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Assessment of Microbial Quality of Soyamilk Sold in Oko Metropolis Anambra State, Nigeria

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ABSTRACT

Four locally prepared soymilk drinks sold in Federal Polytechnic Oko, Anambra State were subjected to microbiological analysis to ascertain their hygienic standard of production. Each of the locally produced soymilk drinks were purchased in some strategic places in Federal Polytechnic Oko, including Back Gate, First Gate, Perm Site and Inside School. Standard biological techniques were used in the enumeration of potential organisms in the samples. The result of the microbial analysis showed that activities of microorganisms were noticeable in the soymilk samples. The total viable count of the samples collected from inside school and perm site were 1.4×10^2cfu/ml and 1.6×10^2cfu/ml respectively. There was no growth in the samples collected from back and first gate. The total microbial count of all the samples ranged from 12.0×10^2cfu/ml in the samples collected from back gate and inside school to 2.8×10^2cfu/ml in the sample collected from perm site. The soymilk drink had an unsatisfactory microbial quality as they contain Lactic acid bacteria (28.33%) E. coli (20.33%), S. qureus (18.33%), Bacillus *spp.* (16.66%) and Pseudomonas spp. (16.66%). The bacterial spectrum isolated from the locally prepared drinks is indicative of exogenous contamination which could cause food poisoning if no care is taken

Keywords: *Soymilk, E. coli, S. qureus, Bacillus spp., Pseudomonas spp., Lactic acid bacteria.*

INTRODUCTION

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. (Onuorah *et al*., 2007). Pathogenic microorganisms can cause disease of plague dimensions with serious economic and environmental consequences (Twizeyimana *et al*., 2009). Pathogenicity represents a form of versatility and specialization that enables certain microorganisms to replicate within a specific host (infectivity) and such hosts shows a sign of disease or eventually die (Odds *et al*., 2001).

The outcome of the infection is dependent on the properties (virulence, invasiveness, toxicity or allergic effects) of the organism but also upon the host's immune status (Vincent, 2005). Pathogenic bacteria include, Salmonella spp, clostridium botulism, staphylococcus and Shigella *spp.* Control of microbial growth and spoilage of product is achieved by restricting and controlling microorganisms from containing the product through good manufacture and handling practice (Ofoefule, 2002)

Soybean (Glycine max) belong to the family Leguminasae. It is native to China and is one of the oldest World crops (Wang *et al.*, 1978). United States is the World's leading producer of soybean followed by China (Wang *et al.*, 1978). There are many varieties of

soybean, the shape and size of seeds vary from small round pea to large elongated beans. The colors also vary from yellow, brown and green to black. The seeds are enclosed in a short hairy pod containing 2-3 seeds attached to the plant. Name of the different varieties of soybean include Glycine max, Glycine ussuriesis (Wild), Glycine garcilus (intermediate) and Glycine soja (Iwe, 2003). Soymilk is defined as an aqueous extract of whole soybeans (dehulled or non-dehulled), closely resembling dairy milk in physical appearance and composition (Pati and Jha, 2008). It is a nutritious beverage rich in high quality protein and contains no cholesterol or lactose. Soymilk is also referred to as a liquid obtained by suspending soybean flour in water, used as fat free substitute for milk. It is inexpensive, highly digestible, it is rich in polyunsaturated fatty acids, linoleic acid (Deshpande *et al*., 2008). It is also non-allergic, can easily be produced with low level technology and serve as good nutrient for vegetarian diet. Soymilk has a great potential to supplement the dairy milk and it is nutritionally comparable with the mother's milk and cow's milk. At the standpoint of nutritional quality, soy protein (soymilk) has many advantages over animal proteins beyond the fact that soymilk are low in saturated fat and cholesterol free, soymilk and cow milk have approximately the same protein content and composition and the amino acid composition show a family close correspondence (Smith and Circle, 1972).

Fresh soymilk has a very short shelf life, which limits consumption to areas close to the production site. Thermal processing is the most common practice used to improve the microbial safety and extend the shelf-life of soymilk because it inactivates vegetative pathogens and many spoilage bacteria. In some conditions, thermal processing, however, detrimentally affects nutritional and quality attributes of soymilk and produces strong off flavors (Lozano *et al*., 2007).

In addition to poor handling and unhygienic practices of local producers of soymilk products, the nutrient composition of soymilk milk makes it an excellent bacteriological medium. These have been implicated in the occurrence and prevalence at of diseases such as typhoid fever and dysentery among soymilk consumers. The sale of soymilk is quite popular in Federal Polytechnic Oko, but there is no report on its microbiological quality, which should have addressed the safety of its consumers in the school. This study was therefore aimed at evaluating the microbial load of soymilk as a means of addressing the safety concerns of its consumer in the study area.

MATERIALS AND METHODS

Study Area:

The study area was Federal Polytechnic Oko, Orumba North Local Government Area of Anambra State in the South East Region of Nigeria.

Collection of Test Samples:

Three Samples of unbranded locally produced soymilk were obtained around Oko metropolis at four different strategic locations, one sample per location. These locations were selected because they were public places where foods are sold to the unsuspecting consumers. The drinks were all freshly prepared. Oral consent were gotten from the sellers of these drinks after we had explained what we intended to use the drinks for. The samples were labeled properly and stored in the fridge in its original container. The four different locations were; 1.) School first gate 2.) Inside school 3.) Old admin and 4.) Perm site.

Microbial Analysis

Total Aerobic Mesophilic Bacteria Count:

A total aerobic mesophilic bacterial count was done according to (FAO, 1997) using plate count agar (Oxoid, CM 0325). The enumeration of bacteria was performed using digital colony counter and the result was expressed as colony forming units per ml (cfu/ml). The number of bacterial colonies of replicate plates was generated using equation (1)

$$\text{No. of cfu} = \text{ml/sample} = \frac{1}{\text{Plate factor} \times \text{Dilution factor} \times \text{Average number of colonies.}}$$

Total Coliform Count:

Total coliform count was done according to standard methods for examination of Dairy products (Michael and Frank 2004). The enumeration of total coliform was performed using digital colony counter and the result was expressed as colony forming units per ml (cfu/ml). The number of bacterial colonies of replicate plates was generated using equation (1) as above.

Lactic Acid Bacteria Count:

Lactic acid bacterial count was done according to standard methods for the examination of Dairy produces (Speck, 1976) using lactobacillus MRS agar (Himedia, M641). Colonies of lactic acid bacteria were counted and expressed as colony forming units per ml (cfu/ml). The number of bacterial colonies of replicate plates was generated using equation (1) shown above.

Bacteria Identification

Gram staining:

They were done using Gram staining reagents which were crystal violent, Lugol iodine, acetone/alcohol and Neutral red/safranine. At each time the smear washed with water. After that the smear air dried and observed under oil immersion objectives. Those that retain crystal violent Gram +ve, those that didn't retain crystal violent Gram -ve.

Motility Test:

Motility test is usually used to differentiate motile organisms from non-motile ones. For this test, the hanging drop technique was employed and the technique was carried out as described by Kirk *et al*., 1975).

Biochemical Tests

Urease Test:

This test was used to demonstrate the ability of the isolates to produces the enzyme urease which splits urea forming ammonia. The test is usually used to differentiate organisms like proteus from other non-urease positive organisms, (Banker *et al*., 1974). The method used was that described by Speck (1976).

Catalase Test:

The test was used to demonstrate which of the isolates could produce the enzyme catalase that release oxygen from hydrogen peroxide. This test is usually used as an aid to

differentiate staphylococci from streptococci and to differentiate other catalase positive organism from catalase negative (Barker, 1976). The method employed here was that described by Speck (1976).

Methyl Red Test:

This test was used to detect which of the isolates could produce and maintain sufficiently a stable acid product from glucose fermentation. This test is usually used as an aid in the identification and differentiation of the Enterobacteriaceae (Barker 1976). This test was carried out as described by Kirk *et al*., (1975).

Indole Test:

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in buffered peptone water. The test was carried out as described by Kirk *et al*., (1975).

Citrate Utilization Test:

This test was used to identify which of the isolates can utilize citrate as the sole source of carbon for metabolism. The test is usually used as an aid in the differentiation of organisms in the Enterobacteriaceae and most other genera (Baker, 1976). The medium used for this test was the Simon's citrate agar.

Sugar Fermentation:

Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. Since most bacteria especially gram negative bacteria utilize different sugars as source of carbon and energy with the production of both acid and gas, or acid only. The test is used as an aid in their differentiation. The growth medium used was peptone water and the method used was that described by Kirk *et al*., (1975).

Coagulase Test:

Slide and tube method was used (Carpenter, 1977). In slide test, a loopful of the isolate was mixed with human plasma and allowed to stand for some minutes. Particles indicating agglutination was used as indication of coagulase reaction. In tube method, plasma was added into a culture of the isolate in peptone water in bijou bottles. The bottles were incubated at 37^0C for 24 hours. A clamping/agglutination of the plasma were used to indicate presence of coagulase.

RESULTS AND DISCUSSION

Table 1
Microbial loads of soymilk sold in Federal Polytechnic, Oko, Anambra State (cfu/ml).

Samples	TVC	TCC	TMC
BAK	No growth	1.40×10^2	2.00×10^2
INS	1.40×10^2	0.90×10^2	2.00×10^2
PER	1.60×10^2	No growth	2.80×10^2
FIR	No growth	No growth	2.40×10^2

Key:BAK: Soymilk collected from Old Admin., INS: Soymilk collected from Inside School., PER: Soymilk collected from Perm Site & FIR: Soymilk collected from First Gate

Table 2
Prevalence of potential bacterial pathogens of their indicators isolated in soymilk samples (%)

Bacterial Pathogens	Soymilk Sample
Staphylococcus Spp.	18.33
Lactobacillus Spp.	28.33
Bacillus Spp.	16.66
Escherichia coli	20.00

Table 1 shows the result of microbial loads of soymilk samples sold in Federal Polytechnic Oko. The soymilk sample collected from perm site had the highest viable count (1.60×10^2 cfu/ml), followed by soymilk sample from inside school (1.40×10^2 cfu/ml). The samples collected from old admin and first gate had no growth in them. The total viable count obtained for every samples analyzed did not exceed the acceptable limit for both pasteurized and ultra-high temperature treated milk (3×10^4 cfu/ml) (Akeem et al, 2011). From the microbial analysis, the sample collected from old admin had the highest coliform count (1.4×10^2 cfu/ml) followed by soymilk collected from inside school (0.9×10^2 cfu/ml). The sample collected from perm site and first gate showed no growth in them. The total microbial count of the analyzed samples ranged from 2.0×10^2 to 2.8×10^2 cfu/ml. Samples from perm site had the highest growth while the one from old admin and first gate had the least microbial loads. The total viable count obtained for every sample analyzed did not exceed the acceptable limit for both pasteurized and ultra-high temperature treated milk (3×10^4 cfu/ml) (Akeem *et al*., 2011). Result higher than the values obtained in this study were reported by Liamngee *et al*., (2013). Contamination of these drinks may be due to source of was used for processing and improper handling during processing (Amusa *et al*., 2005; Nwachukwu *et al*., 2007). A total of five different bacteria were isolated from all the soymilk samples and the different isolates and their prevalence are shown in Table 2. The prevalence of staphylococcus aureus in soymilk samples was 18.33%. The detection of S. aureus poses a serious health hazard to the consumers as it is known to possess heat stable enterotoxin (Stewart, 1974).

Lactobacillus *spp.* is the predominant organism found with occurrence of 28.33% which will spoil the milk and will not produce soymilk yoghurt and not serve as probiotics with health benefits (Farinde *et al*., 2009) but contains other species associated with dental carriers and tooth decay (Anagu *et al*., 2015). Bacillus *spp.* was the least predominant in the samples with 16.66%. Anagu *et al*, (2015) reported similar result for zobo drinks purchased within Awka metropolis Anambra State, Nigeria. Bacillus contamination food poisoning which mainly manifested as diarrhea type illness, which can lead to liver failure or even death due to its pH and heat stable toxins in the contaminated drinks (Anagu *et al*., 2015). Pseudomonas *spp.* in the soymilk was 16.66% while E. coli was 20.00%. Food Association of America (FAO) (1997) suggests that *E. coli* should be totally absent from any soy food, otherwise such products are unsuitable for consumption. The presence of E. coli indicates fecal contamination of the source of water. It has been suggested by Adesokan *et al*., (2013) that proper hygiene and use of portable water in the production of locally made drinks will improve the microbial quality of such food product. But this is hardly achievable in Nigeria as people resident in such rural areas and some resident in urban areas still make use of well water or the popular "tanker" water supply as source of water for cooking and cleaning.

CONCLUSION

This study have shown that the microbial quality of soymilk drinks sold in Federal Polytechnic, Oko is satisfactory but its safety cannot be assured due to the isolation of highly pathogenic organisms like S. aureus, Bacillus spp., E. coli, Pseudomonas spp. These organisms present potential health hazards to consumers, therefore proper good hygienic conditions and portable water should be the practice to prevent bacteria which can present public health crisis.

REFERENCE

1. Adesokan, I. A., Abiola, O. P., Adigun, M. O. and Anifowose, O. A. 2013. Analysis of quality attributes of hibiscus sabdariffa (zobo) drink blended with aqueous extract of ginger and garlic. *Afri. J. Food Sci*, 7:174-177
2. Akeem, A. A., Uduma, E. O., Christian C. O. and Emmanuel, C. I. 2011. Evaluation of microbiology quality of some soybean milk products consumed in Nigeria. *Prime Research on Medicine* (PROM) VN. 1(2):025-030.
3. Amusa, N. A. Ashaye, O. A., Aiyebayo, A. A. and Oladapo, M. O. 2005. Microbiological and nutritional quality of hawked zobo drinks widely consumed in Nigeria. *J. Food Agric. Environ*., 3: 47-50
4. Anagu, L., Okolocha, E., Ikegbunam, M., Ugwu, M., Oli, A. and Esimone C. 2015. Potential spread of pathogens by consumption of locally produced zobo and soymilk drinks in Awka, metropolis, Nigeria. *British Microbiol. Res. J,* 5(5): 424-431.
5. Baker, H., Thomson, A. D., Frank, O., and Leevy, G. M. 1974. Urease test method. *Am. J. Clin. Nutr,*27; 676
6. Barker, H. 1976. Citrate utilization of bacterial contamination. *J. Nutr. Sci*, 22(Suppl): 63-68.
7. Carpenter, P. C. 1977. Microbiology 4th edition W. B. Saunders Company, Philadalphia. *p.* 401-402.
8. Deshpande, S. P., Bargale, P. and Jha, K. 2008. Suitability of soymilk for development of shrink hand. *J. Food Sci. Technol*., 45: 284-286.
9. FAO (Food Agricultural Organization).1997. Annual report on food quality control. 1: 11-13.
10. Farinde, E. O., Adesetan, T. O., Obatolu, V. A. and Oladapo, M. O. 2009. Chemical and microbial properties of yoghurt processed from cow's milk and soymilk. *J. Food Processing Preserv,* 33(2): 245-254.
11. Iwe, M. O. 2003. The science and technology of soybeans. Rojoint Communication services Ltd Enugu, Nigeria. *p.* 20-25
12. Krik, C. J. C., Peel, R. N., Kershew, R. J. 1975. Basic medical laboratory technology. Pitman medical publishing Ltd, London. *p.* 121-122
13. Liamngee, K., Terna, T. P., Bem, A. A., Orpin, J. B., Mzungu, I., Obaje, M. and Anum, T. 2013. Microbial analysis of soybean milk sold in Makurdi metropolis. *J. Environ. Sci. Toxicol. Food Technol,* 3(3):97-104.
14. Lozano, P. R., Drake, M., Benitez, D. and Cadwallader, K. R. 2007. Instrumental and sensory characterization of heat-induced odorants in aseptically packed soymilk. *J. Agri. Food Chem.,* 55(8): 3018-3026.
15. Odds, F. C., Rinaldi, M. G., Sheehan, D. J. and Warnock, D. W. 2001. Antifungal susceptibility testing: Practical aspects and current challenges. *Clin. Microbiol*, 14: 643-658.
16. Ofoefule, S. I. 2002. A textbook of pharmaceutical technology and industrial pharmacy. Samakun, Nigeria Enterprise. *p.* 120-125.
17. Onuorah, C. E., Adejare, A. O. and Uhiara, N. S. 2007. Comparative physiochemical evaluation of soymilk and soya cake produced by three different methods. *Nig. Food J,*25: 2-5
18. Patil, T. and Jha, K. 2008. Optimization and emulsifier requirement for production of soy ice cream by response surface analysis. *J. Food Sci. Technol,* 45: 267-269.

19. Smith, K. and Circle, J. 1972. Soybean chemistry and technology. Vol AVI Publishers, West-Port Connectivity. *p.* 4740.

20. Speck, M. L. 1976. Compendium of method for microbiological examination of foods. American Public Health Examination Association Inc. *p.* 563-567.

21. Stewart, F. S. 1974. Bigger's Bacteriology and Immunology for students of medicine. (9^{th} ed.) ELBS and Bailliere, Tindal and Casell.

22. Twizeyimana, M. O., Ojiambo, P. S., Sonder, K., Ikotun, T., Hartman, G. L., and Bandyopadhay, R. 2009. Pathogenic variation of phakopsora pachyrhizi infecting soybean in Nigeria. Doi: 10.1094/PHYTO-99-4-0353. 99: 353-361.

23. Vincent, C. O. 2005. Principles of the pharmaceutical applications of anti-microbial agents. EL DEMAK (publishers) Enugu State, Nigeria. *p.* 60-65

24. Wang, H., Mustakas, G., Wolt, W., Wang, C., Hesseltine, W., and Bagley, E. 1978. Soybean as human food and simply processed. Unpublished manuscript. U. S. Department of Agriculture, Utilization Research Project No 5.