



Microbial analysis of packaged and exposed soybean flour sold in selected markets in Benin City, Nigeria



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ABSTRACT

Soybean flour is one of the products derived from processing soybean (*Glycine max*) popularly known for its rich protein content. It is usually exposed to microbial contamination during processing and handling which compromise food safety. In this study, microbial assessment of exposed and packaged soybean flour sold in four (4) markets in Benin City was carried out. A total of sixteen (16) samples which comprise of three (3) exposed and one (1) packaged soybean flour were randomly purchased from each market. Two (2) samples of soybean flour (sun-dried and oven-dried) prepared in the laboratory served as control. Analyses of all the samples were performed using Standard microbiological methods. The total heterotrophic bacterial count (THBC) of the exposed product obtained from Oba (OBM), Ogba (OGM), Uselu (USM) and New Benin (NBM) markets were within the range of 6.31-6.86, 0-6.66, 6.02-6.60 and 5.70-6.50 log₁₀CFU/g while the equivalent values for total fungal count (TFC) were 0-4, 5.04-5.81, 4.90-5.18 and 4.48-5.27 log₁₀CFU/g, respectively. The THBC of the packaged product obtained from OBM, OGM, USM and NBM were 6.51, 6.89, 6.54 and 6.73 log₁₀CFU/g whereas the equivalent values for TFC were 5.76, 6.69, 6.72 and 6.43 log₁₀CFU/g, respectively. A total of 25 bacterial and 13 fungal isolates were obtained from all the samples. The bacterial isolates and their percentage occurrence were *Escherichia coli* (12%), *Klebsiella* sp. (12%), *Enterobacter* sp. (20%), *Citrobacter* sp. (20%) and *Bacillus* sp. (36%) while the fungal isolates were *Aspergillus niger* (23%), *Aspergillus clavatus* (8%), *Aspergillus nidulans* (8%), *Aspergillus flavus* (15%), *Aspergillus oryzae* (8%), *Saccharomyces* sp. (8%), *Penicillium* sp. (15%), and *Mucor* sp. (15%). Two exposed soybean flour samples from OGM and the control met the THBC criteria recommended by International Commission on Microbiological Specification for Foods, while others did not. Therefore, proper packaging, personal hygiene practices and good manufacturing practices are recommended to guarantee that soybean flour available in the markets are safe for human consumption.

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INTRODUCTION

Globally, it is estimated that 600 million persons fall sick after consuming contaminated foods of which 420, 000 persons lose their lives including 125, 000 children below 5 years old. There are wide varieties of food products derived from different sources such as soybean which could be contaminated by pathogenic microorganisms

(Waré et al., 2018).

Soybean [*Glycine max* (L.) Merr.] is an important oil seed belonging to the family *Leguminosae* and subfamily *Papilionaceae* (Mateos-Aparicio et al., 2008). It is usually cultivated as a food crop (Dugje et al., 2009; Pratap et al., 2012). Soybean is a stable food and its nutritional value is

of great importance (Chen et al., 2012). In addition, soybean has various non-edible uses (Etiosa et al., 2018). Cultivation of soybean in Nigeria mainly takes place in the savannas (Kamara et al., 2014).

It is estimated that the protein content of soybean range between 20-25% (Ogundele et al., 2015). The protein content of 1 kg of soybean is as much as protein content of 2 kg of boneless meat or 45 cups of cow's milk or 5 dozens of eggs (Dashiell, 2008). Soybean is rich in minerals (potassium, phosphorus, magnesium, sulfur, calcium, chloride, and sodium), vitamins (thiamin, riboflavin, vitamin A, vitamin E, niacin, pantothenic acid, and folic acid) and essential amino acids (lysine, leucine and isoleucine) (Hans et al., 1991; Liu et al., 1997; Naik and Gleason, 2010). However, the presence of antinutritional factors such as protease inhibitors, lectins, glycinin, saponins, oligosaccharides affect the nutritional value, utilization, and digestibility of protein found in soybean (Adeyemo and Onilude, 2013; Cabrera-Orozco et al., 2013; Yasothai, 2016).

In addition to nutritional importance, there are several health benefits associated with the consumption of soybean such as protecting the body against osteoporosis, lowering of cholesterol, reducing the risk of breast cancer, prevent prostate and uterus cancer development, proper functioning of the liver, kidney, heart and stomach as well as help in managing menopausal symptoms (Venter, 1999; Chen et al., 2012; Saha and Mandal, 2019; Asif and Acharya, 2021).

Processing of soybean lead to the production of soyflour, soymilk and soy yogurt. Soybean flour is commonly used to fortify wheat flour, maize flour and cassava flour (Naik and Gleason, 2010). A large portion of soybean flour available in the markets are produced mainly by women in their various homes. However, microbial quality of the product is not a priority to most of the producers and handlers mainly as a result of lack of awareness (Okwori et al., 2010). Although flour is generally regarded as safe due to its low water activity, contamination of the product with pathogenic and non-pathogenic microorganisms could occur during processing which render the product unsafe for human consumption. Foodborne outbreaks due to consumption of flour-based products is not common because they are subjected to kill step such as baking or cooking known to eliminate most microorganisms present in the product (Agwa and Ossasi-Chidi, 2016).

In recent times, limited studies on microbiological quality of soybean flour sold in various markets located in different cities have been reported. It should be a public health concern that results obtained from the studies carried out

indicate high microbial load of soybean flour. The microorganisms identified in the products were *Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyphi*, *Bacillus cereus*, *Proteus* spp., *Pseudomonas aeruginosa*, *Klebsiella* spp., *Proteus* spp., *Yersinia enterocolitica*, yeast and mould (Okwori et al., 2010; Okwori et al., 2010; Nwokocha et al., 2016; Athanase et al., 2021). Although the researchers attributed microbial contamination of soybean flour to poor handling of the products, unhygienic environment and processing machines, they paid little attention to the effect of packaging and non-packaging of soybean flour to microbial contamination.

Soybean flour either exposed or packaged is usually added directly to food without undergoing heat treatment. In many developing countries, soybean flour is widely used as a cheap source of protein to fortify weaning food shortly before feeding infants (Athanase et al., 2021). A large population of pregnant women and lactating mothers use soybean flour as a nutritional supplement (Nwokocha et al., 2016). Soybean flour mixed with crayfish is a popular product in many local markets (Samuel and Otegbayo, 2006). Many nursing mothers purchase the product and use it to fortify weaning food without considering the microbiological quality.

Newborns especially premature babies, low birth weight infants, immunocompromised infants and those living with HIV/AIDS stand a high risk of being sick as a result of consuming weaning food mixed with soybean flour and crayfish contaminated with pathogenic microorganisms (Athanase et al., 2021). The practice of mixing soybean flour with powdered crayfish, prawn, milk and other food additives depending on the choice of the producer without subjecting the final product to a processing method that will drastically reduce its microbial load predisposes the consumers to foodborne diseases. Therefore, this study is aimed at carrying out microbiological analysis of exposed and packaged soybean flour taken into consideration the food additives and preservatives in the packaged products sold in selected markets in Benin City.

MATERIALS AND METHODS

Sample collection

A total of sixteen (16) samples of soybean flour were randomly purchased from Ogba market, Uselu market, Oba market, and New Benin market. Three (3) samples of exposed soybean flour were obtained from each of the four (4) markets using a sterile polythene bag properly labeled for each sample collected. Four (4) samples of soybean flour sealed in plastic bottles by the manufacturer were also randomly purchased from four (4) shops located at Ogba market, Uselu market, Oba market and New Benin market. About 50 Kg of soybean grains were purchased

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from a trader using a sterile polythene bag. All the samples were hurriedly taken to Microbiology laboratory, Wellspring University for microbial analyses within 12 h.

Preparation of soybean flour

A slightly modified method described by Sanful and Darko (2010) was adopted in the preparation of soybean flour in the laboratory. The soybean grains were manually sorted and foreign materials removed. The grains were washed in clean water, and the chaff manually removed. The clean grains were boiled for 45 min at a temperature of 100°C, and then decanted. The boiled soybean grains were divided into two portions - A and B. Portion 'A' was sun-dried for 3 h while Portion 'B' was oven-dried at 150°C for 1 h using hot air oven (Gulfex Medical and Scientific England). The dried portions of the soybean grains were crushed into a fine powder using a laboratory blender (Usha Mixer Grinder, India) properly sterilized with 70% ethanol.

Serial dilution

Ten (10) fold serial dilution of the soybean flour samples were carried out using the procedure described by Jideani (2003). A plastic rack was arranged with ten (10) sterile test tubes containing 9 ml of sterile normal saline (8.5 g NaCl mixed with 1 L of water, autoclaved at 121°C for 15 min at 15 psi). One gram (1 g) of each soybean flour sample was weighed using electronic balance (Metler MT-2000). The weighed sample was carefully introduced into the first test tube using a sterile spatula, vigorously mixed and labelled as dilution 10⁻¹. A sterile pipette was used to transfer 0.1 ml solution from dilution 10⁻¹ to the next test tube (dilution 10⁻²). Subsequent stepwise transfers were carried out using a sterile pipette for each transfer until dilution 10⁻⁷ was reached. The content of each test tube was shaken vigorously before transferring an aliquot (0.1 ml) of the mixture into the next test tube.

Microbiological analysis

Total heterotrophic bacteria count

Total heterotrophic bacteria count (THBC) of the soybean flour samples were determined by culturing the samples on nutrient agar (NA) plates using pour plate method. The 10⁻³ and 10⁻⁴ dilution of the samples were selected, and aliquot (0.1 ml) of the dilutions were separately inoculated in well-labeled sterile Petri dishes in duplicates. Autoclaving of MacConkey agar (L-S Biotech, India), NA (Biomark Laboratories, India) and potato dextrose agar (L-S Biotech, India) was done at 121°C for 15 min at 15 psi

using the autoclave (Lincoln Mark Medical England, Model YX-280A). The autoclaved culture media prepared using manufacturers' instruction were allowed to cool to about 45°C, then poured on the sterile Petri dishes containing the inoculum, gently stirred and allowed to solidify. The inoculated plates were incubated at 37°C for 48 h using the incubator (Axiom Medical LTD UK). After incubation, the colonies observed on the culture plates were manually counted and results obtained were recorded. The bacterial population of the duplicate samples were calculated using the formula below.

$$\frac{\text{Cfu}}{\text{mL}} = \text{No. of colonies} \times \frac{1}{\text{serial dilution}} \times \frac{1}{\text{dilution plated}}$$

Isolation and maintenance of pure culture

The single colonies on the culture plates were identified and streaked as a primary inoculant on freshly prepared NA, incubated at 37°C for 24 h to obtain a pure culture after repeated subculturing. The pure culture obtained was maintained inside NA slants kept inside a refrigerator at 4°C until the isolates were identified.

Characterization and identification of the bacterial isolates

Bacterial isolates from the samples of soybean flour were characterized and presumptively identified based on their cultural and morphological characteristics, followed by Gram staining, motility test and biochemical tests which include catalase, oxidase, citrate, urease, indole and sugar fermentation (glucose) using the methods described by Isu and Onyeagba (2002) and Cheesbrough (2002). The culture media used for the biochemical tests include Christensen's urea agar (Titan Biotech LTD, India), Simmons citrate agar (L-S Biotech, India) and peptone water (L-S Biotech, India). They were autoclaved at 121°C for 15 min at 15 psi. In order to properly identify the bacterial isolates, the characteristics of each isolate were compared with that of known characteristics using the determinative schemes.

Total fungal count

An aliquot (0.1 ml) of dilutions 10⁻³ and 10⁻⁴ of the soybean flour samples were transferred aseptically into Petri dishes containing freshly prepared potato dextrose agar (PDA) in duplicates and properly labeled. The culture plates were incubated at room temperature (28 ± 2°C) for 5 days. On completion of the incubation period, the fungal colonies on the culture plates were manually counted and the results obtained was expressed in colony forming units per millilitre (CFU/ml) using the formula below.

$$\frac{\text{Cfu}}{\text{mL}} = \text{No. of colonies} \times \frac{1}{\text{serial dilution}} \times \frac{1}{\text{dilution plated}}$$

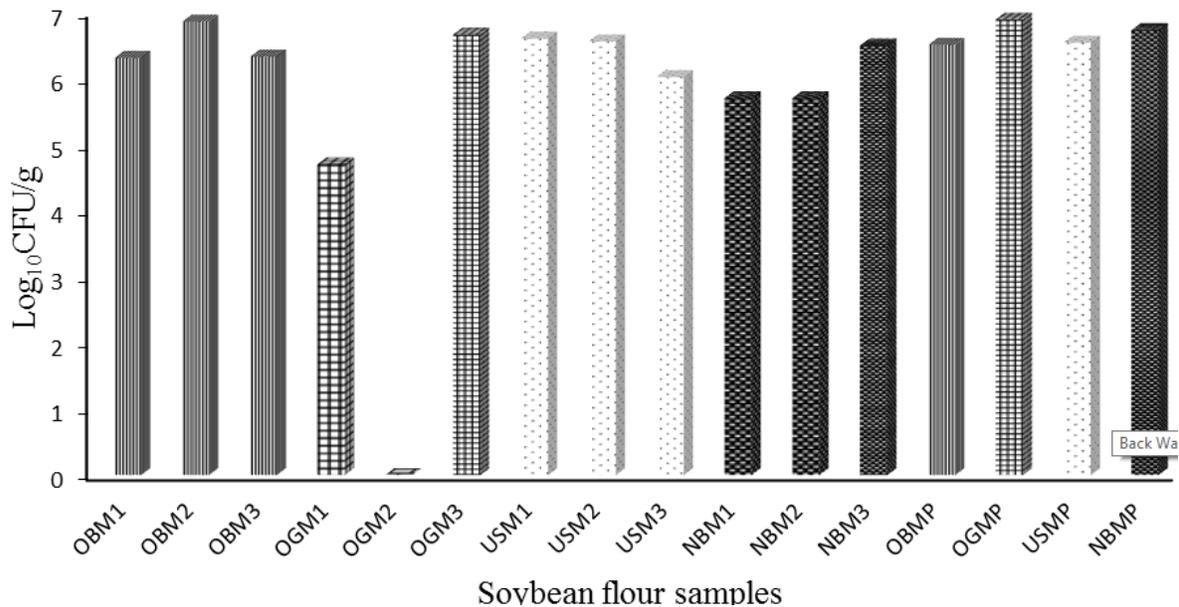


Figure 1. Total heterotrophic bacterial count of exposed and packaged soybean flour obtained from the selected markets. **Key:** OBM1-3 represent the exposed soybean flour from Oba market; OGM1-3 represent the exposed soybean flour from Ogba market; USM1-3 represent the exposed soybean flour from Uselu market; NBM1-3 represent the exposed soybean flour from New Benin market; OBMP represent the Packaged soybean flour from Oba market; OGMP represent the Packaged soybean flour from Ogba market; USMP represent the Packaged soybean flour from Uselu market; NBMP represent the Packaged soybean flour from New Benin market.

Purification of the fungal isolates

A sterile inoculating needle was used to pick discrete colonies on the fungal plates and streaked on freshly prepared PDA plates. The inoculated plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 5 days to obtain pure isolates.

Characterization and identification of the fungal isolates

The fungal isolates were characterized and identified based on colonial morphology and microscopic characteristics. The microscopic morphology of the fungal isolates was determined by viewing their mycelia under the microscope (Olympus binocular microscope) at x40 objectives with lactophenol cotton blue stain. The morphology of the fungal isolates under the microscope were compared with reference standards as described by Ellis et al. (2007) and Geo et al. (2013).

RESULTS

Presented in Figure 1 is the total heterotrophic bacterial count (THBC) of soybean flour obtained from the four

selected markets in Benin City. The THBC of exposed soybean flour obtained from Oba market, Ogba market, Uselu market and New Benin market were within the range of 6.31-6.86, 0-6.66, 6.02-6.60 and 5.7-6.5 $\log_{10}\text{CFU/g}$ whereas the corresponding values for the packaged soybean flour were 6.51, 6.89, 6.54 and 6.73 $\log_{10}\text{CFU/g}$, respectively.

Depicted in Figure 2 is the total fungal count (TFC) of exposed and packaged soybean flour obtained from the selected markets. The TFC of exposed soybean flour obtained from Oba market, Ogba market, Uselu market and New Benin market were within the range of 0-4, 5.04-5.81, 4.9-5.18 and 4.48-5.27 $\log_{10}\text{CFU/g}$ whereas the corresponding values for the packaged soybean flour were 5.76, 6.69, 6.72 and 6.43 $\log_{10}\text{CFU/g}$, respectively.

The cultural and morphological characteristics of bacterial isolates from the soybean flour samples are represented in Table 1. Table 2 shows the result of motility and biochemical tests carried out on the isolates for proper identification. The bacterial isolates identified as depicted in Table 1 and 2 were *E. coli*, *Klebsiella* sp., *Enterobacter* sp., *Citobacter* sp., and *Bacillus* sp.

Presented in Table 3 is the morphology of the fungal isolates from the samples of soybean flour obtained from the selected markets. The fungal species identified were *Penicillium chrysogenum*, *Mucor* sp., *A. flavus*, *A. niger*, *A. oryzae*, and *A. clavatus*.

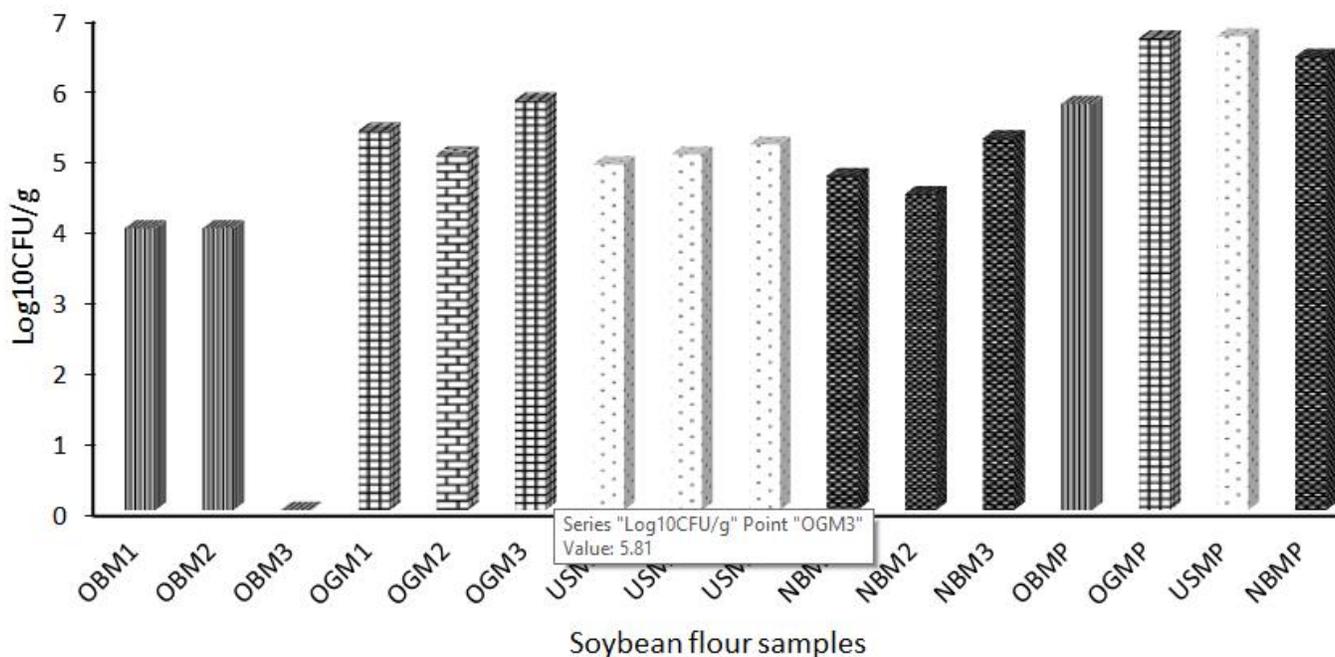


Figure 2. Total fungal count of exposed and packaged soybean flour obtained from the selected markets. **Key:** OBM1-3 represent the exposed soybean flour from Oba market; OGM1-3 represent the exposed soybean flour Ogba market; USM1-3 represent the exposed soybean flour from Uselu market; NBM1-3 represent the exposed soybean flour from New Benin market; OBMP represent the Packaged soybean flour from Oba market; OGMP represent the Packaged soybean flour from Ogba market; USMP represent the Packaged soybean flour from Uselu market; NBMP represent the Packaged soybean flour obtained from New Benin market.

Table 1. Cultural and morphological characteristics of the bacterial isolates.

Isolate code	Margins	Elevation	Surface	Colour	Shape	Gram reaction	Cell type	Cell arrangement	Opacity	Probable microorganism
1	Entire	Low convex	Smooth	Greyish white	Circular	-	Rods	Singly	Opaque	<i>E. coli</i>
2	Smooth	Convex	Mucoid	Greyish white	Rod	-	Rods	Singly	Opaque	<i>Klebsiella</i> sp.
3	Entire	Flat	Mucoid	Whitish	Rod	-	Rods	Singly	Opaque	<i>Enterobacter</i> sp.
4	Entire	Convex	Smooth	Red	Circular	-	Rods	Singly	Translucent	<i>Citrobacter</i> sp.
5	Curled	Slightly convex	Rough	Grey white	Irregular	+	Rods	Chains	Opaque	<i>Bacillus</i> sp.

Key: + represent positive; - represent negative.

The THBC of the soybean flour (sun-dried) prepared in the laboratory is 4.86 log₁₀CFU/g, whereas there was no culturable bacteria found in soybean flour (oven-dried) also prepared in the laboratory. The bacterial isolate found in the soybean flour (sun-dried) prepared in the laboratory is *Bacillus* sp. No culturable fungal specie was detected in both samples of soybean flour prepared in the laboratory.

Presented in Figure 3 is the percentage occurrence of bacterial isolates found in the exposed and packaged soybean flour samples. They include *Citrobacter* sp. (20%), *Bacillus* sp. (36%), *E. coli* (12%), *Klebsiella* sp. (12%) and *Enterobacter* sp. (20%). Figure 4 shows the percentage occurrence of fungal isolates from the exposed and packaged soybean flours which were *A. niger*

Table 2. Biochemical test for identification of the bacterial isolates.

Isolate code	Catalase	Oxidase	Citrate	Urease	Indole	Glucose fermentation	Motility	Probable microorganisms
1	+	-	-	-	+	+	+	<i>E. coli</i>
2	-	-	-	+	-	+	-	<i>Klebsiella</i> sp.
3	+	-	+	-	-	+	+	<i>Enterobacter</i> sp.
4	+	-	+	-	-	+	+	<i>Citrobacter</i> sp.
5	-	-	+	-	-	+	+	<i>Bacillus</i> sp.

Key: + represent positive; - represent negative.

Table 3. Cultural and morphological characteristics of the fungal isolates.

Isolate code	Colour	Surface	Elevation	Margins	Form	Spore	Probable isolates
1	Creamy	Smooth and moist	Flat	Entire	Circular	Ascospores	<i>Saccharomyces</i> sp.
2	Blue-green	Rough and cottony	Craterform	Curled	Irregular (slightly)	Conidia spores	<i>P. chrysogenum</i>
3	Yellowish green	Smooth	Flat	Entire	Circular	Blastopore	<i>A. flavus</i>
4	Dark brown	Powdery	Flat	Filiform	Irregular	Conidial	<i>A. niger</i>
5	Pale yellow	Smooth	Flat	Entire	Circular	Conidial	<i>A. oryzae</i>
6	Blue-green	Smooth	Raised	Entire	Circular	Conidiophore	<i>A. clavatus</i>
7	Brown	Smooth	Flat	Entire	Spherical	Conidial	<i>A. nidulans</i>
8	Brown	Rough and cottony	Flat	Entire	Circular	Sporangiophore	<i>Mucor</i> sp.

(23.08%), *A. clavatus* (7.69%), *A. nidulans* (7.69%), *A. flavus* (15.38%), *A. oryzae* (7.69%), *Saccharomyces* spp. (7.69%), *Penicillium* spp. (15.38%), and *Mucor* spp. (15.38%).

DISCUSSION

The result obtained from this study shows that exposed soybean flour from Oba market (OBM2) had the highest heterotrophic bacterial count (6.86 log₁₀CFU/g) whereas there was no culturable bacteria in the exposed soybean flour (Sample OGM2) obtained from Ogba market. Other samples of soybean flour from the four markets had varying population of heterotrophic bacteria. Unculturable bacteria reported in one out of sixteen samples of soybean flour obtained from the markets could be as a result of the processing method used in producing the soybean flour. It is most likely that extensive heat was applied during preparation of soybean flour (Sample OGM2) which drastically reduced the bacterial population to undetected level in the finished product. In addition, the handler of Sample OGM2 in the market might have applied good

hygienic practices that prevented cross contamination of the product and probably the product was freshly produced (Sanful and Darko, 2010). However, the THBC of eleven (11) samples of exposed soybean flour from different vendors which were within the range of 4.70-6.86 log₁₀CFU/g is an indication of poor handling and unhygienic practices of the vendors and soybean flour processors. The storage condition of soybean flour and shelf life of the product are factors that could also influence the microbiological quality. The result obtained from a six months shelf study of soybean flour showed that the product was contaminated with *Enterobacter*, *Staphylococcus* sp., *Bacillus* sp. and *E. coli* (Akinola and Owoseni, 2017).

It is shocking that majority of the soybean flour samples, both the packaged and exposed did not meet the criteria stipulated by International Commission on Microbiological Specification for Food (ICMSF) with regards to total bacterial count in food which should not be above 6 log₁₀CFU/g (Odu et al., 2019). Meanwhile, soybean contains saponins, phenols, flavonoids, micronutrients and polysaccharides which possess varying levels of antimicrobial potentials. A study carried out by Chaleshtori

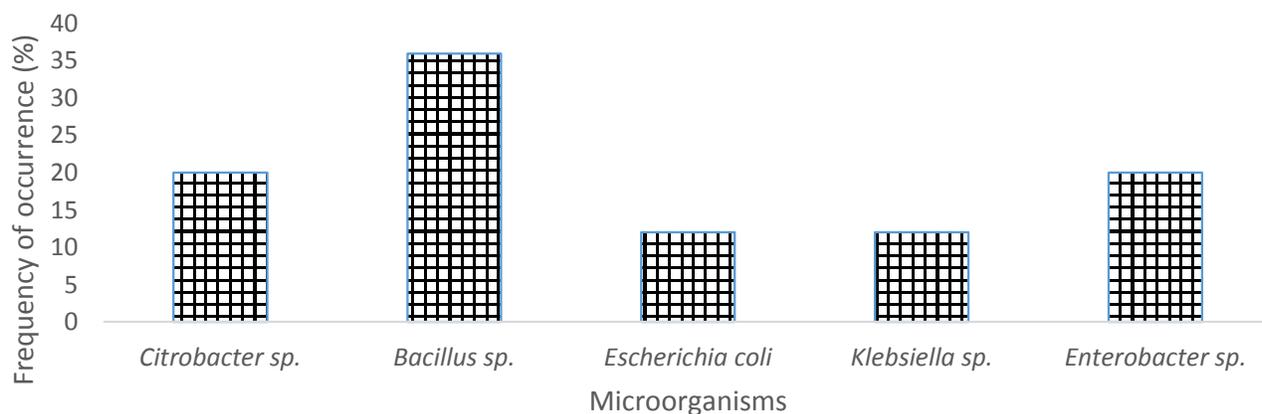


Figure 3. Percentage occurrence of the bacterial isolates from the soybean flour.

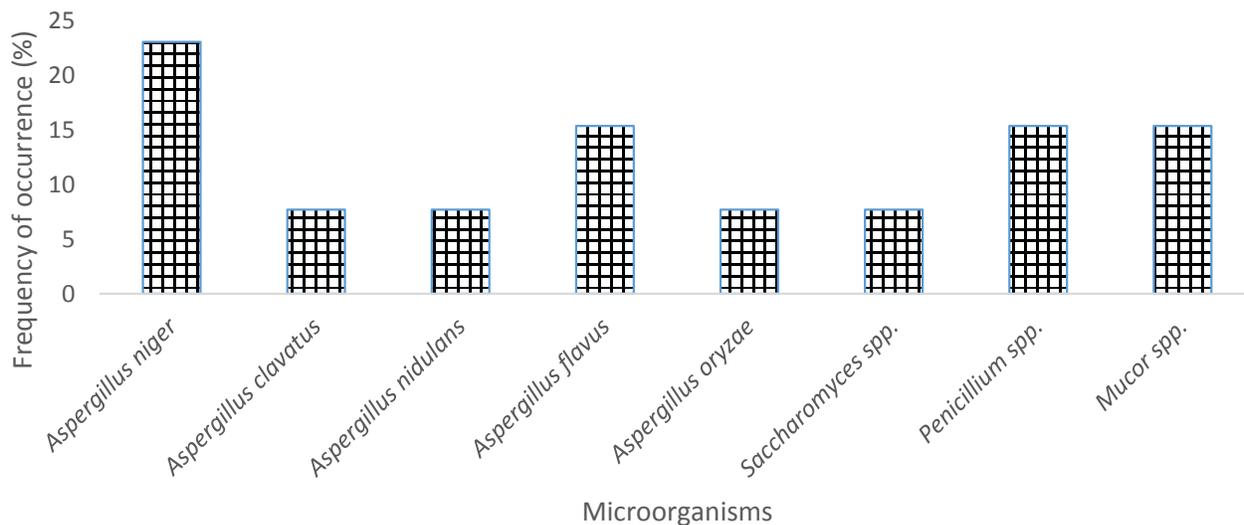


Figure 4. Percentage occurrence of the fungal isolates from the soybean flour.

et al. (2017) reported that methanolic extracts of M7 and M9 varieties of soybean demonstrated significant antimicrobial effect against *Listeria monocytogenes*, *B. cereus*, *S. aureus* and *Klebsiella pneumoniae*. In a related study, Dhayakaran et al. (2015) reported that 10 and 100 µg/mL concentration of isoflavones from soy flour had antimicrobial effect against *L. monocytogenes* and *E. coli*, respectively. Surprisingly, the result obtained from this study shows that chemical composition of soybean flour might not have played a significant role in reducing the microbial load of the product. The method of processing soybean into flour could have affected the chemical compounds in soybean which possess antimicrobial properties.

According to Zumbes et al. (2014), high microbial load in foods is an index of poor personal hygiene and sanitary

conditions during preparation, storage, and marketing of the food products. Exposure of food products to the environment is a critical factor responsible for bacterial contamination of the products. Several studies aimed at assessing the microbial air quality of markets and its environs reported the presence of diverse microorganisms in large numbers. A study carried out by Makut et al. (2014) reported the presence of bacterial species namely *E. coli*, *Bacillus sp.*, *Enterobacter aerogenes*, *S. aureus* and *Streptococcus pyrogenes* while the fungal species reported were *A. flavus*, *A. niger*, *A. fumigatus*, *Penicillium sp.*, *Rhizopus stolonifer*, *Mucor sp.*, *Absidia corymbifera*, *Alternaria alternate* and *Candida albicans* from outdoor air environment. Most of the bacterial and fungal isolates reported by the researchers were also detected in the samples of exposed and packaged soybean flour

evaluated in this study.

As for the packaged soybean flour obtained from the markets, the sample obtained from Ogba market had the highest THBC (6.89 log₁₀CFU/g) whereas the lowest value was encountered in packaged soybean flour obtained from Oba market (6.51 log₁₀CFU/g). It is surprising that THBC of all the packaged soybean flour is higher than the values reported in most of the soybean flours exposed in the markets. What could be considered as one of the sources of microbial contamination of the packaged soybean flour is the additives soybean processors mixed with the product to add value to it. A feedback from the traders revealed that most of their customers prefer packaged soybean flour than exposed soybean flour because of attractive packaging, labelling and additives inside the packaged product. They also stated that customers have the impression that packaged soybean flour is safer and less contaminated by germs than exposed soybean flour. Finally, the traders reported that nursing mothers that are low income earners living in their environs patronize soybean flour either exposed or packaged more than powdered milk because it is a cheap source of protein. They directly add it to weaning food to fortify it and feed their babies. Many children, adolescents and adults also add soybean flour (exposed or packaged) to their diet. Research findings from this study indicates that consumption of either packaged or exposed soybean flour contaminated with potentially pathogenic microorganisms exposes infants, children, adolescents and adults to risk of foodborne diseases.

The result obtained from this study shows that soybean flour (sun-dried) prepared in the laboratory had a lower heterotrophic bacterial count compared with soybean flour either exposed or packaged with the exception of Sample OGM1 and OGM2. High bacterial count in most samples of the commercialized soybean flour (packaged and exposed) could be attributed to microbial contamination of soybean seeds in the field and during storage, poor handling of the seeds by vendors, dirty environment, unhygienic practices during processing of soybean flour, microbial contamination of food additives mixed with soybean flour and exposure of the product to the environment without proper packaging. However, the application of hygienic practices during processing of soybean flour and excluding food additives in the product prepared in the laboratory could be responsible for lower microbial load of the product when compared with majority of the commercialized soybean flour.

From the result obtained from this study, exposure of soybean seeds for three (3) hours under the sun before milling could not eliminate all microorganisms present in the seeds. *Bacillus* sp. was isolated from soybean flour prepared using the sun-dried soybean seeds. This could be attributed to the ability of *Bacillus* sp. to form spores resistant to harsh environmental conditions. However, the absence of culturable bacteria in the soybean flour

prepared using soybean seeds oven-dried at 150°C for one (1) hour could be attributed to the effectiveness of dry heat in eliminating culturable bacteria present in the soybean seeds. In a related study, Ansah et al. (2020) reported that yeast and mould count of plantain flour subjected to sun-drying is 3.9 × 10⁵ CFU/g whereas the sample subjected to indirect sun-drying (solar cabinet drier) is 8.6 × 10⁴ CFU/g.

Findings from this study show that total fungal count of exposed soybean flour obtained from Oba market within the range of 0-4 log₁₀CFU/g is lower than the values encountered in similar products obtained from other markets. There was no culturable fungi found in soybean flour (Sample OBM3) obtained from Oba market. According to Amadi et al. (2014), food becomes unacceptable if the total fungal count exceed the standard threshold of 10⁴ CFU. Based on the Microbial Standards of the Public Health Laboratories of the Advisory Committee for the Food and Dairy Products which categorized certain foods as unsatisfactory, only soybean flour obtained from Oba market is considered safe for human consumption. The total fungal count of exposed soybean flour obtained from Ogba, Uselu and New Benin markets were within the range of 5.04-5.81, 4.90-5.18 and 4.48-5.27 log₁₀CFU/g, respectively. Among the packaged soybean flour obtained from the markets, the sample from Oba market had the lowest fungal count (5.76 log₁₀CFU/g) compared with the corresponding values for packaged soybean flour from Ogba market (6.69 log₁₀CFU/g), New Benin market (6.43 log₁₀CFU/g) and Uselu market (6.72 log₁₀CFU/g). The lowest fungal count encountered in the packaged soybean flour from Oba market compared with other packaged products could be as a result of calcium propionate added to the product which was excluded in packaged soybean flour obtained from New Benin and Uselu markets. According to Inetianbor et al. (2015), calcium propionate is a food additive which serves as an antimicrobial agent. The use of potassium sorbate in snacks is often recommended to prevent the growth of yeasts and mould (Amadi et al., 2014).

The product label of packaged soybean flour obtained from New Benin market (NBMP) stated that sugar and vanilla flavour were added to soybean flour. Dried fish, prawn, egg, dehydrated carrot, banana, sucrose, salt and vanilla powder were added to packaged soybean flour obtained from Uselu market (USMP) according to the information on the product label. As for packaged soybean flour obtained from Ogba (OGMP) and Oba market (OBMP), the products were mixed with sugar, vanilla flavour, milk, sucrose and calcium propionate. Although the products in the market were not exposed to the environment which harbours ubiquitous microorganisms, the chemical composition and microbiological quality of food additives, food preservatives and materials used in fortifying soybean flour could influence its microbial load. A recent study carried out by Ugwu (2019) reported that

dried crayfish obtained from Ogbete main market in Enugu metropolis was contaminated with microorganisms which include *Staphylococcus* sp., *E. coli*, *Bacillus* sp. and *Klebsiella* sp. Fresh prawn properly dried is nutritious. It has a sweet and unique flavour remarkably not the same with fresh prawn. According to Haque et al. (2012), bacteria isolated from dried prawn were *Micrococcus varians*, *Micrococcus radiodurans*, *S. aureus*, *E. coli*, *Klebsiella edwardsii*, *K. ozaenae*, *Bacillus subtilis*, *Bacillus megaterium* and *Aeromonas hydrophila*. Research findings by Pitchiah (2004) stated that carrot powder prepared as a convenient food is also contaminated with microorganisms. In a related study, Wanjera (2020) reported that banana flour was contaminated with yeast and mould (6.65 CFU/g), *E. coli* (3.03 CFU/g) and coliforms (4.46 CFU/g).

According to Pal et al. (2016), post-process contamination of dried milk powder predisposes the product to microorganisms such as *Salmonella*, *B. cereus* and *S. aureus*. Sugar is mainly used as a food sweetener. It also adds flavour to food and functions as a food preservative. However, excessive intake of sugar could lead to obesity, increase in risk of diabetes and cancers (Zaitoun et al., 2018). Research findings published several decades ago reported that refined sugar could be contaminated with thermophilic spoilage bacteria (Wolk and Smith, 1944). Improper handling of milk powder and sugar which is eventually added to soybean flour could contaminate the product (Inetianbor et al., 2015). Pure sucrose is literally known to contain no nutrient. Despite the reasons behind the use of synthetic additives in food, Inetianbor et al. (2015) warned against using them indiscriminately especially in products meant for children due to adverse health effects. The effect of food additives manifest early signs which include headaches, change in energy levels, mental concentration and behaviour while increased risk of cancer, cardiovascular disease as well as other degenerative conditions could manifest after many years. A recent study carried out by Habiba et al. (2019) reported the absence of pathogenic microorganisms in nearly 90% samples of food additives sold in super shops located in different parts of Dhaka City. The result obtained shows that total viable bacterial count of food flavours which include vanilla, chocolate, strawberry, mango, lemon and orange were 2×10^1 , 2.1×10^1 , 4.1×10^2 , 3.6×10^2 , 4.4×10^2 and 2.5×10^2 cfu/ml, respectively. Among the flavours, vanilla exhibited the highest level of antimicrobial activity against *E. coli*, *Bacillus* spp., *Pseudomonas* spp., *Klebsiella* spp., *Salmonella* spp. and *Staphylococcus* spp. Salt (NaCl) added to food functions as a preservative and also improves its flavour. In addition to kitchen use, many food industries add salt to their products. In recent times, the increase in daily intake of salt is becoming a source of concern to health professionals because excessive intake of sodium is associated with hypertension which might result in

cardiovascular diseases. Daily intake of salt recommended is 6 g NaCl (Ecem-Akan and Kink, 2017).

This study has shown that soybean flour prepared in the laboratory (sun-dried and oven-dried samples) had no fungal growth. This could be as a result of hygienic practices put in place during preparation of soybean flour in the laboratory which might not be strictly applied during processing and handling of soybean flour available in the markets. Non-inclusion of crayfish, prawn and other food additives reported to harbour microorganisms into soybean flour prepared in the laboratory under hygienic conditions could also have contributed to absence of culturable fungi in the product. A total of five (5) bacterial species identified from the exposed and packaged soybean flour were *E. coli*, *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp. and *Bacillus* sp. Only *Bacillus* species was detected in soybean flour (sun-dried) prepared in the laboratory. This could be as a result of spores formed by *Bacillus* sp. which could be resistant to food processing conditions. In a related study, Okwori et al. (2010) reported the presence of *E. coli*, *S. aureus*, *Proteus* sp., *Bacillus* sp., *P. aeruginosa*, *Klebsiella* sp., *Y. enterocolitica* and *Salmonella paratyphi* from samples of soybean flour sold in Makurdi metropolis. This is in agreement with the findings from this study. The presence of *E. coli* and *Klebsiella* sp. in the soybean flour samples could be as a result of faecal or sewage contamination of water, equipment and environment where soybean flour was processed (Bukar et al., 2009). Flies perching on exposed soybean flour in a dirty market environment, food additives already contaminated with microorganisms, poor storage conditions, unhygienic practices of soybean processors and traders could be responsible for bacterial contamination of the product.

E. coli is a Gram-negative bacterium which cause gastroenteritis mainly in infants and children. It is a well-known enteric microorganism (Akinola and Owoseni, 2017). In a related study, Elenwo et al. (2019) attributed microbial contamination of yam flour, plantain flour and cassava flour exposed in the market to too much handling of the products by the personnel, the use of poor quality water and undue exposure of the flour to the environment. A study carried out by Waré et al. (2018) reported the presence of *Enterobacter* sp. and *Klebsiella* sp. in flours used in the preparations of weaning food. This corroborates the findings from this study. *Enterobacter* sp. and *Klebsiella* sp. are implicated in structural abnormalities affecting the urinary tract as well as recurrent urinary tract infections (UTIs).

The source of contamination of packaged and exposed soybean flours with *Staphylococcus* sp. could be from the normal flora of the skin of the food handlers. *Staphylococcus* sp. is implicated in food poisoning due to enterotoxin produced by the bacterium which manifest symptoms such as diarrhea and vomiting. *Bacillus* sp. also isolated from the soybean flours could produce

enterotoxins which cause food poisoning (Akinola and Owoseni, 2017). *Bacillus* sp. is a known spore former found in water, soil, air and on vegetation. This could be the reason *Bacillus* sp. had the highest frequency (36%) of occurrence among the bacterial species isolated from soybean flour either packaged or exposed including soybean flour prepared in the laboratory. Among the bacterial isolates, *E. coli* and *Klebsiella* spp. had the least frequency of occurrence (12%). This result is not in agreement with research findings from a similar study that involved bacteriological assessment of soybean flour sold in Markurdi metropolis which reported that *E. coli* had the highest frequency (32.7%) of occurrence among the bacteria isolates (Okwori et al., 2010). In a related study involving microbiological analysis of exposed and packaged cassava, plantain and yam flour sold in selected markets, Odu and Maduka (2019) reported that *Bacillus* sp. and *E. coli* had the highest (46.67%) and least (10%) frequency of occurrence among the bacterial isolates, respectively. A total of four (4) fungal genera were identified in the exposed and packaged soybean flour obtained from the four markets. The fungal isolates were *Aspergillus niger*, *A. clavatus*, *A. nidulans*, *A. flavus*, *A. oryzae*, *Saccharomyces* sp., *Penicillium* sp., and *Mucor* sp. According to Odu and Maduka (2019), infections caused by *Penicillium* sp. include rhinocerebral mucormycosis, genitourinary, mucocutaneous, gastrointestinal, pulmonary and disseminated infections. The source of the fungal genera isolated from soybean flours could be from air around the environment during processing and marketing of soybean flour. Some species of *Saccharomyces* are opportunistic pathogens.

In a related study, Waré et al. (2018) reported the presence of *Mucor* sp., *Penicillium* sp., *Fusarium verticillioides*, *A. flavus*, *Aspergillus carbonarius*, *Aspergillus tamari*, *A. oryzae* and *Aspergillus sydowii* in exposed flours used in formulating weaning food. They further reported that some species of *Aspergillus* found in the flour were capable of producing mycotoxins. Some of the fungal isolates reported by Waré et al. (2018) were also identified in soybean flour sampled from four markets in Benin City. Among the fungal isolates encountered in the soybean flour either exposed or packaged, *A. niger* had the highest frequency of occurrence (23%). This result should be a public health concern because some species of *Aspergillus* could produce aflatoxins. Fungal isolates with the least frequency of occurrence (7.69%) were *A. clavatus*, *A. nidulans*, *A. oryzae* and *Saccharomyces* sp. Consumption of soybean flour contaminated with pathogenic fungi could result in chronic diseases especially in immunocompromised individuals.

RECOMMENDATION

Strict compliance with GMPs during preparation of

soybean flour is advocated. Soybean flour sold in the market should be properly packaged to prevent cross contamination of the product by microorganisms and foreign materials. Indiscriminate and unregulated use of synthetic food additives by some producers of soybean flour should be discouraged on health grounds. Parents and guardians are advised not to purchase unbranded soybean products containing food additives with the intention of adding it directly to weaning food of their children. Since the microbiological quality of 100% soybean flour prepared under strict hygienic condition is better than soybean flour mixed with crayfish, prawn and other materials, it is recommended for everyone. To improve the microbiological quality of soybean flour mixed with additives from different sources, the product should be subjected to gamma irradiation.

Conclusion

This study revealed that soybean flour either exposed or packaged randomly purchased from Oba, Ogba, Uselu and New Benin markets were highly contaminated with bacteria and fungi except Sample OGM2 and OBM3. The THBC of the exposed and packaged soybean flour were within the range of 0-6.86 and 6.51-6.89 log₁₀CFU/g whereas the equivalent values in terms of total fungal count were 0-5.81 and 5.76-6.72 log₁₀CFU/g, respectively. The bacterial species isolated from the products were *E. coli*, *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp. and *Bacillus* sp. while the fungal isolates were *A. niger*, *A. clavatus*, *A. nidulans*, *A. flavus*, *A. oryzae*, *Saccharomyces* sp., *Penicillium* sp., and *Mucor* sp. The THBC of soybean flour (sun-dried) prepared in the laboratory is 4.86 log₁₀CFU/g, whereas no culturable bacteria were found in the oven-dried sample. No culturable fungi was found in both products. Only *Bacillus* sp. was found in the sun-dried sample whereas no culturable bacteria was found in the oven-dried sample. Therefore, soybean flour prepared in the laboratory under hygienic conditions were less contaminated by microorganisms when compared with soybean flour either exposed or packaged obtained from the markets with few exceptions.

Competing interests

The authors have declared that no competing interests exist.

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