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Microbial quality of raw and processed groundnut (*Arachis hypogaea*) and cashew nuts (*Anacardium occidentale*) in Rivers State, Nigeria

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ABSTRACT: The cashew and groundnut seeds are commonly considered snack nuts eaten on their own, used in recipes, or processed into cashew cheese, cashew butter peanut butter, groundnut biscuit etc. In this study, the contamination level of raw and roasted groundnut and cashew nuts was determined using standard microbiological methods. For nut samples, the total bacteria count ranged from Log $_{10}$ cfu/g 3.5 -7.8, the total fungi count ranged from Log $_{10}$ cfu/g 2.5 -5.8, the total *Staphylococcus* count ranged from Log $_{10}$ cfu/g 2.6-5.5. *Enterococcus* sp, *Serritia* sp, *Klebsiella* sp, *Bacillus* sp and *Staphylococcus* sp were bacteria isolated from groundnut and cashew. The highest percentage occurring bacteria observed was *Enterococcus* sp and *Bacillus* sp with 33.33% while *Serritai* sp was the least occurring with a percentage occurrence of 3%. *Aspergillus* sp had a percentage occurrence of 69.91%, *Fusarium* sp. had the least percentage occurrence of 3%. Due to the levels of microbial contamination of most of the samples and the kind of microbial species involved, proper hygiene standards must be adopted during the postharvest handling of the nuts.

Keywords: Cashew nuts, groundnut, microbial contamination, moisture content, pH.

INTRODUCTION

Due to the excellent nutritional value and the delicious nature of cashew and ground nuts, it is eaten by many individuals (Bhat and Vasanthi, 2013). In addition to being consumed directly, nuts may be used for several industrial processes, including the extraction of oils for cooking, body lotion, soap, and other domestic and commercial uses (Abdulla, 2013). Nuts are very sensitive to microbial invasion, particularly fungal infection since they are rich in proteins, lipids, minerals, and low water content (Pitt and Hocking, 2009; Bhat and Vasanthi, 2013). Types of nuts, hazelnut, pistachio, almonds, cashew nuts, and peanuts, are rich in high-quality proteins, unsaturated good fats, minerals, and vitamins, and they have low water content; therefore, nuts are highly vulnerable to microbial spoilage especially fungal attack (Beuchat, 1996; Olayinka *et al.,* 2016). Microorganisms may enter into the nuts' shell while still on the trees and this usually occurs when the pods or hard shells of the nuts are split open and the seeds are attacked by insects or pests which make space for the microbial spores to access the developing seeds (Frisvad and Samson, 2004). Other possible ways of contamination of nuts by microorganisms include the harvesting process, sorting practices, and washing of the nuts before storage. If the nuts are not properly treated amid these stages, it may lead to bacterial and mould growth especially when nut seeds are not properly dried to a safe moisture content before storage or distribution (Adetunji *et al.*, 2014). The fungal contamination occurs without necessarily showing any form of moldiness, molds create mycotoxins as secondary metabolites, which are consumed by people and lead to different forms of diseases such as cancers, spontaneous fetus removal, cirrhosis, and other liver infections (Alice, 1976; Frisvad and Samson, 2004). Nuts can become contaminated with foodborne pathogens at any stage of production, collecting preparing, distribution, or consumption. Proper food safety measures ought to be

received to avoid contamination issues. Other bacterial foodborne pathogens associated with dry foods, including tree nuts and peanuts, are *Bacillus cereus, Clostridium botulinum, Clostridium perfringens, Cronobacter, Escherichia coli O157:H7, L. monocytogenes,* and *Staphylococcus aureus* (Beuchat *et al*., 2013; Dubey and Maheshwari, 2013). Groundnuts and cashew nuts are among the most common nuts that are consumed in open places. Cashew nuts are fried/roasted and are sold in sachets or bottles. Groundnuts, on the other hand, are fried/roasted and bundled in sachets, and bottles, additionally sold raw either hull or dehulled which are consumed. It may be a known truth that individuals purchase and consume some processed food sold in open places without respect to the condition of these commodities whether they are safe for consumption or not (Bhat and Vasanthi, 2013). Groundnut and cashew products are produced locally and for domestic use. This may pose a health risk due to the high risk of microbial contamination through the variable methods of production that lack standard quality supervision. Microbial contamination can be from the improper handling of the product or poor storage methods. These contaminations can pose serious health problems to consumers. The study is aimed at determining the bacterial and fungal contamination levels of raw and roasted groundnut and cashew nuts sold.

MATERIALS AND METHODS

Description of the study area

The study was carried out in vendors within and around Choba and Rivers State University campus, in Port Harcourt, Rivers State. The choice of both locations is associated with the population of people including students and staff of host tertiary institutions located around these study areas. The University of Port Harcourt is located in Choba in Port Harcourt, Rivers State, it is located in the Niger Delta region of Nigeria. It was established in 1975 as University College, Port Harcourt and was given university status in 1977. The Rivers State University (RSU) Port Harcourt was established in October 1980 from the Rivers State College of Science and Technology which was itself established in 1972. It is located at Nkpolu-Oroworukwo in Port Harcourt, the capital of Rivers State, Nigeria.

Sample collection

A total of 60 samples from different types of vendors of groundnut and cashew nuts was purchases at random., comprising of 30 raw and 30 roasted nuts. The samples were transported to the microbiology laboratory.

Microbiological analysis of the nuts samples

Enumeration of bacteria

Representative 25 g of the nuts were aseptically weighed and homogenized with 225 mL sterile saline using a Stomacher 400 laboratory blender (Seward Ltd, UK). A 6-10 fold dilution tube containing 9 ml of sterile saline was used and 1 ml was transferred from the residue homogenate aseptically using a sterile syringe, to the first dilution tube, the same procedure was used. 1 ml was aseptically withdrawn from the first dilution bottle to the second dilution bottle. This was repeated until the dilution was completed. Using a new 1 ml pipette, 0.1 ml was aseptically transferred from the dilution tubes labelled 10^{-4}, 10^{-5}, 10^{-6}, aseptically to freshly prepared and serial dilutions were made for Total Bacteria Count (THC), and Total Staphylococcus Count (TSC) using Plate count agar plates and Mannitol salt agar plates and incubated at 37°C for 24-48 hours.

The number of colonies was counted and the average was taken, the colony forming unit of each average was calculated using the average divided by the dilution factor, multiplied by the volume plated. The total population was expressed as Colony Forming Units per gram (Cfu/g) (Adetunji *et al*., 2018)

Enumeration of fungi

A homogenate was prepared by measuring 25 g of each of the samples and was transferred into 225 ml of normal saline and homogenized for 30 seconds aseptically. A 6-10 fold dilution tube containing 9 ml of sterile saline was used and 1 ml was transferred from the residue homogenate aseptically using a sterile syringe, to the first dilution tube, the same procedure was used. 1 ml was aseptically withdrawn from the first dilution bottle to the second dilution bottle. This was repeated until the dilution was completed. Using a new 1 ml pipette, 0.1 ml was aseptically transferred from the dilution tubes labelled 10^{-4}, 10^{-5}, and 10^{-6}, aseptically to a freshly prepared and dried Potato Dextrose agar (for Total Fungi count) and spread with a sterile bent rod aseptically. The plates were then incubated at an ambient temperature of 25°C. This was done in duplicate.

The number of colonies was counted and the average was taken, the colony forming unit of each average was calculated using the average divided by the dilution factor, multiplied by the volume plated. The total population was expressed as Colony Forming Units per gram (Cfu/g) (Adetunji *et al*., 2018)

Isolation of microorganisms

Pure culture of isolates obtained was repeated subculture

on freshly prepared nutrient agar, Mannitol salt agar and Potato Dextrose agar. The isolates further were stored on slants at 4°C refrigeration temperature for identification. Identification of characteristic bacteria isolates was based on colonial morphology, microscopy and biochemical tests (Ellen and Sydney, 1990).

Characterisation and identification of isolates

Colonies of different bacteria species were then picked out using a sterile inoculating loop and subcultured for purification by streaking on plate count agar and incubated at 30°C for 24 hours. Individual colonies were characterized based on their colony morphology, microscopic examination, and biochemical characteristics.

Identification of fungi

Identification of fungi isolates was based on gross morphology and microscopy. For fungal identification, a mash of hypha of the test organism was made on slides containing Lacto phenol cotton blue, covered with a coverslip and observed in X 40 objective of the microscope.

Proximate analysis

The moisture contents of the groundnut and cashew nuts were determined using standard methods (AOAC, 2003).

Data analysis

Statistical analyses were conducted with the help of a social science statistical program (SPSS Version 23.1). When detecting significant variations among sample values, analysis of variance (ANOVA) was utilized.

RESULTS AND DISCUSSION

Microbial quality of cashew nut and groundnut

Nuts and nut products in general are profoundly vulnerable to microbial contamination basically due to their high nutritional content as well as their pH which is conducive to microbial growth and activities (Fraizer and Westhoff, 1978). Contamination of nuts by molds may also occur early within the field, and deterioration could develop during delayed storage (Freirea and Bargail, 2001). Concurring to Pixton (1982), most stored agricultural products, including nuts are hygroscopic and will retain moisture f rom the surrounding environment until they reach equilibrium, and thus the storage environment is a vital concern in preserving this food. This coupled with the high ambient temperature and relative humidity in the absence of proper processing might lead to the issue of microbial growth in handling nut and nut products (Ogundero, 1987). This study investigated the microbial load and diversity of groundnut and cashew nuts sold in the market. Total bacteria counts of roasted and raw nuts ranged from Log $_{10}$ cfu/g 3.5 -7.8 as shown in Figure 1. The result of the total viable count of the nuts samples, some were within the microbial limits/standard of 10^4 to less than 10^6 cfu/g of ready-to-eat food products as reported by Fylde Borough Council in the manual of PHLSG (2008) and some also exceeded the stated limits (PHLSG, 2008). Total staphylococcus counts on roasted and raw nuts ranged from Log $_{10}$ cfu/g 2.6 -5.5 as shown in Figure 3. The level of microbial contamination recorded in this study might be a result of the contaminated water used to wash the nuts, the unhygienic conditions of the storage containers, the packaging material used for selling them, and direct hand contact by the personnel shelling them (Moore and Griffith, 2002). Microbial contamination of food is a public health concern and must be addressed at every point of consumption. FDA (2013) recommended that total viable or aerobic plate counts per gram for nuts and seeds should be 5 $\times 10^3$ cfu/g. This is an indication that the contamination levels of the majority of the samples were beyond the FDA threshold. The raw cashew nut had significantly high microbial counts compares to other nut samples ($p<0.05$) , this may be due to the high nutritional values of cashew nuts, which are subject to microbial contamination. Total fungi count on roasted and raw nuts ranged from Log $_{10}$ cfu/g 2.5 -5.8 as shown in Figure 2. The International Commission on Microbiological Specifications for Foods (1989) set fungal tolerances for flour, cereal and packaged nut products in the range of 10^2 to 10^4 /g. However, for most strict companies, acceptance standards of less than 10^3 /g are adopted (King *et al*., 1981). From the nut samples investigated, some were fit for human consumption while some were not fit as they exceeded 10^3 and 10^4 /g acceptable fungal levels.

Identified bacteria

Five (5) bacterial genera; These genera include *Staphylococcus* sp, *Enterococcus* sp, *Bacillus sp*, *Klebsiella* sp, *Serritia* sp as shown in Table 2 were observed. Staphylococcus contamination has been previously reported in nuts outside Nigeria (King and Jones, 2001). Processing prerequisites such as heating (roasting) and salting would have resulted in reducing S*. aureus* count in addition to the presence of anacardic acid. According to Shebuski and Vilhelmsson (2000), *S. aureus* is the most osmotolerant food-borne pathogen and outbreaks of staphylococcal meal poisoning are regularly

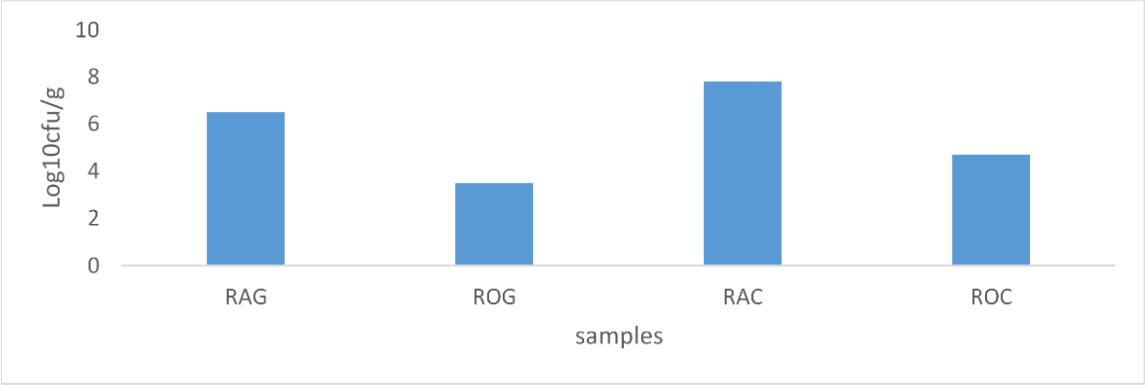

Figure 1. Mean Total Bacteria Counts of the different Nuts Samples (**Legend:** RAG = Raw Groundnut, ROG = Roasted Groundnut, RAC = Raw Cashew nut, ROC = Roasted Cashew nut).

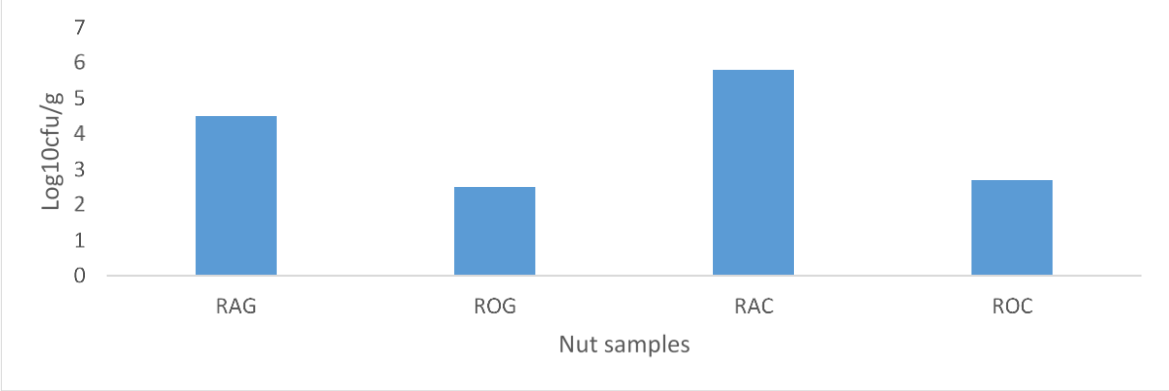

Figure 2. Mean Total Fungi Counts of the different Nuts Samples (**Legend:** RAG = Raw Groundnut, ROG = Roasted Groundnut, RAC = Raw Cashew nut, ROC = Roasted Cashew nut).

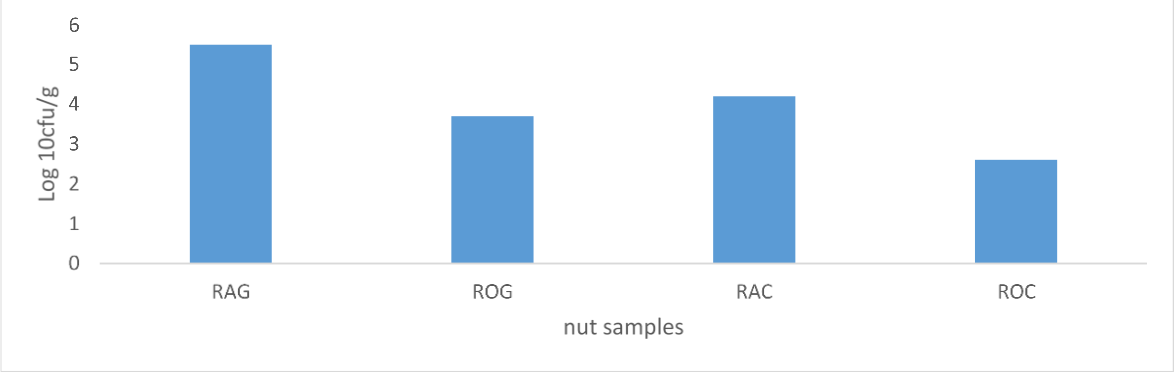

Figure 3. Mean Total Staphylococcus Counts of the different Nuts Samples (**Legend:** RAG = Raw Groundnut, ROG = Roasted Groundnut, RAC = Raw Cashew nut, ROC =Roasted Cashew nut).

linked to ingredients of decreased water activity values (Yoon and Kim, 2003; Han *et al*., 2005). Also, the presence of *Escherichia coli* and *Enterobacter sp* suggests the presence of faecal contamination (Izah *et al*., 2015). Many *E. coli* and *Enterobacter* sp strains are enterotoxigenic (Ezekiel *et al*., 2011). Bacillus sp is generally observed in the soil (Odu and Okonko, 2012), they would have entered the samples when they come in contact with soil.

Table 1. Organism isolated from the different samples**.**

Sample code	Probable organism
raw groundnut 1	*Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp *Candida* sp,
raw groundnut 2	*Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp *Candida* sp
raw groundnut 3	*Serritia* sp, *Enterococcus* sp, *Klebsiella* sp, *Bacillus* sp, *Aspergillius* sp
raw groundnut 4	*Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp, *Candida* sp
raw groundnut 5	*Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp *Aspergillius* sp
raw groundnut 6	*Bacillus* sp, *Enterococcus* sp, *Staphylococcus* sp, *Penicilium* sp
raw groundnut 7	*Bacillus* sp, *Staphylococcus* sp
raw groundnut 8	*Bacillus* sp, *Enterococcus* sp, *Staphylococcus* sp, *Aspergillus* sp
raw groundnut 9	*Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp, *Penicillium* sp
raw groundnut 10	*Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp *Aspergillius* sp
Roasted groundnut 1	*Staphylococcus* sp, *Klebsiella* sp, *Penicilium* sp
Roasted groundnut 2	*Staphylococcus* sp, *Candida* sp, *Aspergilius* sp
Roasted groundnut 3	*Bacillus* sp, *Staphylococcus* sp
Roasted groundnut 4	*Bacillus* sp, *Enterococcus* sp, *Staphylococcus* sp *Penicillium* sp
Roasted groundnut 5	*Bacillus* sp, *Enterococcus* sp, *Staphylococcus* sp, *Aspergillius* sp
Roasted groundnut16	*Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp, *Aspergilius* sp
Roasted groundnut 7	*Bacillus* sp, *Enterococcus* sp
Roasted groundnut 8	*Bacillus* sp, *Enterococcus* sp, *Staphylococcus* sp,
Roasted groundnut 9	*Bacillus* sp, *Enterococcus* sp, *Staphylococcus* sp *Penicillium* sp
Roasted groundnut 10	*Bacillus* sp, *Enterococcus* sp, *Staphylococcus* sp, *Aspergillius* sp
raw cashew 1	*Staphylococcus* sp, *Enterococcus* sp, *Klebsiella* sp, *Penicilium* sp
raw cashew2	*Bacillus* sp, *Enterococcus* sp
raw cashew 3	*Bacillus* sp, *Enterococcus* sp
raw cashew 4	*Bacillus* sp, *Enterococcus* sp
raw cashew 5	*Bacillus* sp, *Enterococcus* sp, *Aspergillius* sp
raw cashew 6	*Klebsiella* sp, *Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp, *Penicilium* sp
raw cashew 7	*Bacillus* sp, *Enterococcus* sp
raw cashew 8	*Bacillus* sp, *Enterococcus* sp
raw cashew 9	*Bacillus* sp, *Enterococcus* sp, *Penicillium* sp
raw cashew 10	*Bacillus* sp, *Enterococcus* sp,
Roasted cashew 1	*Staphylococcus* sp, *Enterococcus* sp, *Penicillium* sp
Roasted cashew 2	*Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp, *Aspergilius* sp, *Fussarium* sp, *Aspergillus fumigatus*
Roasted cashew 3	*Staphylococcus* sp, *Bacillus sp Enterococcus* sp, *Aspergilius* sp
Roasted cashew 4	*Bacillus sp Enterococcus* sp, *Aspergilius* sp
Roasted cashew 5	*Bacillus sp Enterococcus* sp
Roasted cashew 6	*Staphylococcus* sp, *Bacillus* sp, *Klebsiella* sp,
Roasted cashew 7	*Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp, *Aspergillus niger*, *Aspergillus fumigatus*, *Candida* sp
Roasted cashew 8	*Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp, *Klebsiella* sp, *Penicillium* sp
Roasted cashew 9	*Staphylococcus* sp, *Bacillus sp Enterococcus* sp, *Aspergilius* sp
Roasted cashew 10	*Staphylococcus* sp, *Bacillus sp Enterococcus* sp, *Penicilium* sp

Table 2. Percentage occurrence of fungi isolated from nuts samples.

Sample code	Frequency	Percentage
Penicilium sp	6	19.00%
Fussarium sp	1	3.00%
Aspergilius sp	22	69.9I%
Candida sp	3	9.00%
Total	30	100%

Table 3. Percentage occurrence of bacterial isolated from nuts samples

Sample code	Frequency	Percentage
Staphylococcus sp	24	25.%
Enterococcus sp	31	33.33%
Bacillus sp	31	33.33%
Klebsiella sp	6	6.00%
Serritia sp	3	3.00%
Total	95	100.00%

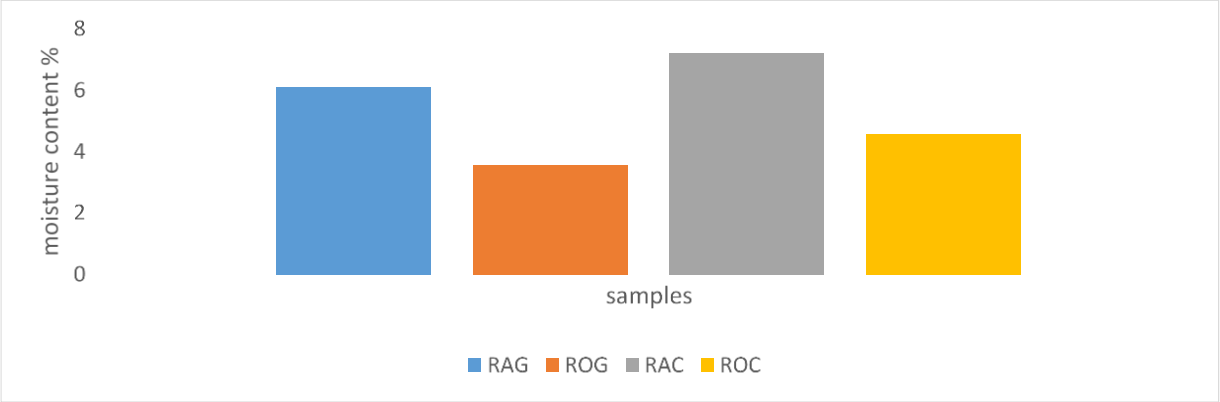

Figure 4. Mean moisture content of the raw and roasted nuts (**Legend:** RAG = Raw Groundnut, ROG = Roasted Groundnut, RAC = Raw Cashew nut, ROC = Roasted Cashew nut).

Identified fungi

Four fungal species were identified in this research: *Aspergillus spp*., *Candida sp*., *Penicillium spp*. and *Fusarium spp* as indicated in Table 3. These fungi were among the microorganisms isolated from previous findings; Fagbohun and Faleye (2012) isolated *Aspergillus niger, Aspergillus flavus, Rhizopus spp. Mucor spp.* and *Aspergillus fumigatus* in sundried groundnut, Adebesin *et al*. (2001) isolated *Aspergillus flavus, Aspergillus niger, Aspergillus tamarii, Penicillium Citrinum, Rhizopus stolonifer* and *Macrophomina phaseolina* in roasted groundnut sold in Bauchi State. From this study, *Aspergillus spp.* was more prevalent in the samples while *Fusarium spp*. was less prevalent. This finding is in line with Abuga (2014), Akinnibosun and Osawaru (2015) and Kigigha *et al.* (2016) who identified *Aspergillus spp*. as the predominant fungi that affect the quality of groundnut seeds sold in Benin City and Yenagoa Metropolis respectively. *Aspergillus spp*. was prominent because it contaminates nuts at various stages right from harvest to production (Dange and Patel, 1984). Table 1 indicate the different microorganism isolate and theiroccurrence in the different samples.

pH and moisture content of the nuts

The moisture contents of the raw groundnut and cashew nuts were above the permissible recommended moisture

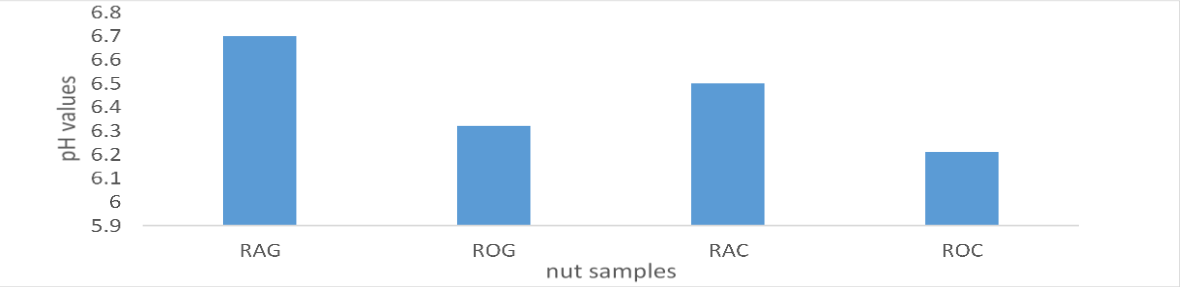

Figure 5. Mean pH values of the raw and roasted nuts (**Legend:** RAG = Raw groundnut, ROG = Roasted groundnut, RAC = Raw cashew nut, ROC = Roasted cashew nut).

limit of 5% (Ramadhani *et al*., 2014). The moisture content of the nuts samples ranged from 3.3 – 7.1 as shown in Figure 4. In comparison to this study, similar moisture content, 5.10-7.2%, and 6.48-7.05% were reported for peanuts from different states of Nigeria by Atasie *et al*. (2009); Adetunji *et al*. (2018) and Oyedele *et al*. (2017), respectively. Also, the moisture content of raw and roasted nuts observed in this study was slightly similar to the results of Adebajo and Diyaolu (2003) who reported a range of moisture content of 4.1–6.8% and higher than the findings of Oluwafemi *et al.* (2009) who reported a low moisture for cashew nuts during the dry and raining seasons. Different from this study was reported by Adetunji *et al*. (2018) who obtained 5.2 to 8.6% moisture content for cashew nuts. As nuts are hygroscopic materials, they absorb moisture from the surrounding atmosphere. The greater moisture content of the raw samples could be a result of their raw nature as they have not yet been handled with any form of the drying process. The excessive moisture contents of the samples may also be a result of inappropriate packaging, use of improper packaging compositions, and harvesting techniques in the farms (Oladapo *et al.,* 2014). Nuts are prone to fungal attacks at different stages of cultivation, harvesting, sorting, processing, and storage. If the nuts are not properly handled at these stages, it could result in mold development especially when they are not dried enough to be considered at a safe moisture level and stored under conditions suitable for mold growth such as excessive humidity and temperature. Figure 5 shows that the mean pH recorded for the samples was (6.32 to 6.7). Thus, the nut is a low-acid food product and without exception, all the samples had pH values conducive to microbial increase and activities including the elaboration of toxic secondary metabolites (Smith and Moss, 1985).

Conclusion

Nuts can become contaminated with foodborne pathogens at any stage of production, harvesting, processing, distribution, or consumption. Proper food safety measures should be adopted to prevent contamination issues.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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