

MICROBIOLOGICAL AND PROXIMATE ANALYSES OF HOME AND INDUSTRIAL MADE SOYMILK SAMPLES CONSUMED IN UMUAHIA METROPOLIS, ABIA STATE, NIGERIA.

Dr. Clifford Obi*

Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, P.M.B 7267, Umuahia, Abia State, Nigeria.

Article Received on
09 October 2014,

Revised on 04 Nov 2014,
Accepted on 25 Nov 2014

***Correspondence for**

Author

Dr. Clifford Obi

Department of
Microbiology, College of
Natural Sciences, Michael
Okpara University of
Agriculture, Umudike,
P.M.B 7267, Umuahia,
Abia State, Nigeria

ABSTRACT

Microbiological and proximate analyses of 50 Home and Industrial Made soymilk samples were carried out using standard analytical procedures. The two soymilk types were contaminated with *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* with *E. coli* having the highest occurrence level (42%) and *Klebsiella pneumoniae* being the lowest (8%). Two fungi: *Aspergillus niger* and *Penicilium notatum* were also recovered from the soymilk samples with *A. niger* being higher in occurrence (66.7%) than *P. notatum* (33.3%). The total viable bacterial count (TVBC) for the Home made soymilk was in the range $6.2 \times 10^6 - 4.0 \times 10^5$ CFU/ml while the TVBC for the Industry made was in the range $2.0 \times 10^2 - 1.0 \times 10^2$ CFU/ml. The total fungal count (TFC) for the Home made soymilk was in the range $4.1 \times 10^6 - 3.0 \times 10^5$ CFU/ml while the TFC for the Industry Made was in the range $2 \times 10^2 - 1.0 \times 10^2$ CFU/ml. Antibiotic sensitivity

screening result showed that the only Gram positive isolate (*S. aureus*) was sensitive to all the antibiotics tested but was most sensitive (80%) to Ampiclox. Among the Gram negative isolates, *E. coli* was sensitive to all the antibiotics tested, but was most sensitive to Augmentin (71%). *Klebsiella pneumoniae* was also sensitive to all the antibiotics but most sensitive to Ampicillin (75%). *P. aeruginosa* was completely sensitive (100%) to Nalidixic acid and Septrin respectively and completely resistant (100%) to Tarivid, Augmentin, Streptomycin, Ceporex and Ampicillin respectively. The Proximate analyses showed that Homemade sample was statistically of the same with the Industrial made sample in moisture

content, dry matter, ash, protein, crude fibre, carbohydrate and pH values ($P < 0.05$), but was significantly higher in energy giving value (392.06 ± 2.05) than the Industrial made (369.06 ± 1.93 ; $P < 0.05$). The Industrial made sample was significantly higher in fats (17.55 ± 0.64) than the Home made (3.37 ± 1.08 ; $P < 0.05$). The Home made samples competed very well with the Industrial made samples in nutritional aspects, but had higher bacterial and fungal counts, thus, depicting health risk challenges associated with the consumption of the Home Made soymilk samples since they are not pasteurised or given any form of heat treatment before consumption.

KEYWORDS: Antibiotics, total microbial count, pathogens, proximate, soymilk.

INTRODUCTION

Soybean is a high-protein legume grown as food for both humans and livestock. Although indigenous to Eastern Asia (Dinakin, 1985), it is known by different names in different parts of the world, but in Nigeria it's known as soya beans. The use of soy beans is traced back to the orient where it was consumed in form of fermented foods (Keshun *et al.*, 1999). Today, its uses range from the preparation of tofu, tempeh and natto, to the fortification and enrichment of foods such as in soy fortified wheat bread and soy blends, soy sauces, soy yoghurt and soy cream cheese and soya milk (Crowley and Dale 1975; Yang, 1979). Soybeans are a perfect source of protein both in quality and quantity. Approximately 35 – 40% of the total dry matter content of the whole soybeans is protein compared to cowpeas' 23% to 24% protein. Soybeans contribute approximately 20% fat to the diet (Ayo *et al.*, 2011). The fat from the soybean is unsaturated type unlike saturated fats from animal origin and hence is good for heart disease patients. Soybean milk (soymilk) is one of the food preparations produced from the activity of microorganisms (Adegoke *et al.*, 2002). It's an aqueous, white, creamy extract produced from soybeans which resembles cow milk both in appearance and consistency. It is a highly nutritious food drink which contains protein, fat, carbohydrates vitamins and minerals. It is because of this nutritious value and comparative low cost, (Wilson, 1995), that soymilk plays an important role in the dietary pattern of people in most developing countries. Recently, the consumption of soymilk has greatly increased for reasons which include poverty alleviation and because it is recommended for people that cannot tolerate lactose since it does not contain lactose. It is continuously being used as a substitute to cow milk in most remote areas of Nigeria and indeed Africa.

The nutritious nature of soymilk however, makes it prone to microbial attack if not properly processed and stored as the nutrients it contains are also required for the growth of most spoilage organisms. A large number of microorganisms such as mesophilic aerobic bacteria, coliforms, yeasts and moulds are known to be responsible for the spoilage of soymilk, producing undesirable changes in the milk (Osuntogun and Aboaba, 2004). The sale of soymilk is quite popular in Umuahia, but there is so far no or recorded data on its microbiological quality, which should have addressed the safety of its consumers in Umuahia.

OBJECTIVES

- Evaluation the microbiological quality of Home and Industrial made soymilks consumed in Umuahia;
- Determination of the proximate composition of the soymilk samples and
- Determining the antibiotic susceptibility pattern of the soymilk contaminants.

SAMPLE COLLECTION

Samples of Home Made and Industrial Made soymilks were purchased from the retail sellers and Super markets at Umuahia Main Market (popularly called “Isi gate”) and taken to the laboratory for analyses.

PREPARATION OF MEDIA

Nutrient agar (NA; Titan Biotech, India), Sabourand dextrose agar (SDA; Titan Biotech, India) and MacConkey agar (MA; Titan Biotech, India) were used for microbial isolation. These were prepared according to the manufacturers’ instructions.

SAMPLE PREPARATION AND INOCULATION

The samples were serially diluted (Dhawale and LaMaster, 2003) and 0.1ml aliquots from suitable dilutions was inoculated into the agar plates and incubated aerobically at 37°C for 24-48 hrs (for bacterial growth) while SDA plates were fortified with Streptomycin (500mg/ml) and incubated at 22°C for 72-120hrs for fungal growth. Developed microbial growths were counted and recorded as colony forming unit (CFU/ml). Isolated colonies resulting from pure plate cultures on NA plates were preserved on agar slants and subjected to Gram staining and biochemical tests (Cowan, 1974).

ISOLATION OF FUNGI

A portion of each fungal colony which developed was aseptically sub-cultured into fresh SDA and the plates were kept as stock cultures for identification tests and other uses (Cheesbrough, 2005).

PROXIMATE ANALYSIS

The proximate analysis of soymilk samples was carried out quantitatively to determine the moisture, crude protein, fat, crude fibre, ash and carbohydrate content of the samples.

Moisture Content Determination

This was carried out using the conventional method (A.O.A.C; 1990). Two moisture cans were dried in the oven and then put into desiccators to cool. 5g of the sample was put in each of the moisture can and placed in the oven and dried at 105°C for 3 hours. It was brought out and transferred into desiccators to cool before weighing. The cycle of heating, cooling and weighing was repeated until a constant weight was obtained which was determined by weight difference and expressed as a percentage of the sample weighed.

It was given by:

% moisture = $(W_2 - W_3) / (W_2 - W_1) \times 100$; where:

W_1 = weight of empty can; W_2 = weight of can + sample before drying; W_3 = weight of can + sample at constant weight.

Ash Content Determination

The method recommended by A.O.A.C. (1990) was used. The crucibles were dried and cooled in a desiccator before use. 5g of the sample was weighed into the crucibles, covered and placed in a muffle furnace at temperature of 55°C. This temperature was maintained for 2 hours until a whitish ash is maintained. The muffle furnace was switched off and the crucible were removed and placed in a desiccator to cool. The crucibles containing the samples were weighed and the percentage ash content was determined.

% Ash = $(W_2 - W_3) / (W_2 - W_1) \times 100$

Where W_1 = weight of crucible; W_2 = weight of sample + crucible; W_3 = weight of crucible + ash.

Fibre Determination: Crude fibre was determined by the method described by (Joslyn, 1990). Exactly 2g of the sample was treated with 20ml of 1.25M H_2SO_4 and boiled for 30

minutes. The resultant mixtures was filtered under suction, washed with hot distilled water and boiled again for another 30 minutes with 1.25M NaOH. The digested sample was then washed severally with hot distilled water. The washed sample was scrapped into a crucible, dried at 100°C for 1 hour, cooled and weighed. The loss in weight on incinerator was taken as the weight of the crude fibre.

Thus % crude fibre = loss in weight on incineration/weight of original sample x 100.

Fat determination (BY SOXHLET EXTRACTION METHOD)

About 2g sample was placed into a soxhlet extractor. The extractor was placed into a pre-weighed dried distillation flask. Then the solvent (acetone) was introduced into the distillation flask via the condenser end attached to the soxhlet extractor. The setup was held in place with a retort stand clamp. Cooled water jet was allowed to flow into the condenser and the heated solvent was refluxed as a result, the lipid in the soxhlet chamber was extracted in the process of continuous refluxing. When the lipid was observably extracted; to concentrate the lipid; the flask was then dried with the air oven at 600°C to constant weight and re-weighed to obtain the weight of lipid. This was by continuous extraction in a soxhlet reflux apparatus (Pearson, 1976).

$$\% \text{ Ash} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where: W_1 = weight of empty flask; W_2 = weight of sample; W_3 = weight of flask + oil extract.

Crude protein determination (kjeldahl method).

About 0.1g of sample was weighed and added into a clean conical flask of 250ml capacity. 3gdigestion catalyst was added into the flask and 20ms concentrated sulphuric acid was also added and the flask was heated to digest the content from black to sky blue colouration. The digest was cooed to room temperature and was diluted to 100ml with distilled water.

Distilation

About 20ml diluted digest was measured into a distillation flask and the flask was held in place on an electro-thermal heater hot plate. To the distillation flask was attached condenser, 40% Sodium hydroxide is injected into the digest via a syringe at the head to the micro arm steel head until the digest becomes strongly alkaline. The mixture was heated to boil and

distill the ammonia gas via the condenser into the receiver beaker. The colour of the acid changed from purple to greenish. Ammonia distillate was introduced into the acid.

Titration

The distillate was titrated with 0.1N Hydrochloric acid back to purple colour from greenish. The volume of hydrochloric acid added to effect the change was recorded as titre value.

CALCULATION: %N = titre value $\times 1.4 \times 100$

Carbohydrate determination

Carbohydrate was determined by weight difference. Thus: % carbohydrate = 100 – (% moisture + % ash + % fat + % crude + % fibre + % crude protein).

pH Measurement

This was determined using a hand held pH/mV/Temperature meter (Model 1Q240, San Diego, USA) attached to a stainless steel.

ANTIMICROBIALSUSCEPTIBILITY TESTING

Preparation of Turbidity Standard Equivalent to McFarland 0.5

One percent (1%) v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water. 1% of barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride in 50ml of distilled water. 0.6ml of Barium chloride solution was added to 99.4ml of the sulphuric acid solution and mixed properly. The solution was preserved in the fridge.

SENSITIVITY TESTING

The Kirby-Bauer disc diffusion technique was used (CLSI, 2009). Pure colonies of isolates were obtained from agar slants and sub-cultured on already prepared culture plates. After growth within 24 hours, isolates from each of the plates were adjusted to 0.5 McFarland standards in 0.9% saline. Swab sticks were dipped into the solution of the isolate and normal saline and streaked uniformly on Muller-Hinton agar plates to obtain confluent growth. Multi antibiotics sensitivity discs were placed on the surface of the media, using a pair of forceps, little force was applied to ensure firm contact with agar plate. The plates were then inverted and incubated aerobically at 37°C for 18-24 hours.

MEASUREMENT OF ZONE OF INHIBITION

The plates were examined for zone of inhibition. Using a ruler, the diameter of zone of inhibition was obtained (mm) from the reverse of the plate. The zone sizes of each plate (both Gram positive and negative plates) was compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant (NCCLS, 1999).

STATISTICAL ANALYSIS

The results obtained from proximate analyses were subjected to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The Home made (HM) and Industrial made (IM) samples of soymilk analysed in this work were contaminated with bacteria (*E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) and fungi (*Aspergillus niger* and *Penicillium notatum*; Tables 1 and 2). The total viable bacterial count (TVBC) for the Home made soymilk was in the range 6.2×10^6 - 4.0×10^5 CFU/ml while the TVBC for the Industry made was in the range 2.0×10^2 - 1.0×10^2 CFU/ml (Table 3). Liamngee *et al.*, (2013) reported a higher microbial load of 8.5×10^7 in some soymilk samples. The total fungal count (TFC) for the Home made soymilk was in the range 4.1×10^6 - 3.0×10^5 CFU/ml while the TFC for the Industrial made was in the range 2×10^2 - 1.0×10^2 CFU/ml. There is also a great disparity in microbial quality of the two samples with the home made samples showing higher fungal load than the Industry made samples. This result predisposes the consumers to some fungal infections like aspergilosis and aflatoxicoses caused by *A. Niger*. The various isolates occurred at various degrees. *E. coli* has 42% occurrence (highest) among the bacteria while *K. pneumoniae* has the lowest occurrence (8%; Table 4). The predominance of *E. coli* among the samples is of public health importance as the pathogen is an indicator of faecal contamination and by implication acts as a pointer to the very possible presence of other enteric pathogens like *Salmonella* spp, *Vibrio cholerae*, *Shigella dysenteriae*. This result is justifiable from the fact that many communities and households in the rural areas where the Home Made soymilks are locally produced lack potable water and do neither subject these products to heat treatments such as pasteurization nor add suitable preservatives/antimicrobial substances to the soymilks they produce. Furthermore, they do not carry out any hazard analysis on their products and the production processes. So, in a typical local production setting, the plastic bottles used in packaging their products are just washed with water (sometimes without soap) and the soymilk thus produced

is filled into the plastic containers and the bottle covers are screwed back manually. This would also explain the 30% occurrence of *S. aureus* in the samples. *S. aureus* is a normal flora found mainly on the skin and the external nares. So, cross-contamination between the products and the pathogen after touching one's external nares is a certainty. Staphylococcal food poisoning was reported by Hobbs (1955) from the consumption of soymilk contaminated with *S. aureus* which is known to produce potent enterotoxin (Meyrand *et al.*, 1998). It is therefore, of paramount importance to subject soymilk production to a reasonable degree of hygiene.

Presence of *E. coli* and *S. aureus* are potential threats to adults, immune-compromised patients and infants who are fed soymilks as weaning food. Obi and Nwozor (2012) had reported the presence of the pathogens in some weaning foods consumed in some localities. Moore (1983) also reported the presence of *S. aureus* in samples of soymilk. Another locally processed soymilk product (nono and wara) was reported to contain *E. coli* and *S. aureus* (Akinyele, 2000). The presence of coliform bacteria in soymilk generally provides an index of the hygienic standard of soymilk and its keeping quality (Sara and Hilda, 1990). The bacterial species frequently encountered from industrial bottled soymilk and causing a risk infection include *P. aeruginosa*, *Aeromonas hydrophila*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* and *S. aureus*. *P. aeruginosa* is usually regarded as a secondary contaminant, not originating from the source of water, but *S. maltophilia* and *B. cepacia* can be found from source waters, and they have the ability to grow with very small concentrations of organic matter (Sydner *et al.*, 1978; Frazier and Westhoff, (1988), If pathogenic bacteria are present, they may persist for long periods in bottled industrial soymilk. Depending on the production environment and level of inoculum, inoculated *Escherichia coli* O157 has been shown to persist even in bottled soymilk (Kerr *et al.*, 1999; Warburton *et al.*, 1992). Attachment to bottle walls and biofilm formation may help bacteria to survive (Warburton *et al.*, 1992). *P. aeruginosa* is a notorious food spoiler and will proceed with the spoilage of the soymilk once it is contaminates the drink. *P. aeruginosa* produces pigments like pyocyanin and pyoverdin (Prescott *et al.*, 2005) and these metabolic by-products can deleteriously affect the colour of the soymilk leading to possible rejection of the drink by a suspecting consumer. *Aspergillus niger* had 52% occurrence (appeared in more than half of the total samples; Table 5). This pathogen is implicated in some health problems like aflatoxicosis and respiratory tract infection (aspergilosis) Prescott *et al.*, (2005). This pathogen poses a threat to the unsuspecting consumers especially the aged and

infants where soymilk is used as a weaning food. *P. notatum* is a potential food spoiler especially foods like soymilk which is rich in protein and other nutrients. Furthermore, the Home made products are not stored in a fridge, so, any unsold product will most like lose its organoleptics owing to the spoilage activities of *P. notatum*. Another health risk is the possible mycotoxins produced by moulds. *Alternaria alternate* and *P. citrinum* found from bottled soymilk waters are potential producers of mycotoxins, and *P. citrinum* has been shown to produce citrinin in soymilk (Criado *et al.*, 2005).

The antibiotic sensitivity test showed mixed bacterial sensitivity responses. The only Gram positive isolate (*S. aureus*) was most sensitive (80%) to Ampiclox, then Levofloxacin, Amoxil, and Norfloxacin (60% respectively; Table 6). The pathogen was not completely resistant to any of the antibiotics tested and this is a welcome development because the organism has not developed resistance to the drugs. Among the Gram negative isolates, *E. coli* was most sensitive to Augmentin (71%) and least sensitive to Tarivid (14%). There was no complete resistance from *E. coli* and this too is a positive development due to high rate of development of antibiotic resistance being witnessed nowadays among many bacteria which poses a great challenge in antimicrobial therapy. *K. pneumonia* was most sensitive to Ampicilin (75%) and least sensitive to Reflacine, Ceporex, Augmentin and Nalidixic acid (25%) respectively. There was no complete resistance here. However, *P. aeruginosa* showed complete resistance to Tarivid, Augmentin, Streptomycin, Ceporex and Ampicilin respectively and was also completely sensitive to Seprin and Nalidixic acid respectively. This is the only isolate that showed total resistance and that to five of the ten antibiotics tested (Table 7). *P. aeruginosa* has been developing resistance to a lot of antibiotics recently and this is partly attributed to its ability to acquire resistance plasmids from within the environment especially in hospitals.

Results of proximate composition (Table 8) showed that the HM was higher in moisture content (81.62 ± 9.65 versus 69.38 ± 1.25), although the two values are also statistically not different ($P < 0.05$). By this, the HM sample is a good supply of body fluid and thirst quencher. Amanze and Amanze (2011) recorded a moisture value of 91.63%. This could be due to the level of dryness of the soy bean seeds used in the research. The dry matter content of the IM sample was not statistically different from that of the HM sample indicating that the HM sample can compete favourably in nutritional quality with the IM sample.

TABLE 1: Colonial morphology and biochemical characteristics of bacteria isolates

Isolate code	Cell Morphology	Colonial morphology	Gram stain	Spore formation	Catalase activity	Coagulase activity	Motility test	Fructose	Glucose	Lactose	Maltose	Sucrose	Citrate utilisation	MR-VP test	Indole test	Vp test	Probable organisms
1	Singly dispersed short fat rods	Flat, yellow opaque, undiluted colonies with slightly deeper colour centre	-	-	+	-	-	+	++	+	+	-	-	+	+	-	E. coli
2	Single rods	Flat, extremely mucoid, transparent colonies varying from white to yellow colour with rhizoid edges	-	-	+	-	-	+	++	+	+	+	+	-	-	+	Klebsiella pneumoniae
3	Single short rods	Large, smooth, with flat edges and an elevated appearance.	-	-	+	-	+	+	+-	-	-	-	+	-	-	-	Pseudomonas aeruginosa
4	Cocci in single and clusters	Deep, yellow convex opaque colonies with entire edges	+	-	+	+	-	+	+-	-	+	+	-	-	-	-	Staphylococcus aureus

KEY: + = Positive reaction; - = Negative reaction; ++ = Acid & gas present; +- = Acid with no gas present

The two soymilk types were also not significantly different in ash content ($P < 0.05$). However, a lower ash value was recorded by Amanze and Amanze (2011). Similarly, Odu *et al.*, (2012) reported much lower ash content result in the range 0.40-0.22 among eight samples they worked on. This difference could be due to differences in species peculiarity and soil nutritional status where the soybean seeds were planted. The lipid content of the IM sample was statistically higher than that of the HM ($P < 0.05$). This could be due to the method of extraction used by the industry which released more lipids from the seeds. This result was higher than the values recorded by Amanze and Amanze (2011) and Odu *et al.*, (2012) respectively. Ayo *et al.*, (2011) reported that fat from the soybean is unsaturated type unlike saturated fats from animal origin and hence is good for heart disease patients. The protein contents of the two samples were not statistically different ($P < 0.05$). This means that the HM soymilk could be a good substitute for the IM ones in terms of protein supply to the body, but the results of Amanze and Amanze (2011) and Odu *et al.*, (2012) were lower than that recorded in this work. This could be due to varieties differences. Min *et al.*, (2005) and Gesinde *et al.*, (2008) reported that soybean varieties greatly affect the protein content and colour of soymilks. The two samples were not statistically different in carbohydrate, crude fibre and pH values ($P < 0.05$). The results fell in range with the findings of Amanze and Amanze (2011) and Odu *et al.*, (2012) (2012). The pH values obtained fell within the range of good quality yoghurt (Tamine, 1977). The HM sample was higher in energy giving value (392.06 ± 2.05) than the IM counter ($P < 0.05$). This most likely justifies the consumption of HM soymilk by the local inhabitants as a good source and supplier of energy especially among those engaged in menial jobs that have much demands on energy needs.

RECOMMENDATION

The National Agency for Food and Drug Administration Control (NAFDAC) in Nigeria should do more in monitoring the production processes of Home Made soymilk as well as organize awareness programmes to inform the local producers of the microbiological challenges associated with local production of soymilks.

Table 2: Colonial Characteristics and Identification of Fungal Isolates

MACROSCOPY	MICROSCOPY	PROBABLE FUNGI ISOLATE
White filamentous colony which later turns black dotted surfaces	Septate hyphae on branched conidiophores with swollen vesicles covered entirely with the phialides or sterigmate	<i>Aspergillus niger</i>
Greenish white surface which later turns bluish	Septate hyphae, branched conidiophore with chains of conidia produced by phialides with bluish penicilliate	<i>Penicillium notatum</i>

Table 3: Total Viable Counts for Bacterial and fungal Isolates

SAMPLE CODE	1	2	3	4	5	6	7	8	9	10
HM (NA)	6.1x 10 ⁵	5.0 x 10 ⁶	5.0 x 10 ⁵	6.2 x 10 ⁶	5.0 x 10 ⁵	5.0 x 10 ⁶	5.0 x 10 ⁵	5.7 x 10 ⁶	4.0 x 10 ⁵	4.1 x 10 ⁶
HM (SDA)	3.1 x 10 ⁵	2.0 x 10 ⁶	3.0 x 10 ⁵	3.2 x 10 ⁶	3.3 x 10 ⁵	4.1 x 10 ⁶	4.5 x 10 ⁵	2.7 x 10 ⁶	3.0 x 10 ⁵	3.1 x 10 ⁶
IM (NA)	1.0 x 10 ¹	2.0 x 10 ¹	2.0 x 10 ²	2.0 x 10 ¹	1.0 x 10 ²	2.0 x 10 ²	1.0 x 10 ¹	1.0 x 10 ²	2.0 x 10 ²	1.0 x 10 ²
IM (SDA)	2.1 x 10 ²	2.0 x 10 ³	2.0 x 10 ²	2.2 x 10 ²	2.3 x 10 ²	1.1 x 10 ²	1.5 x 10 ²	2.1 x 10 ²	1.0 x 10 ²	1.1 x 10 ²

KEY: HM= Home-made soymilk; IM= Industry made soymilk; NA= Nutrient Agar, SDA = Sabourand Dextrose Agar

Table 4: Distribution of bacterial isolates in Soymilk Samples

SAMPLE CODE	11	12	13	14	15	16	17	18	19	20
HM (NA)	6.1x 10 ⁵	5.0 x 10 ⁶	5.0 x 10 ⁵	6.2 x 10 ⁶	5.0 x 10 ⁵	5.0 x 10 ⁶	5.0 x 10 ⁵	5.7 x 10 ⁶	4.0 x 10 ⁵	4.1 x 10 ⁶
HM (SDA)	3.1 x 10 ⁵	2.0 x 10 ⁶	4.3 x 10 ⁵	3.2 x 10 ⁶	3.3 x 10 ⁵	4.1 x 10 ⁶	4.5 x 10 ⁵	2.7 x 10 ⁶	3.0 x 10 ⁵	3.1 x 10 ⁶
IM (NA)	2.0 x 10 ²	2.0 x 10 ¹	1.0 x 10 ²	1.5 x 10 ²	2.2 x 10 ²	2.3 x 10 ²	2.0 x 10 ²	2.0 x 10 ¹	1.0 x 10 ²	2.0 x 10 ²
IM (SDA)	2.1 x 10 ²	1.2 x 10 ³	2.0 x 10 ²	1.2 x 10 ²	2.2 x 10 ²	1.1 x 10 ²	1.35 x 10 ²	2.2 x 10 ²	1.0 x 10 ²	1.3 x 10 ²

KEY: HM= Home-made soymilk; IM= Industry made soymilk; NA= Nutrient Agar, SDA = Sabourand Dextrose Agar

Table 5: Distribution of fungal isolates in Soymilk Sample

ISOLATES	NUMBER	% OCCURENCE
<i>E. coli</i>	21	42.0
<i>Staphylococcus aureus</i>	15	30.0
<i>Pseudomonas aeruginosa</i>	10	20.0
<i>Klebsiella pneumoniae</i>	4	8.00
TOTAL	50	100

Table 6: Antibiotic Sensitivity profile of Gram positive isolates

ISOLAT E	N	CPX (30µg) n %	NB (10µg) n %	CN (30µg) n %	AML (10µg) n %	S (30µg) n %	RD (30µg) n %	E (10µg) n %	CH (10µg) n %	APX (30µg) n %	LEV (10µg) n %
<i>S. aureus</i>	15	6 40	9 60	3 20	9 60	3 20	6 40	6 40	3 20	12 80	9 60

KEY: N= Total number of isolates

n= number of sensitive organisms

%= percentage sensitivity (n/N x 100).

CPX - Ciproflox

NB - Norfloxacin

CN - Gentamycin

AML - Amoxil

S - Streptomycin

RD - Rifampicin

E - Erythromycin

CH - Chloramphenicol

APX - Ampiclox

LEV - Levofloxacin

TABLE 7: Antibiotic Sensitivity profile of Gram negative isolates

ISOLATES	N	OFX (10µg) n %	PEF (30µg) n %	CPX (10µg) n %	AU (30µg) n %	CN (30µg) n %	S (10µg) n %	CEP (30µg) n %	NA (10µg) n %	STX (30µg) n %	PN (10µg) n %
<i>E. coli</i>	21	3 14	9 43	6 29	15 71	12 53	6 29	9 43	9 43	12 57	9 43
<i>Klebsiella pneumoniae</i>	4	2 50	1 25	1 25	1 25	2 50	2 29	2 50	1 25	2 50	3 75

<i>Pseudomonas aeruginosa</i>	10	0 00	5 50	5 50	0 00	5 50	0 00	0 00	10 100	10 100	0 00
-------------------------------	----	------	------	------	------	------	------	------	--------	--------	------

KEY: N= Total number of isolates

n= number of sensitive organisms

%= percentage sensitivity (n/N x 100).

OFX - Tarivid

PEF - Reflacine

CPX - Ciproflox

AU - Augmentin

CN - Gentamycin

S - Streptomycin

CEP - Ceporex

N - Nalidixic acid

STX - Septrin

PN - Ampicilin

Table 8: PROXIMATE COMPOSITION OF SOYMILK SAMPLES

S/N	SAMPLES	Moisture (%)	Dry Matter (%)	Ash (%)	Fat (%)	Protein (%)
1.	Home Made (HM)	81.62±9.65 ^a	18.38±9.65 ^a	0.85±0.06 ^a	3.37±1.08 ^a	6.36±0.60 ^a
2.	Industrial Made (IM)	69.38±1.25 ^a	30.62±1.25 ^a	0.99±0.01 ^a	17.55±0.64 ^b	7.38±0.54 ^a

^{a,b} means with the same superscript in same column are not significantly different. Means with different superscripts in the same column are significantly different.

S/N	SAMPLES	Crude fibre (%)	CHO (%)	P ^H	Energy (cal)
1.	Home Made (HM)	0.13±0.04 ^a	7.69±8.06 ^a	5.56±0.21 ^a	392.06±2.05 ^a
2.	Industrial Made (IM)	0.33±0.04 ^a	4.38±1.39 ^a	5.38±0.04 ^a	369.06±1.93 ^b

^{a,b} means with the same superscript in same column are not significantly different. Means with different superscripts in the same column are significantly different.

CONCLUSION

The poor handling and unhygienic practices of local producers of soymilk products has placed the HM sample analysed here below the IM sample in terms of microbiological quality. Thus, there is the urgent need to introduce proper hygienic and quality assurance

consciousness in the production of Home Made soymilks. However, the nutritional composition of the Home Made sample competed favourably with the Industry Made samples. Thus, with adequate monitoring, adherence to quality control measures during production, proper packaging and branding, the Home Made sample will compete very well with the Industry sample in the market.

REFERENCES

1. Amanze, K. O. And Amanze, J. O. (2011): Quality evaluation of yoghurt from cowmilk, soymilk and cow/soymilk. *Jorind*. 2011; 9(2): 44-47.
2. Association of analytical chemists. (1990): Official Method of Analysis 15th edition, Washington, D.C., 1990 pp: 223-225.
3. Adegoke, G. O, Gbadamosi, R., Evwoerhurhoma, F., Uzo-peters, P. I, Falade, K. O, Itiola, O., Moody, O., and Skura, B. (2002): Protection of maize (Zeamays) and soybeans (Glycine max) using *Aframomum danielli*. *Journal of European Food Resources and Technology*, 2002; 214: 408-11.
4. Akinyele, B. J. (2000): Microorganisms Associated With Some Locally Processed Milk Products, *Journal of Techno Science*, 2000; 4; 28-30.
5. Ayo, J. A, Oluwalana, I. B., Idowu, M. A., Ikuomola, D. S., Ayo, V. A., Umar, A., Yusuf, E. (2011): Production and evaluation of millet- egg-soybean hull composite flour: A weaning food. *Journal of Food Nutrition*, 1: 7-13.
6. Cheesebrough, M. (2005): District laboratory practice in tropical countries part 2, 2nd edition. New York: Cambridge university press. 64 – 70.
7. Cowan, S. T. (1974): Cowan and steel's Manual for the identification of Medical bacteria. 2nd edition, Harper and Row London. Pp: 39.
8. Clinical Laboratory Standard Institute, CLSI. (2009): Performance for antimicrobial susceptibility. 10th edition. Vol 29: Pp1-3.
9. Criado, M. V., Fernández Pinto, V. E., Badessari, A. and Cabral, D. (2005): Conditions that regulate the growth of moulds inoculated into bottled mineral water. *International Journal of Food Microbiology*. 99; 343–349.
10. Crowley, L. R. and Dale, D. C. (1975): Effect of Heat Treatment of the Nutritive Value of Soymilk Protein Fed to weaning rats. *Journal of Food Science*, 30, 723-728.
11. Dhawale, S. and LaMaster, A. (2003): Microbiology Laboratory Manual. The McGraw Hill companies incorporation USA. Pp: 13-15.

12. Dinakin, J. (1985): Soybean production in Nigeria (London: Academic Press). Pp: 85 - 99.
13. Frazier, W. C. and Westhoff, D. C. (1988): Food Microbiology. 3rd edition. Tata McGraw- Hill Publishing Company Ltd., New Delhi. Pp: 54-56.
14. Gesinde, A. F., Oyawoye, O. M. and Adebisi, A. (2008): Comparative Studies on the Quality and Quantity of Soymilk from Different Varieties of Soybean. Pakistan Journal of Nutrition 7 (1): 157-160.
15. Hobbs, B. C. (1955): Dried Milk In: Milk Hygiene FAO/WHO Geneva. Journal of Applied Bacteriology, 18-484.
16. Ismali, I. (2004): The Fulani Milk Maid and Problems of Dairying in Nigeria, African commune. Pp 98 Josyln, M. A. (1990): Method in food analysis, 2nd edition. New York academic press 411- 413.
17. Kerr, K., Fitzgerald, M., Sheridan, J. J., McDowell, D.A., Blair, I.S. (1999): Survival of Escherichia coli O157:H7 in bottled natural mineral water. Journal of Applied Microbiology, 87: 833–841.
18. Keshun, A. H., Marth, E. H., and Turner, R. H. (1999): Growth and Acidity of Lactic Acid Bacteria in Soymilk, Journal of Clinical Microbiology, 33: 34-37.
19. Meyrand, A., Boutrand-Loei, S., Ray-Gueniots, S., Mazuy, C., Gaspard, C.E., Jourbert, G., Perrin, G., Lapeyere, C., and Vernozy-Rozand, C. (1998): Growth and enterotoxin production of Staphylococcus aureus during the manufacture and ripening of Camembert-type cheeses from raw goat's milk. Journal of Applied Microbiology 85: 537-544.
20. Min, S., Y. Yu, and S. S. Martin, (2005): Effect of Soybean Varieties and Growing Locations on the Physical and Chemical Properties of Soymilk and Tofu. Journal of Food Science, 70: 8–21.
21. Liamngee, K., Terna, P., Bem, A. A., Orpin, J. B., Mzungu, I., Obaje, M and Anum, T. (2013) Microbial Analysis of Soyabean Milk Sold In Makurdi Metropolis. Journal of Environmental Science, Toxicology and Food Technology. 3 (3): 97-104
22. Moore, N. H. (1983): Oil seed handling and preparation prior to solvent extraction. Journal of American Oil Chemistry Study 60: 141.
23. National Committee for Clinical Laboratory Standards Guidelines. (NCCLS) (1999): Performance standards for antimicrobial susceptibility testing: mint informational supplement. Wayne, pensilvania document M100 – 59, vol 19. No 1, table 21.

24. Obi, C. N and Nwozor, N. C (2012): Bacteriological Analyses of weaning foods consumed in Umuariaga community, Ikwuano L.G.A. Abia State. *Research Journal of Applied Sciences*. 7(4) 208-211.
25. Odu, N. N., Egbo, N. N and Okonko, I. O (2012): Assessment of the effect of different preservatives on the shelf-life of Soymilk stored at different temperatures. *Researcher* 4(6): 62-69.
26. Osuntogun, B. and Aboaba, O. O. (2004): Microbiological and Physico-chemical Evaluation of some Non-alcoholic beverages. *Pak. Journal of Nutrition*. 3(3): 188-192.
27. Pearson, D. I. (1976): *The Chemical Analysis of Food*. Church Hill Living Stone Press, Edinburgh. Pp:59-63.
28. Prescott, L. M., Hurley, P. J and Klein, A. D. (2005): *Microbiology*. 6th ed. McGraw-Hill Publisher, Singapore. pp 1126.
29. Sara, F. and Hilda, N. (1990): Fate of pathogenic and non-pathogenic *Escherichia coli* strain in two fermented milk products. *Journal of Applied Bacteriology*. 69(6): 814-821.
30. Sydnor, I.S., Johnson, W. and Zoltala (1978): Significant pathogen in dairy product (Marth, E.H. ad.) *Public Health Association*, Washington D.C. pp. 11-32.
31. Tamine, A. Y. (1977): Effects of temperature on Micro Organisms in yoghurt Production. *J. Food Science and Technology*; 30; 772-773.
32. Warburton, D.W., Dodds, K.L., Burke, R., Johnston, M.A. and Laffey, P.J. (1992): A review of the microbiological quality of bottled water sold in Canada between 1981 and 1989. *Canadian Journal of Microbiology*. 38:12-19.
33. Wilson, L. (1995): Soyfoods. In: Erickson, D.L. (Ed.), *Practical Handbook of Soybean Processing and Utilization*. AOCS Press, Cha. Pp: 119-121.
34. Yang, O. H. (1979): *Easy to Prepare Soybean Foods for Villagers*. Tropical Foods Chemistry and Nutrition. Vol. 2 London: Academic Press. Pp: 99-101.