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Determination of Shelf Life of Locally Produced Soymilk

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Abstract: ***The study was carried out to access the shelf life of soymilk and soymilk yogurt sold in Onitsha Urban 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 bought from different markets from 50 different soymilk vendors and one laboratory prepared soy milk and was used for the study. The laboratory prepared samples were used as control. The shelf life of soymilk was evaluated; samples were stored at various temperatures for 7days. Sensory evaluation of the samples was taken. Standard microbiological methods were utilized to isolate the microorganisms involved, biochemical analysis was used to identify the isolates. Results revealed the presence of E.coli, and members of the genera Staphylococcus, Streptococcus, Klebsiella, Bacillus, Salmonella, Pseudomonas sp., and fungi isolates of the genera Aspergillus, Candida, Rhizopus, Penicillum sp., and Saccharomyces cerevisiae. Almost all the samples obtained from different markets were contaminated with E.coli , coliform and Staphylococcus sp., Bacteria counts of days 0, 3 and 7 are 0.9 x 10^3 – 7.7 x 10^3 , 1.1 x 10^3 - 8.0 x 10^3 and 4.6 x 10^3 - 0.8 x 10^3cfu/ml respectively. Samples prepared under aseptic condition showed no growth of microorganisms on the day zero, but on day 3 there was growth of Streptococcus sp. in one sample ranging from 1.3 x 10^3- 2.0 x 10^3 cfu/ml, on day 7, Streptococcus sp. was seen in all the samples ranging from 2.0 x 10^3 - 3.6 x 10^3. Bacillus was isolated in soymilk stored at room temperature. The study shows that the use of sterile processed water and pasteurization of the end product extends the keeping quality. Soymilks were 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, this subjects the populace to multiplicity of adverse health implications which includes salmonellosis, diarrhea and other diseases caused by Staphylococcus, Pseudomonas, Bacillus, Salmonella and Aspergillus. Soymilk vendors should be educated on the effect of food poisoning on human health and importance of producing their products hygienically.***

Keywords: Soy milk, shelf life, contaminated, Staphylococcus, Saccharomyces, Microorganism.

1. 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 (Hua, 2007). It is made by soaking soybeans in water before grinding and straining. Creamy white soy milk resembles cow's milk but differs from its dairy counterpart in a number of ways (Williams and Akiko, 2000). Not only is it higher in protein and iron content, it is cholesterol-free, low fat, and low sodium. It is, however, lower 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. Yoghurt is a semi-solid fermented milk product consumed in most part of the world and the changes in the physical, chemical and microbiological structure of yoghurt determine the storage and shelf life of the product (Sofu and Ekinci, 2007). Preservatives are added to improve yoghurt shelf life. These are generally additives, which prolong the life span of foods and drinks by preventing microbial attack. Technically, preservatives are chemicals used to poison micro-organisms and prevent the food onto 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.

2. Materials and Methods

Study area

The study was conducted in Chukwuemeka Odemuegwu Ojukwu University Uli 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), were also purchased. Already prepared soy milk was purchased from fifty different 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

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 hour intervals. 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 clean 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 at 85°C for an hour 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.

Procedure for addition of chemical preservatives in soymilk

About 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 80^{o}C for 30 mins. then lactic acid bacteria was added on the soymilk and was allowed to stay at the temperature of 42-45^{o}C. The soymilks were stored in sample bottles with label on each bottle for easy identification of each sample.

3. Methods

Isolation of Bacteria

Serial dilution

One milliliter 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

Thirty three 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 121^{o}C 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 inculum 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 24hrs medium that was obtained from serially dilution was placed on colony counter and the reading was 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.

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.

4. Result, Discussion and Conclusion

Table 1 presents enumeration of bacterial isolates from laboratory prepared soymilk during determination of shelf life, on zero day there was no growth of bacteria or fungi in all the samples. On day three, there was growth of bacteria on the soymilk with preservatives and soymilk stored at room temperature and there was no growth on the refrigerated sample. Fungi were isolated from soymilk milk stored at room temperature. On the seventh day there was growth of both bacteria and fungi on all the samples. From this study, it is obvious that there is more contamination of the soymilk by bacteria than by fungi. Shelf life for lab. Prepared soymilk was 3days while shelf life of commercially available ready-to-drink yogurt is matter of hrs (see Table 2)

Table 1: Total Microbial Viable Count of the Soymilk Prepared In the Lab.

0 day	Soymilk Stored at Room Temperature (27^{o}C),	Soymilk with Preservatives	Soymilk Stored in Refrigerator
Bacterial count	-	-	-
Fungi count	-	-	-
3rd day			
Bacteria count	2.0 x 10^{3}	1.3 x 10^{3}	-
Fungi 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}
Fungi count	3.3 x 10^{3}	1.2 x 10^{3}	0.8 x10^{3}

Table 2 presents Enumeration of bacterial isolate from laboratory prepared soymilk and soymilk yogurt at 7th day in which there was growth of Streptococcus sp. in all the samples, Bacillus was only isolated from soymilk at room temperature.

Table 2: Enumeration of Bacterial Isolate from Lab. Prepared Soymilk during Determination of Shelf Life on 7th day

Isolates	A	B	C
Staphylococcus auerus	-	-	-
***Streptococcus* sp.**	3.6 x 10^3	3.3x10^3	2.0 x10^3
E.coli	-	-	-
Klebsella	-	-	-
***Pseudomonas* sp.**	-	-	-
Salmonella	-	-	-
Bacillus	3.2 x 10^3	-	-
Micrococcus	-	-	-

Key: - = absent
A= Soymilk Stored At Room Temperature (27°c),
B= Soymilk with Preservatives
C= Soymilk Stored In Refrigerator

Table 3 presents total viable counts of bacterial isolates from samples purchased from different market at 0 day. Their microbial loads ranges from 2.1 x 10^3 to 6.2 x 10^3 . the samples purchased from Onitsha main market have the least microbial loads and soymilk purchased Ogbo ogwu market bridge head have the highest bacterial loads.

Table 3: Total Viable Count of Bacteria Isolates From Soymilk Samples Purchased From Different Market at 0 Day

S/N	*SNM*	*SOMM*	*SRM*	*SAM*	*SOBH*
S1	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

Key:
SNM; Soya from Nkpor market.
SOMM; Soymilk from Ontisha main market.
SAM; Soymilk from Awka Market,
SOBH; Soymilk from Ogbo Ogwu market bridge head Onitsha.

Figure 1 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.

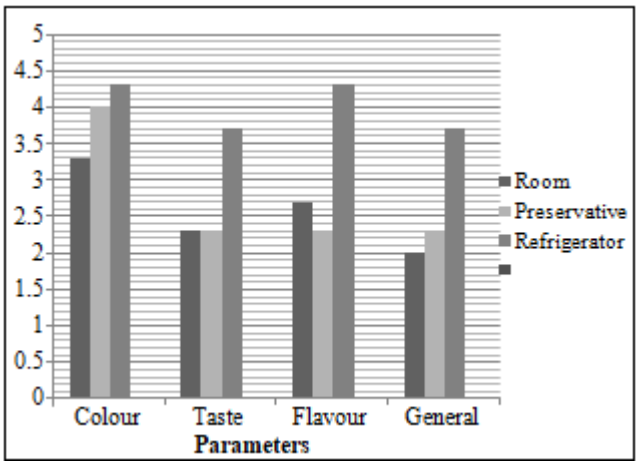

Figure 1: Sensory Evaluation of Soymilk

5. Discussion

The microorganisms isolated from soymilk in this project work tally with already known organism isolated from previous studies on microbiological quality of local soymilk, (Adeleke *et al*.,2010).

The bacteria isolated from soymilk belong to the following general *E.coli*, *Staphylococcus*, *Streptococcus, Klebsiella*, *Bacillus*, *Salmonella* and *Pseudomonas* as represented in table 1. Proper pasteurization of this product is very necessary for it help 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 and this agrees with the work done by Momoh, *et al*., (2011). 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 (Mbajiuka, 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 stored in the refrigerator there was no isolate in the soymilk from 0day till 7th day.

The sensory evaluation conducted on the samples 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.

6. Conclusion

It has been concluded that addition of preservatives and storage in refrigerator helps to extend the shelf life of soymilk.

7. Acknowledgement

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