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Public Health Implication Of Ready-To Drink Soymilk And Soymilk Yogurt Sold In Onitsha Urban Anambra State, Nigeria

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***Abstract*—The study was carried out to access the vulnerability of the populace to microbial infections. The shelf life of soymilk and soymilk yogurt sold in Onitsha Urban was evaluated to assess the public health implication of these drinks visa vies food safety and health of the populace. The microbial analysis was carried out in the project Laboratory of Chukwuemeka Odumuegwu Ojukwu University Uli Anambra State. Fifty (50) samples of soymilk were bought from different markets from 50 different soymilk vendors. Soymilk and soymilk yoghurt were also prepared under laboratory conditions as control. Samples were stored at various temperatures for 7days. Sensory evaluation of the samples was also taken. Standard microbiological methods were utilized to isolate the microorganisms involved; biochemical analysis was used to identify the isolates. Pathogenicity testing was carried out. Polymerase chain reaction was used to further establish the identity of the organisms. Proximate analysis of soymilk and soymilk yogurt were also determined. Microorganisms isolated from this work were E.coli, Staphylococcus sp, Streptococcus sp, Klebsiella sp, Bacillus sp, Salmonella sp, Pseudomonas sp. and fungi isolates were Aspergillus sp, Candida sp, Rhizopus, Saccharomyces cerevisiae, Penicillum sp. The results show that almost all the samples bought from different markets were contaminated with E.coli , coliform and Staphylococcus sp., they ranges from $0.9 \times 10^3 – 7.7 \times 10^3$, $1.1 \times 10^3 - 8.0 \times 10^3$ and $4.6 \times 10^3 - 0.8 \times 10^3$cfu/ml respectively. Samples prepared under aseptic condition showed no growth of microorganisms on the zero day, on 3rd there was growth of Streptococcus sp. in soymilk. Bacillus spp. were isolated only in soymilk stored at room temperature. The mice inoculated with E.coli showed most significant clinical manifestations, while minor or no clinical signs were seen on others. Soymilk and soymilk yogurt show statistical significance in Protein, ash and pH for both samples, but only soymilk yogurt showed significant difference in fat. Sensory evaluation show that soy yogurt of different storage method were generally acceptable. The protein content of both soymilk and soymilk yogurt is high. The study showed that the use of sterile processed water and pasteurization of the end product extends the keeping quality. Soy yogurt used for this study was stable for 30days whereas soymilk was only stable for 5days. The microbial load of ready-to- drink yogurt sold in our local markets is very high, their keeping quality is matter of hours these subjects the populace to a multiplicity of adverse health implications which includes salmonellosis, diarrhea and other diseases caused by Staphylococcus, Pseudomonas etc. Soymilk vendors should be educated on effect of food poisoning on human's health and importance of producing their products hygienically.**

***Keywords*—Polymerase chain reaction; Pathogenicity; populace; pasteurization; food poisoning.**

INTRODUCTION

Soy milk is a water extract from whole soy beans. It is an emulsion containing water soluble proteins, carbohydrate and oil droplets. Soy milk is a high protein, iron-rich milky liquid produced from pressing ground, cooked soybeans (Singh et al., 2011). Soymilk is made by soaking soybeans in water before grinding and straining. Creamy white soy milk resembles cow's milk but in fact differs from its dairy counterpart in a number of ways. Not only is it higher in protein and iron content, but it is cholesterol-free, low fat, and low sodium. It is, however, lowers in calcium and must be fortified with calcium when given to growing children. Those who are allergic to cow's milk or are unable to digest lactose, the natural sugar found in cow's milk, find soy milk easy to digest since it is lactose-free (Wikipedia, 2010). Those who are calorie-conscious can purchase reduced fat soy milk (called lite soy milk) but this is often lower in protein as well. Some do not enjoy the taste of original soy milk, so manufacturers now offer flavored soy milk. Soy milk can be substituted for milk in nearly any recipe. Those who merely want to boost protein intake often add powdered soy milk to other beverages; others find it economical to purchase it in powder form and then make soy milk when they add water to the powder. Children under one year of age should be given a formula of soy milk specifically developed with their nutritional needs in mind. Soy milk that is intentionally curdled is known as tofu (Klein et al., 2010).

Soya bean (*Glycine maxima*), the primary material for Soya milk production has been identified to be one of the most important legumes of the tropics with high protein content. Soybean is used in various forms in many parts of the world. Soybeans and products derived from them have

served as an important source of protein in the diet of millions of oriental people for nearly 5,000 years (Vij et al., 2011). It is a potential food material that contains all essential amino acids that are very important for the proper development of the body, indeed Soya beans, has a higher content of Lysine in comparison to other plant proteins. Soybean contributes approximately 20% fat to the diet (Norooz 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 (Anderson and Bush, 2011). Other than the high protein content, it also has good amount of calories and fat. Soya bean contains 43 grams of protein per 100gms, which is the highest among the pulses. It also contains 19.5gms of fat, 21gms carbohydrate and provides 432 kcal per 100gms (Ayo, 2011). The protein of soybean contains all the essential amino acids in adequate amount except methionine and cystine. It is one of the best vegetarian food items as far as protein content is concerned it is a good source of riboflavin. Soybean contains a factor that inhibits the action of the digestive enzyme trypsin and this factor can be destroyed by heat (Anderson and Bush, 2011). Soybean should be cooked well for digestion and absorption. Studies have shown that in type II hypercholesterolemia, patients already on low lipid, low cholesterol diets, eight weeks substitution of animal protein by soybean protein reduced plasma cholesterol by 23 to 25 percent (Hara et al., 2012). Soya bean when processed they give Soya beans milk which can be converted to yoghurt which is valuable protein supplement or substitute for adult and infant feeding. Soya milk is lactose-free and can be consumed by the lactose-intolerant people as a substitute to milk.

Yoghurt is a semi-solid fermented milk product consumed in most parts of the world and the changes in the physical, chemical and microbiological structure of yoghurt determine the storage and shelf life of the product [13]. Preservatives are added to inhibit the growth and metabolism of microorganism. These are generally additives, which prolong the life span of foods and drinks by preventing micro-organisms attack. Technically, preservatives are chemicals used to poison microorganisms and to prevent the food onto which it is added from fermentation and spoilage without causing any harmful effect to the person who consumed the food. The uses of chemical preservatives enhance food quality, reduce waste and enhance consumer acceptability. Recent reports indicate that some probiotic bacteria could better compete with yoghurt cultures in a soy-based substrate. Soy has been examined as a substrate for the lactobacillus species: L. Casei, L. helveticus, L. fermenti, L. fermentum and L. reuteri (Garro et al.,2010;Murti et al.,2013b; Chumchuere and Robinson, 2009;Garro et al., 2014;Tzortzis et al., 2014).Documented information indicates that soymilk has a significant amount of raffinose and stachyose but does not contain lactose and that lactic acid bacteria (LAB) from different sources are quite different in their efficiencies in soy yoghurt fermentation (Tuitemwong and Tuitemwong, 2013). The problems of soymilk can be improved by lactic fermentation, so production of fermented soymilks such as soy yoghurt is important (Nsofor et al., 2012).

Pathogenic microorganisms are microorganisms capable of causing disease, although they represent only a small part in the total microbial world; they receive much attention because they represent a threat to the human or animal health and to agriculture (Ofoefule,2012) . Pathogenic microorganisms can cause disease of plague dimensions with serious economic and environmental consequences. Pathogenic bacteria include, *Salmonella* spp., *Clostridium botulism*, *Staphylococcusand shigella* spp.. This work is however aimed at studying the public health implications of ready to drink soya milk produced in Onitsha Urban and environs.

Materials and Methods

The materials used for this experiment include;

Equipment and apparatus

Weighing balance, Petri dishes, conical flask, autoclave, incubator, cotton wool, aluminum foil, measuring cylinder, beaker, microscope, wire loop, slides, oven, water bath, crucible, test tubes, blender, sieve, sorxhet, kjeldahl, muffle furnace, hot plate, pot, pipette, pH meter, retort stand and test tube rack.

Media

Nutrient agar, nutrient broth, agarose agar, Mannitol salt agar (MSA) for *S. aureus*, Eosin methylene blue agar for *E. coli* , Desoxycholate agar (DCA) for *Salmonella*spp., Sabouraud dextrose agar (SDA) for moulds and fungi. Chloraphenicol was added to inhibit the grow of bateria, MaConkey broth for colifrom count, Buffered peptone water for pre enrichment, Nutrient agar for viable count. Media was sterilized by autoclaving at 121^{o}C for 15 minutes except DCA which involved only boiling over gauze. In all cases of colony counts, the resulting colonies following inoculation and incubation were counted.

Reagents

Distilled water, Peptone water, crystal violet, safranin, Lacto phenol blue, surphric acid, petroleum ether, alcohol, iodine reagent, immersion oil, Chloramphenicol, glucose peptone water, malachite green.

Study area

The study was conducted in Chukwuemeka Odumuegwu Ojukwu University Uli (COOU) Anambra State, Nigeria.

Sample collection

Soya beans (*Glycine Maxima*) yellow seeds was purchased at modern Market, Onitsha, Anambra State, Nigeria; a starter culture (developed from skimmed powdered milk); flavouring agent; sugar and chemical preservatives (Sodium benzoate and Potassium metabisulphate), was also purchased and already prepared soy milk was purchased from fifty different locally produced soya milk vendors, ten samples from different market within Onitsha and environs. The samples were taken to the laboratory for analysis, the microbial load of all the samples were determined at the interval of the 0day, 3rd, and 7th day and all the samples are kept in the refrigerator during the time of the analysis.

Procedure for the production of soya milk and yoghurt

Soymilk was produced locally using the method of Lee et al., (1990) for soymilk production. 200g of the soybean seed was cleaned, sorted (to remove cracked, damaged and discolored seeds) and winnowed. This was then soaked in two liters of clean water for 8 – 10 hours. The water was changed at three hours interval. The beans were then parboiled in water for 45 minutes with constant agitation.

The boiled beans were then allowed to cool, dehusked, thoroughly washed and homogenized with sterile water into a paste using electric blender. The paste obtained was sieved using a clean muslin cloth to separate the milk (filterate) from the paste. The extracted milk was transferred into a pot and pasteurized or rather heated to 80°C for 30mins. and allowed to cool gradually to a temperature of about 42-45°C. The yellowish wad appeared on the surface were continually packed off using a clean spoon. The cooled homogenized milk was incubated with the already prepared starter culture yoghurment (lactic acid bacteria). The mixture was stirred properly and kept to stand at a temperature of about 42-45ºC for a period of 24h. At the expiration of the incubation period, the output is yoghurt.

Procedure for addition of chemical preservatives in soymilk and soymilkyogurt

0.01g each of Sodium benzoate and Potassium metabisulphate was dissolved in 3ml of distilled water and shaken thoroughly inside a beaker 3ml of the solution was added to 200ml of the prepared soya milk after which they were pasteurized at 80ºC for 30mins. then lactic acid bacteria was added on the soymilk and was allowed to stay at the temperature of 42-45ºC. The soy milk yoghurt and soymilk were stored in sample bottles with label on each bottle for easy identification of each sample.

METHODS

Sterilization of materials

All glassware used in the course of this research work were washed and sterilized in an oven. The grinding machine used for the processing of the soybean seeds was rinsed with sterile water, cleaned with ethanol and re-washed with sterile water. Other materials were sterilized using ethanol. The cloth used for filtration was soaked in ethanol before being rinsed in clean water.

Determination of shelf life

Soymilk and soymilk yoghurt produced in the laboratory under aseptically method were divided into three, one of each samples contain preservatives, they were poured into different bottles with labeled, after pasteurization they were store at different temperature, one from each samples were stored at room temperature of 25-27ºC, the second were stored in the refrigeration at 4ºC and the third bottles contained preservatives and was stored at room temperature. Their microbial loads were determine for seven days and there sensory evaluation were also determine from day 1, 3rd day and 7th day.

Isolation of Bacteria

Serial dilution

1ml of the sample was picked with the aid of sterile pipette after vigorous shaking. A metal rack was arranged with sterile test tubes containing 9ml of sterile distilled water. A tenfold serial dilution (Dhawale and LaMaster, 2003), was carried out by dropping 1ml of the first test tube labeled 10-1. This was mixed properly. 1ml was again taken from the 10-1 dilution tube and transferred into the next rest tube labeled 10-2. The dilutions continued to dilution 10-5. Each test tube was vigorously shaken before each transfer.

Isolation of Fungi

Preparation of Sabouraud dextrose Agar

33g of dehydrated Sabouraud Agar was weighed and dissolved in 500ml of distilled water. The solution was poured into a conical flask and the mouth plugged with cotton wool, then it was covered with aluminum foil and autoclaved at 121ºC for 15mins, Chlorophenicol was added into the culture medium to suppress the growth of bacteria. It was allowed to solidify. Inoculation was done by introducing 0.1ml of inoculum on Sabrouraud agar by spread plate method after which the plates were incubated at 22-25 ^{0}C for 3 days suitable for the growth of molds and yeast. The colonies that developed were isolated and sub-cultured. The pure cultures obtained were transferred into agar slants from where they are identified using morphological biochemical characterization.

Identification of isolates

Isolates were identified with the aid of keys and diagrams presented by Frazier and Westhoff (2004), Kogan (2001) Bernette and Hunter (1987); the following test were carried out: Gram staining, spore staining, catalase test, citrate test, methyl red test, indole test, urea test, coagulase test, sugar fermentation test, oxidase test, lactose test, glucose test,mannitol test and motility test.

Bacterial Counting

The Petri dishes containing the overnight culture that was obtained from serially dilution was placed on colony counter and the readings were taken. The number of colonies counted on the plates was recorded taking into consideration the dilution factor and used to calculate colony forming units (cfu) per ml. Cfu/ml= (No. of colonies x dilution factor)

Volume of culture plate

Characterization and Identification of fungal isolate

Fungal isolates were characterized and identified according to their cultural morphology and microscopy such as colour, consistency and growth pattern of mycelia, A wet mount method (Dhawale and LaMaster, 2003) was done before viewing the isolates under X40 objective of the microscope. each morphological structure of each isolate was matched with a mycology atlas (Bernette and Hunter, 1987) for identification.

Molecular Characterization

Extraction of DNA

5ml of the bacteria culture was grown overnight in agar broth at 37ºC, 1.0ml culture f 10,000 x g for 2mins) in a microcentrifuge, the supernatant was discarded.

The cell pellet was resuspended in 567µl TE buffer by repeated pipetting.

30µl SDS (10%w/v) and 3µl proteinase k (20mg/ml were added and mixed, it was incubated for 1hr at 37ºC. 100 µl of 5MNacl was added and was properly mixed, 80µl of CTAB/ Nacl solution were also added and was mixed and incubated for 10mins at 65ºC. equal volume of chloroform and isoamyl alcohol were added and was gently mixed. The sample was centrifuged at 10,000 x g for 5mins, white precipitate appeared at the interface of two aqueous layer. The upper aqueous layer was transferred to fresh tube. Equal volume of

phenol, chloroform and isoamyl alcohol were added, it was properly mixed and centrifuged at 10.000 x g for 5mins. The upper aqueous layer was again transferred to a fresh tube. 0.6volume isopropanol was added and was gently mixed, until white thread like material appeared, it was centrifuged at 10,000 x g for few min and the supernatant was discarded. The precipitate was washed with 1ml ethanol (70% v/v) for a few seconds, it was centrifuged for a minute and the supernatant was discarded. The DNA pellet was allowed to air dry to evaporate ethanol. The DNA pellet was dissolved in 100 µl of TE buffer.

Preparation of Agarose gel

1.0% agarose was added in the electrophoresis buffer and it was boiled in a hot plate to dissolve the agarose, it was cooled to 55ºC, the agarose solution was poured in a sealed gel-casting platform, the gel comb was inserted closed to one end of the gel-casting platform. After gel has hardened, the seal was removed from gel casting platform and the gel comb was with drew.

The gel was placed in electrophoresis tank containing sufficient electrophoresis buffer to cover the gel up to 1mm. DNA samples were prepared with an appropriate amount of 10x loading dye. Total volume of the sample should be around 10-20µl.The samples were loaded into wells with autopipette by ensuring that appropriate DNA molecular weight markers were put in a separate well.

The gel was connected with power supply so that DNA migrates from cathode to anode. When dye reached the end of the gel the power supply was turned off. The gel was placed in ethidium bromide solution for about 10mins

Pathogenicity Test

Four to five weeks old 13 albino mice weighing 23-28g were used to determine the pathogenicity of the different bacterial and fungal isolates. The microbial isolates were inoculated in nutrient agar and wereincubated at room temperature for 24hrs. the resulting microbial suspension was centrifuged at 3000rpm for 10mins.the supernatant was discarded the microbial pellet was resuspended in normal saline. The resulting solution was finally adjusted to a final concentration of 10^8 cells/ml using a spectrophotometer (Enamul and John, 2005). 0.5ml normal saline suspension of 10^8cells/ml was inoculated orally into 12 albino mice. Each of the albino mice were fed with a particular organism isolated, one mouse was fed with their normal feed and no microorganism was added to its food, it serves as control. The mice were observed for 14days to detect changes in the autonomic or behavioral response like spontaneous activity, irritability, urination, breathing rate and mortality (Kumar *et al*., 2009).

Proximate Analysisof Soya Milk And Soy Milk Yoghurt

Method of AOAC, 2005 was used to determine the protein, ash. fiber, moisture and fat content of the samples

Determination of carbohydrate

(Differential method)

100-(% protein + Moisture +Ash + fat + fiber)

pH determination

The pH of the samples were determined using digital pH meter by inserting the electrode directly, inside undiluted sample and the readings was taken, this was done on the soymilk and soymilk yogurt for 7 days.

Sensory evaluation

All the samples were evaluated for organoleptic characteristics and overall acceptability by 10 panelists that comprised undergraduate students, teaching and non-teaching staff members of Anambra State College of Agriculture Mgbakwu, Anambra State, Nigeria; using five point hedonic scale ranging from excellent (score = 5) to very poor (score = 0) as extremes (Obi *et al*., 2010). Prior to each assessment, the panelists were informed about the task of the test. In addition to the information, a detailed set of written instruction on testing method was available in each table. A 10ml portion of soymilk was served to each panelist and asked to freely evaluate, Comment and score the samples and asked to taste, color, flavor, texture. Scale used was as follows, 5- excellent, 4- very good. 3- good, 2- fair and 1- poor, 0- very poor/ unacceptable. To eliminate bias, un-labeled samples were presented to the panelist individually with sufficient privacy to guarantee independent judgment. The acceptability of the samples was based on the scores and remarks made by the panelists. The result of the test was assessed using the Hedonic preference test. The scores for the samples were analyzed statistically using the method of analysis- Anova (Snedecor and Cochran, 1976). Mineral and water were available as neutralizers. The test was performed under conditions of standard light and temperature 20ºC. The same subjects were used in all the steps of the sensory evaluation, so accurate data collection could be obtained.

The sensory evaluation of the samples was done on 0day, 3rd day and 7th day.

Data analysis

All the results in this study are reported as mean of three replicate analysis of soymilk and soymilk yogurt on different days. One way analysis of variance (ANOVA) was used to determine difference between the mean scores. Differences between means obtained from ANOVA were ascertained using Ducan's multiples range tests. Significant was accepted at $P<0.05$. Results presented as tables and graph.

TABLE 1: Total viable count of bacteria isolates from soymilk samples purchased from different market at 0 day (cfu/ml)

S/N	SNM	SOMM	SRM	SAM	SOBH
SI	3.4 x 10^3	3.1 x 10^3	4.2 x 10^3	2.9 x 10^3	3.0 x 10^3
S2	3.8 x 10^3	4.2 x 10^3	3.2 x 10^3	3.3 x 10^3	2.4 x 10^3
S3	5.6 x 10^3	5.8 x 10^3	3.7 x 10^3	3.9 x 10^3	4.2 x 10^3
S4	**3.0** x 10^3	**2.1** x 10^3	3.9 x 10^3	**4.0** x 10^3	**3.0** x 10^3
S5	**3.1** x 10^3	**4.2** x 10^3	**3.8** x 10^3	**3.2** x 10^3	**4.9** x 10^3
S6	**3.7** x 10^3	**3.1** x 10^3	**3.5** x 10^3	**3.7** x 10^3	**3.5** x 10^3

S7	**4.6** x 10^3	**5.1** x 10^3	**2.4** x 10^3	**3.1** x 10^3	**6.2** x 10^3
S8	**3.0** x 10^3	**3.9** x 10^3	**3.4** x 10^3	**4.0** x 10^3	**2.9** x 10^3
S9	**3.4** x 10^3	**3.6** x 10^3	**3.9** x 10^3	**2.7** x 10^3	**3.8** x 10^3
S10	**3.0** x 10^3	**3.8** x 10^3	**2.4** x 10^3	**3.2** x 10^3	**3.1** x 10^3

SNM; soya from nkpor market. SOMM; soymilk from ontisha main market. SAM; soymilk from Awka Market, Soymilk from Ogbo Ogwu market bridge head Onitsha.

TABLE 2: Total microbial viable count of the soymilk and soy yoghurt prepared in the lab. during determination of shelf life (cfu/ml)

0day	A	B	C	D	E	F
Bacterial count	-	-	-	0.6 x 10^3	-	-
Fungal count	-	-	-	-	-	-
3rd day						
Bacterial count	2.0 x 10^3	1.3 x 10^3	-	1.4 x 10^3	-	-
Fungal count	1.2 x 10^3	-	-	-	-	-
7th day						
Bacterial count	3.6 x 10^3	3.3 x 10^3	2.0 x 10^3	2.8 x 10^3	3.6 x 10^3	2.2 x 10^3
Fungal count	3.3 x 10^3	1.2 x 10^3	0.8 x10^3	3.4 x 10^3	2.8 x 10^3	1.0 x 10^3

KEY:A= Soymilk Stored At Room Temperature (27°c),
B= Soymilk with Preservatives
C= Soymilk Stored In Refrigerator
D= Soymilk Yogurt Stored At Room Temperature
E= Soymilk Yogurt with Preservatives
F= Soymilk yogurt Stored in The Refrigerator

TABLE 3: Total Viable Fungi Count from soya milk bought from different market 0 day (cfu/ml)

S/N	SNM	SOMM	SRM	SAM	SOBH
S1	2.4 x 10^3	3.2 x 10^3	-	2.6 x 10^3	1.0 x 10^3
S2	-	**1.4** x10^3	**3.0** x10^3	**2.8** x10^3	-
S3	**3.0** x10^3	**3.6** x10^3	**2.8** x10^3	**2.0** x10^3	**2.8** x10^3
S4	**1.5** x10^3	**2.0** x10^3	**1.1** x10^3	**1.4** x10^3	**0.9** x10^3
S5	**1.8** x10^3	**1.6** x10^3	**2.5** x10^3	**3.0** x10^3	-
S6	-	**1** .6 x10^3	**3.4** x10^3	**4.4** x10^3	**2.6** x10^3
S7	**3.2** x10^3	**3.4** x10^3	**2.9** x10^3	**1.6** x10^3	**1.8** x10^3
S8	**1.9** x10^3	**2.7** x10^3	**2.4** x10^3	-	**3.4** x10^3
S9	**2.5** x10^3	-	-	**2.6** x10^3	**3.0** x10^3
S10	**2.7** x10^3	**1.6** x10^3	**2.4** x10^3	**3.5** x10^3	**2.8** x10^3

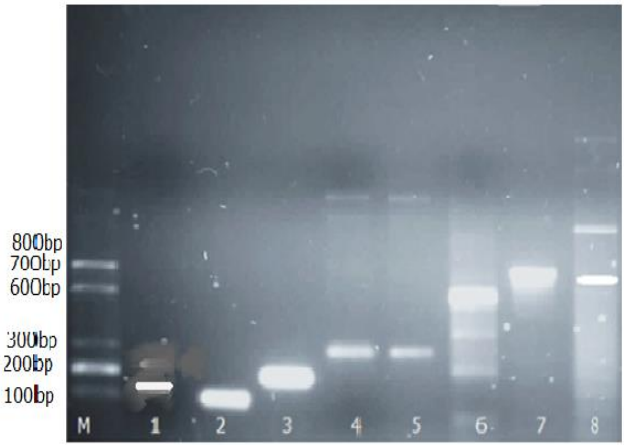

Figure1: Molecular characterization of bacteria isolates
Agarose gel showing restriction profile of PCR amplified region of isolates used in this work. M, marker gene (lane 1), *Staphylococcus aureus* (lane 2), *Streptococcus pneumoniae* (lane 3), *E.coli* (lane4), *Pseudomonas aregiunosa* (lane 5), *Micrococcus* sp. (lane 6), *Samonella typhi* (lane 7), *Klebsella*sp.(lane 8).

Pathogenicity Test

The mice fed with *E.coli* started stooling and vomiting continuously after 2days of feeding, diarrhea suspected.

The mouse fed with *Staphylococcus* sp. after 1hr was uncomfortable, moving up and down and making unpleasant noise.

The mouse fed with *Streptococcus* sp. shows no noticeable sign.

The mouse fed with *Bacillus* sp. shows neither noticeable sign nor symptom. The mouse fed with *Salmonella* sp. after 3hrs started showing signs of weakness, stooling continuously, moving to and fro.

Other mice fed with *Pseudomonas* sp, *Micrococcus* sp, *Aspergillus* sp, *Candida* sp, *Penicillin* sp, *Saccharomyce cerevisiae* shows no clinical manifestations.

The mouse that served as control didn't show any clinical manifestation.

Table 4, presents proximate analysis of soymilk yogurt within days (7days) in which protein content ranges from 10.087±0.143 – 7.78 and it shows that protein is highest in the nutritional content of soymilk yogurt, and there was decline in protein, fat and an increase in carbohydrate. There was also decline in pH which ranges from 4.89-4.577.

TABLE 4: Proximate Analysis of Soymilk Yoghurt (%)

days	Protein	Moisture	Fibre	Ash	Fat	carbohydrate	pH
1	10.067 ± 0.143	77.2 ± 0.653	1.03 ± 0.34	0.56 ± 0.00	3.15 ± 0.00	8.21 ± 2.819	4.897 ± 0.000
2	10.095 ± 0.128	78.3 ± 0.040	0.6 ± 0.000	0.27 ± 0.000	3.15 ± 0.000	7.682 ± 0.219	4.890 ± 0.000
3	10.087 ± 0.000	78.4 ± 0.493	0.53 ± 0.003	0.39 ± 0.000	3.12 ± 0.000	8.554 ± 0,383	4.813 ± 0.000
4	10.067 ± 0.010	78.9 ± 0.63	0.5 ± 0.030	0.57 ± 0.006	2.99 ± 0.000	7.709 ± 2.079	4.753 ± 0.000
5	10.057 ± 0.000	78.9 ± 2.10	0.63 ± 0.003	1.22 ± 0.004	3.08 ± 0.012	7.427 ± 0.152	4.167 ± 0.000
6	10.077 ± 0.000	78.23 ± 0.160	0.63 ± 0,003	1.33 ± 0.004	2.57 ± 0.012	7.8 ± 0.152	4.590 ± 0.000
7	10.083 ± 0.017	78.4 ± 0.210	0.5 ± 0.010	2.03 ± 0.001	2.99 ± 0.021	7.147 ± 0.423	4.577 ± 0.000
Between groups signif.	0.000	0.159	0.000	0.163	0.000	0.201	0.000

Values are means of triplicate determinations □± = standard deviation

Table5 presents proximate analysis of soymilk. There was a decrease in the protein which ranges from 10.47-7.78 the fiber content remained constant throughout the period of analysis there was an increase in carbohydrate and ash content of the sample. Decrease in the pH was observed which ranges from 6.607 at the 0 day to 4.640 at 7th day.

Table 5: Proximate Analysis of Soy Milk

Days	Protein	Moisture	Fiber	Ash	Fat	Carbohydrate	pH
1	10.47 ± 0.058	78.1 ± 0.003	0.63 ± 0.003	0.55 ± 0.005	1.76 ± 0.343	8.54 ± 0.192	6.607 ± 0.000
2	10.46 ± 0.000	78.3 ± 0.093	0.6 ± 0.000	0.27 ± 0.073	1.44 ± 0.233	8.896 ± 0.851	6.457 ± 0.000
3	10.421 ± 0.003	78.4 ± 0.040	1.7 ± 0.003	0.386 ± 0.001	1.7 ± 0.036	9.492 ± 0.061	6.380 ± 0.000
4	10.42 ± 0.007	78.9 ± 0.063	1.7 ± 0.010	0.57 ± 0.010	1.67 ± 0.004	8.52 ± 0.034	5.770 ± 0.000
5	10.26 ± 0.005	78.2 ± 0.030	1.7 ± 0.003	1.22 ± 0.024	1.89 ± 0.031	7.856 ± 0.065	4.740 ± 0.000
6	10.13 ± 0.010	78.2 ± 0.023	0.5 ± 0.030	1.6 ± 0.040	1.97 ± 0.003	7.57 ± 0.009	4.667 ± 0.000
7	10.43 ± 0.003	78.4 ± 0.370	0.6 ± 0.010	2.03 ± 0.000	1.98 ± 0.088	7.567 ± 0.088	4.640 ± 0.000

Values are means of triplicate determinations □± =S.D

Figure 2 representative of soymilk yogurt , which shows that refrigerated soymilk yogurt was highest in all parameters determined followed by soymilk yogurt with preservatives, the sample stored at room temperature have the least general acceptability.

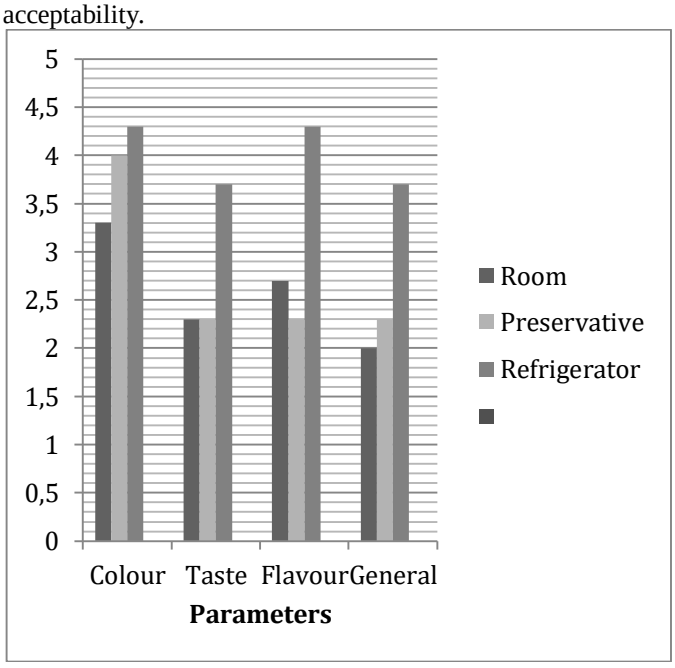

Figure 2: Sensory Evaluation of Soymilk Yogurt

Figure 3 presents sensory evaluation of soymilk of different days in which refrigerated soymilk have highest acceptability color, taste ,and flavor, followed by soymilk with preservatives and lastly soymilk stored at room temperature have the least general acceptance.

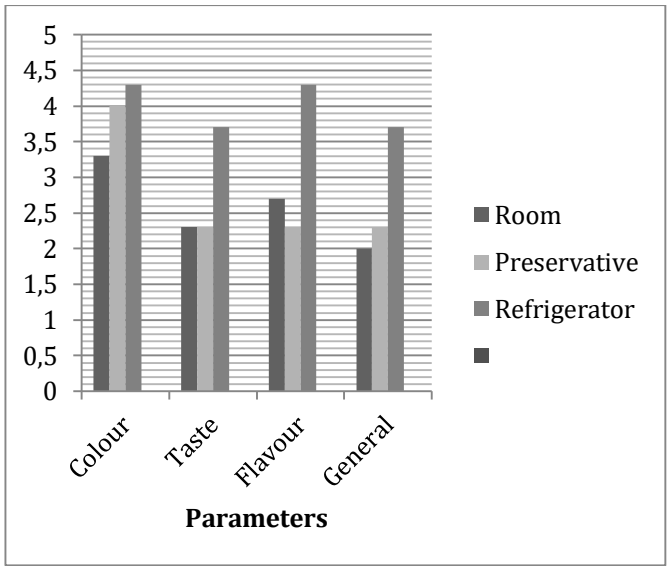

Figure 3: Sensory Evaluation of Soymilk

Discussion

Soymilk and soybean products have served as an important source of protein in the diet of millions of people for nearly 5,000 years (Ng et al., 2011). Its high nutrient value has made it so irresistible that it is recommended very highly by nutritionists as a substitute to cow milk. The increase in the consumption rate of soybean milk due to its high protein content has encouraged low scale production of the soymilk under house hold condition with little or no regard to the quality control measures (Chukwu, 2012).

All soymilk samples used in the study contained one form of microorganism or more, each samples contained different contaminants. The ubiquity in the hawking of locally produced soymilk packaged in different forms was considered a public health concern (Vij et al., 2011). The presence of *Salmonella*spp., *Staphylococcus aureus* and *E.coli* reflects a poor hygienic standard in the production process of soymilk and most of the strains of *Staphylococcus aureus* are known to be pathogenic. As for the soymilk and soy yogurt produced organisms was isolated until day seven showed that the soymilk vendors did not produce their products under hygienic conditions or rather they use contaminated water which may be the cause of the product contaminated with coliform and the presence of all this organisms poses a serious health threat to the consumers. The microbial loads of this organisms increased by the day as can be seen in table 1,table 9 and 10, but a reduced in microbial load of *Staphylococcus* sp. was observed and a high increase in *Bacillus* sp was observed this implies that bacillus may be one of the organisms that induces spoilage. Proper pasteurization of this product is very necessary to destroy most of the growing organisms in the soymilk product that would have caused the spoilage earlier than observed in soymilk purchased from the market in which spoilage was observed from the zero day of purchased comparing to laboratory prepared soymilk stored at room temperature in which spoilage was noticed from fourth day. The soymilk with chemical preservative showed no fungi growth on the 0day to 3rd day until 7th day when the growth of *Saccharomyces cerevisiae* was detected; this can be as the result of the antimicrobial properties of benzoic acid this corresponded with the work of Momoh et al., (2011). Chemical preservatives are included in food and pharmaceutical preparations to prevent microbial spoilage of the products and minimize the risk of the consumer acquiring an infection when the preparations are taken (Sean *et al*., 2014). These chemical agents affect microorganisms by disrupting critical cell factors e.g. they may damage the plasma membrane or denature various cell proteins while others interfere with the functioning of nucleic acids this inhibiting cell reproduction (Lansing et al., 2012). For the soymilk and soymilk and soy yogurt stored in the refrigerator there was no microbial growth in the soymilk from 0day till 7th day and soymilk yogurt, the isolation of *Streptococcus* sp. may as the result of inoculation of starter culture (lactic acid bacteria) to enhance fermentation.

The sensory evaluation conducted on the three packaging methods indicates that on the 7th day that soymilk stored in the refrigerator and preservatives was generally accepted, this may be because of the introduction of chemical preservatives which have antimicrobial properties that inhibits the growth of microorganism and the sample stored in the refrigerator might also be as the cause of reduced temperature which deactivate the growth of some microorganisms. There was no changes found in the sample during storage in the refrigerator, a slight increase was seen in the ash content and that might also be the cause of slight increase seen in carbohydrate content of the sample as can be seen in Table 4 and 5.

The pH values recorded for the soymilk and soy milk yoghurt for seventh day ranges from 6.60-4.64 and 4.89-4.58 respectively, this decrease in pH of soymilk yoghurt is 4.89 – 4.58 which is acidic and this might be caused as the inoculation of lactic acid bacteria. The decrease in pH of both samples maybe as the result of acid production from the hydrolysis of milk sugar during the storage period. It could be inferred that changes in the pH was enhanced by storage conditions and that the pH declining pattern was similar in both soymilk and soymilk yogurt.

Conclusion

It has been concluded that locally produced soy milk in Onitsha market and environ are all contaminated with one or more microorganisms. The storage of soymilk and soymilk yogurt in the refrigerator, addition of chemical preservatives and proper pasteurization contributed in the extension of soymilk and soymilk yogurt shelf life. Soymilk yogurt have a longer shelf life as to compare to soymilk.

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