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Research Article

Risk Assessment of Grounded Egusi (Citrullus Colocynthis) Sold in Selected Markets in Yenagoa

Opara Christiana Ngozi*

Department of Microbiology, Federal University Otuoke, Bayelsa State, Nigeria.

***Corresponding Authore:** Opara Christiana Ngozi, Department of Microbiology, Federal University Otuoke, Bayelsa State, Nigeria.

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Abstract

This study assessed the microbial quality and risk assessment due to toxic metal contamination of grounded egusi collected from three different markets, (Kpansia, Swali and Tombia) in Bayelsa state. The sample where microbiologically analysed using spread plate technique. Agar dilution method was used to isolate fungi. Havey metal analyses were carried out by atomic absorption spectrophotometer (Agilent, UK). These organisms following the order of predominance were isolated Vibrio parahemolyticus (4.3%), Proteus mirabilis (4.3%), Salmonella typhimarum (8.7%), Shigella spp (8.7%), Enterobacteriaceae (8.7%), Escherichia coli (17.4%), Staphylococcus aureus (13%), Staphylococcus epidermidis (13%), Staphylococcus saprophyticus (4.3%), Bacillus cereus (4.3%), Bacillus megaterium (4.3%),, Enterobacter aerogenes (4.3%), and Vibrio cholera (4.3%). The total viable count of bacteria in ground melon seeds from kpansia market (AKG) was 20.9 X 109 CFU/g, Swalli market (BSG) 9.4 X 109 CFU/g, tombia market (CTG) 15.6 X 109 CFU/g respectivly. The percentage occurrences of fungi isolated were Penicillum chrysogenum(18%), Aspergillus niger (55%), Cladosporium spp (18%), fusarium solani(9%). Level of zinc was higher (1.01-2.09 ppm) than other metals such as chromium (0.01-0.03 ppm), lead (0.01-0.06) and copper (0.02-0.03 ppm). The results of this study suggest that grounded melon seeds accumulate relatively higher amounts of Zn than the other metals studied. This study highlights the need for proper handling of grounded melon seed during processing, storage, and distribution, to safeguard its quality for consumers.

Key Words: grounded melon seeds; microbial activities

Introduction

Melons are major food crops in sub-Saharan Africa and tropical regions. They belong to the Citrullus family which consists of a wide variety eaten as fruits, and their seeds are used in many dishes (FAO, 2007). Melon seeds are used as a major source of ingredients in preparation of a traditional soup called egusi soup (Arthur et al., 2020).

Foodborne illnesses, often caused by the consumption of contaminated food products, pose a significant public health challenge worldwide. Bacterial contamination is a major contributor to such illnesses, and its prevention is crucial to safeguarding consumer health (Lee and Yoon, 2021). Melon seeds, due to their composition and use, can potentially harbor various types of bacteria, including pathogens that might pose health risks when consumed (Giwa and Akanbi, 2020). The process of harvesting, processing, storing, and utilizing melon seeds can introducecontaminants and facilitate bacterial growth, making microbial analysis an essential aspect of ensuring food safety (Riley, et al., 2020).

The fungal load refers to the quantity of fungi present in a melon seed and there maybe diverse of these organisms in the melon seed.In the case of grounded and ungrounded melon seeds sold in the market, understanding the fungal load of these food substance is prerequisite to ensure the products quality and minimize the health risks (Bankole et al., 2004). Melon seeds deteriorate quickly in storage due to fungal infection which may result in decreased nutritive value, color changes, increased peroxide value, reduced seed germination, and mycotoxin production (Bankole et al., 2004).

The accumulation of trace metals compromises the safety of food because they interfere with the proper function of the nervous system, kidneys, and other vital organs in the body. The presence of many of these elements has been reported in many foods. For instance, arsenic, lead, nickel, copper, chromium, and cadmium have been detected in products of fruit and vegetables, cereals, root tubers, and meat (Ofori et al., 2016). Although some of these (e.g., nickel, copper, and chromium) are essential elements, excessive amounts induce toxicity (Senesse et al., 2004). Processing, handling, and distribution of grounded melon seed may affect its chemical and or microbial quality. This study was to investigate some heavy metal and microbiological properties of grounded melon seed sold in Yenagoa.

Materials And Methodology

Grounded melon seeds were obtained from three different markets. Swalli, Opolo and Tombia markets from Yenagoa Bayelsa state, Nigeria and taken to Federal University Otuoke for further analysis.

Samples preparation

Ten grams of sample was aseptically weighed into 90mL of sterile peptone water and homogenized in a Stomacher (model 4001, Seward Medical) for 30 seconds at normal speed. The homogenized egusi was used as stocked.

Serial Dilution

Ten-fold serial dilution of the samples was carried out as described by Cheesbrough (2005). 1ml from each stock was transferred into a test tube containing 9ml of sterile water. This was continuously repeated until the tenth test tube (10-10).

Inoculation and Incubation

The 10-1 dilution obtained was vortex for about 2 min to ensure uniform mixing. One microliter of the 10-1 dilution was pipette into 9mL of sterile salt peptone water to obtain 10-2 dilution. This procedure was repeated for 10-3, 10-4, 10-5, and 10-6 dilutions. An aliquot (1mL) of each dilution was inoculated into sterile plates of Mannitol Salt agar (MSA), Thiosulphate Citrate Bile salt Sucrose agar (TCBS), Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB), and Salmonella Shigella Agar (SSA). Using spread plate method, a flame sterilized bent glass rod was used to spread the aliquot over the surface of the solidified agar. After which the plates were incubated in an inverted position for 24hrs at 37°C. Visible discrete colonies were counted and expressed as colony forming units per gram (CFU/g). The bacteria were identified by their morphology, gram staining and Biochemical test.

Enumeration of fungi The isolation of fungi was carried out according to the agar dilution method as described by Pal et al., (2015). One (1) gram from each sample were homogenized with 90 ml of buffer peptone water and serial decimal dilutions (10-1 to 10-4) were performed. Fungal species were isolated on the Potato dextrose agar. The medium was poured into sterile Petri dish and 0.1 ml of each sample suspension was spread-plated onto the PDA media. The plates were incubated for 5 to 7 days at 25°C. Fungal isolates were sub-cultured on Sabouraud Dextrose agar and incubated for 5 to 7 days at 25°C for purification. Fungi were identified by using taxonomic schemes based on macroscopic and microscopic observation. The total fungal count for each plate was expressed as colony-forming units per gram of sample (CFU/g). Each genus or species identified was then expressed as percentage (%) of the total isolated fungi.

Identification of Fungi Identification of fungal Genera and the determination of each species of fungi were done using a modified form of Rood et al., (2018) procedure which involves identifying fungi isolates through the use of morphological and microscopic examination.

Heavy metal determination

The heavy metal content of the prepared samples was determined using calibrated flame atomic absorption spectrometry (FAAS) (USA at varying wavelength viz: 213.9 nm, 324.70 nm, 228.8 nm,

357.9 nm, 217 nm, 279.5 nm and 248.3 nm for zinc, copper, cadmium, chromium, lead, manganese, arsenic and iron respectively. The analysis of zinc, iron, copper, cadmium chromium, lead, arsenic and Manganese was carried out with a Buck Scientific Model 210 VGP atomic absorption spectrophotometer, USA. In all cases, air- acetylene was the flame used and hollow cathode lamp of the individual metals was the resonance line source. The calibration plot method was adopted for the analysis (Izah and Ohimain, 2015)

Data Analysis

The One-way ANOVA test was used, comparison of means of TFC across sampling and overall (%) for fungal species. The means were separated for test of significance by the Duncan's Multiple Range Test at P = 0.05.

Results and Discussion

Table 1 shows the number of colonies and their respective bacterial loads obtained from ground melon seeds from Kpansia (AKG), Swali (BSG), and Tombia (CTG) markets.

Sample code	Number of counts/colonies	Cfu/g	Log CFU/g
AKG	209	20.9×10^9	10.32
BSG	94	9.4×10^9	9.97
CTG	156	15.6×10^9	10.19

Table 1: *total viable count of bacteria in ground melon seeds*

The total occurrence of bacteria isolates from grounded Egusi samples revealed that. Escherichia coli ha the highest percentage distribution The total occurrence of bacteria isolates from grinded melon seeds samples revealed that E.coli had 17.4%, Stapphylococcus aureus had 13.0%, Staphylococcus epidermidishad 13.0%, Proteus mirabilis had 4.54%, Bacillus megalerium had 4.3%, vibrio cholera had 4.3%, Enterobacter cloacae had 4.3%, shigella had 8.7%. (Table 3). From the table, the bacterial specie that was most isolated from ground melon seeds was Escherichia coli (4) with 17.4% composition. This is similar to the report of Ronice et al. (2022) in which Escherichia coli was the most isolated bacterial specie from ground melon seeds. This could be as a result of the environment in which the ground melon seeds were processedwhich led to contamination with Escherichia coli. Cross- contamination could also occur during the process of grinding the melon seeds if the equipment or utensils used was not properly sanitized, which led to the transfer of Escherichia coli to the melon seeds (Ronice et al., 2022). These isolates include both Gram- negative and Gram-positive bacteria, with distinct characteristics. The isolation of Bacillus sp. from the grounded melon seed was in agreement with the study by Ekundayo et al.,(2013) who isolated these organisms and some other pathogens from grounded egusi seeds. The presence of Escherichia coli, Vibrio cholera, Salmonella typhimurium, Shigella spp, and other pathogenic bacteria in the samples is of significant concern as these organisms are known to cause severe gastrointestinal infections which supports the study of Ojieh et al., 2007. The detection of these pathogens emphasizes the potential public health risks associated with the consumption of contaminated melon seeds. Furthermore, the presence of Staphylococcus aureus, Staphylococcus epidermis, and Staphylococcus saprophyticus suggests the possibility of staphylococcal contamination, which can lead to food poisoning, conforms the work of Kings et al. These findings underline the importance of implementing stringent hygiene and quality control measures during the production, processing, and handling of melon seeds in local markets.

Fungal organisms found in grinded melon seed were identified as Penicillum chrysogenum(2), Aspergillus niger (6), Cladosporium spp (2), fusarium solani(1). Aspergillus niger are the prevalent species of fungal isolated in this study. Table 5, presents the rates of occurrence of the isolated fungi. The data revealed that 55% of the analysed samples were contaminated with Aspergillus niger specie

Metal	Mean concentration(ppm)			
	AKG	BSG	CTG	WHO limits
Zinc (Zn)	1.01	2.09	1.37	0.05
Copper (Cu)	0.02	0.02	0.03	0.10
Chromium (Cr)	0.01	0.03	0.03	0.01
Lead (Pb)	0.01	0.06	0.04	0.01
Manganese (Mn)	0.07	0.08	0.06	1.30

Table 2*: Mineral and heavy metal properties of Grounded melon seed sold in yanagoua*

Elements of heavy metals are either absorbed from the soil or released during food processing, which will affect the body when consumed contaminated food. Their toxicity may affect mental and nervous systems and other vital organs (Jurdziak et al., 2015). The five metals, namely, lead (Pb), Chromium (Cr), Zinc (Zn) and copper (Cu) and Manganese (Mn) were detected (Table 5). Among the metal detected, levels of Cr were the lowest and ranged between 0.01 and
0.03 ppm, with no significant differences (p > 0:05) between levels observed for the various markets. This concentration is below the 0.1 ppm suggested for legumes (JECFA, 2010). Chary et al., (2008) explained that because Cr is highly mobile and poorly adsorbed in soils, it is easily absorbed by plants. The concentration of Pb and Cu was 0.01–0.06 ppm and 0.02–0.03ppm correspondingly for the two metals. Pb may occur through absorption from the soil or contamination through processing equipment. Pb accumulation in plants is slower and occurs when soils contain high concentrations because it is tightly bound to soil colloids (Chary et al., 2008). This observation suggests that the milling of seeds into powder may have contributed to the levels of these elements in the powder. Indeed, previous studies of Sampare, (2006) reported that the use of a disc attrition mill contributes to heavy metal accumulation in food. Kwofie et al., (2011) explained that the grinding discs in these attrition mills are fabricated by local artisans using unalloyed cast iron. This material is not resistant to wear and corrosion and therefore its usage may result in the release of some of the metals into food. An analysis of discs in previous studies revealed considerable levels of Pb and other heavy metals, and these gradually wear off into food at a high rate (Adeti et al., 2015).

Conclusion

This study demonstrated that grounded Egusi samples marketed in yanagoea were of poor microbiological quality. The presence of pathogens such as Vibrio parahemolyticus, Proteus mirabilis, Salmonella typhimarum, Shigella spp, Enterobacteriaceae, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Bacillus cereus,Bacillus megaterium, Enterobacter aerogenes, and Vibrio cholera suggests a potential health risk for consumers. This study reveals theoccurrence of eleven fungi associated with grounded melon seed. Occurrence of the fungi represents a statement of unhygienic matrix in which the grounded melon seed is normally stored. Studies on themicrobial load of household blenders used in blending grounded melon seed samples should be carried out so as to ascertain, if the isolated microorganisms and heavy metals were from the food samples or from the environment where grounded melon seeds werekept.

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