

Microbial Evaluation Of Locally Produced Soybean Products

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Abstract: This study is aimed at the microbial evaluation of locally produced soybean products such as soymilk and soy yeast produced by a Nigerian manufacturing company in Benin City, Edo State, Nigeria. The microbial evaluation procedures were done using the Pour plate method and streaking. The media used were Nutrient Agar (NA), MacConkey Agar (Mcc), Blood Agar (BA) for isolation of bacteria and Potato Dextrose Agar (PDA) for isolation of fungal organisms. Incubation was carried out in an inverted position at 25⁰C for five days for fungal isolates and 37⁰C for bacterial isolates. Statistical analysis was done using the SPSS 10.0 package software and excel. The result of the mean bacteria count ranged from 0.85×10^2 – 20.3×10^2 cfu/g. *Staphylococcus* sp, *Bacillus* sp, *Enterobacter Bacillus*sp had the highest frequency of occurrence rate of **4 (36.36%)** and **4 (36.36%)** respectively. Antibiotic sensitivity test showed that almost all the bacteria isolates were susceptible to the antibiotics used except for Ampiclox and Amoxicillin to which they were resistant. The fungal counts ranged from 0.45×10^2 – 15.3×10^2 cfu/g. The fungal isolates were *Trichoderma* sp, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani*, *Alternaria alternata* and *Candida* sp. *Aspergillus flavus* had the highest frequency of occurrence rate of **5 (29.41%)**. The microbial population obtained from this study was below the acceptable limit of 2.0×10^4 cfu/g recommended for the general bacterial count by the Soy Food Association of America (SFAA). It is therefore, recommended that adequate monitoring and strict adherence to quality control measures and good manufacturing practice (GMP) during production should be strictly adhered to, as this will ensure that products are free from pathogenic microorganisms.

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Key words: Microbial evaluation, Microbial contaminants, Soybean products

1. Introduction.

Soya bean (*Glycine max*) the primary material for soy yeast and soy milk production has been identified to be one of the important legumes of the tropics with high protein content (Liu, 2004). It is a potential food material that contains all essential amino acids that are very important for the proper development of the body and indeed has a higher lysine content in comparison to other plant proteins (Daniyan and De, 2011). Soybean has been consumed in several ways in various parts of the world. There is a growing awareness in the production and utilization of soybean in Nigeria within the last decade (Daniyan and De, 2011). An expanding market is due largely to the popularity of soybean as a miracle crop to address problems of malnutrition (Michelfelder, 2009). Soybean seed actually has hundreds of uses from industrial products like engine oil or crayons to food products and animal feeds (Ayo *et al.*, 2011). It is widely used for the production of soy yeast and soymilk as a food condiment incorporated into local Nigerian dishes to improve the nutritional value (Hua *et al.*, 2007) Soy yeast is a mixture of soy flour and

yeast concentrate whereas soymilk is a mixture of soybean and milk flavoring agent (Daniyan and De, 2011).

Soybean is an alkaline food and as such is a good medium for the breeding of bacteria. Among the microbes present in the contamination and spoilage of locally produced soy flour are *Bacillus* sp, *Micrococcus* sp, *Klebsiella* sp, *Escherichia coli* and *Staphylococcus* sp. The presence of spore-formers like the *Bacillus* is of particular importance because their presence could produce toxins (Micheal and Larry 2007) and these toxins have detrimental effects on the human health (De Nadita, 2002).

Many people may say that attacks on the quality of locally produced food products are simply scare tactics designed to increase profit for food products manufactured in the large-scale industries. Yet, large increases in cancers and other diseases related to poor food quality point to a large problem in the current quality of what we take in as food (Micheal and Larry 2007). Despite government regulations of food safety through the Hazard Analysis Critical Control Point (HACCP), dangerous contaminants may still be in the

food product even after ensuring safe procedures of food production. Despite all its numerous uses for which soy bean flour has been recognized, little or no attention has been paid to the microbiological content of its products during storage and production. There is scanty or no literature as regards the presence of these contaminating microbes in Nigeria. This study, therefore, focuses on the determination of the microbial load of the soybean products. The importance of this study cannot be overemphasized as it will address the paucity of information on the microbes associated with soybean production in Nigeria. The specific objectives of this study, therefore are to determine the microbial load of the soybean products and identify the bacteria and fungi genera isolated from the soybean products.

2. Materials And Methods.

Hand gloves, Petri dishes, tips, L-shaped glass rods, syringe, incubator, antibiotic sensitivity disks, cotton wool, disinfectants (Savlon) aluminum foil, masking tape, McCartney bottles, marker pens, test tubes, Durham tubes, conical flasks, beakers, measuring cylinders, canisters, Gram stain reagents, glass slides, standard wire loops, light microscope, weighing balance and spatula.

Sterilization Of Materials

All glass wares such as conical flasks and test tubes used in this study were thoroughly washed with detergent and rinsed in clean water to ensure that they were grease-free. They were allowed to drip-dry and arranged in canisters. The glass wares were then properly sterilized in an autoclave at 121°C for 15mins. at 15 lbs. Those that had screw caps were sterilized with their caps relatively loose around the glass mouth. The inoculating loops were sterilized by the red heat method with the aid of the Bunsen burner flame before and after use.

Preparation And Sterilization Of Media

All media were prepared from commercially available products and made up according to the manufacturers' instructions. They were sterilized by autoclaving at 121°C for 15min at 15lbs except otherwise stated. The culture media used were: Nutrient-Agar (NA); MacConkey Agar (Mcc), Blood Agar (BA) for bacterial isolates and Potato Dextrose Agar (PDA) for the fungal isolates.

Sampling Site And Collection Of Samples

The sampling site is the Amen Jan Nigeria Limited, a small manufacturing enterprise that deals with the production of Soybean food such as golden meal soymilk and soy yeast. Its factory is located in one of the incubation sections in Technology Incubation Centre in Benin City, Edo State, Nigeria.

Samples were collected five times between January and February 2016. They were transported to

the microbiology laboratory in sterile polythene bags for microbial evaluation. All samples were processed within 24hours. Samples collected were analyzed for the following; to estimate the total microbial count, isolate bacteria and fungi from the soy samples and identify the bacterial genera and fungi group isolated from the soy samples

Isolation Of Bacterial And Fungal Isolates

Pour plate method was used. One milliliter of the serially-diluted sample ($10^1, 10^2, 10^3$) was dispensed aseptically into sterile petri-dishes each containing the appropriate media for isolation of bacteria species and fungal species. Incubation was carried out in an inverted position at 25°C for five days for the fungal isolates and at 37°C for 24hrs for the bacteria isolates.

Enumeration Of Bacteria

Nutrient agar was used for the estimation of total bacterial count by the pour plate method. Empty sterile Petri dishes were inoculated with 1ml of the appropriately diluted samples. About 15ml of the molten nutrient Agar (45°C) was then poured into the inoculated Petri dish. The Petri dish gently rotated clock-wise and anti-clock-wise to ensure uniform mixing of the inoculum with the agar medium. The agar was allowed to set and the culture plates were then incubated at 37°C for 24-48hr. After incubation, bacterial colonies formed on the agar-plates were counted. Only plates with bacterial colonies of between 50 and 300 were used for this purpose.

Identification And Characterization Of Isolates

The identification of bacteria isolates was based on their morphological, cultural and biochemical characteristics. Gram reaction, oxidase, catalase, sugar fermentation (glucose, arabinose, dulcitol and mannose), indole production, urease, citrate, methyl red (MR) and Voges-Proskauer (VP) tests were carried out. The identification of the isolates was carried out using the method of Cowan and Steel (1974) Manual for the Identification of Medical Bacteria. For fungal isolates, pure cultures of the isolates obtained were used for identification. The identification of the fungal isolates was based mainly on the structural features as observed from the growing colonies in mounts seen under the microscope. The plate identification involved, color, presence of mycelia, type of spores and production of fruiting bodies in the microscopic examination. A wet amount of each isolate was prepared on a microscopic slide, covered with a cover slip, stained with lactophenol-cotton-blue and viewed under x10 and x40 magnification to ascertain the features of the isolates. The features of the isolates were compared with those described in standard fungi manuals.

Morphology And Cultural Characteristics Of Bacterial Isolates On Media

Twenty four (24) to 48 hr. agar cultures of each isolate were used in determining their cultural characteristics. The features examined in the colonies include; - edge, shape, colour, opacity surface appearance and microcopy.

Gram's reaction

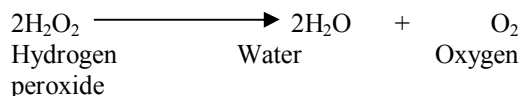
Gram staining reaction was carried out according to the method of Harrigan and McCance (1976). A smear of the organism was made on a clean, grease-free slide with the aid of a sterile wire loop. The smear was fixed by passing the slide through a Bunsen burner flame. Thereafter the smear was covered with crystal violet dye for about 30 to 60 sec. Lugol's iodine (a mordant) was applied to the slide for 30 sec. This was washed over the sink using distilled water. The next step involved decolorization with acetone. After decolorization, a counter-stain (saffranin) was applied onto the slide and allowed to stay for about 30 sec and washed off over the sink with slow-running distilled water. This was done for each of the isolates. The slides were allowed to air dry and then viewed with the aid of a light microscope using the oil immersion objective.

Oxidase test

The procedure of Steel (1961) was used. One end of a filter paper was soaked with 1% solution of the reagent, tetra-methyl-para-phenylene-diaminedihydrochloride. A 24 hr nutrient agar culture of the test organism was smeared on the soaked filter paper, using a sterile bent glass rod. A deep-blue purple color observed on the smeared filter paper within 5-10 sec indicates a positive reaction, while no color change on the filter paper indicates a negative oxidase test.

Catalase test

A drop of distilled water was placed on a clean glass slide. With the aid of a sterile wire loop, a bit of the isolate was emulsified onto the slide. A drop or two of 3% solution of hydrogen peroxide (H₂O₂) was added to the suspension of the organism. The production of gas bubbles indicates a positive test, while a negative test was indicated by no gas bubble (Harrigan and McCance, 1976).



Sugar fermentation test

The sugars tested were glucose, lactose and manitol. The sugar fermentation medium was constituted according to manufacturer's instruction. The medium was dispensed into 5ml Bijoux bottles containing Durham tubes and sterilized using the

autoclave at 121°C for 15 min. The bottles were allowed to cool after sterilization and inoculated with the test organisms. The culture was then incubated at 37 °C overnight. After overnight incubation, a color change from red to yellow indicated acid production while air bubbles found within Durham tubes indicated gas production. Controls were set up appropriately with tubes containing sugar, basal medium, and indicator but lacking the test organism.

Indole test

The test was carried out according to the description of Kovacs (1928). The test organism was inoculated into sterile nutrient broth and incubated at 37°C for 48hr. After incubation 0.3-0.5 ml of Kovacs' reagent (p-dimethylaminobenzaldehyde, amyl alcohol and conc. HCL) was added to the culture. A pink colour ring formation in the tube indicated a positive test, while no coloration showed a negative test.

Urease test

The test organism was inoculated onto sterile urea agar slant containing urea and the indicator, phenol red. The culture was then incubated at 37°C for 4-24 hr. After incubation, if the organism utilizes the urea, the medium would turn cherry-red. This shows that the organism is urease positive; otherwise it is urease negative.

Citrate utilization test

The ability of each isolate to utilize citrate as its only source of carbon and ammonia as its only source of nitrogen was tested using Koser citrate medium. The medium was prepared, dispensed into McCartney bottles, sterilized and allowed to cool in the slant position. It was then inoculated with a 24-hr agar culture of each isolate and incubated at 37°C for 24-48 hr. At the end of the incubation period, a change in color from green to deep-blue, due to alkaline reaction indicated citrate utilization and was recorded as positive. Absence of color change indicated a negative result.

Methyl red and Vogues-Proskauer test

This was carried out by inoculating tubes containing MR-VP broths with 24-hr culture of the test organism and incubated at 37 °C for 48 hr. After 48 hr. a few drops (about 3-5) of MR (Methyl red indicator) were added to the tubes. A positive result shows a pink color in the tube due to the production of acid during dextrose fermentation while an orange or yellow color indicates a negative result.

Also, VP reagent (5% Alpha-Naphtol and Ethanol; Potassium hydroxide (KOH); deionized water) was added to the tubes. The tubes were oriented in the slant position and left for 15mins to 1 hr. for the reaction to take place. A positive VP reaction shows a reddish color at the top of the broth, while a negative reaction shows a brownish color.

Coagulase Test

The slide test method for bound coagulase was conducted to determine if any of the microbial isolates could elaborate the enzyme coagulase. A drop of water was placed on a clean grease free slide, upon which a bit of the isolate was emulsified onto the slide with the aid of a sterile wire loop. A drop of plasma was placed on the microbial suspension and mixed gently. Immediate clumping (20secs to 2min) was recorded as positive result (Obulie *et al.*, 1998).

Motility Test

Motility test was by stab inoculation. The sterilized medium was allowed to cool to about 45°C. The medium in each tube set in vertical position, was then stab inoculated with 24hrs culture of the isolate, using a straight wire to about half a depth of the medium. The test tube was incubated at 37°C for 24hours and then observed. Non motile bacteria gave growth that were confined to the stab line, having sharply defined margins and leaving the surrounding medium clearly transparent. Motile bacteria gave diffuse growth which extended as a zone of turbidity from the stab line (Obulie *et al.*, 1998).

Antibiotic Sensitivity

Antimicrobial agents Ten antibiotics for gram positive organisms commonly used in human therapy were employed in the antibiogram test. The paper disks containing the antibiotics were obtained from Abtek Biologicals Ltd. (Liverpool L9 7AR, United Kingdom) and include: Ceftriaxone (10 µg), Ciprofloxacin (5 µg), Gentamycin (10 µg), Cotrimoxazole (25 µg), Perflaxacin (5ug) Ampiclox (10ug) Erythromycin 5ug, Cefuroxime (10ug) and Streptomycin (25ug) Amoxicillin (25ug).

Antibiotic susceptibility test

The antibiotic susceptibility test was performed and interpreted based on the disk agar diffusion method as described by Clinical Laboratory Standard Institute (CLSI, 2006) using Mueller-Hinton agar (Biotek laboratories, Lightwater, Surrey, United Kingdom) plates. To determine the sensitivity of the organisms to the antibiotics, a sterile swab was placed into a 24-hour broth culture of the test organism and then excess liquid was removed by gently pressing the swab against the inside of the tube. The swab was then

used to streak onto a Mueller-Hinton agar plate three times in different directions; and then the plate was allowed, to dry for approximately 5 minutes. Using flame-sterilized forceps, each multi-disk antibiotic was pressed gently onto the agar surface to ensure that the disk adheres to the agar, and then the plates were incubated overnight at 37° C. The inhibition zone diameters (IZD) were measured in millimetres. Using a zone size interpretive chart (CLSI Performance Standards for Antimicrobial Disk Susceptibility Tests, 2006) each isolate was then declared susceptible or resistant depending on the size of the zone of inhibition to the various antibiotics.

3. Results.

The result presented in Table 1 shows the mean bacterial counts from soybean products. The highest mean bacterial count was 20.3×10^2 and the lowest mean bacterial count was 0.85×10^2 . The highest mean is below the reference point of 2.0×10^4 CfU/g. Table 2 shows the cultural, morphology and biochemical characteristics from which the bacteria isolates were identified. The bacterial genera isolated are *Staphylococcus* sp, *Bacillus* sp, *Enterobacter* sp, *Lactobacillus* sp and *Serratia* sp. Table 3 shows the percentage frequency of occurrence of bacterial isolated from soy samples from different sampling period. *Staphylococcus* spp. had the highest frequency. Table 4 shows the Antibiotic susceptibility pattern of bacteria isolates from soybean products manufactured at Amen Jan Nigeria limited. Table 5 shows the average mean counts of fungi with the highest mean count of 1.53×10^2 and the lowest mean count of 0.45×10^2 . The average mean count of fungi exceeds the reference point of 1.0×10^2 . Table 6 shows the Cultural and microscopic characteristics of the fungal isolates. The fungal groups isolated are *Trichoderma* sp, *Penicillium notatum*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani*, *Alternaria alternata* and *Candida* sp. Table 7 shows the percentage frequency of occurrence of the fungi group isolated from the soy samples from the different sampling period. *Aspergillus flavus* had the highest frequency.

TABLE 1: Total Bacterial Counts In CfU/G

SAMPLES	1 ST BATCH	2 ND BATCH	3 RD BATCH	4 TH BATCH	5 TH BATCH	MEAN
SOYMILK($\times 10^2$)	20.3×10^2	1.1×10^2	3.25×10^2	3.8×10^2	1.4×10^2	5.97×10^2
SOY YEAST($\times 10^2$)	10.8×10^2	2.55×10^2	2.2×10^2	1.11×10^2	0.85×10^2	3.5×10^2
TOTAL COUNT	15.6×10^2	1.83×10^2	2.73×10^2	2.46×10^2	1.13×10^2	4.74×10^2

Table 2: Cultural, Morphological And Biochemical Characteristics Of Bacteria isolates from Soymilk and Yeast

Colour On Media Of Isolation	Gram	Motility	Coagulase	Urease	Methyl Red	Catalase	Oxidase	Citrate	Indole	Fructose	Sucrose	Mannitol	Glucose	Lactose	Voges Proskauer	Probable Organisms
Cream	+ Rod	-	+	+	+	+	+	+	-	A	A	-	AG	-	-	<i>Bacillus</i> spp
Yellow	+ cocci	-	+	+	+	+	-	+	-	A	A	A	A	A	-	<i>Staphylococcus</i> spp
Pink	-short rods	+	-	+	+	+	-	+	-	-	A	A	AG	A	+	<i>Enterobacter</i> spp.
Yellow	+ rod	-	-	+	+	+	+	+	-	-	A	-	AG	A	+	<i>Lactobacillus</i> spp
Red	-Rods	+	+	+	-	+	-	+	-	A	A	A	A	A	-	<i>Serratia</i> spp.

Key: - negative, + positive, AG Acid and Gas, A acid, G gas

Table 3: Frequency of occurrence of bacterial isolated from soybean milk and soy yeast

Bacteria Isolate	No. of isolate	Frequency of occurrence (%)
Staphylococcus sp.	4	36.36%
Bacillus sp.	4	36.36%
Enterobacter sp.	1	9.09%
Lactobacillus sp.	1	9.09%
Serratia sp.	1	9.09%
Total	11	100%

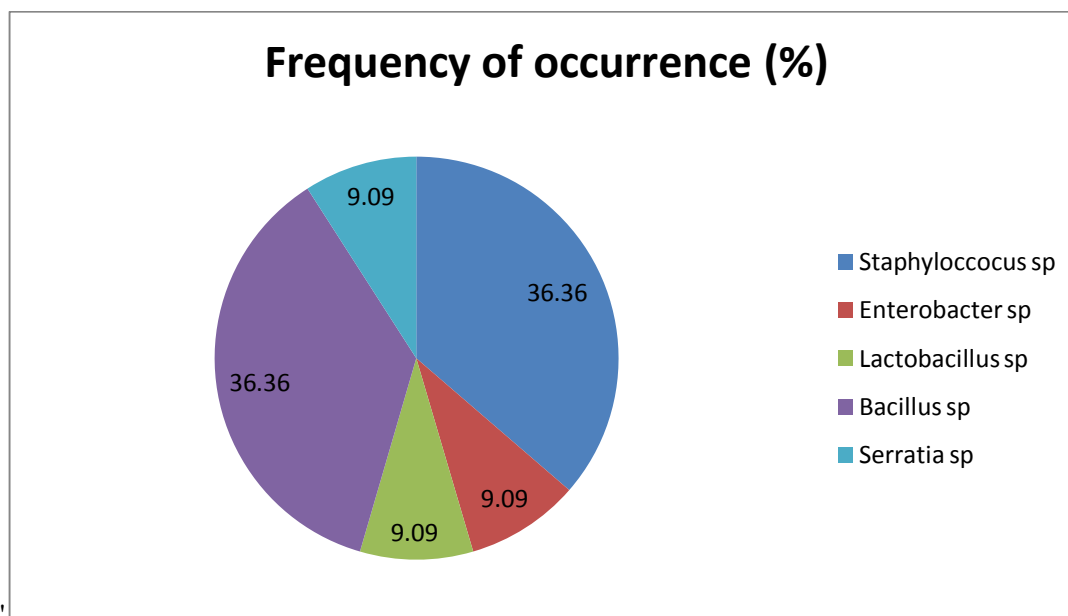


Figure 1: Pie chart showing frequency of occurrence

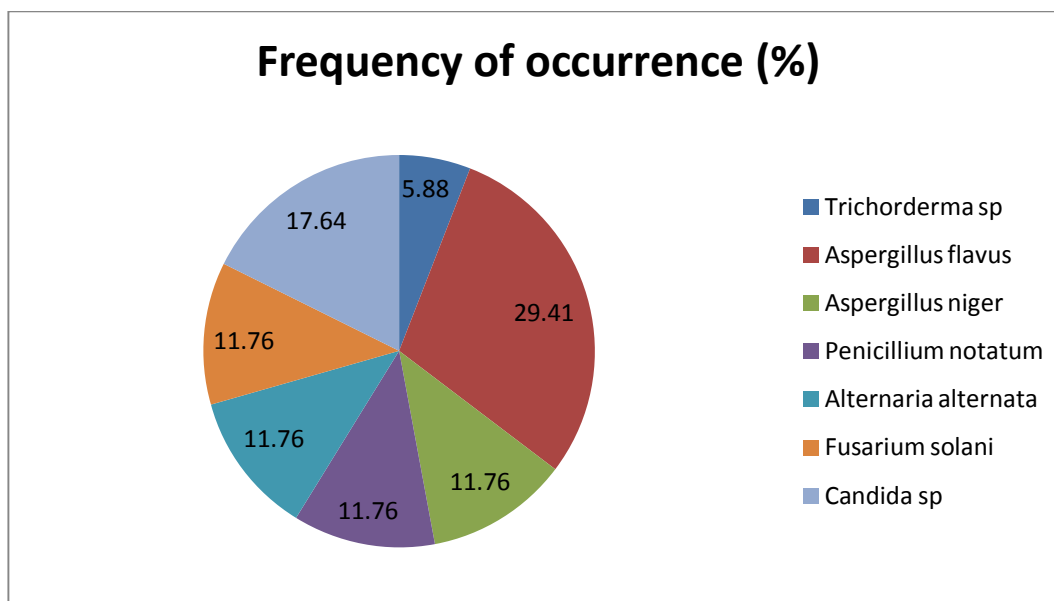


Figure 2: Pie chart showing frequency of occurrence of Fungal isolates

Table 4: Antibiotic susceptibility pattern of isolates from soymilk and yeast

Organisms	Isolates	percent susceptibility									
		CRO	CIP	S	SXT	E	PEF	CN	APx	CMX	AM
<i>Staphylococcus</i> sp	4	3(75)	4(100)	4(100)	4(100)	3(75)	4(100)	4(100)	0(0)	2(50)	0(0)
<i>Bacillus</i> sp	4	0(0)	4(100)	4(100)	4(100)	3(75)	3(75)	3(75)	0(0)	3(75)	0(0)
<i>Enterobacter</i> Sp	1	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)
<i>Serratia</i> Sp	1	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	0(0)
<i>Lactobacillus</i> Sp	1	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	0(0)

Key: CRO- ceftriazone, CIP- Ciprofloxacin, S- Streptomycin, SXT- cotrimoxazole, E- Erythromycin, PEF- Pefloxacin, CN- Gentamycin, APX- Ampiclox, CMX- Zinnacef, AM- Amoxicillin.

Table 5: Fungi counts from the soy samples

Samples	1 st Batch	2 nd Batch	3 rd Batch	4 th Batch	5 th batch	Mean
SOYMILK($\times 10^2$)	5.35×10^2	0.45×10^2	2.35×10^2	1.36×10^2	1.75×10^2	2.25×10^2
SOY YEAST($\times 10^2$)	6.8×10^2	0.5×10^2	2.3×10^2	1.53×10^2	0.65×10^2	2.36×10^2
TOTAL COUNT	6.08×10^2	0.48×10^2	2.32×10^2	1.45×10^2	1.2×10^2	2.31×10^2

Table 6: Cultural and microscopic characteristics of the fungal isolates

Isolate Code	Morphological Characteristics	Microscopic Characteristics	Possible Organism
F1	Yellowish-green colony	Conidiophores are repeatedly branched. Conidia heads clustered at tips of the phialides	<i>Trichoderma</i> sp
F2	Brownish cotton-like	Long, erect conidiophores. Round shaped conidia	<i>Penicillium notatum</i>
F3	Light brown powdery	Long septate hyphae with conidiophores. Round shaped conidia heads with both uniseriate and biseriata arrangement of phialides	<i>Aspergillus flavus</i>
F4	Black fluffy colony	Conidiophores smooth walled and separte hyphae. Biseriate arrangement of phialides	<i>Aspergillus niger</i>
F5	Cream cotton-like	Short septate hyphae with banana-shaped conidiospores	<i>Fusarium solani</i>
F6	Black colony	Elongated septate hyphae with branched conidiophores with beak macroconidia	<i>Alternaria alternata</i>
F7	Creamy/white, wet/dry colony	Non septate. Spherical to subspherical blastoconidia	<i>Candida</i> sp

Table 7: Frequency occurrence of fungi group isolated from soymilk and yeast

Fungi Isolate	No. of isolate	Frequency of occurrence (%)
<i>Trichoderma</i> sp	1	5.88%
<i>Aspergillus flavus</i>	5	29.41%
<i>Aspergillus niger</i>	2	11.76%
<i>Penicillium notatum</i>	2	11.76%
<i>Alternaria alternata</i>	2	11.76%
<i>Fusarium solani</i>	2	11.76%
<i>Candida</i> sp	3	17.64%
Total	17	100%

4. Discussion

The attendant increase in the consumption of soybean food is due to its high protein content and has encouraged low scale production under household conditions with little or no regards for quality control measures (Hua *et al.*, 2007).

Results of this present study showed the microbial evaluation of soy bean products obtained from Amen Jan Nigeria Limited. Varying microbial counts were observed from the results in this study; and this could be attributed to the presence of the nutritional composition of the soybeans as an excellent growth medium for microorganisms. This in accordance with Agboke *et al* (2011), who reported 1.40×10^8 cfu/g and 1.32×10^2 cfu /g for total bacterial counts and fungal counts respectively while Adebayo-Tayo *et al* (2008) reported 1×10^5 cfu /g for bacterial counts and De nadita (2002), reported 9.9×10^{10} cfu/g for fungi counts. In this study, the microbial count was 4.74×10^2 cfu/g and 2.31×10^2 cfu/g for total bacterial count and fungal counts respectively. The difference from the above reports showed that the sampling source of this study was contamination cautious recording low microbial counts, unlike the other sources from the literature with high microbial counts.

A total of five different bacteria species were isolated from the ten (10) soy samples analyzed and they are *Staphylococcus*, *Bacillus*, *Enterobacter*, *Lactobacillus* and *Serratia* sp. The isolation of these organisms is supported by the work of De Nadita (2002) who isolated *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis* and *Escherichia coli* from untreated soy flour samples. This is further supported by Adebayo-tayo *et al* (2008) who isolated *Bacillus*, *Micrococcus*, *Staphylococcus*, *Pseudomonas*, and *Streptococcus* from powdered soymilk sold in Akwa Ibom, South Southern Nigeria.

The incidence of the different bacteria isolated showed that *Staphylococcus* (36.36%) and *Bacillus* (36.36%) are the bacteria frequently isolated from

soybean products. The bacteria least isolated was *Serratia* with an incidence of 9.09%. Adebayo-tayo *et al* (2008) recorded low level of *Pseudomonas* sp. *Staphylococcus* sp is a possible contaminant from the handlers and the occurrence of *Bacillus* sp can be as a result of their spores in the environment, and their spores could survive high temperature of processing.

The result of the antimicrobial sensitivity test showed that many of the bacteria isolates were sensitive to most of the antibiotics tested. All the Gram positive organisms were susceptible to ciprofloxacin, streptomycin and septrin but were resistant to ampiclox and amoxicillin. Anagu *et al* (2015) reported that *Staphylococcus* and *Bacillus* sp were susceptible to gentamycin and ampiclox but resistant to septrin, although the findings in this work showed that *Staphylococcus* and *Bacillus* sp were sensitive to septrin and gentamycin but resistant to ampiclox. In the Gram negative organisms, *Serratia* was susceptible to zinnacef and *Enterobacter* was resistant to it.

A total of seven (7) fungal groups were isolated from the soy samples and they are *Trichoderma*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum*, *Alternaria alternata*, *Fusarium solani* and *Candida* sp. The isolation of these organisms is in accord with of Agboke *et al* (2011) who isolated *Candida* sp in soybean milk products consumed in Nigeria. De Nadita (2002) also isolated *Aspergillus* sp, *Penicillium*, *Rhizopus* and *Candida* sp. The incidence of the different fungal groups isolated showed that *Aspergillus flavus* (29.41%) and *Candida* sp (17.64%) are the fungal groups frequently isolated from soybean products. The fungi least isolated was *Trichoderma* sp with an incidence of 5.88%. The incidence of these organisms is supported by the work of Adebayo-tayo *et al* (2008) who isolated *Aspergillus flavus* with the highest frequency of occurrence of 20% and also Brooks *et al* (2003) who isolated *Aspergillus flavus* with the highest frequency of occurrence of 16.4%.

The presence of *Aspergillus* could be attributed to the prevalence of their spores in the atmosphere. This organism can be easily trapped during the post-harvest processing and handling of soybean grains. Since most fungi spores are found in the air, the spores must have contaminated the grains during drying. Dongo and Ayodele (1997) showed that *Aspergillus* occurred and highest in the number of colonies identified from air spores of some localities.

The presence of most of the organisms in this study may be attributed to several factors which include initial contamination of the raw materials to the poor handling of finished products, the utensils and sanitary conditions of the processing environment. The different genera of microorganisms obtained in this work is a reflection of nutrients present in the soybean and their ability to withstand high temperature during processing of the soybean products. Adebayo *et al* (2008) and Agboke *et al* (2011) stated that the nutritional composition of soybeans has provided an excellent growth medium for microorganisms to flourish.

Conclusion: Conclusively, the quest for cheap source of protein has encouraged small scale production of vegetable protein products of which the soymilk or soy yeast is an example. Soybean consumption has encouraged small scale production of the product under household condition with little or no regard to quality control measures. The soybean products sampled in this study had microbial count below the acceptable limit of 2.0×10^4 cfu/g recommended for the general bacterial count by the Soy Food Association of America (SFAA) and this further suggests that contamination could be as a result of poor handling of the final product and its processing environment. Therefore, National Agency for Food and Drug Administration Control (NAFDAC) in Nigeria should develop microbiological and chemical standards for soybean products in order to ascertain its quality to avoid the cumulative effects of microbial contaminants in the human systems.

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