UJMR, Vol. 7 Number 1, June, 2022, pp 15-21 *E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668*

https://doi.org/10.47430/ujmr.2271.004

Received: 8th Feb, 2022 **Accepted:** 5th Mar, 2022

Microbiological Assessment of Groundnut Paste Sold in Jimeta Markets, Yola, Adamawa State Nigeria

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Abstract

Groundnut paste is frequently associated with food-borne illness due to contamination traceable to food handlers, processing materials as well as environmental conditions and this therefore necessitated the microbiological quality examination of groundnut paste. The percentage occurrence of bacteria isolates and moisture content were determined using standard laboratory techniques. The percentage moisture content of the groundnut pastes was between the range of 0.8% and 4.8%. Total bacteria count fell between 1.8 ×10^{14} and 12.4 × 10^{14}CFU/mL with organisms such as Proteus species (spp.), Pseudomonas spp., Bacillus spp., Salmonella spp., Klebsiella spp., Stapylococcus aureus, Escherichia coli, Shigella spp., Alcaligenes faecalis and Enterobacter spp. isolated. Total fungal count was between 2×10^{7} and 4×10^{7}CFU/mL with identified organisms such as Aspergillus niger, Aspergillus flavus, Rhizopus spp. and Penicillium spp. Proteus spp. was the most prevalent with a percentage of 19.23 % while Escherichia coli, Alcaligenes faecalis and Enterobacter spp. showed the least prevalence of 3.85%. The results also show that fungi species spreads across all the samples with Aspergillus niger and Aspergillus flavus obtained in two of the samples, Rhizopus spp. in three other samples while Penicillum spp. were obtained in four samples. It is apparent from the result of this study that the groundnut paste examined were highly contaminated with microbial isolates sufficient enough to be a public health hazard in Jimeta markets and Adamawa State at large, therefore caution must be applied in its uses and consumption.

Key Words: Groundnut paste; Food; Contamination; Bacteria; Fungi; Percentage occurrence.

INTRODUCTION

Groundnut (*Arachi hypogea*), or peanut, species in the family of fabaceae that originates from South America, Mexico and Central America (Dill hay and Tom 2007). It is an economically important oilseed, feed, and food crop and widely cultivated in tropical and subtropical regions of the world. It is known by many local names such as earthnut, goober pea, pindas, jack nut and monkey nut (Variath and Janila, 2017). Groundnut is a staple food for the majority of people in sub-Sahara Africa and consumed in several forms including seeds, oil, boiled, flour, cake, and paste (Boli*et al.*, 2020). Groundnut, a nutrient dense agricultural produce is very high in energy due to its high fat and protein contents, the seeds contain 44-56% oil and 22-30% protein on a dry seed basis and are a rich source of mineral such as phosphorus, calcium, magnesium and potassium and vitamins A, E, K and B group; the carbohydrate content of groundnut is relatively low and accounts for less than 30% of the whole nut and the nut has relatively high content of fiber (Karunarathna *et al.*, 2014). It is an industrial crop whose major utilization is as a source of oil (Elegbede, 1998). Ayoola and Adeyeye (2010) described groundnut as an inexpensive source of high quality dietary protein and oil. Groundnut is recorded to be the 13th most important food crop, 4th most important source of edible oil and 3rd most important source of vegetable protein in the world. Furthermore, it is one of the most popular commercial crop in Nigeria which accounted for 70 percent of the total Nigeria export earning between 1956 and 1967 and later declined around mid-1980s due to the combined effect of drought and disease (Girei *et al.*, 2013).

The major groundnut producing states in Nigeria are Kano, Katsina, Kaduna, Jigawa, Sokoto, Zamfara, and Kebbi in the Northwest; Adamawa, Bauchi, Yobe and Borno in the Northeast; and Benue, Plateau, Nasarawa, FCT Abuja, Kogi, Niger and Kwara in the Central Zone (Ajeigbe *et al.*, 2014). Groundnut paste can be processed with or without additives. Some of the additives used in processing groundnut paste include pepper, Calabash nutmeg also known as ehuru, ginger and/or onion. In Nigeria, groundnut paste is produced traditionally on a small scale and as such has been given little or no attention on the microbiological quality and safety.

MATERIALS AND METHODS

Sample collection and processing

Different traditionally processed and packaged (in polythene bags) groundnut pastes designated as GPJM (Groundnut paste without additive from Jimeta main market), GPBM (Groundnut paste without additive from Bypass market), GPOM (Groundnut paste without additive from Old market), GPSC (Groundnut paste without additive from shopping complex), GPJMA (Groundnut paste with additive from Jimeta main market) and GPSCA (Groundnut paste with additive from shopping complex) were purchased and transported to the Microbiology laboratory of the Modibbo Adama University, Yola for analysis.

Determination of Moisture Content

The moisture content was calculated as the percentage of the original weight of dry paste divided by weight of wet paste multiplied by 100 as illustrated below:

$$\text{Moisture content\%} = \frac{\text{Wt. of wet Sample-Wt. of dry sample } x \text{ 100}}{\text{Weight of wet sample}}$$

Determination of Total bacterial count (TBC)

To determine the bacterial load of each processed groundnut paste sample, serial dilution of each of the samples were prepared. Using a weighing balance, 25grams of each sample was transferred into a glass test tube containing 225 ml of sterile distilled water to prepare 300 ml sample suspension. From each of the sample suspension, serial dilution up to 10^{-14} were prepared. Plating of 0.1 ml of 10^{-13} on Nutrient, Mac Conkey and Salmonella-Shigella, incubating at 37 °C for 24 hours.Colonies were counted using Leica Quebec dark field colony counter. The counts were expressed in colony forming unit per millimeter using the formula.

$$\text{CFU/mL} = \frac{\text{No of colonies x dilution factor}}{\text{Volume of sample}}$$

Determination of Total fungal count (TFC)

Determination of total fungal count was carried out by inoculating 0.1ml of 10^{-6} dilution from samples into potato dextrose agar (PDA) plates. Each plate contained 0.1 ml of chloramphenicol to inhibit the growth of bacteria and was incubated at 30 °C for 5-7days. Total fungi count was obtained by counting the number of colonies on potatoes dextrose agar (PDA).The technique of James and Natalie (2001) was adopted for identification of the unknown isolated fungi using lacto phenol cotton blue stain.The bacteria and fungi isolates were sub-cultured onto appropriate media to obtain pure isolates.

Identification of bacterial Isolates

Morphological characteristics on the petri dish, Grams reactions, microscopic examination were carried out according to the methods described by Chessbrough (2002). Furthermore, various biochemical tests such as coagulase, catalase, oxidase, Simon's citrate, indole, methyl red, voges-prokauer and urease test were carried out for identification.

Determination of percentage occurrence of bacteria isolate

The percentage occurrence of each bacteria isolates was determined by dividing the occurrence of each bacterium in the samples from the markets with the total number of all the bacteria in the samples from the markets and expressed as a percentage using the formula below:

$$\text{Percentage occurrence} = \frac{X}{Y} x\ 100$$

Where

X = Total number of each bacteria in the samples from the markets.

Y = Total number of all the bacteria in the samples from the markets.

Determination of percentage frequency of fungal occurrence

This was determined as described by Atanda (2005). The percentage frequency of fungal occurrence was determined by dividing the occurrence of individual fungal isolates with the total number of occurrence of the fungal isolates and expressed as a percentage using the formula below:

$$\text{Percentage occurrence} = \frac{X}{Y} x\ 100$$

Where

X = Total number of each organism in the samples from the market.

Y = Total number of all organism in the samples from the market

RESULTS

GPOM and GPSC have the same and highest moisture content with the percentage of 4.8% while GPBM have the least moisture content with the percentage of 0.8%. GPJM have thepercentage moisture content of 1.4%, GPJMA and GPSCA have the percentage moisture content of 1% and 4.4% respectively (Table 1). GPOM had the highest viable count of $12.4x10^{14}$cfu/ml while GPJM had the lowest viable count of $1.8x10^{14}$ cfu/ml. GPBM, GPSC, GPJMA and GPSCA had the viable count of $4.1x10^{14}$, $7.6x10^{14}$, $3.3x10^{14}$ and $7.9x10^{14}$cfu/ml respectively. For the fungal counts,GPJMA had the highest viable count of 4.0 x 10^7 cfu/ml while GPOM, GPSC and GPSCA had the same viable count of 2.0 x 10^7 cfu/ml which is the lowest viable count. GPJM and GPBM also had the same viable count of 3.0 x 10^7cfu/ml (Fig 1).The morphology and biochemical test carried out indicated that the likely organisms present in the samples are Proteus spp., *Pseudomonas* spp., *Bacillus* spp., *Salmonella* sp., *Klebsiella* spp., *Stapylococcus aureus, Escherichia coli, Shigella* sp., *Alcaligenes faecalis and Enterobacter* spp.(Table 2). *Proteus* spp. is the most prevalent organism isolated from the different location with a percentage occurrence of 19.23 % while *Escherichia coli, Alcaligenes faecalis* and *Enterobacter* spp. were the least having the same percentage occurrence of 3.85 %. *Pseudomonas* spp. and *Klebsiella* spp. have the same percentage (11.54%), *Bacillus* spp. and *Salmonella* spp. have the same percentage (15.38%), *Stapylococcus aureus and Shigella* spp. alsohave the same percentage occurrence of 7.69 % (Table 3). Growth appearances and microscopic examination indicate that likely fungi isolates include *Aspergillus niger, Aspergillus flavus, Rhizopus* spp. *and Penicillum* spp. (Table 4).The presence of *Aspergillus niger, Rhizopus* spp. and *Penicillum* spp. were isolated from GPJM, *Aspergillus niger, Aspergillus flavus* and *Rhizopus* spp. were isolated from GPBM, *Aspergillus niger* and *Penicillum* spp. were isolated from GPOM, *Aspergillus niger, Rhizopus*spp. and *Penicillum* spp. were isolated from GPSC; *Aspergillus niger were isolated from* GPJMA, *Aspergillus niger, Aspergillus flavus* and *Penicillum* spp. were isolated from GPSCA. *Aspergillus niger* is the most prevalent organism with six isolates, *Aspergillus flavus* is the least with two isolates. Others include *Rhizopus* spp. three isolates and *Penicillum* spp. with four isolates (Table 5).

Table 1: Percentage Moisture content of groundnut paste

Sample	Weight of Wet Sample (g)	Weight of Dry Sample (g)	Moisture content (%)
GPJM	5	4.93	1.4
GPBM	5	4.96	0.8
GPOM	5	4.76	4.8
GPSC	5	4.76	4.8
GPJMA	5	4.95	1
GPSCA	5	4.78	4.4

Key: GPJM: Groundnut paste without additive from Jimeta main market, GPBM: Groundnut paste without additive from Bypass market,GPOM: Groundnut paste without additive from Old market,
GPSC: Groundnut paste without additive from shopping complex,GPJMA: Groundnut paste with additive from Jimeta main market,GPSCA: Groundnut paste with additive from shopping complex.

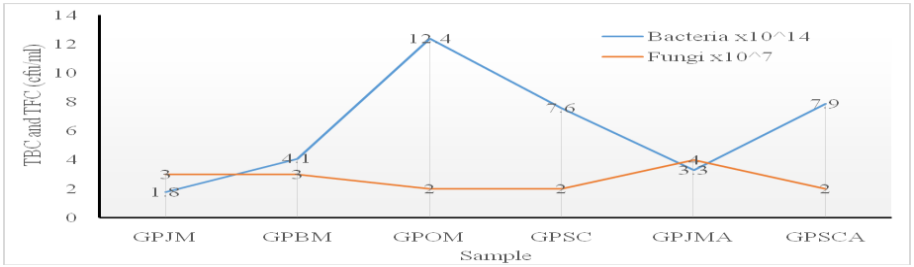

Fig 1: Total bacterial count (TBC/ml) and Total fungal count (TFC/ml) of the groundnut paste samples collected from Jimeta markets Yola, Adamawa State Nigeria.
Key: TBC: Total bacteria count, TFC: Total fungal count, CFU: Colony forming unit, GPJM: Groundnut paste without additive from Jimeta main market, GPBM: Groundnut paste without additive from Bypass market, GPOM: Groundnut paste without additive from Old market, GPSC: Groundnut paste without additive from shopping complex, GPJMA: Groundnut paste with additive from Jimeta main market, GPSCA: Groundnut paste with additive from shopping complex.

Table 2: Morphological and biochemical characteristics of bacterial isolates

Parameters	A	B	C	D	E	F	G	H	I	J
Gram reaction	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Cellular morphology	Rod in single	Rod in single	Rod in chain	Rod in single	Rod in single	Cocci in cluster	Rod in single	Rod in single	Rod in single	Rod in single
Coagulase test	-	-	-	-	-	+	-	-	-	-
Catalase test	+	+	+	+	+	+	+	+	+	+
Oxidase test	-	+	-	-	-	-	-	-	+	-
Simon's citrate	+	+	+	+	+	+	-	+	+	+
Indole test	-	-	-	-	-	-	+	-	-	+
Methyl red test	+	-	-	+	-	+	+	-	-	-
Vogesproskauer	-	-	-	-	-	+	-	-	-	+
Urease test	+	+	+	-	+	+	-	+	-	+

Most likely organism

A: *Proteus* spp.
B: *Pseudomonas* spp.
C: *Bacillus* spp.
D: *Salmonella* spp.
E: *Klebsiella* spp.
F: *Staphylococcus aureus*
G: *E coli*
H: *Shigella* spp
I: *Alcaligenes faecalis*
J: *Enterobacter* spp.

Table 3: Percentage occurrence of each bacteria isolates obtained from the Jimeta markets Yola.

Bacteria isolates	GPJM	GPBM	GPOM	GPSC	GPJMA	GPSCA	No of isolates	Occurrence (%)
Proteus spp.	+	+	+	+	+	-	5	19.23
Pseudomonas spp.	+	-	-	-	+	+	3	11.54
Bacillus spp.	+	+	+	+	-	-	4	15.38
Salmonella spp.	+	+	+	+	-	-	4	15.38
Klebsiella spp.	+	-	-	+	+	-	3	11.54
Staphylococcus aureus	-	-	+	+	-	-	2	7.69
E coli	-	-	+	-	-	-	1	3.85
Shigella spp.	-	-	+	+	-	-	2	7.69
Alcaligenes faecalis	-	-	-	-	-	+	1	3.85
Enterobacter spp.	-	-	-	-	+	-	1	3.85
Total							**26**	**100**

Key: GPJM: Groundnut paste without additive from Jimeta main market, GPBM: Groundnut paste without additive from Bypass market, GPOM: Groundnut paste without additive from Old market, GPSC: Groundnut paste without additive from shopping complex, GPJMA: Groundnut paste with additive from Jimeta main market, GPSCA: Groundnut paste with additive from shopping complex.

Table 4: Morphological characteristics of fungi isolates

Growth on Potatoes Dextrose Agar (PDA)	Microscopy	Likely Organism.
Black appearance on culture.	Conidial/heads are dark brown to black.	*Aspergillus niger*
Yellow green appearance on culture.	Spreading yellow green colonies, rough walled stipes, mature vesicle bearing phialides over their entire surface and conspicuously echinulate conidia.	*Aspergillus flavus*
colonies with shade of green	Hyphomycete, flask-shaped phialide arranged in group from metulae forming a penicillus	*Penicillum* spp.
White colonies (dense cotton) that turn yellowish brown with sporulation	Mass of non-septate hyphae bearing sporangia on sporangiospore Rhizoids present	*Rhizopus* spp.

Table 5: Percentage Occurrence of each fungi isolate obtained from the markets

Fungal isolate	GPJM	GPBM	GPOM	GPSC	GPJMA	GPSCA	No of isolate	Occurrence %
Aspergillus niger	+	+	+	+	+	+	6	40
Aspergillus flavus	_	+	_	_	_	+	2	13.33
Rhizopus spp.	+	+	_	+	_	_	3	20
Penicillum spp.	+	_	+	+	_	+	4	26.67
Total							15	100

Key: GPJM: Groundnut paste without additive from Jimeta main market, GPBM: Groundnut paste without additive from Bypass market, GPOM: Groundnut paste without additive from Old market, GPSC: Groundnut paste without additive from shopping complex, GPJMA: Groundnut paste with additive from Jimeta main market, GPSCA: Groundnut paste with additive from shopping complex.

DISCUSSION

The results obtained from the investigation showed that the groundnut paste examined contained high bacterial load including those with additives. Manufacturing units of most commercially produced groundnut paste are located in remote areas from where they are transported to various places. Lack of safe transportation practices as well as packing in a dirty dusty environment can lead to an increase in the microbial load even though the product is of low moisture content. Gupta and Dudeja 2017 noted that lack of safe transportation practice of ready-to-eat meals and use of unclean food processing equipment such as homogenizer, mixing vats, conveyor etc. leads to an increase in the microbial loads.

The presence of *Escherichia coli*, *Proteus* spp. and *Salmonella* spp. in groundnut paste samples indicates the possibility of a microbial hazard and fecal contamination. This finding is similar to the report by Carminati *et al.,* 2016, where *Escherichia coli* and *Salmonella* spp. were dictated in a peanut confectionery and it was concluded that manufacturing environment and food handlers were the main sources for possible contamination. Coliforms are considered as normal flora of the intestinal tract of humans and animals. They have been used as indictor organisms for bacteriological quality of food and water. *Escherichia coli*, *Proteus* spp. and *Salmonella* spp. have been used to assess microbiological safety, sanitization condition during processing and keeping quality of ready-to-eat food (Okoronkwo and Disegha 2020).The isolation of these organisms from groundnut paste is also in line with the findings of Odun and Okonko (2012) which reported that *Escherichia coli* and *Salmonella* spp. are among the most important food borne bacteria pathogens in ready-to-eat food. In the pathogenesis of *Salmonella* infection, it is known that the main route of entry into the host is the mouth (Sokari, 1991). *Escherichia coli* cause dysentery (Nester, 1995; Adebayo-Tayo 2012).Isolation of *Bacillus* spp. and *Staphylococcus aureus* from the groundnut paste was similar with the findings of Muhammad *et al.,* 2020, where different species of *Bacillus* were isolated from groundnut cake in Sokoto State, North-Western Nigeria. *Bacillus* spp. which is known to be one of the highest occurring bacterial isolate causes toxin medicated disease rather than an infection (Adebayo-Tayo *et al* 2019). *Bacillus* spp. is a normal inhabitant of the soil and a poisoning organism associated with animals. This is however because of the survival advantage which the spores have in air and in other harsh condition. Contamination could be from the water and materials used in processing the groundnut paste traditionally. The enterotoxins produced by this organism are stable at pH 8-10. Sokari (1991) also reported the isolation of *Bacillus* spp. in ready-to-eat food in Nigeria. The occurrence of *Staphylococcus aureus* in groundnut paste samples may be a reflection of repeated hand contact with these foods at the point of sale. Outbreaks of staphylococcal food poisoning have been reported to occur as the result of contamination of precooked food, often through unsanitary handing and holding food at temperatures that allow the growth and toxin production (Sophia *et al.,* 2015).*Pseudomonas* can also cause diseases in animals including humans. It is also found in soil, water and skin. Its versatility enables the organism to infect damaged tissue of those with reduced immunity. People infected with *Shigella* develop bloody diarrhea, fever and stomach cramps starting a day or two after they are exposed to the bacterium (Rogawski *et al.,* 2020). *Alcaligenes faecalis* has been reported to cause sepsis, meningitis, peritonitis, enteric fever, endocarditis etc. (Huang 2020). *Enterobacter* spp. can cause a range of infections such as bacteremia; lower respiratory tract infection, skin and soft tissue infection (Davin-Regli *et al.*, 2019).

Fungi are common environmental contaminants and the molds bear resistant spores that easily

contaminate groundnut paste which contribute to the spoilage of groundnut paste. Results showed that groundnut paste samples were all contaminated regardless of their collection location or additive content. The aflatoxin producing fungi such as *Aspergillus flavus* and *Aspergillus niger* are ubiquitous in nature. The contamination of food by Aflatoxin has been a global concern because of their carcinogenic, teratogenic and mutagenic compounds. (Nii*et al.*, 2021). *Rhizopus* spp., *Penicillium* spp., *Aspergillus niger* as well as *Aspergillus flavus* have been identified. This result is similar to the work carried out by Boli*et al* 2020 where *Penicillium, Fusarium, Alternarium, Clasdosperious* and *Aspergillus* were isolated from the groundnut paste sold in Abidjan (Xing *et al.,* 2016).The high fungal and bacterial counts may be due to non-hygienic handling, poor storage, use of contaminated water, and inadequate general hygiene during processing and/or poor quality raw materials (Susheela and Melissa 2016).

Microbial deterioration is a concern of groundnut paste because the ubiquitous nature of microorganisms dictates that they can easily contaminate the product if the necessary precautions during processing and by food handlers are neglected (Solomon *et al.,* 2012). The assessment of the quality and safety of food is important in human health. Food that is unfit for human and animal consumption may not necessarily be spoiled but may contain high number of food poisoning bacteria. Furthermore, old people, pregnant women, immunodeficiency individuals as well children less than 5 years of age could be more vulnerable to foodborne diseases; high bacterial load suggests poor storage and inadequate general hygiene during processing and/or contaminated raw materials (Susheela and Melissa 2016).

CONCLUSION

In conclusion, it is apparent from the result of this study that the groundnut paste examined were contaminated with microbial isolates which include bacterial species such as *Proteus* spp., *Pseudomonas* spp., *Bacillus*spp., *Salmonella* spp., *Klebsiella* spp., *Staphylococcus aureus*, *E. coli*, *Shigella* spp., *Alcaligenes faecalis* and *Enterobacter* spp., while fungal isolates comprises of *Aspergillus niger*, *Aspergillus flavus*, *Penicillum* spp. and *Rhizopus* spp. The total microbial counts were not within acceptable standard for human consumption, this is to say that the level of bacterial and fungal count did not conform to the standard specifications of National Agency for Food and Drug Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON). Furthermore, contamination level was sufficient to be a public health hazard. Therefore, it is clear from this study that there are microorganisms in groundnut paste that are capable of causing human diseases.

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