

THE EFFECT OF DIFFERENT PACKAGING MATERIALS ON THE SANITARY QUALITY AND PROXIMATE COMPOSITION OF TRADITIONALLY PRODUCED PEANUT BUTTER

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ABSTRACT

The study was conducted to determine the microbial and proximate composition of Peanut butter using standard microbiological methods. A total of 40 samples from different vendors of peanut butter were purchased at random. 20 samples were purchased in plastics while 20 were purchased in nylons. Nylon peanut butter was more contaminated than the plastic peanut butter. The organisms isolated include *Bacillus* sp, *Escherichia coli*, *Serratia* sp, *Enterococcus* sp, *Staphylococcus* sp and *Klebsiella* sp. *Bacillus* sp had the highest percentage of occurrence of 35.5% followed by *Klebsiella* sp and *Staphylococcus* sp with a percentage frequency of occurrence of 19.4%, respectively. *Enterococcus* sp had a frequency of 12.9% and *Serratia* sp had a frequency of 9.7%. While *Escherichia coli* was the least with a frequency of 3.2%. For fungi, *Aspergillus* sp, and *Penicillium* sp were isolated. *Aspergillus* sp had the highest Percentage of occurrence of 60% and *Penicillium* sp a percentage occurrence of 40%. The occurrence of pathogenic bacteria in traditionally made peanut butter can be a potential health risk to the consumers, so proper handling of peanut butter during production is encouraged and also proper storage condition and safe usage while using for a prolonged period time should be maintained properly to minimize the risk of microbial contamination of the peanut butter as well as the foodborne diseases of the consumers.

Keywords: Peanut butter; microbial load; food-borne pathogens; packaging materials.

INTRODUCTION

“Peanut (*Arachis hypogaea*) is a leguminous crop and it is the 13th most important food crop and 4th important oilseed crop in the world after soybean, cottonseed and rapeseed” [1]. Peanuts are grown globally mostly in developing countries, where it is made up about 97% of the total land area under cultivation and 94% of the global production [2]. The major producers of peanut, also known as groundnuts are China, India, Mali, Ghana and Nigeria [3]. They are also widely consumed in Central and South America [4]. It was introduced in to Nigeria in the 16th century and it has been estimated that about

1.4 million hectare is cultivated for groundnut in Nigeria. “Peanut butter is a food paste made from ground dry roasted peanut (groundnut) and is common in the Philippines, North America, the Netherlands and the United Kingdom. Peanut butter has been known to be linked with food illness in which initial contamination is linked to food handlers. In Nigeria, peanut butter is made traditionally on a small scale and as such has been given little or no attention on the sanitary quality and safety of traditionally made Nigerian peanut butter is lacking in literature” [5]. “Also, Pathogens can be transferred in several ways to food, such as through contaminated water and equipment, poor worker hygiene, and pests” [6],

“Various epidemiological reports and studies have linked foods of ready to eat origin as the main vehicles linked with illness caused by food-borne pathogens. human to human transmission has also been described” [5]. This research will provide information on the microbial quality of traditionally processed peanut butter which will help in food quality and safety decision-making.

MATERIALS AND METHODS

Sample Site

A total of 40 samples were purchased from some markets located at Okigwe, Imo state. 40 samples comprising of 20 plastics samples and 20 nylon samples. The samples were transported in a Ziploc bag to the Microbiology Laboratory.

MICROBIAL ANALYSIS OF THE PEANUT SAMPLES

A 10 g of peanut butter samples was homogenized in 90 ml of buffered peptone water and shaken vigorously according to the method of [7]. Serial dilutions were made for Total Bacteria Count (THC), total coliform count and total fungi count using nutrient agar plates, Plate count agar, MacConkey agars and Potatoes dextrose agar respectively and incubated at 37°C for 24-48 hours and 48-72 hours for Total fungi count. The number of colonies were counted and the average taken, the colony forming unit of each average was calculate using average divided by the dilution factor, multiplied by the volume plated. Total population were expressed as Colony Forming Units per gram (C f u/g). The

bacterial isolates obtained were 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. The cultural characteristics of each fungi isolates were identified according to their colour, shape and the cell morphology was done based on mycelia, hyphae, septate, spore formation using lactophenol blue. A piece of the mycelium from the Petri plates was mounted on a clean grease free slide using a sterile wire loop and covered with a cover slip, after which a drop of lactophenol cotton blue was added and examined with the microscope The resultant microscopic characteristics were compared with the scheme provided by [8,9,10].

Antibiogram Screening

“This was carried with methods as recommended by Clinical and Laboratory Standard institute (CLSI, 2012). A loop full of test organism was inoculated on nutrient broth and incubated for 24 hours. Exactly 0.2 ml from the 24 hours culture of the organisms was dispensed into 20 ml sterile nutrient broth and incubated for 3-5 hours to standardize the culture to 0.5 McFarland standards (10^6 cfu/ml). Antibiotics susceptibility testing using the Kirby-bauer method as described by” [11].

Proximate Analysis

The moisture, crude protein, crude fat, crude fiber, Carbohydrate and ash contents of the peanut butters were determined using standard methods [12].

RESULTS

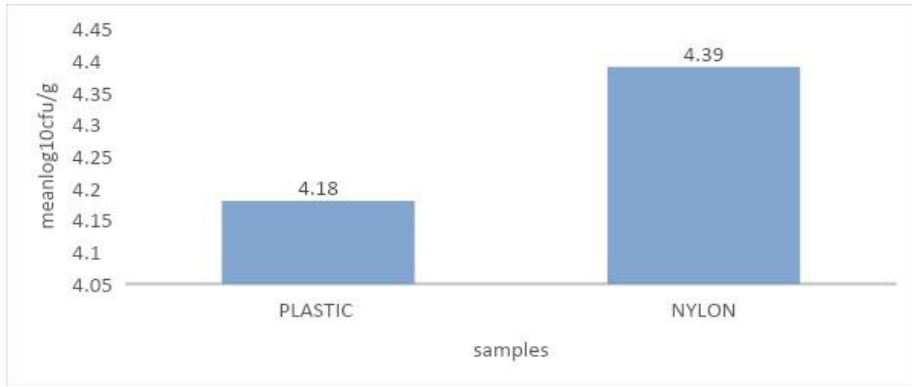


Fig. 1. Mean Total Bacteria count of Peanut butter

KEY : Nylon peanut butter, Plastic peanut butter

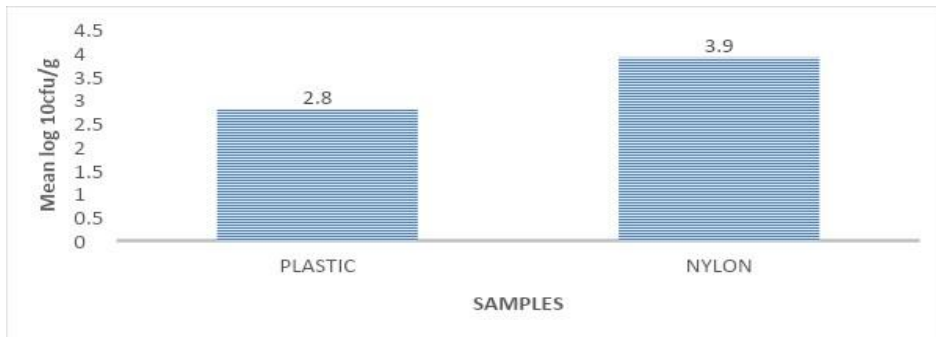


Fig. 2. Mean Total Fungi count of Peanut butter

Key : Nylon peanut butter, Plastic peanut butter

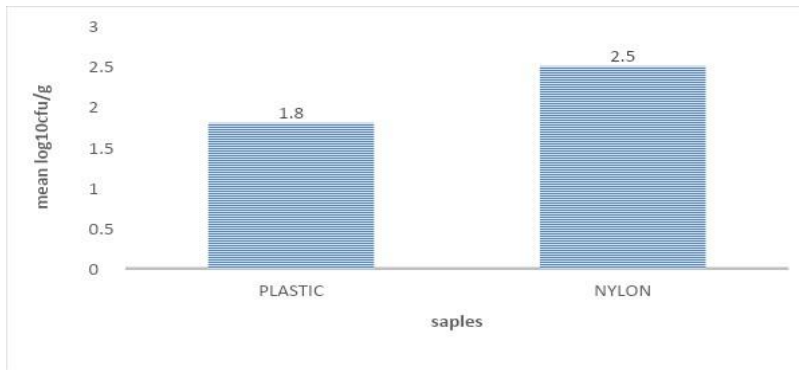


Fig. 3 Mesn Total Coliform count of Peanut butter

Key :Nylon peanut butter Plastic peanut but



Fig. 4. Percentage Frequency of organism isolated from peanut butter samples

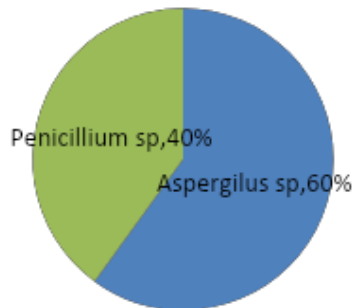


Fig. 5. Percentage occurrence of fungi isolated from Peanut butter samples

Table 1. The antibiotic susceptibility testing (mm) gram positive isolated from peanut butter

Sample c	Ode organism	AM	R	CPX	S	SXT	E	PEF	CN	APX	Z
PL	<i>Staphylococcus sp</i>	R	12	25	20	20	17	16	20	17	20
NY	<i>Staphylococcus sp</i>	7	R	23	19	21	14	20	18	16	25
PL	<i>Bacillus sp</i>	8	R	21	22	19	16	19	15	13	23
NY	<i>Bacillus sp</i>	R	8	20	18	20	15	22	16	R	19
PL	<i>Bacillus sp</i>	10	R	25	20	20	18	18	14	R	18
NY	<i>Bacillus sp</i>	R	10	25	22	23	19	15	20	12	20
PL	<i>Bacillus sp</i>	8	9	24	18	22	15	20	19	14	24
NY	<i>Bacillus sp</i>	9	10	22	20	16	16	19	15	18	22
PL	<i>Bacillus sp</i>	R	R	20	19	21	18	22	19	R	15
NY	<i>Bacillus sp</i>	10	R	25	21	20	19	15	15	7	14
PL	<i>Bacillus sp</i>	R	11	25	20	20	17	16	20	8	17
PL	<i>Enterococcus sp</i>	12	10	23	19	21	14	20	18	10	20
NY	<i>Enterococcus sp</i>	15	12	21	22	19	16	19	15	18	25
NY	<i>Enterococcus sp</i>	9	16	20	23	24	21	16	15	13	20
PL	<i>Enterococcus sp</i>	10	15	20	18	20	15	22	16	15	23

Key: AM= Amoxicillin, R= Ropephin, CPX= CIPROFLOXIN, S=Streptomycin, SXT=Seprin, E=Erythromycin, PEF=Pefloxacin, CN=Gentamycin, APX, Amipiclox, Z=Zinaape peanut butter Key:NY- Nylon peanut butter, PL- Plastic peanut butter

Table 2. The Antimicrobial susceptibility testing (mm) gram Negative isolated from Peanut butter

Sample Code	Organism	AM	AU	CN	PEF	OFX	S	SXT	CH	SP	CPX
PL	<i>Klebsiella sp</i>	16	17	20	25	26	10	16	25	20	30
PL	<i>Klebsiella sp</i>	17	18	22	19	21	R	20	18	18	25
NY	<i>Klebsiella sp</i>	18	15	18	20	19	16	19	20	18	23
NY	<i>Klebsiella sp</i>	14	11	16	18	23	R	22	19	16	20
NY	<i>Serratia sp</i>	10	13	22	24	20	15	18	18	21	27
NY	<i>Serratia sp</i>	17	20	19	22	23	20	30	20	22	25
NY	<i>Serratia sp</i>	21	16	20	21	21	17	18	23	18	19

KEY: AU=Augmentin, OFX= Ofloxacin, S= Streptomycin, CH=Chloramphenicol, AM= Amoxicillin SP= Sparfloxacin, EF=Pefloxacin, CN=Gentamycin, CPX=Ciprofloxacin, KEY: NY-Nylon peanut butter, PL- Plastic peanut butter

Table 3. Mean Proximate Composition of Peanut butter

Sample	Plastic Peanut butter	Nylon Peanut butter
Moisture content (%)	6.06	5.30
Ash (%)	5.5	3.58
Crude fiber (%)	6.58	4.51
Crude protein (%)	35	21.45
Crude lipid (%)	25	18.1
Carbohydrate (%)	24.09	31.56

DISCUSSION

Microbial Quality of Traditionally made Peanut Samples with different Wrapping materials

“The assessment of the quality and safety of food is vital to human health. An increased bacteria contamination alone does not make food unsafe but it reflects poor handling, poor storage, inadequate general hygiene during processing and/or poor-quality raw materials” [5,13] This study was done to evaluate the safety of the consumption of the traditionally made peanut butter. Microbial contamination in food items and its link to health risk in both animals and humans continue to be of great concern over the years. Peanut butter are a good source of protein and edible oil; it known to be mostly contaminated by microorganisms.

The mean total bacteria count of nylon peanut butter is log 10 4.39 while that of plastic is 4.18.cfu/g, This is slightly higher than counts obtained by (Odu and Okonko 2012) where the total bacteria count ranged from 2.54og CFU/g (3.5×10^2 cfu/g) – 3.56og CFU/g (2.3×10^3 cfu/g) and similar to the study conducted by [14] on RTE food. Traditional made, peanut butter is characterized by unknown hygienic conditions to our knowledge, The microbial count of heterotrophic bacteria obtained in this study is above the limit set by [15], for total viable bacteria the acceptable limit which is 5×10^4 – 10^5 cfu/g .This might be due to bad processing and prolonged storage as the pastes were produced in large quantity and might not be immediately sold because of the large quantity overshadowing the demand, forcing the producers to ensure long storage which can be regarded as lag storage resulting in an increased level of organisms contrary to the practice. The

mean total coliform count of nylon peanut is log [15] 2.5 while that of plastic is 1.8 cfu/g. This is similar to the counts obtained by [5].

The Organisms isolated include *Bacillus* sp (35.5%), *Escherichia coli* (3.2%), *Serratia* sp of (9.7%) *Enterococcus* sp (12.9%), *Staphylococcus* sp .(19.4%) and *Klebsiella* sp .(19.4%) *Bacillus* sp had the highest percentage of occurrence of 35.5%. While *Escherichia coli* was the least with a frequency of 3.2%. Existence of the genus *Bacillus* in this study, reports that these bacteria are associated with toxin production, consistent with [16]. It leads to food poisoning. These bacteria are normal inhabitant of the soil and can cause illness to humans and animals. These organisms can withstand harsh conditions such as roasting and mixing processes [17] The presence of *E. coli* indicates that the products are unsanitary processed directly from source, storage, and packaging. This can have a negative impact on consumer health [18]. *E. coli* causes dysentery [19,20]. This shows that fecal contamination of the peanut butter sample used in this study and the unsanitary handling of products that affect consumer health [21,22,23].

“The isolation of *E. coli* in the pastes was in line with the findings of [5] who also isolated *E. coli*, *Bacillus* sp, *Serratia* sp, *Proteus* sp, *Micrococcus* sp, and *Staphylococcus* sp. The presence of *Staphylococcus* sp in samples could be attributed to contamination from human handling, the surrounding air and environment during processing and display for sale in the markets. *Staphylococcal* sp in peanut paste can occur through the grinders and other utensils used in the processing. The sanitary condition of the markets can also lead to product contamination. The presence of these microorganisms

indicates the use of non-portable water mainly used in local food processed” [23,24].

Staphylococcus aureus is known to produce heat-stable enterotoxins, and when they enter living tissue [25], there is a potential for multiple antibiotic resistance. Microorganisms can grow surviving in the roasting process [26] if the sample is not kept at the proper temperature [23,24]. The presence of *Staphylococcus aureus* may also be due to aerosols transmitted from the human body and other inanimate objects, as approximately 40% of humans have *Staphylococcus aureus* in their nostrils. This species is commonly found on the skin, nose and mucous membranes of humans and linked to a variety of diseases such as urinary tract infections [27].

In addition, repeated manual contact with these foods can be reflected at the time of sale. Also, since retailers who purchased samples often encounter temperatures of 26-38°C, hence, growth of this organism may have been favored, cleaning the cooking utensils, grinders, and other utensils may have promoted the growth of this organism. The sanitary surrounding of these markets can also lead to food contamination. The presence of these microorganisms indicates a potential commination resulting from the use-of well water mainly used in the local food processing industries are not free from microbial contamination

The genus *Klebsiella* is associated with urinary tract infections in individuals with weakened immunity.

“Their isolation in this study calls for additional appropriate control measures. The presence of *E. coli* in peanut butter

samples indicates a microbial hazard and potential fecal contamination. Coliforms are thought to be part of the normal intestinal flora of humans and animals. They have been used as indicator organisms for food and water bacteriological quality. *E. coli* and *Klebsiella sp* were used to evaluate microbiological safety, hygiene during treatment, and ready-to-eat food” [28]. “The isolation of these organisms in peanut butter is in line with the findings” of [27].

Fungi were recorded in peanut butter samples studied, a finding in accordance with the report of [29,30,31]. The mean total fungal count for plastic is Log 10 cfu/g 2.8 while nylon peanut butter is 3.9 cfu/g. This work is in accordance with the work done by [31] although slightly higher where the total fungi count ranged from 10^4 to 10^6 CFU/g. The fungi count obtained in the sample was within the limit set by the Standard Organization of Nigeria which states that the maximum tolerance limit of 10^4 cfu/g for foods.

Aspergillus sp, and *Penicillium sp* were isolated. *Aspergillus sp* had the highest Percentage of occurrence of 60% and *Penicillium sp*, a percentage occurrence of 40%. These organisms were also found in the work of [31]. Other studies in Kenya, Benin, and Mali have also shown that peanut butter and other peanut products reveals the occurrences of *Aspergillus*, *Fusarium* and *Penicillium* [32,33,34,35]. It is reported to be common with (peanuts) among other materials, in their spores in the field during plant breeding, nut storage, product preparation, packaging, or final product storage. They can also initiate deterioration of the food material and/or produce spores which assist their survival [36].

Existence of the genus *Aspergillus*, It means the risk of mycotoxin formation and poses a health risk to consumers [37]. According to Pittet [38], the mycotoxin produced by *Aspergillus sp*. include aflatoxin and ochratoxin A (OTA). Mycotoxins are attracting worldwide attention due to the significant losses associated with their effects on human and animal health. Mycotoxins can be acute, chronic, or both toxic, depending on the type and intake of the toxin. Acute human illnesses include liver and kidney damage, attacks on the central nervous system (CNS), skin disorders, and hormonal disorders. Indeed, microbial-origin food-borne disease is a major international health problem associated with food safety in developing countries [39]. The susceptibility to peanut contamination is primarily due to nutritional content and is useful for many fungi. If the hulls, which protect the seed against invasion by fungi, become damaged, the underlying cotyledons become susceptible to attack.

Bacillus sp, *Escherichia coli*, *Serratia sp*., *Enterococcus sp*, *Staphylococcus sp* and *Klebsiella sp*, were isolated and it showed varying rates of resistance and susceptibility to the tested drugs. The Gram-positive Bacteria isolates were more susceptible to Ciprofloxacin, followed by Zinaapex, followed by Septrin, then Streptomycin, Pefloxacin, Gentamycin and Erythromycin respectively. But was more resistant to Amoxicillin and Ropephin, and least susceptible to Ampiclox [40]. The Gram negative Bacteria isolates were more susceptible to Ciprofloxacin, followed by Ofloxacin, followed by Pefloxacin, Chloramphenicol, Gentamycin, Spreflolcatin, Streptomycin, Amoxicillin respectively, the least susceptibility was seen in Augmentin, but was also more

resistant to Streptomycin. This is also similar to work done by [11].

PROXIMATE COMPOSITION OF TRADITIONALLY MADE PEANUT SAMPLES WITH DIFFERENT WRAPPING MATERIALS

“The proximate composition, the value of the mean moisture content was 5.30 - 6.06 for the peanut butter wrapped differently. these values are lower than (7.48) of raw peanuts” [41] and “this fact could be explained by the reduction of moisture content during the roasting which is an important step of peanut butter processing” [36]. “The water content or moisture of food affects its physical as well as chemical properties such as the structure, appearance and taste of the food product. These properties become important in determining the food’s susceptibility to spoilage, shelf life and the processing conditions required. Fat is one of the ingredients in peanut butter. The mean fat content of the peanut butter was reported to be 18 -.25 % for the peanut butter wrapped differently which is less than the value given by” [43]. The mean carbohydrate content of peanut was 24 -31 % for the peanut butter wrapped differently. The value of carbohydrate in peanut butter is comparable to data given by [31]. FSA [44] reported negligible values for carbohydrates in both butter and margarine.

The mean value of the ash content of this study was 3.5-5.5% for the peanut butter wrapped differently and is similar to the work done by [45] and the ash content of peanut spread was found to be 4.23% as indicated by [43]. The relatively higher Ash value might be as a result of the salt. The mean crude fibre content in these results (4.5- 6.5%), was within the range of the study of 24 who recorded 5%. This indicates

an ability of peanut butter to maintain a normal intestinal tract because diet low in crude fibre may cause constipation and colon diseases, The protein content is 21-35 % for the peanut butter wrapped differently which is slightly similar to the study of [31]. the studied peanut butters could be regarded as a valuable source of protein in improving the nutrition status of humans. In addition, the lipid content of the marketed peanut butters falls within the range (41-48 %) reported by [46] for peanut paste prepared with two Nigerian cultivars of *Arachis hypogea*. This result suggested that peanut it is an interesting oleaginous crop for which the implementation should result in the economic wellbeing of rural people. It can be deduced that peanut spread is a good source of protein and should be used in conjunction with other foods as protein supplements.

CONCLUSION

This study showed that the peanut butter was contaminated with pathogenic bacteria and fungi thereby posing health risk to consumers. Routine inspection and evaluation of peanut butter should be encouraged in Nigeria. Isolation of microorganisms such as *Staphylococcus sp*, *Escherichia coli*, *Bacillus sp*, *Salmonella sp*, which have public health concerns with traditionally made peanut butter samples, do not only pose health hazards to indigenous consumers but also to visitors who are exposed to its consumption. Enforcing proper sanitation and monitoring of products by relevant regulatory bodies should be encouraged This is to prevent contamination from environmental and human sources. Also control measures to prevent bacterial cross-contamination of raw and processed nuts must be enforced to maintain the hygienic quality of the processing environment and equipment. Application of

good agricultural manufacturing and Storage Practices and Combination Hazard Analysis and the application of a Hazard Analysis Critical Control Point (HACCP) system covering all stages of production, processing, packaging, and distribution reduces microorganisms. Useful for Edible peanut.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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