ECRONICON OPEN ACCESS

NUTRITION
Research Article

Microbial Profile of Soymilk Whey/Pineapple Juice Blend

Ojukwu M*, Osuji C.M, Ogueke C.C and Ahaotu N.N

Department of Food Science and Technology, Federal University of Technology, Owerri, Imo State , Nigeria

***Corresponding Author:** Ojukwu M, Department of Food Science and Technology, Federal University of Technology, Owerri, Imo state, Nigeria.

Received: April 05, 2015; **Published:** April 24, 2015

Abstract

The microbial quality of pasteurized pineapple juice/soymilk whey beverage formulation was evaluated. Soymilk whey was prepared by acidic isoelectric point coagulation of soymilk and centrifugation to separate the clear whey from the curd. A beverage comprising a blend of concentrated soymilk whey and pineapple juice was formulated by dissolving sucrose syrup (15% total solids) into concentrated whey and stirring to achieve complete dissolution. The sweetened whey (13% total solids) was blended into a single strength pineapple juice (13% total solids) at a ratio of 25% parts of sweetened whey into 75% part of pineapple juice. The microbial quality of the pasteurized (65°C for 30 min) ambient stored (25°C - 27°C) were investigated. Microbial contaminants of the beverage blend were below 10^6CFU/ml thus within acceptable limit for human consumption The following microorganisms were present in the stored beverage ; *Staphylococcus sp; Bacillus spp Micrococcus luteus; Enterococcus sp Serratia sp; Penicillium notatum; Saccharomyces cerevisae; Rhizopus stolonifer; Mucor sp; P. notatum*. Total microbial count declined after three weeks of storage from 0.66 X 10^4 to 0.58 X 10^4CFU/ml and 1.03 X 10^5 to 0.33 X 10^5CFU/ml for bacterial and fungal counts, respectively. The use of soymilk whey/pineapple juice beverage as a healthy means of promoting isoflavone consumption is feasible

Keywords: *Soybean; Soymilk Whey; Pineapple juice; Microbial Count*

Introduction

Soymilk is an aqueous extract of soybeans. It is an off white emulsion/suspension containing the water soluble proteins and carbohydrates, and most of the oil in the soybean. It is prepared by cooking dehulled, ground soybeans in water and filtering of the solid matter (okara). Rich in B vitamins, protein and iron. Soymilk whey is the clear and watery upper phase formed when soymilk coagulates [1]. Osuji and Anyaiwe [2] reported the benefits of utilizing soymilk whey as an ingredient for other foods. Soymilk whey is an excellent source of proteins, vitamins and minerals [3]. Osuji and Anyaiwe [2] also reported the possibility of commercializing the production of soymilk whey and Pineapple juice blend. The Pineapple/soymilk whey beverage could be a viable means of increasing the consumption of isoflavones. The two fluids are similar in colour and appearance and are expected to blend very well [4].

Pineapple has long been one of the most popular of the non-citrus tropical and subtropical fruits, largely because of its attractive flavour and refreshing sugar-acid balance [5]. It is very juicy perhaps more than oranges and juice extraction from it is not encumbered as much as that of oranges.

Soymilk being an excellent medium for microbial proliferation will inadvertently alter the pH of the beverage blend and thus the affect its keeping quality. Therefore the purpose of this research is to determine the microbial shelf stability of the soymilk whey/pineapple juice blend.

Materials and Methods

Material Source

Three varieties of soybean (Glycine max); Samsoy1, Samsoy2, and TGX were obtained from Department of Crop Science, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Soymilk Production

Different varieties of Soybean were separately used to prepare soymilk (figure 1) in batches by following an adaption of the process described by Nsofor and Osuji [6]. Each batch was processed by washing and then blanching 150 grams of cleaned soybean in a cooking pot with 2.0 liters of tap water for 15 minutes. The blanched soybeans were hand dehulled and the hulls were removed by flotation. The blanched dehulled coytledons were then used for soymilk extraction by placing them, in a Somimax™ soymilk-making machine (Model No.Ns-360D New Brook Corporation, USA) using 1.2 liters of tap water.

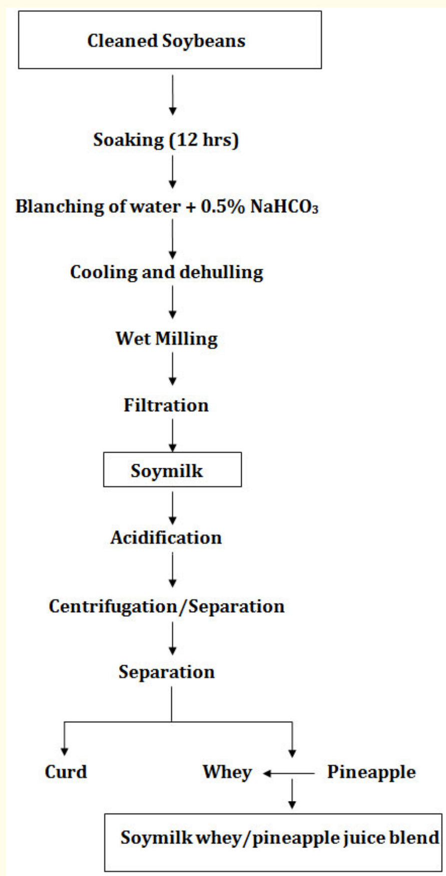

Figure 1: *Process flowchart for the production of soymilk pineapple juice blend.*

Whey Production

All soymilk samples from the three soybean varieties were separately used for the production of whey. The pH was adjusted to pH 4.6 by the step wise addition of 1molar citric acid before it was centrifuged (1000 rpm for 10 minutes) and the whey was collected in glass bottles corked, sterilized (121°C for 15 min) and used for further analysis.

Concentration of the Whey

The whey produced from soybeans of different varieties was concentrated by boiling in a heated sauce pan over an electric stove with continuous stirring to evaporate water until the desired total solids content (concentration) was attained. The concentrated whey was packaged in glass bottles and sterilized. It was later used in the formulation of the fruit juice/soymilk whey blend.

Determination of Total Solids

The total solid content of all soymilk whey samples, sweetened whey samples and pineapple juice/soymilk whey beverage samples were evaluated by pipetting 3 ml from each sample into a previously weighed porcelain dish, the porcelain dish and its content were placed in a moisture extraction oven maintained at 90°C. After five hours, the samples were weighed. This process was repeated until the dish attained a constant weight. The percentage total solid was calculated as the percentage residual dry matter after evaporation of moisture.

Formulation of Pineapple Juices/Soy Whey Blend

A beverage comprising a blend of concentrated soymilk whey and pineapple juice (extracted using a juice extractor) was formulated by dissolving sucrose into concentrated whey and stirring to achieve complete dissolution. The quantity added was enough to achieve 13% total solids in the sweetened whey. The sweetened whey was blended into single strength pineapple juice (13% total solids) at a ratio of 25% parts of sweetened whey into 75% part of pineapple juice. The blend was packaged in a glass bottle, sterilized (121°C for 15 min) and stored at ambient temperature for three weeks, before it was used for microbial determination.

Microbial Analysis of Pineapple Juice/Soymilk Whey Blend

Reagents

Crystal violet (gentian violet) 0.5% in water (M & B England) Basic safranin (Merck, Germany), Alcohol (BDH, England), Iodine reagent (BDH, England) Immersion oil (CVI, Nigeria Ltd), Malachite green (M & B, England), Nutrient Agar powder (BDH, England), Sabouraud dextrose Agar (BDH, England), Chloramphenicol succinate (Laborate Pharmaceuticals India), Glucose peptone water (Oxoid Ltd, UK) Lactose powder (Oxoid Ltd, U.K), Maltose powder (Oxoid Ltd, U.K), Glucose powder (Oxoid Ltd, UK) Sucrose powder (Oxoid Ltd, UK) Galactose powder (Oxoid Ltd, U.K) Phenol red (BDH, England) Starch (BDH, Nigeria Ltd) Nutrient Agar (Becton and Dickson Co, USA) Starch (Oxoid Ltd, U.K) Iodine reagent (BDH, England) Plasma was supplied by Anthony Van Leeuwenhoek Research and Development Centre ,Nekede, Owerri West, Imo state.

Culture Media

The culture media used include Nutrient agar (Becton and Dickson Co, USA), Sabouraud dextrose Agar (BDH, England), McConkey agar, nutrient broth No.2, mannitol salt agar, deoxycholate citrate agar, selenite F broth (Oxoid). All media were prepared according to manufacturer's instructions.

Isolation and Cultural Characterization

Preparation and Inoculation

One millilitre (1 ml) of each sample was dispersed into 9 ml of sterile distilled water in a bijou bottle. The content was shaken vigorously and serially diluted (10-folds dilution). Aliquot portion (0.1 ml) of different dilutions was transferred onto freshly prepared surface dried media (Nutrient Agar, MacConkey Agar, Eosine Methylene Blue Agar, Salmonelle-Shigella Agar, Mannitol Salt Agar and Potato Dextrose Agar) [7].

Inoculum was spread evenly with a sterile spreader and incubated. Media for bacteria cultivation was incubated at 37°C for 24-48h. Potato dextrose agar plate was incubated at ambient temperature (280± 02°C) for fungal growth [8].

Microbial Count

Bacterial count was done with a colony counter. Hand lens was used to determine fungal count. Total microbial count was expressed in colony forming unit per millilitre (CFU/ml).

Characterization and Identification of Microbial

Isolates

This was done on the basis of colonial, microscopic and few biochemical methods [7,8]. Colonial morphology, pigmentation and spore and mycellial arrangement was used in the characterization and identification of fungi [9,10]. The identities of the isolates were confirmed with reference to standard manuals [11].

Characterization of Microbial Isolates

Microscopic Characterization

Gram Staining Test

The Gram staining technique was used for the bacterial isolates as described by Cheesbrough [7]. A smear of the isolate was made on grease free glass slide with a drop of water and allowed to dry. The smear was fixed by mild heating, flooded with crystal violet and allowed to stand for 30 seconds. The crystal violet was rinsed off with water; Lugol's iodine was added and allowed to stand for 30 seconds. This was washed off with water and acid alcohol, till discoloration. It was counter stained with Safranin for 10 seconds and rinsed with water. The wet slide was allowed to air dry. A drop of oil immersion was added on the slide and viewed using X100 objective lens of the microscope.

Spore Staining Test

The spore stain was used to confirm the presence of spores when indicated in the Gram stain. Isolates were heat fixed on a slide and flooded with 5% malachite green. It was steamed for 3 minutes (without allowing it to boil), dried and cooled. It was then rinsed off and stained with Safranin for 30 seconds. This was rinsed, dried with filter paper and viewed under the microscope using oil immersion tens. The positive spores showed green while the vegetative cells were stained pink.

Motility Test

This test was used to determine the motility of bacteria isolated. The test was carried out on a semi-solid agar medium in which motile bacteria swarm and gave a diffuse spreading growth. The medium was dispensed into test tubes, sterilized and allow setting in an upright position. It was then inoculated using an inoculation needle by stabbing it into the medium in the test tube. This was incubated at 37°C for 24 hours. Diffuse growth from the straight line of inoculation was recorded as positive result [7].

Biochemical Characterization of Bacteria Isolates

Microorganisms that were not identified by the colonial and microscopic characteristics were further subjected to few biochemical tests described by Cheesbrough and Beishir [7,8].

Catalase Test

The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria. Catalase has one of the highest turnover numbers of all enzymes such that one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen in a second. Catalase activity can be detected by adding the substrate H_2O_2 to an appropriately incubated (18-24 hours) tryptic soy agar slant culture. Organisms which produce the enzyme breakdown the hydrogen and the resulting O_2 production produces bubbles in the reagent drop indicating a positive test. Organisms lacking the cytochrome system also lack the catalase enzyme and are unable to breakdown peroxide into O_2 and water and are catalase negative.

Coagulase Test

Coagulase is enzymes that clot blood plasma by a mechanism that is similar to normal clotting. The coagulase test identifies whether an organism produces this exoenzyme. This enzyme clots the plasma component of blood. The only significant disease causing bacteria of humans that produce coagulase are *Staphylococcus aureus*. Thus this enzyme is a good indicator of *S. aureus*. In the test, the sample is added to rabbit plasma and held at 37°C for four hours for Formation of clot within four hours is indicated as positive result and indicative of a virulent *Staphylococcus aureus* strain. The absence of coagulation after 24 hours of incubation is a negative result indicative of a virulent strain.

Oxidase Test

Oxidase test is an important differential procedure that should b performed on all gram negative bacteria for their rapid identification. The test depends on the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and ∞-naphthol. This method uses N, N-dimethyl-p-phenylenediamine oxalate in which all Staphylococci are oxidase negative. In the presence of the enzyme cytochrome oxidase (gram negative bacteria) the N, N-dimethhyl-p-phenylenediamine oxalate and ∞-naphthol react to indophenol blue. *Pseudomonas aeruginosa* is an oxidase positive organism.

Sugar Fermentation/Oxidation

This test is used to differentiate between bacteria groups that oxidize carbohydrate such as members of *Enterobacteriaceae*. One milliliter (1 ml) of 10% glucose, maltose, lactose, fructose, mannitol, and sucrose were separately under aseptic conditions transferred into duplicate tubes containing 9 ml of sterile Hugh and Leifson's medium to obtain a final concentration of 1% of each of sugar. The tubes were stab-inoculated in duplicates while two uninoculated tubes serve as control. Vaseline was used to cover one set of the duplicate tubes, one control to discourage oxidative utilization of sugar. All tubes were incubated at 37°C for 48h. After the incubation, they were observed for acid production in the culture. Yellow coloration indicates acid production in the open tubes only suggesting oxidative utilization of the sugar while acid production in the sealed tubes suggests a fermentative reaction.

Hydrogen Sulphide Production (H_2S) Test

The test isolates were aseptically inoculated into a tube containing triple sugar iron agar started by stabbing the agar to the bottom and streaking the surface of the slant. The inoculated tube was incubated at 37°C for 72h and was examined daily. Black precipitation and yellow coloration was checked for. Black precipitate indicates H_2S production and yellow coloration for sucrose, lactose and glucose fermentation.

Urease Test

Urease Agar slant in McCartney bottle was inoculated with the bacteria isolate at 30°C for 4 hours and then overnight. A pink colour in the medium indicated a positive result.

Imvic Test

This test consists of four different test; they are Indole production, Methyl-Red test, Voges Proskaeur test and Citrate utilization test. This test is specifically designed to determine the physiological properties of microorganism. They are especially useful in the differentiation of Gram-negative intestinal bacilli, particularly *Escherichia coli* and the *Enterobacter-Klebsiella* group.

Indole Test

This test demonstrates the ability of certain bacteria to decompose the amino acid-Tryptophan to Indole. The bacteria isolates were inoculated into the medium and incubated at 37°C for 48 hours. At the end of incubation period, 3 drops of kovac's reagents (see appendix) was added and then shaken. A red colour ring at the interface of the medium denotes a positive result.

Methyl red and Voges-Proskaeur test must be considered together since they are physiologically related. Opposite test is usually obtained from the MR and VP test, that is, MR+, VP-, or MR-, VP+.

Methyl-Red Test

Methyl red test was preformed to demonstrate the capacity of different organisms to produce acid from the fermentation of sugar (dextrose). Methyl-red positive organisms produce a red coloration when five drops of methyl-red indicator is added into 48h old MR-VP broth culture.

Voges-Proskaeur Test

The Voges-Proskaeur test demonstrates the ability of organisms to produce acetoin from glucose metabolism. Some organisms metabolize glucose to produce pyruvic acid which is further broken down to yield Butane-diol and acetyl-methyl carbinol as an intermediate product. Into one milliliter of the culture add one milliliter of six percent alcoholic solution of alpha-naphtol and one milliliter of 16% KOH and stand for 15-20 minutes. Development of red to pink colour is a positive test.

Citrate Utilization Test

This is one of the several techniques used to assist in the identification of *Enterobacteria*. Principle of the test is based on the ability of an organism to use citrate as its only source of carbon. The test was carried out using Simmon's citrate agar.

The slopes of the media were prepared in bijou bottles as recommended by the manufacturers. A sterile straight wire was used to the slope with a saline suspension of the test organisms before stabbing the butt. The bottles are incubated at 35°C for 48h. Bright blue colours in the medium means positive test while no change in colour of medium indicates negative citrate test [7].

Procedure for Staining Fungi

Place a drop of mounting fluid (lactophenol cotton blue) on a clean grease free slide. Carefully lie out a bit of the specimen on the fluid and place a cover slip. The slide was warmed to remove air bubbles and to enhance the action of the staining fluid. The preparation was allowed to stand for 10 minutes to remove air bubbles. Excess mounting fluid was blot dried to render the organisms easier to see. The slide was examined with a high dry objective (40x).

Data Analysis

The data obtained were subjected to Analysis of Variance (ANOVA) [12]. The difference between the means was determined using Fishers Least significant difference test. Significance was accepted at 5% probability level.

Results and Discussion

Table 1 shows the bacterial and fungal counts obtained during storage of the juice blend. Their numbers ranged from 0.58×10^4 to 3.2×10^4 CFU/ml for bacteria and 0.33×10^5 to 3.33×10^5 CFU/ml for fungi. Microbial contaminants of the beverage blend were below 106 CFU/ml thus within acceptable limit for human consumption [13] There was an initial increase in bacterial count which dropped subsequently from week 2. The sudden fall of the count could be attributed to the production of some metabolites which inhibited the growth of microorganisms. The high fungal counts suggest the presence of fermentative organisms of fungal origin [14]. This is confirmed by the presence of yeast, Saccharomyces sp. which is also isolated in some packaged fruit blend samples (Table 1).

Total Heterotrophic Bacterial and Fungal count (CFU/ml)		
Weeks of Storage	Bacterial Count ($\times 10^4$)	Fungal Count ($\times 10^5$)
0	0.66 ± 0.36^b	1.03 ± 0.75^a
1	3.2 ± 0.36^a	3.33 ± 3.21^a
2	2.5 ± 0.62^a	0.33 ± 0.06^b
3	0.58 ± 0.4^b	0.33 ± 0.35^b
LSD	0.95	2.44

Table 1: *Mean values of the Total Heterotrophic Bacterial and Fungal count (CFU/ml) during the storage of Pineapple Juice Soymilk whey blend.*
Values are the means of triplicate determinations ± = Standard deviations.
A b c: Means within the columns followed by the same letter(s) are not significantly different from each other.

Identification of isolates (Table 1) showed the presence of *Bacillus* sp, *Micrococcus* sp., *Penicillum* sp. Of particular importance are the *Bacillus* sp. *Bacillus* sp. are known causative agents of food poisoning and intoxication [15]. The presence of some of these organisms are not surprising as most of them are known to thrive in medium rich in fermentable substrates such as sugars which often lead to the production of acids after fermentation. *Bacillus* species are spore formers whose spores could survive high temperatures of processing [16]. The thermophilic nature of the spores of these microbes ensures survival at pasteurization temperatures. Apart from being resistant to heat, substances contained in the blends such as proteins, sugar etc make the microorganisms resistant to heat as the medium is rich for their proliferation. Sugar reduces the water activity of the system and in turn results in increase in heat resistance [17].

The presence of *Bacillus* sp. in almost all the samples may be attributed to its ability to form spores which are heat resistance. *Bacillus* sp. are ubiquitous in nature, thus they may have contaminated the product from the processing equipment or may have originated from one of the ingredients of the fruit blend e.g. sugar. *Bacillus* sp. was also reported in bottled drinks and juice in a study conducted by Abdalla *et al*.

Several food samples have been reported to contain some of these organisms [10,18-20]. Blackey and Priest [18,19] reported that *Bacillus* species is common in soils and vegetation and has been isolated in several countries from wide variety of routine samples of food. Its presence therefore could be due to the contamination from many sources, which may include soil, air and water [19]. The organism might have come in during processing; an observation that goes to support Pederson [21] according to whom spores of molds and Bacillus abound in air and water.

Weeks of Storage	Bacterial Isolates	Fungal Isolates
Week 0	*Staphylococcus* sp; *Bacillus* spp *Micrococcus luteus; Enterococcus* sp	*Saccharomyces cerevisae; Mucor* sp
Week 1	*M. luteus; Staphylococcus* sp, *Bacillus* sp; *Bacillus* spp; *M. luteus; M. roseus Enterococcus* sp	*Penicillium notatum; Saccharomyces cerevisae; Rhizopus stolonifer; Rh. stolonifer; Mucor* sp *P. notatum; Aspergillus* sp;
Week 2	*Bacillus* spp; *M. luteus; Serratia* sp; *Staphylococcus* sp	*Saccharomyces cerevisae; Mucor* sp *P. notatum;*
Week 3	*Bacillus sp; Enterococcus sp; Staphylococcus* sp	*Saccharomyces cerevisae; P. caseicolum; Mucor* sp

Table 2: *Dominant Bacteria and Fungi isolated from samples.*

Micrococcus sp were among the organisms isolated from the fruit blends. Odunfa and Adeyeye [22] cited in Essien., *et al*. [16] reported that *S. lactis* and *Micrococcus acidiophilis* are known to be involved in fermentation of agricultural produce. The surrounding air, packaging materials and the personnel concerned with the packaging processes could all serve as sources of these contaminants [19]. This agrees with Kawo and Abdulmumin, Aboloma and, Akinyosoye and Nwosisi [19,20,23] who isolated these organisms and reported that they could be contaminants from air or materials used in processing. *Penicillium* and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions [24,25]. Although they can be useful to humans in producing antibiotics and blue cheese production, many species are important spoilage organisms, and some produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem) [24,25].

These microorganisms could have survived the time - temperature regime employed in the pasteurization of the fruit blend, as they could be thermophiles such as Bacillus sp. or thermodurics such as *Micrococcus* sp. Spores of *Pennicilium* sp., *Aspergillius* sp., *Mucor* and *Rhizopus* sp. can also survive pasteurization temperatures [15]. The generally observed high microbial counts in this study could be attributed to the presence of sugars which causes an increase in the heat resistance of microorganisms suspended therein. Corry [17] found that sucrose increased the heat resistance of *S. Senftenberg* more than any of four other carbohydrates tested such as glucose, sorbitol, fructose and glycerol. Also, proteins are known to offer some protection against heat [26]. This may have contributed to the ability of the organisms in the fruit blend to survive the pasteurization temperatures. Also the larger the number of organisms, the higher is the degree of heat resistance. It has been suggested that the mechanism of heat protection by large microbial populations is due to the production of protective substances excreted by the cells, and some investigators claim to have demonstrated the existence of such substances [26]. However, no coliforms were isolated in all fruit blend samples. None of the *Salmonella Shigella* Agar plates showed any black colonies of Salmonella or pale pink colonies of Shigella.

Conclusion

Soymilk whey/pineapple juice blended beverage has good keeping qualities. The beverage is very nutritious and does not contain pathogenic microbes. The development of a soymilk whey/pineapple juice beverage as a means of promoting soybean isoflavone consumption is feasible.

Recommendation

Soymilk whey can be used for making soy based beverages with potential health benefits. I recommend further work on regulation of the pH of soymilk whey beverage blend to enhance shelf stability.

Bibliography

1. Osuji CM., *et al*. "Stability, yield, and chemical properties of soymilk whey from sprouted soybeans (*Glycine max*) of different varieties". *Nigerian Food Journal* 29.2 (2011): 78-85.
2. Osuji CM and Anyaiwe UC. "Stability, yield, and chemical properties of soymilk whey from sprouted soybeans (*Glycine max*) of different varieties". *Nigerian Food Journal* 28.2 (2010): 154-161.
3. Iwe MO. "The Science and Technology of Soybean". Rejoint Communication Services Limited, 65 Adelabu St, Uwani Enugu, Nigeria.
4. Iwe MO and Onuh JO. "Functional properties of Soybean and sweet potato flour mixtures *Lebensm-Wiss. U.-Technology*". *Journal of Nutrition* 126:161-167.
5. Bartolome AP., *et al*. "Pineapple fruit: morphological characteristics, chemical composition and sensory analysis of red spanish and smooth cayenne cultivars". *Food Chemistry* 53.1 (1995): 75-79.
6. Nsofor LM and CM Osuji. "Stability, rheology and chemical properties of soymilk concentrates developed from sprouted soybeans". *Journal of Food Science and Technology* (1997) 34: 33-40.
7. Cheesbrough M. "District Laboratory Practice in Tropical Countries". (2000) Part. Cambridge University Press, UK. pp 35-38, 62-69.
8. Beishir I. "Microbiology in practice. a self- instruction laboratory course". (1987) 4th ed. Harper and Row Publishers, New York. pp 96-111, 120-130, 238-272.
9. Harrigan WF and McCance ME. "Laboratory methods in food and dairy microbiology". (1990) 8th ed. Academic Press Inc. London. pp 7-23, 286-303.
10. Frazier WF and Westhoff DC. "Food Microbiology". (1987) 3rd ed. Tata McGraw-Hill Publishing Co. Limited, New Delhi, India. pp 17-64, 454-456.
11. Barnett HI and Hunter BB. "Illustrated genera of imperfect fungi". (1987) 4th ed. Macmillian Publishing Company, New York, USA. pp 106, 130.
12. Steel RDG and Torrie JH. "Principles and procedure for statistics: A Biomedical approach". (1980) 2nd ed. McGraw Hill Co., Newyork, p.623.
13. ICMSF (International Commission on Microbiology Specification for Food). "Sampling for microbiological analysis". Principles and Specific Application, University of Toronto Press, Toronto pp 1-18.
14. Zocklein B. "Production of wine and analysis". (1990) Van Nostrand publishing company limited, USA.

15. Adams MR and Moss MO. "Food Microbiology". 3rd ed. Chapter 3 (1995) 20-62. RSC Publishing Company.
16. Essien EC and Monago EA. "Evaluation of the Nutritional and Microbiological Quality of Kunun (A cereal based non-alcoholic beverage) in rivers state, Nigeria". *The Internet Journal of Nutrition and Wellness* 10.2 (2009).
17. Corry JEL. "The effect of sugars and polyols on the heat resistance of salmonellae". *Journal of Applied Bacteriology* 37.1 (1974): 31-43.
18. Blakey LJ and Priest FG. "The occurrence of Bacillus cereus in some dried foods including pulses and cereals". *The Journal of Applied Bacteriology* 4.8 (1980): 297-302.
19. Kawo AH and Abdulmumin FN. "Microbiological quality of re-packaged sweets sold in metropolitan Kano, Nigeria". *Bayero Journal of Pure and Applied Sciences* 2.1 (2009): 154-159.
20. Aboloma RI. "Microbiological analysis of bread samples from bakery to sale points in Ado-Ekiti, Ekiti State, Nigeria". *Biological and Environmental Science Journal for the Tropics* 5.3 (2008): 77-81.
21. Agboola S.A. "An Agricultural Atlas in Nigeria". (1979) Oxford Publication. 34-78.
22. Odunfa SA and Adeyeye S. "Microbiological changes during the traditional production of Ogi- aaba, a West African fermented Sorghum grel". *Journal of Cereal Science* 3 (1985): 173-180.
23. Akinyosoye FA and Nwosisi NL. "Microorganisms associated with the production of 'kunun zaki', a Nigerian cured drink". *Bioscience Research Communications* 6.2 (1994): 133-137.
24. Doyle EM. "FRI BRIEFINGS: Microbial food spoilage: losses and control strategies. A Brief Review of the Literature". (2007) Food Research Institute, University of Wisconsin Madison.
25. Adebayo-Tayo BC., *et al*. "Microorganisms associated with spoilage of stored vegetables in Uyo Metropolis, Akwa Ibom State, Nigeria". *Nature and Science* 10.3 (2012): 23-32.
26. James MJ., *et al*. "Intrinsic and extrinsic parameters of foods that affect microbial growth". *Modern Food Microbiology* (2005) 7th ed. 3: 34-56.

Volume 1 Issue 3 April 2015