Microbial quality of peanut butter manufactured by small-scale producers at two popular open-air markets in Lusaka

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ABSTRACT

To contribute to the safety and quality of peanut butter manufactured by small-scale producers and consumed in Zambia, six samples from two popular local markets in Lusaka and six others from commercial retail outlets were procured and analyzed for microbial quality. Various fungal genera including *Mucor, Alternaria, Cladosporium, Penicillium, Trichothecium* and *Trichophyton* were identified, with *Cladosporium* being the most predominant. The main bacterial genus isolated and identified was *Bacillus*, and for both fungi and bacteria, total microbial loads in peanut butter samples produced by small-scale manufacturers were found to be significantly higher than those in samples from commercial retail outlets. The high microbial loads present a public health challenge necessitating an urgent need for good manufacturing and hygiene practices to help minimize fungal and bacterial contamination, improve the quality of the products and forestall the potential for food-borne disease outbreaks.

1 Introduction

Groundnut, (*Arachis hypogea* L.) also popularly known as peanut, belongs to the family Fabaceae, is a major annual oilseed crop and a significant source of protein with high lysine content, making it a good complement for cereal food products (Eke-Ejiofor *et al.*, 2012; Okaka, 2005). Mature groundnut seed has been approximated to contain mean values of moisture (1.81%), protein (18.42%), total lipids (49.66%), carbohydrate (21.3%), total sugars (4.9%), dietary fibre (8.4%), calcium (92 mg), magnesium (168 mg), phosphorous (376 mg) and iron 4.6 mg (reviewed in Bonku and Yu (2019); USDA (2010). Peanuts are sometimes considered as street food satisfying the needs of urban populations due to being readily available and comparatively cheaper than many processed snack foods (Donkor *et al.*, 2009). In Zambian urban areas, peanuts are sold in informal, open air markets where they are processed in different ways including being roasted as quick snacks eaten on their own eaten together with roasted cassava. Recent calls aimed toward value-addition have led to a rise in numbers of small-scale peanut butter manufacturers in Zambian urban open air markets. Small-scale manufacturers however face many constraints including limited financial resources to enable them procure facilities and equipment for good manufacturing practices. They consequently do not usually pay much attention to potential sources of contamination and for this reason, peanuts as raw material for peanut butter are one of the most likely sources of fungal and bacterial contamination especially during handling, storage and raw material transportation.

Microbial contamination has been estimated to increase along the marketing chain due to poor handling practices (Polixeni and Panagiota, 2008) and bacteria including Staphylococcus and Enterobacteriaceae and fungi including members of the genus Aspergillus have been reported in various studies on peanut product

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quality (Britton *et al.*, 2021; Carminati *et al.*, 2016). In a study on microbial assessment of peanut-processing confectionery factories in Brazil (Carminati *et al.*, 2016), *Salmonella* spp, *Escherichia coli* and *Enterobacteriaceae* were reported, while quality analysis of dakuwa, a Nigerian groundnut product (Ocheme *et al.*, 2014) revealed the presence of various fungal species including *Mucor* spp, *Aspergillus niger* and *A. flavus*. Bacteria including *Staphylococcus aureus*, *S. pyogenes* and *Bacillus subtilis* were also identified as microbial contaminants. The presence of *Aspergillus flavus* was suggested to be mainly due to contamination of peanuts during harvesting and post-harvest storage. Akinnibosun and Osawaru (2015) identified various fungal genera and species including *A. flavus, A. niger*, *Neurospora* spp., *Mucor* spp., *Penicillium* spp., *Trichoderma* spp. and *Fusarium* spp. from peanut butter samples in their study. They also identified *Staphylococcus aureus, Bacillus subtilis*, *Micrococcus* spp., *Streptococcus* spp. and *Proteus vulgaris* as contaminants of peanut butter samples. In a related study, **?** isolated *Mucor* spp., *Alternaria* spp., *Helminthosporium* spp., *Geotrichum* spp., *Fusarium* spp., *Cladosporium* spp., *Penicillium* spp. and *Aspergillus* spp. from peanut butter samples collected randomly and aseptically from different sellers in Nigeria while Gachomo *et al.* (2004) identified *Fusarium* sp, *Aspergillus* sp, *Rhizopus* sp, *Penicillium* sp., *Sclerotium* sp. and *Rhizoctonia* sp. in another study.

The major factor identified as a source of microbial contamination in groundnuts are shell damage, kernel splitting, poor harvesting practices and drought (Ukwuru and Acholo, 2010). Contamination of street-vended food such as peanut butter has been attributed mainly to exposure to polluted environment, poor sanitation and hygienic practices by vendors (Mensah *et al.*, 2002). Peanut butter is also vulnerable to aflatoxigenic fungal contamination (Rachaputi *et al.*, 2002) and this remains a serious health concern not least of which are concerns over aflatoxin-B1-producing *Aspergillus* species, which have been implicated in carcinogenesis or stunting in children (Bankole and Adebanjo, 2003; Mupunga *et al.*, 2017).

Msekera groundnut variety 4 (MGV4), MGV5 and Chishango are among the major varieties used in Zambia in various types of food and manufacturing products including vegetable oil and peanut butter. Peanut butter is made by extruding dry roasted groundnuts into a paste and small-scale manufacturers work under conditions that appear to consider quality control an unimportant factor. The levels of bacterial and fungal contamination have never been systematically assessed and records on microbiological quality of marketed peanut butter produced by small-scale manufacturers in the country are hard to come by. We observed with concern, the challenges faced by small-scale peanut butter producers of Lusaka particularly in unregulated factories and set out to examine the microbial quality of products selected at random. Results from this study suggest an urgent need for technical assistance to small-scale peanut butter producers to improve the quality of their products for the sake of both consumers and producers.

2 Materials and methods

2.1 Sample collection

Six peanut butter samples were purchased and aseptically collected from different street vendors at Soweto and City markets of Lusaka respectively, where sizeable quantities of groundnuts are sold by traders from various parts of the country. Some of the groundnuts are processed into peanut butter at factories located within the peripheries of market areas. Six commercially-produced peanut butter samples were also purchased from local supermarket outlets in Lusaka and samples were transported to the microbiology laboratory at the Department of Biological Sciences in a cooler box and stored at $4^{\circ}C$ until analysis. Potato dextrose agar, (PDA) for fungal culture and nutrient agar (NA) for bacterial growth (Sigma Aldrich, US) in powder were procured from Kansma Investments Ltd., Lusaka, Zambia. Culture media were prepared according to manufacturer's instructions with inclusion of 1.5% (w/v) agar and sterilized by autoclaving using standard conditions ($121^{\circ}C$ and 15 pounds per square inch, psi) for 15 minutes.

2.2 Microbial isolation

Peanut butter samples from the two public markets were aseptically and evenly mixed to make one composite sample and the same was done for the commercial samples. The isolation of both fungi and bacteria was carried out using an agar dilution method as described by Collado *et al.* (2007). From each sample, 1 g was aseptically weighed and transferred to 10 ml of sterile distilled water to make the first dilution. Serial dilution ($10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}$ and 10^{-6}) of respectively composited samples was performed by transferring 1 ml of the immediate previous dilution to 9 ml of sterile distilled water and mixing was done using a benchtop vortex at each step to make a suspension. From each dilution sample suspension, $100\,\mu$l was spread-plated on NA and PDA plates respectively. Bacterial cultures were incubated at $37°C$ for 24 hr. and discrete bacterial colonies on nutrient agar were counted and expressed in colony-forming units (CFU/ml) by dividing the number of countable colonies by the product of the dilution factor and the spread-plated volume.

Primary fungal cultures on PDA plates were incubated at $25°C$ until colony growth was visible after which they were sub-cultured on freshly-prepared PDA plates. Estimations of fungal quantities were performed by plating 0.1 ml of the serially-diluted samples on PDA plates and colonies were counted as the case was for bacteria.

2.3 Characterization and identification of bacterial and fungal isolates

Single bacterial colonies with distinct morphologies were picked up from primary Petri dishes and transferred to freshly-prepared Petri plates of nutrient agar medium. Identification was carried out on selective media as described by Prescott *et al.* (2002) and Sherman and Cappuccino (2005). Isolated bacteria were characterized by Gram-staining as described by Holt *et al.* (1994) and Sherman and Cappuccino (2005), and selected biochemical tests were conducted for tryptophan conversion to indole (indole test), citrate utilization, catalase activity, mannitol utilization and motility test as described (Sherman and Cappuccino, 2005; Holt *et al.*, 1994).

Fungi were identified using taxonomic schemes through comparisons of microscopic and macroscopic structures including colony morphology (mycelium colors, colony margins, mycelium texture) and microscopic structure (conidia shape, hyphae organization and nature of spore-bearing structures and spore types where possible).

2.4 Statistical analysis

All analyses were performed in Microsoft Excel and R Statistics Software. Differences between means were evaluated by a two sample T-test with significance difference being determined and established at $p = 0.05$.

3 Results

3.1 Various pre- and post-harvest fungi contaminate peanut butter samples

To determine the quality of peanut butter samples with reference to fungal content, serial dilutions of compounded samples were Petri-plated on PDA and fungal colony and mycelium characteristics were visually and microscopically examined. Fungi with varying colony morphologies producing pigments of various colors were isolated from peanut butter samples produced by small-scale producers and those produced by commercial manufacturers even though the levels of contaminations in commercially-produced samples were low, Figure 1. Some isolates grew as flat colonies and others as raised colonies. Colony margins were regular for some colonies and irregular for others and texture and pigment production also varied with some appearing wrinkled, compact or fluffy. Colonies of some of the fungal isolates produced red pigments in the growth medium (Fig. 1A and Table 1) while others had thali with radial folds or crater-like depressions in the center (Fig 1D).

Examination of microscopic structures revealed notable variations in conidia, hyphae, mycelia septation and sporangial formation in the isolates, Figure 2A-2F. Some isolates produced septate hyphae with short conidiophores

perpendicular to vegetative hyphae and others produced erect conidiophores with black to grey pigments or round to oval sporangia, Figure 2. Based on hyphal septation, sporangiophores and sporangium shapes, isolate A was identified as *Trichophyton tonsurans* while isolates B, C and F with short erect sporangiophores were identified to belong to the genus Cladosporium. The genus for isolate D could not be determined due to limited morphological features, Table 1 and Fig. 2. Taken together, these results suggest that different types of fungi occur as contaminants in peanuts used as raw materials by small-scale peanut butter producers in Lusaka.

Small-scale peanut butter producers described their production methods as involving roasting and mechanically peeling the skins of roasted peanuts before producing the final product. The results from this study would suggest that either the processing methods were not sufficient to reduce the levels and types of fungi with potential to contaminate the raw materials or the equipment used is not sufficiently cleaned to minimize contamination of final products. To therefore determine the effects of processing on fungal quality, quantitative comparisons of fungi in samples produced by small-scale producers and commercial samples were made through serial dilutions and the numbers of fungal colonies in the two sample sets were determined. Peanut butter samples produced by small-scale producers at the two markets had comparatively higher amounts of fungal contaminants than commercially-produced samples from retail outlets (Figure 3). These results support those from the qualitative analyses reported above, backing the need for quality control assistance to small-scale peanut butter manufacturers.

3.2 Bacteriological contamination was higher in samples from Lusaka markets

When analyzed for bacterial contamination, commercially-produced peanut butter samples showed comparatively lower levels of bacterial contamination than samples from the Lusaka open air markets sampled in the study. At the lowest dilution factor involving taking one-tenth of the undiluted peanut butter suspension to make a final volume, bacterial cell counts were observed to be high in both commercial and Lusaka market peanut butter samples. However, at the next lowest dilution factor of one-hundredth, samples from Lusaka markets had colony numbers which were too numerous to count (TNTC) (results not shown), while colony numbers in peanut butter samples from Lusaka markets were observed to be twice higher than those in commercial samples at 10^{-3} and 10^{-4}, again demonstrating that samples produced by open market producers needed quality control improvement.

To determine the variability in bacterial colony morphologies, selected colonies from both small-scale and commercial peanut butter samples with visibly distinct features (color, sizes and shapes) were examined visually and characterized. Similarities and variations in bacterial colonies from both peanut butter sample groups were observed, Table 2. Samples from both peanut butter sample groups had bacterial colonies which grew with flat undulate margins while others were raised or convex, Table 2. These results found no qualitative differences in bacterial contaminants from the two peanut butter sample groups while quantities of bacteria were higher in peanut butter samples from small-scale producers. Peanut butter samples from both sample sets had bacteria with hemolysis zones, and in terms of colony elevation, both flat and convex colonies were observed and some colonies has irregular and undulating margins while others had umbonate margins Table 2. Colony colors varied and included white, cream and bright yellow colonies.

To determine the variability in the biochemical properties of bacterial isolates, Gram-staining and selected biochemical tests were conducted on selected colonies. Rod-shaped bacteria were isolated from both peanut butter sample sets and all of them were positive for the Gram stain, Figure 4. In addition, all bacterial isolates were positive for the catalase test and hydrolyzed starch while some were positive for mannitol salt utilization and others were negative. Based on Gram stain and bacterial cell shape, the main bacterial contaminant in the peanut butter samples belonged to the genus Bacillus, Table 3.

4 Discussion

Microbial quality of foods produced by small scale producers is a subject or public concern particularly because producers in resource-poor countries tend to overlook the need for quality against production costs. The occurrence of bacteria and in some cases aflatoxin-producing molds in peanut butter samples has been reported in various studies (Britton *et al.*, 2021; Mupunga *et al.*, 2014, 2017). The quality of peanuts as raw materials for food products has been demonstrated at least in one study (Britton *et al.*, 2021) where bacterial and fungal contamination in peanuts from Senegal was analyzed. Other studies have demonstrated bacterial contamination with *Salmonella tennessee* and *Listeria monocytogenes* to be a challenge in peanut butter samples creating health concerns (reviewed Chang *et al.* (2013); Carminati *et al.* (2016)). We sought to identify some of the microbial quality issues faced by peanut butter producers in two open markets in Lusaka and have identified various molds belonging to the genera Cladosporium, Penicillium sp, Trichophyton, Mucor, Trichothecium and Alternaria. The genus that was most frequently isolated was Cladosporium species followed by Penicillium species. The bacteria isolated from the peanut samples were all Gram-positive rods and all were identified as bacillus species (*B. subtilis* and *B. cereus*). Enterobacteriaceae were identified in one study on peanut microbial quality and the results from Carminati *et al.* (2016) showed bacterial contamination to be a concern.

Fungal colony count in peanut butter samples from the two sample set, showed comparatively higher fungal contamination in samples from small-scale producers than that from samples from commercial retail outlets, Figure 3. The mycoflora was mainly represented by seven genera: Mucor, Alternaria, Trichothecium, Ulocladium, Cladosporium, Penicillium and Trichophyton and the predominant isolate belonged to Cladosporium genus. Our results are in agreement with those from one study (Ding *et al.*, 2015) in which PCR was conducted using primers targeting the internal transcribed spacer sequence to determine the mycoflora of peanut samples. Among the molds that were identified were Aspergillus, Cladosporium, Penicillium and Alternaria. Our study has identified Cladosporium, Mucor, Penicillium and Ulocladium species in peanut butter samples from Lusaka markets but these were not in commercial peanut butter samples. Trichothecium sp. was only isolated from commercial samples and not in peanut butter samples made by small-scale manufacturers. Results similar to ours were also reported from a study in Ivory Coast where Mucor, Alternaria, Cladosporium and Penicillium were identified in peanut butter and other peanut products (Boli *et al.*, 2013).

The loads of fungi in peanut butter samples from small-scale producers was higher (approximately 50% more) than those in samples from commercially-produced retail outlets peanut butter samples, even though Student's T-test analysis showed no significant difference in the fungal load between samples from the two markets in this study. The observed high microbial loads of peanut butter samples by small-scale producers indicate microbial contamination posing a health hazard to consumers and which may expose them to risks of acquiring food borne diseases. *Cladosporium* spp are generally considered non-pathogenic, while some *Alternaria* species have been shown to be opportunistic pathogens in immune-compromised patients and may cause allergic fungal sinusitis or other allergenic reactions as the case has been demonstrated for *A. alternata* (Loghmani *et al.*, 2017) or osteomyelitis (Chhabra *et al.*, 2013). We identified *Trichophyton tonsurans* as one of the isolates and this was of interest to us since this species has been demonstrated to cause Tenia capitis, an infection of the scalp at least in one study (Peixoto *et al.*, 2019) while Penicillium sp (Pitt, 1979), has been demonstrated to cause allergic alveolitis (Moise *et al.*, 2018).

Total bacterial counts enumerated from the peanut butter samples ranged from 105 to 107 CFU/ml with the highest value (4.6×10^7 CFU/ml) recorded in samples collected from Soweto and City market (local samples). The bacterial counts for the individual markets ranged from $1.1 \times 10^5 - 3.2 \times 10^6$ CFU/ml for commercially produced peanut butter samples and $1.3 \times 10^6 - 4.6 \times 10^7$ CFU/ml in samples produced by small-scale manufacturers. The total plate count for both markets was observed to be beyond the microbiological standard for legume products (1×10^4 CFU/ml). This is probably due to handling and processing process of peanut butter in Zambia. The bacterial loads of peanut butter samples for the local markets included in this study were comparatively higher than those of samples from commercial manufacturers. This could be due to handling and processing which could

be exogenously introducing microbes from the hands and mouth to the peeled groundnut. Bacillus species such as *B. cereus* are food poisoning bacteria while *Bacillus subtilis* causes miscellaneous problems. These bacteria are thus potential pathogens as they are ubiquitous in nature and occur as residents of soil, dust, bodies of insects, animals and humans that handle the groundnuts and its products (Frazier and Westhoff, 1978).

Food-borne illness of microbial origin are a major international health problem associated to food safety in developing countries (WHO, 2002). Contamination of the peanut butter has been attributed to exposure to polluted environment, poor sanitation and poor hygienic practices by the producers and the presence of most of the isolated organisms in the peanut butter samples highlight a potential public health problem concerning the consumption by the consumers.

5 Conclusion

The results of this study revealed the presence of bacterial and fungal contaminants in Zambian peanut butter samples. The fungi isolated include members of the genera Mucor, Alternaria, Cladosporium, Penicillium, Trichothecium and Trichophyton tonsurans while for bacteria only Bacillus species were isolated from the peanut butter samples. The potential origins and sources of the microbes that contaminated the peanut butter samples from this study including raw materials, workers and factory utensils were beyond the scope of the study.

6 Recommendations

Several suggested interventions to improve the quality of peanut butter produced by small-scale producers at Zambian open markets may be made. First among these, is the need to educate and train producers on the long-term benefits of good manufacturing and hygiene practices. Producers could be educated to understand that better quality products could improve their profits. Quality improvement could be achieved through cleaning of production equipment, and preparation, storage and processing of raw materials. The second recommendation would be to local authorities to provide adequate sanitation and refuse disposal facilities at open air markets. Lastly it would be helpful to producers and consumers alike if regular monitoring for adherence to basic standards was conducted together with efforts to assist small-scale peanut butter producers through financing and product quality certification.

Declaration of conflict of interest

The authors declare no conflict of interest. Funding for this research was provided by the researchers.

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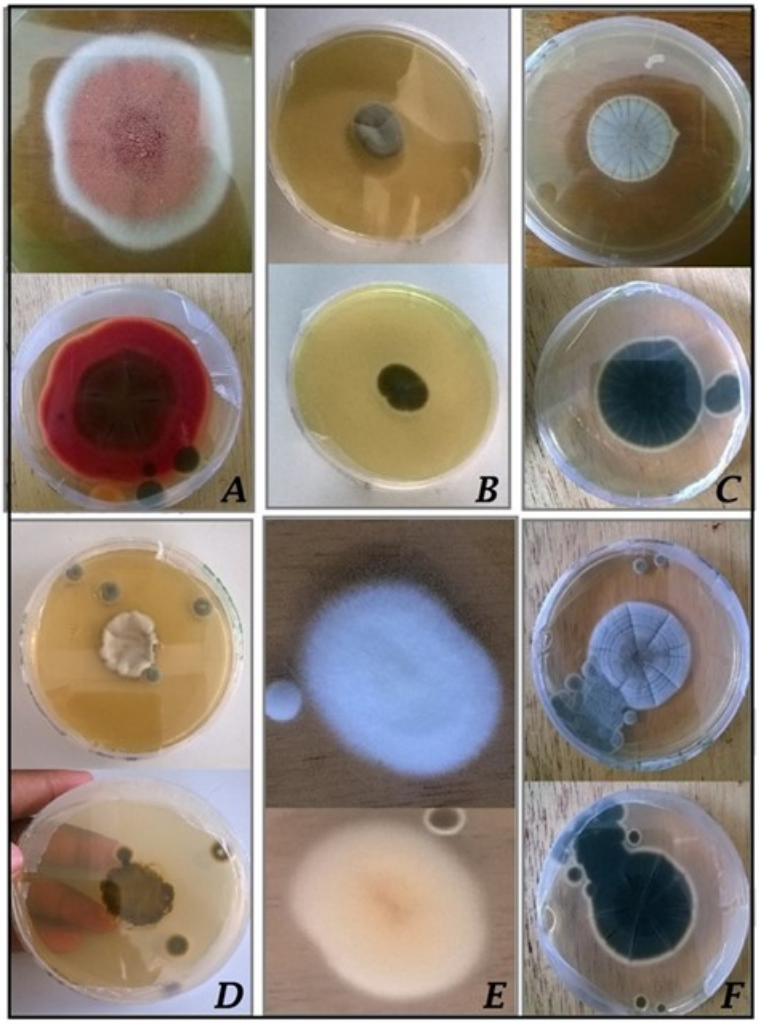

Figure 1: Variability in fungal colony morphologies of isolates from peanut butter samples with top plate view (upper panel of each lettered isolate) or back side view of the plate (lower panel of each lettered isolate). A, an isolate producing a pink colony with white margins and an intense-red pigment in growth medium. Variability in colony growth was observed in B (slow growth with olive-green color) or crinkled radial growth pattern, C. Colony showing lobate, D, zonate growth fluffy white, E and zonate growth pattern, F.

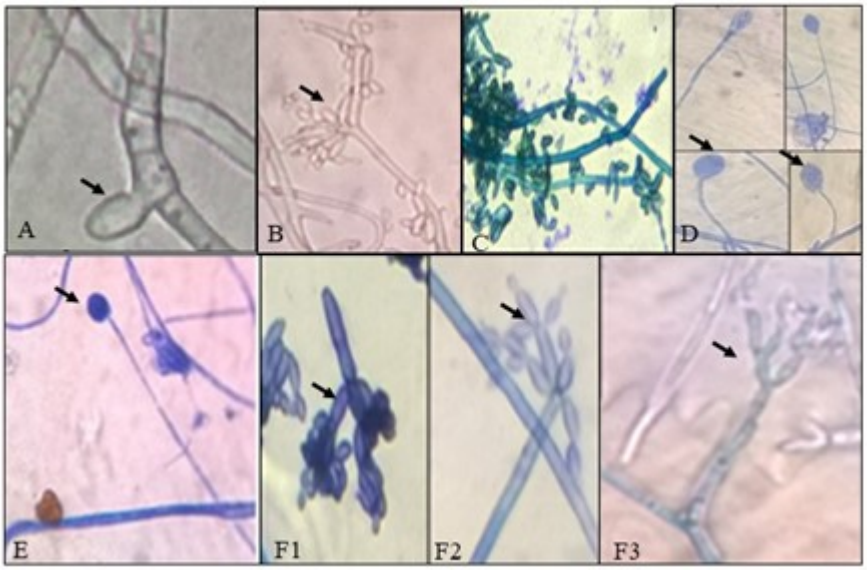

Figure 2: Variability in microscopic structures of fungal isolates from peanut butter samples. Arrows show distinct spores and spore-bearing structures.

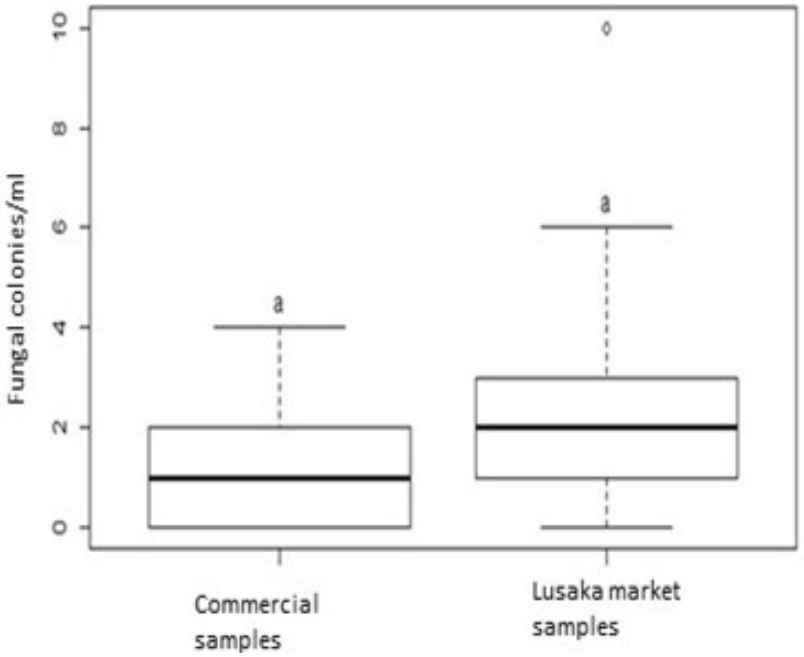

Figure 3: Differences in fungal quantities in peanut butter samples from commercial and Lusaka open air markets. Results were from two experimental replicates.

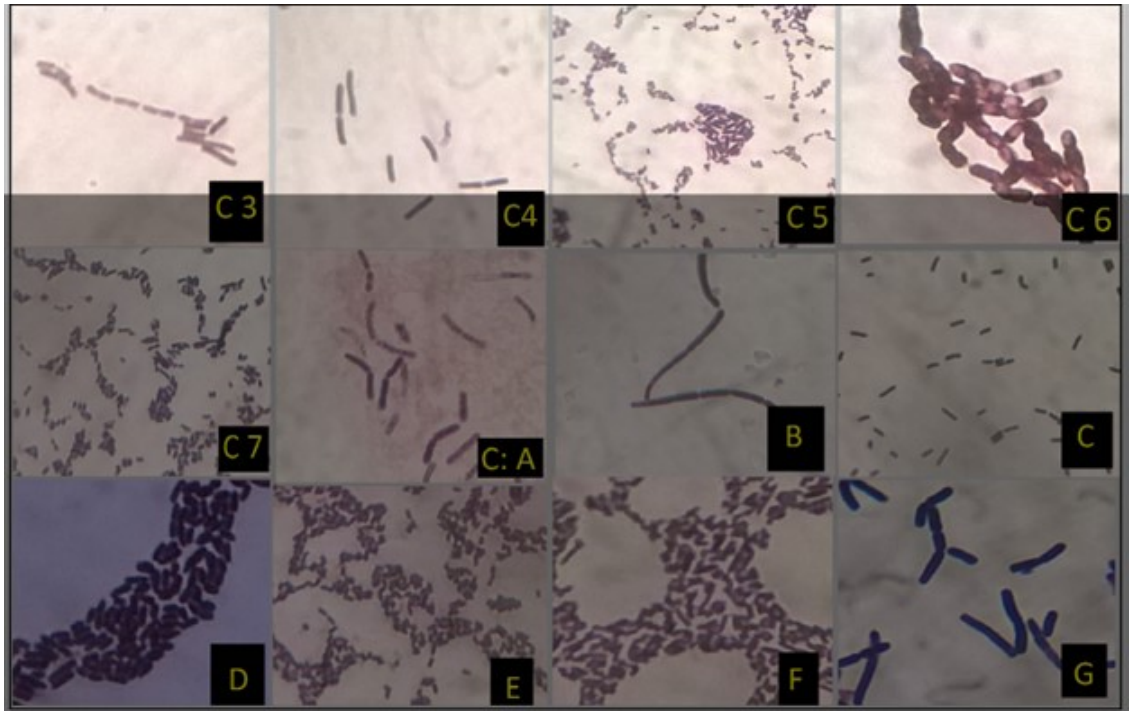

Figure 4: Gram-stain results of bacterial isolates from peanut butter samples. Colonies labeled C3 to C7 were for bacteria from peanut butter from commercial retail outlets while B to G were from samples manufactured by small-scale manufacturers and C:A was found in both sample types.

Table 1: Characteristics of selected fungal colonies isolated from peanut butter samples.

Colony	Morphology	Elevation	Margin	Surface	Color	Genus
A	Circular	Crateriform	Entire	Smooth	Red top with dark red area on the back side	*Trichophyton*
B	Oval	Raised	Entire	Smooth	Dull olive suede-like with dark green reverse	*Cladosporium*
C	Circular	Raised	Curled	Suede-like with gentle folds	Olive green with dark green reverse	*Cladosporium*
D	Circular	Wrinkled	Undulate	Wrinkled	Cream white top with olive brown reverse	*Unknown*
E	Oval	Raised	Entire	Smooth	White cottony top with white brown reverse	*Mucor*
F	Circular	Raised	Curled	Suede-like with gentle folds	Olive green with dark green reverse	*Cladosporium*

Table 2: Comparison of selected bacterial colonies from peanut butter samples.

Colony no.	Peanut butter source	Description					
		Form	**Elevation**	**Margin**	**Color**	**Size**	**Other**
1	Small-scale	Circular	Flat	undulate	Clear	Large	Haemolysis zone
1	Commercial	Irregular	Flat	umbonate	white	medium	dry colony
2	Small scale	Irregular	Flat	umbonate	Cream white	large	Dry colony
2	Commercial	Circular	Flat	undulate	clear	large	Haemolysis zone

Table 3: Biochemical characteristics of selected bacterial colonies from peanut butter samples from both commercial and small scale peanut butter producers.

Colony no.	S	GS	CT	SH	SIM	SCA	MSA	Microorganism
1.	Rods	+	+	+	Motility +; Indole −	−	−	*Bacillus* sp.
2.	Rods	+	+	+	Motility −; Indole −	+	+	*Bacillus* sp.
3.	Rods	+	+	+	Motility +; Indole −	+	+	*Bacillus* sp.
4.	Rods	+	+	+	Motility −; Indole −	+	−	*Bacillus* sp.
5.	Rods	+	+	+	Motility +; Indole −	+	+	*Bacillus* sp.
6.	Rods	+	+	+	Motility −; Indole −	+	−	*Bacillus* sp.
7.	Rods	+	+	+	Motility −; Indole −	+	+	*Bacillus* sp.

Key: *S = shape; GS = Gram stain; CT = catalase test; SH = starch hydrolysis; SIM = sulfur indole motility test; SCA = Simmons citrate agar for citrate utilization test; MSA = mannitol salt agar for mannitol test.*