* sp., *Klebsiella* sp., Actinomycetes and Diphtheroids. The distribution of bacteria in shells of cooked conophor nuts from different centres in Owerri are shown in Table 2. *Staphylococcus aureus* and *Lactobacillus* sp. were detected in all the samples with the later being more predominant. Contamination by *E. coli* occurred in samples from Arugo motor park and Okigwe road motor park. *Salmonella* sp.

and *Shigella* sp. were detected in samples from Relief market, Arugo motor park and Ekeonunwo market. *Enterobacter* sp. *and Klebsiella* sp. were confirmed in samples from New market, Relief market and Ekeonunwo market. An average of about six isolates was recovered in samples from each of the five sampling centres. Ekeonunwo market recorded the highest diversity of isolates while the lowest diversity of isolates was observed in samples from Okigwe road motor park and New market.

Table 1. Bacterial population of shells of cooked conophor nuts retailed in Owerri

	Viable counts (log_{10} cfu/g)	
	TAHB	Total coliform
Sampling location		
Ekeonunwo market*	5.40	4.18
New market	4.85	2.00
Arugo motor park	4.20	3.32
Okigwe road motor park	5.15	3.49
Relief market	6.30	1.04

* Same as Owerri main market; TAHB, total aerobic heterotrophic bacteria.

Table 2. Distribution of bacteria in shells of cooked conophor nuts retailed in Owerri

	Sample code*				
Isolate	AMP	EM	ORMP	NM	RM
Escherichia coli	+	–	+	–	–
Salmonella sp.	+	+	–	–	+
Shigella sp.	+	+	–	–	+
Enterobacter sp.	–	+	–	+	+
Klebsiella sp.	–	+	–	+	+
Staphylococcus sp.	+	+	+	+	+
Lactobacillus sp.	+d	+d	+d	+d	+d
Bacillus sp.	+	+	+	–	–
Actinomycetes	–	+	–	–	+
Diphtheroids	–	+	–	+	–

* AMP, Arugo motor park; EM, Ekeonunwo (Owerri main) market; ORMP, Okigwe road motor park; NM, New market; RM, Relief market; +, present; –, absent; d, dominant colonies in nutrient agar plate

DISCUSSION

The presence of aerobic heterotrophic bacteria and coliforms in the samples may be a reflection of the inappropriate cooking of the conophor nuts, unhygienic exposure to open air and unhygienic handling by hawkers' resulting in feacal contamination. The high counts of aerobic heterotrophic bacteria and the presence of coliform as shown in Table 1 place the consuming public at health risk since most of the isolates cause food infection (Frazier and Westhoff, 2000). The bacterial load of the shell of cooked conophor nuts from each location differs. While the load of aerobic heterotrophic isolates ranges from 4.20–6.30 $\log_{10}$ cfu/g, the total coliform ranges from 1.04–4.18 $\log_{10}$ cfu/g of the samples. The latter is slightly higher than the values reported by Adebesin *et al.* (2001) who studied the microbiological quality of some groundnut products hawked in Bauchi. This may be as a result of locational differences, handling and environmental factors. The low count of total coliform recorded for Relief market sample could be as a result of difference in hygienic practices while the high coliform count associated with Ekeonunwo market could be due to high level of feacal contamination. On the other hand, the absence of *E. coli* from the EM, NM and RM samples; *Enterobacter* sp. and *Klebsiella* sp. from the AMP and ORMP samples; and absence of *Salmonella* sp. *and Shigella* sp. from the ORMP and NM samples could be due to proper cooking and prevention of recontamination through hygienic handling . The presence of coliform especially feacal coliform above the acceptable limits in foods have been reported to be significant (Mensah *et al.*, 2002).

The isolation of ten bacterial genera identified as *Escherichia coli, Enterobacter* sp., *Klebsiella* sp., *Salmonella* sp., *Shigella* sp., *Bacillus* sp., *Staphylococcus aureus, Lactobacillus* sp., Actinomycetes and Diphtheroids makes the cooked conophor nuts microbiologically unacceptable for consumption. This is an indication of bacterial contamination resulting from inadequate cooking or post process recontamination from flies, unsterile wrapping material, dust or poor hygienic handling. The Gram reaction shows that the bacterial isolates were both Gram positives and Gram negatives. In a similar investigation, Little *et al.* (2009) reported the same bacterial genera in edible nut kernels samples retailed in United Kingdom. The presence of these bacteria in food samples poses a serious health concern to the consuming public (Vural and Erkan, 2008).

Of all the microbes, *Lactobacillus* sp. was reported as the most dominant bacteria on *T. conophorum* nuts (Table 2). Under favourable ambient temperature of storage of the nuts while still on sale, this lactic acid bacterium would grow, increase in their numbers and produce acid that may be antagonistic to some contaminating organisms in the food. However, in nuts of *T. conophorum* kept beyond 24 h at ambient temperature, higher levels of lactic acid bacteria may result in spoilage (sour and acid odour). In countries with high ambient temperature conditions, street foods kept for a sufficient period of time have been reported to encourage high population of bacteria that may lead to

spoilage and food illness (Bryan and Bartleson, 1985). A similar study on edible nuts reported *Staphylococcus aureus, Lactobacillus* sp. and *Bacillus* sp. as being dominant isolates (Vural and Erkan, 2008). Of significant note is the absence of *Streptococcus* sp. an agent of food spoilage in sample studied

The presence of *Staphylococcus aureus* can be as a result of contamination from the skin, mouth and nose of the nut handlers, which is an indication of poor personal hygiene (Nester *et al.*, 2004). *Staphylococcus aureus* has previously been isolated from walnut kernels (Riyaz-UL-Hassan *et al.*, 2003). *Bacillus* sp. was detected in the *T. conophorum* nut shells and this was not unexpected since the *T. conophorum* nut samples may have been contaminated by *Bacillus* endospores from dust (which may not be destroyed by heat) especially since the nuts are usually wet and are sold and handled at open space. This may also constitute a health risk since *Bacillus* sp. are known to be associated with food poisoning. Although, selective *Bacillus* sp. agar was not used to evaluate the level of *Bacillus* sp. in the samples, a low count of *Bacillus* in foods has been reported to be insignificant (Beumer, 2001).

The identification of *Salmonella* sp. from the sample is of public health concern as the organism is known to cause food poisoning (Nester *et al.*, 2004). However, it is believed that appropriate heat treatment the nuts are subjected to during proper cooking will kill the vegetative cells of the organism.

The presence of *E. coli* in the result indicates feacal contamination of the nuts. The presence of *Enterobacter* and *Klebsiella* which are enteric bacteria also indicates feacal contamination (Nester *et al.*, 2004)

The occurrence of Diphtheroids and Actinomycetes shows that contamination was also from the dust particles may be during exposure to open air in the different local markets, since they are common inhabitants of soil (Nester *et al.*, 2004). The presence of Actinomycetes may be because of endospores, which are highly heat-resistant (Nester *et al.*, 2004). The presence of *Shigella* sp. is unacceptable in ready-to-eat *T. conophorum* nuts because it is a pathogenic bacterium which causes dysentery.

Conclusion

The result obtained in this study demonstrated the microbial diversity and population in the shells of ready-to-eat conophor nuts on retail sales in Owerri, Imo State, Nigeria and supports previous microbiological reports on other ready-to-eat street edible nuts. The presence of feacal coliforms and some pathogenic contaminants in the samples make them microbiologically unacceptable and indicates the need for urgent improvement in the hygienic conditions during the processing and post-processing handling of conophor nuts. Further findings on the risk factors that could pre-dispose ready-to-eat conophor nuts to microbial contamination would assist in the education of food handlers on how to improve the microbial quality of ready-to-eat conophor nuts. Special attention should be given to effective communication on microbiological food risk, proper instruction on handling procedures during and after cooking *T. conophorum* nuts, consumer education on transmission of food borne diseases

and more vigilant monitoring by public health authorities (food inspectors and control staff).

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