BACTERIOLOGICAL PROFILE OF SOYA MILK DRINK SOLD IN MAJOR GOVERNMENT SECONDARY SCHOOLS IN ENUGU STATE

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Abstract:*This researchinvestigatedthe bacteriological quality of locally processed soymilk sold in major government secondary schools in Enugu metropolis, with the objective toIsolate and determine the prevalence and distribution of bacteria in soymilk drinksprior tothe susceptibility of bacteria isolates to 5 easily accessible antibiotics. In an ice pack soya milkSamples were collected from women and children who hawks the product in each secondary school premises and environs . Data on soymilk production andprocessingwas collected using questionnaire method. Pour plate method and relevant biochemical tests were used for isolation, identification and characterisation of bacteria. All samples were contaminated with members of the enterobacteriaceae,including E. coli, Klebsiella spp, Salmonella typhi,streptococcus faecalis, and Staphylococcus aureus. Soymilk sold in thestudyareaswhich was packaged in polyethylene bags and used disposable plastic bottleswere highly contaminated with faecal microbes indicating poor hygiene of handlers andpoorsanitary quality ofprocessing water., it was recorded that women of the age 25-30 with secondary school education are the highest producers and sellers of the soyamilk drinks in the 7 major government schools in Enugu metropolis.It was clearly recorded that the major source of water for these producers are well and rain water which are not considered to be cleanfor consumption.The result revealed that soya milk drinks sold in these schools are potential vehicles for transmitting food bornediseases, thus the need to develop good manufacturing,post production preservation and packaging techniques.*

I. Introduction

Soyabean (*Glycine max*) is a high-protein legume grown as food for both humans and livestock. Although indigenous to Eastern Asia [1], it is known by different names in different parts of the world; soyabeans (Nigeria), Chinese pea (China), Churiabean (Manchuria) [2].

The use of soyabeans is traced back to the orient where it was consumed in form of fermented foods. Today, its uses range from the preparation of tofu, tempeh and natto, to the fortification and enrichment of foods such as in soy fortified wheat bread and coy soy blends, soy sauces, soy yoghurt and soy cream cheese [3; 4]. Soyabeans, which has been cultivated and considered as a miracle bean by many people, is the main source of protein for all of East Asia, particularly to vegetarians. In Nigeria, although the cultivation of soyabeans has been successfully established, a greater percentage of the whole produce until now had been cultivated and exported as cash crop. Presently, soyabeans is incorporated into so many food formulation of both children and adults to enhance nutritional value of foods [5], in preparations such as ''dawadawa'', allele, moi-moi, akara, soy-ogi and most recently as soymilk [1]. Other commercial uses in animal feed production and the vegetable oil industry cannot be overemphasized [6].

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 [5]. These have been implicated in the occurrence and prevalence rate of diseases such as typhoid fever and dysentery among soymilk consumers [7; 8].

The sale of soymilk is quite popular in Makurdi, but there is no report on its microbiological quality, which should have addressed the safety of its consumers in Makurdi. This study was therefore aimed at evaluating the microbial load and nutritive value of soymilk as a means of addressing the safety concerns of its consumers in the study area.

II. Materials and Methods

2.1 Survey of Soymilk Production and Consumption

The study was carried out in Wurukum, Wadata, North Bank and High Level areas of Makurdi, Benue State. A questionnaire was designed to collect data on soymilk production and consumption. A total of 280 questionnaires were given out to men, women, students, house wives, civil servants and children in the survey

areas. Data collected included Gender of respondents, Age of consumers, Educational status of producers, Occupation of respondents and Consumption rate.

2.2 Collection of Soymilk Samples

The soymilk samples which were already packaged in polyethylene sachets or plastic bottles were collected from women and children that hawk the product round Makurdi and taken to the Microbiology laboratory of the Department of Biological Sciences, Benue State University, Makurdi, and preserved at 4°C for further analysis.

2.3 Microbial Analysis of Soymilk Samples
2.3.1 Inoculation of Samples

The pour plate method of Downes and Ito [9] was used. Plate count agar (Biotech) was used for total aerobic counts. Eosin Methylene Blue Agar (Biotech) was the choice for isolation of coliforms. Whereas Nutrient and MacConkey agar were used for other bacteria. Incubation was in an aerobic incubator (Nino/30/CLAD) for 24 hours at 37°C. After 24 hours, the bacteria colonies that appeared on plates were counted using a digital colony counter (Digincont 02236). The average colony counts from duplicate plates were obtained and expressed as colony forming units (c.f.u.) per millilitre of sample.

2.3.2 Isolation

All pure cultures of bacteria isolated were maintained on appropriate agar slants. The isolates were kept in the refrigerator (4-8°C) prior to identification. Cultural characteristics of discrete colonies such as colour, shape elevation, pigmentation, opacity and nature of edges of the colonies were observed and noted.

2.3.3 Characterization of Isolates
i) Catalase test

A small amount of pure bacterial growth from appropriate agar slant was transferred to the surface of a clean glass slide with a sterile wire loop. A few drops of 3% hydrogen peroxide (H_2O_2) were immediately added, and observed for any evolvement of gas.

ii) Oxidase test

The method reported by Gaffa and Azoro [10] was used. A freshly prepared 1% solution of tetra-methyl-p-phenylene-diamine dihydrochloride was poured onto the culture of the test bacteria on the plate so as to cover the surface. This was then decanted and observed for either rapid or non development of purple colour.

iii) Lactose fermentation test

Lactose broth was dispensed in 9ml amounts into test tubes containing inverted Durham's tubes. The tubes were further autoclaved at 121°C for 10 minutes at 15lb pressure. Each tube was then inoculated with 1ml of soymilk sample and incubated at 37°C for 18-24 hours. Tubes were examined for any changes in colour and presence of gas.

iv) Slide agglutination test

The method reported by Gaffa and Azoro [10] was used. A drop of sterile water was placed on a clean slide and a small amount of the test organism emulsified in the fluid on the slide to form a smooth milky suspension. Using an inoculating wire loop, a small portion of undiluted plasma preserved at room temperature was placed on the bacterial suspension on the slide. The slide was observed for coarse agglutination within five to ten minutes. A slower reaction meant a negative result.

v) Motility test

This was carried out using the procedure outlined by Gaffa and Azoro [10]. A thin layer of Vaseline was applied around the edge of the well in a concave slide. A loopful of the bacterial suspension was put in the centre of a cover slip. The concave slide was inverted and pressed on top of the cover slip so that the cover slip completely covered the well. The preparation was quickly turned right side up again. The edges of the cover slip were checked to ensure that the vaseline sealed the edges properly. Bacterial suspension on the underside of the cover slip was observed under the microscope for bacterial cells in motion.

vi) Spore test

A thin film of the bacteria culture was made, dried and heat fixed with a minimal amount of heat. The film was then stained with Zeihl Neelsen's carbol fuchsin for 3-5 minutes. The preparation was heated until steam was observed. The stain was washed in water and treated with 0.5% of H_2SO_4 for few minutes. The preparation was decolorized by dipping the slide in a 2% solution of nitric acid in absolute ethyl alcohol and

immediately washed off in water. The counter stain, aqueous methylene blue was applied and left for about 3 minutes. The preparation was washed in water, dried and viewed under the microscope for possible presence of spores.

v) Tests for coliforms
v (a). Presumptive test
1ml of thoroughly mixed soymilk samples were inoculated into lactose broth tubes containing 9mls of the media. The tubes were incubated at 37°C and observed after 24hours for any evidence of gas production.
v (b). The confirmed test
Samples showing positive lactose fermentation were transferred into Eosin Methylene Blue Agar (EMBA) on plates using sterilized wire loop and incubated for 24 hours at 37°C. Growth was monitored for the either the presence (metallic sheen) or absence of coliforms (colourless colonies).
v (c). The completed test
Organisms showing dark and pinkish mucoid colonies were inoculated into lactose broth. The production of gas in lactose broth was considered as evidence of coliforms, while microscopic identification of Gram negative, non-sporing short bacilli showed a positive completed test.

2.4 Proximate analysis of soymilk
2.4.1 Determination of moisture content in soymilk
The method of Pearson [11] was used. The weight of an empty aluminium dish can (W_0) was determined before the sample was introduced into it. About 20mls of the soymilk sample was measured out and further weighed (W_1) in the aluminium dish can. The aluminium dish can was then dried in a hot air oven for 24 hours and cooled in a dessicator and weight (W_2) measureded. Percentage of moisture content was determined as follows:

Percentage of moisture content $= \frac{(W1-W0) - (W2-W0)}{(W1-W0)} \times 100$

Where: W0 = Weight of empty moisture can
        W1 = Weight of moisture can and sample
        W2 = Weight of dessicated sample
        (W1 – W0) – (W2 – W0) = Weight loss
        (W1 – W0) = Weight of sample

2.4.2 Determination of ash content in soymilk
The method reported by AOAC [12] was used. An empty clean crucible dish was weighed and the weight noted as W0. Soymilk sample in 2ml amount was measured and weighed in the empty clean crucible; the weight (W1) was noted. The crucible containing the sample was further placed in a muffle furnace and ashed at 500°C - 600°C for 3 hours. The crucible was removed from the muffle furnace and cooled in a dessicator, and weighed (W2) using a weighing balance. Percentage ash content was determined as follows:

Percentage of ash content $= \frac{(W2-W0)}{(W1-W0)} \times 100$

Where: W0 = Weight of empty crucible
        W1 = Weight of sample and crucible
        W2 = Weight of ash and crucible

2.4.3 Determination of crude fibre content in soymilk
The method reported by AOAC [12] was used. 20mls of soymilk sample were weighed in a 1 litre conical flask and the weight (W0) noted. 200mls of boiling 1.25% H_2SO_4 were added into the conical flask containing the sample and boiled gently for 30minutes. The content was filtered through a muslin cloth and the residue scraped into a clean conical flask using a spatula. 20mls of boiling 1.25% NaOH were added and allowed to boil gently for 30 minutes. Further filtration was done using using the buckner pressurized filter through a muslin cloth spread over its funnel. The resulting residue was thoroughly washed with distilled water and rinsed once with 10% HCl and twice with ethanol. Final rinsing with petroleum ether was done thrice and allowed to drain. The residue was scraped into a flat silica dish and dried overnight in the oven at 105°C. Afterwards, it was cooled in the dessicator and the sample weighed (W1). The sample was further ashed at 55°C for 90 minutes in a muffle furnace, cooled again in the dessicator and reweighed. Percentage fibre content was determined as follows:

Percentage fibre content $= \frac{(W1-W2)}{W0} \times 100$

Where: W0 = Weight of sample
        W1 = Weight of insoluble material

W2 = Weight of ash

2.4.4 Determination of lipid content in soymilk

The method reported by AOAC [12] was used. A 250ml round bottom flask was dried in an oven, allowed to cool in the dessicator and the weight measured. 200ml of petroleum ether were introduced into the dry 250ml round bottom flask. 10mls of the sample were measured using a measuring cylinder and weighed in a labelled porous thimble, which was covered with a clean white cotton wool. The covered thimble was placed into the soxhlet extractor (Model: EME 60250/CE) fitted into the round bottomed flask. The extraction apparatus was set up with the flask on the hot water bath of the soxhlet extracting unit. The content of the round bottom flask was then heated. As the ether evaporated, it condensed dropped into the thimble where it extracted the ether soluble constituent into the round-bottomed flask. The extraction lasted for 5-6 hours during which all the petroleum ether moved up to the extractor, leaving behind the fats in the round bottom flask. The porous thimble was removed with care and the petroleum ether collected from the top for re-use. The round-bottomed flask containing the lipids was then removed and oven dried for 1 hour at 105 - 110°C. After drying, it was further transferred into a dessicator (containing silica gel) where it was cooled and also weighed.

Percentage of lipid content $= \frac{(W3 - W2)}{W1 - W0} \times 100$

Where: W0 = Weight of empty porous thimble
W1 = Weight of thimble and sample
W2 = Weight of empty extraction flask
W3 = Weight of extraction flask and extract
W1 – W0 = Weight of sample
W2 – W3 = Weight of extract

2.4.5 Determination of protein content in soymilk

The soymilk sample was measured in 5ml amounts in a measuring cylinder and introduced into a 100ml micro kjeidahl nitrogen digestion flask. The following were further added to the flask: 5mls of distilled water, one tablet of mercury kjeidahl catalyst, half spatula full of K_2SO_4, 5mls of concentrated H_2SO_4.

The flask was placed on a 6 – unit digester (model: 65500) inside the fume cupboard and digested for 3 hours. After the digestion was completed, the content was cooled; leaving behind a colourless residue. Some quantity of distilled water was added to the colourless digest and the flask swirled until everything was dissolved. The content was emptied into a 100ml volumetric flask.

The Malkam distillation apparatus was set up and nitrogen distillation carried out as follows: 5ml of the extract were pipetted into the distillation pot, followed by 5ml of 55% NaOH and distillation carried out for 15 minutes. During the distillation, a 100m/conical flask containing 5ml boric acid was kept under the condenser to collect the distillate. Boric acid was observed for any change in colour.

2.4.5.1 Titration

The method reported by AOAC [12] was used. A 50ml burette was filled with 0.01N HCl and titrated against the ammonia distillate until colour change was observed. The second burette reading was also noted and titre value calculated. Percentage of protein content was determined as follows:

Percentage of protein content $= \frac{0.014 \times vol.\ of\ titre\ \times NHCl\ \times vol.\ of\ extract}{Weight\ of\ Sample} \times 100$

Where: 0.014 = nitrogen factor
Vol. of titre = Volume read from burette
NHCl = Normality of HCl acid used
Vol. of extract = Capacity of the volumetric flask
100 = A constant

2.4.6 Determination of carbohydrate content in soymilk

The method of Obsorne and Voogt [13] was used. Carbohydrate content was determined indirectly by subtracting the crude protein, crude fibre, crude lipid, ash and moisture content from 100% as follows:
Percentage carbohydrate content (% CC) = 100 − (% Protein + % Fibre + % Ash + % Moisture + % Lipid)

III. Results

3.1 Soymilk production and consumption in Makurdi

The demographic characteristics of respondents on soymilk production and consumption in Makurdi metropolis are presented in TABLE 1. 73.57% of respondents were males while 26.43% were females. The highest number of respondents (28.46%) was aged between 26 – 30 years, followed by age group 31-35 (25.00%). Persons above 40 years of age represented the least number of respondents (4.61%). All producers of

soymilk in the study were female and educated. 54.55% of producers had primary education, while 27.27% had secondary education. Only 7.14% of producers were graduates. 33.57% of producers (majority) were mainly house wives. 61.79% of respondents were daily consumers, 31.07% occasional consumers and 7.14% non-consumers.

TABLE 1: Demographic characteristics of respondents on soymilk production and consumption in Makurdi metropolis

Demographic characteristics	Number of Respondents	Percentage (%)
Gender		
Males	206	73.57
Females	74	26.43
Total	280	100
Age (years) of consumers		
Below 20	15	5.77
21 – 25	58	22.31
26 – 30	74	28.46
31 – 35	65	25.00
36 – 40	36	13.85
Above 40	12	4.61
Sex of producers		
Male	0	0.00
Female	11	100
Educational status of producers		
No School	-	-
Primary School	6	54.55
Secondary School	3	27.27
Graduates	2	18.18
Consumption rate		
Daily consumers	173	61.79
Occasional consumers	87	31.07
Non – Consumers	20	7.14
Occupation of respondents		
Civil servants	46	16.43
Housewives	94	33.57
Students	63	22.50
Self – employed individuals	77	27.50

3.2 Microbial load of soymilk sold in Enugu state metropolis

The microbial load of soymilk sold in Makurdi metropolis is presented in TABLE 2a. The highest microbial load (8.5×10^7 c.f.u./ml) was observed in the Wadata area while the least (3.0×10^7 c.f.u./ml) was at High level. A slight decline in microbial load was noted after the first week of sample collection. Microbial load of soymilk samples was significantly different (TABLE 2b) across the sampling locations as well as sampling periods (P = 0.05).

Table 2a: Microbial load of soymilk (in c.f.u./ml) sold in Enugu state metropolis

Location	Week 1 (c.f.u./ml)	Week 2 (c.f.u./ml)	Week 3 (c.f.u./ml)
New lay	7.6×10^7	6.9×10^7	7.1×10^7
Obiagu	5.6×10^7	4.1×10^7	4.5×10^7
GRA	4.7×10^7	3.8×10^7	3.0×10^7
New Haven	8.5×10^7	6.6×10^7	6.0×10^7

TABLE 2b: Analysis of Variance (ANOVA) for the Microbial Load of Soymilk Sold in Enugu metropolis

Source of Variation	Degree of Freedom (df)	Sum of Squares (SS)	Mean Squares (MS)	F – Calculated (F – cal)	F – Tabulated (F – tab)
Weeks	2	4.94	2.47	10.74	5.14
Location	3	25.34	8.45	36.72	4.76
Error	6	1.38	0.23		
Total	11	31.66			

F – cal > F – tab = There is significant difference (P = 0.05)
F – tab > F – cal = No significant difference (P = 0.05)

3.3 Biochemical characteristics of organisms

Biochemical characterisation of microbes isolated from soymilk samples (TABLE 3) showed that the samples were contaminated with coliforms and other bacterial species. Organisms isolated include *Escherichia coli*, *Klebsiella spp*, *Salmonella typhi*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus* (C-ve), and *Aerobacter aerogenes* as presented.

TABLE 3: Biochemical Characteristics of Organisms

Test \ Name of organism	*Escherichia coli*	*Klebsiella spp*	*Salmonella typhi*	*Streptococcus faecalis*	*Staphylococcus aureus*	*Staphylococcus (C-ve)*	*Aerobacter aerogenes*
Gram reaction	-	-	-	+	+	+	-
Motility	-	-	+	-	-	-	+
Spore	-	-	-	-	-	-	-
Catalse	+	+	+	+	-	-	+
Lactose fermentation	+	+	+	-	+	-	+
Oxidase	-	-	-	+	-	+	-
Slide agglutination	-	-	-	-	-	+	-

- = Negative or absent
+ = Positive or present
C – ve = Coagulase negative

3.4 Test for coliforms

The cultural characteristics and results of test for coliforms are presented in TABLE 4. Probable organisms were *E. coli, Klebsiella spp, Salmonella spp, Streptococcus faecalis, Staphylococcus aureus, Staphylococcus (Coagulase –ve),* and, *Aerobacter aerogenes.*

TABLE 4: Cultural characteristics and result of test for coiforms

Cultural Growth on Nutrient Agar	Cultural Growth on MacConkey Agar	Growth Characteristics on Eosin Methylene Blue Agar (EMBA)	Microscopic Appearance	Probable Organism
Flat, yellow, opaque, undulate colonies with slightly deeper coloured centers about 1.25mm diameter.	Red, opaque, flat colonies with undulate edges about 1.75mm diameter.	Dark almost black centered undulate colonies with metallic sheen about 1.25mm diameter	Single, fat, short rods	*E. coli*
Flat, extremely mucoid, transparent colonies, varying from white to yellow in colour, with rhizoid edges about 1.75mm diameter.	Red, flat colonies, opque with rhizoid edges about 1.75mm in diameter	-	Single, fat, short rods	*Klebsiella spp*
Flat, yellow, opaque3 colonies with undulate edges about 0.65mm diameter.	Red, flat, opaque colonies with rhizoid edges about 0.75mm in diameter.	-	Single, fat, short rods	*Salmonella spp*
Dome shaped yellow opaque colonies with edges crenated about	Raised, red, opaque colonies with undulated edges about	-	Single chains	*Streptococcus faecalis*

0.5mm diameter.	0.85mm diameter			
Deep yellow, convex papillate, opaque colonies with entire edges about 0.75mm diameter.	-	-	Spherical clusters	*Staphylococcus aureus*
Yellow, convex paillate opaque colonies with entire edges, about 0.75mm diameter	-	-	Spherical clusters	*Staphylococcus (Coagulase –ve)*
-	-	Flat, purple to pinkish mucoid colonies, opque with crenated edges about 0.75mm diameter	Single, fat, short rods	*Aerobacter aerogenes*

-         = No growth

3.5 Proximate composition of soymilk

The proximate composition of soymilk (TABLE 5) showed that the samples had high moisture content and very low content of fibre and ash. Samples from Wadata had the highest ash (1.00%), fibre (0.35%) and lipid (18.00%) contents respectively, while samples from Wurukum, High level and North Bank had the highest moisture (91.49%), protein (7.76%) and carbohydrate (13.39%) contents respectively. Soymilk samples from Wurukum, had the least fibre (0.10%), lipid (1.56%), protein (2.86%) and carbohydrate (3.09%) contents respectively. Ash content was least in soymilk samples collected in Northbank (0.80%), while moisture content was least in soymilk samples collected in High level (68.50%).

TABLE 5: Proximate composition of soymilk

Percentage Compositon (%)

Area Sampled	Moisture	Ash	Fibre	Lipid	Protein	Carbohydrate
North Bank	74.80	0.80	0.10	4.13	6.78	13.39
Wurukum	91.49	0.90	0.10	1.56	2.86	3.09
Highlevel	68.50	0.98	0.30	17.10	7.76	5.36
Wadata	70.26	1.00	0.35	18.00	7.00	3.39

3.6 Consumer acceptability of soymilk

Results for consumer acceptability test of soymilk in the study area (TABLE 6) showed that acceptability of soymilk by consumers ranged from moderate. to extreme

TABLE 6: Consumer acceptability of soymilk

acceptability

Parameters	North Bank	Wurukum	Highlevel	Wadata
Colour	9.0	8.0	8.0	8.4
Sweetness	8.0	7.0	7.0	7.1
Favour	8.0	6.0	7.0	7.0
Mouth feel	8.0	7.0	7.0	7.0
Overall acceptability	8.0	7.0	7.1	7.3

Scale

9 = like extremely   4 = Dislike slightly
8 = Like very much   3 = Dislike moderately
7 = Like moderately   2 = Dislike very much
6 = Like slightly   1 = Dislike extremely
5 = Neither like nor dislike

IV.   Discussion

other hand, soymilk production in the study area was exclusive to females. This can be explained by the fact in this part of the world, cooking is mostly seen as the sole responsibility of females.

Soymilk on sale in Makurdi is contaminated by various microorganisms such as *E. coli, Klebsiella spp, Salmonella typhi, Streptococcus faecalis, Staphylococcus aureus, Staphylococcus* (Coagulase negative). This is in agreement with findings of Soomro [14] who reported the presence of *Staphylococcus aureus* in samples obtained from different locations in India. In a similar work on microorganisms associated with locally processed milk products (nono and Wara) at Ilorin, *E. coli,* and *Staph aureus* were also isolated [15]. *Streptococcus faecalis* was isolated in this study but absent in reports earlier mentioned. This may be attributed to the culture media used and differences in environmental conditions.

The contamination of this soymilk product be members of the *Enterobacteriaceae* is mostly as a result of poor handling, use of contaminated raw materials, unhygienic processing environment and lack of good manufacturing practices by processors. This is also evidenced by the low literacy level of producers in the study. A similar report has been published by Ismail [16].

Results for proximate analysis of moisture content of Northbank (74.80%), High level (68.50%) and Wadata (70.26%) were identical to result (74.50%) reported by Goodman [17] and the moisture content of Wurukum (91.49%) close to the value (91.4%) reported by Asiedu [18]. The fibre content of all soymilk samples in this study areas were however within the range 0.1% - 0.35%. This was in contrast to 0.00% reported by Asiedu [18] and Goodman [17]. Moreso, the lipid content (4.13%) in soymilk from Northbank was same as that (4.13%) reported by Goodman [17], while that of Wurukum (1.56%) was slightly higher than that (1.50%) reported by Asiedu [18]. Those of Highlevel (17.10%) and Wadata (18.00%) were slightly lower than the value (19.89%) reported Habeeb [19].

Differences in nutrient content observed in the study in comparism to others reported by various researchers may be due to differences in soybean varieties used in milk production as well as slight variations in assay techniques used.

V. Conclusion

Soymilk which is highly consumed in Makurdi is commonly contaminated with bacteria of faecal origin whose source may be traced to poor hygiene of producers, unsanitary conditions of processing equipment and raw materials. Observation of strict sanitary practices and application of Hazard Analysis Critical Control Points (HACCP) at necessary points will reduce the level of contamination and enhance consumer safety.

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