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Full Length Research Paper

Microbial and physico-chemical quality of powdered soymilk samples in Akwa Ibom, South Southern Nigeria

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Branded and unbranded powdered soymilks were evaluated for microbial quality, physicochemical parameters and aflatoxin level. The total bacterial count ranged from 4×104 to 1.1×105 cfu/g and 2.0 × 104 to 7.2 × 104 cfu/g for branded and unbranded powdered soymilk samples, respectively. Coliform were not detected in the branded and unbranded powdered soymilk samples. The fungal count ranged from 2.1×104 to 4.9 ×104cfu/g and 1.5 ×104 to 2.6 ×104 cfu/g for branded and unbranded soymilk, respectively. The isolated microorganisms were *Micrococcus* sp., *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas* sp., *Streptococcus* sp., *Aspergillus flavus*, *Candida pseudotropicalis*, *Saccharomyces cerevisae*, *Penicillium citrium* and *Cladosporium* sp. in branded samples while *Pseudomonas* sp., *B. subtilis*, *Yersinia enterocolitica*, *Streptococcus* sp., *A. flavus*, *Aspergillus niger*, *C. pseudotropicalis*, *Saccharomyces* sp. were isolated from unbranded samples. The pH ranged from 7.9 – 8.0 and 7.8 – 8.0 while the lactic acid ranged from 90.08 – 144.13 mg and 81.07 – 162.144 mg for branded and unbranded samples in that order. The moisture content ranged from 12.76 – 13.05%. The proximate analysis revealed that the crude protein ranged from 16.68 – 17.74% branded and unbranded powdered soymilk samples. Aflatoxin B1 were detected in the samples and it ranged from 7.87 – 19.76 µ/kg and 4.58 – 16.74 µ/kg for branded and unbranded samples respectively while Aflatoxin B2 ranged from 3.43 – 11.74 µ/kg and 2.57 – 9.79 µ/kg. The microbial population in terms of numbers and types reflected poor standard of production constituting a serious health hazard among populace. Thus, this calls for proper monitoring and quality assurance.

Key words: Powdered soymilk, branded, unbranded, microorganisms, Aflatoxin B1

INTRODUCTION

The soybean (Glycine max (L) Merrill) is a member of the family Leguminosae, sub-family Pailionaceae (Hermann, 1962). It is an annual plant of varying heights. The number of pods and seeds are strongly influenced by environmental factors (Smith, 1972). It is prone to fungal infection on farm and after harvest. Nasir (2003) observed that fungicides reduced in vitro growth of fungi isolated from soybean seeds. The oldest written reference to soymilk surfaced from China in a poem titled 'Ode to Tofu' by Su Ping at about A.D. 1500 (William and Akiko, 2000). Soymilk was first referred to in the United States by Trimble in 1896 in the American and the first commercial soymilk in the US was produced by J.A Chard soy products in New York (Gavin and Wettstein, 1990). pathogen to the teaming consumers (Nester et al., 2004).

Soymilk, which is a water extract of whole extract of whole soybean, is rich in water soluble protein, carbohydrate and oil. So, its benefits when compared to cow milk include cost effectiveness and larger quantity can be produced. Soymilk can be produced by traditional method, whole bean method, defatted method or extruder method (Harrigan and McCance, 1976). It is lactose-free unlike dairy milk and can be taken by lactose intolerant people (Poskitt, 1993; Samona, 1993; Nsofor et al., 1997). It is also non-allergic; can be easily produced with low level technology and serve as good nutriment for

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vegetarian diet (Samona, 1993; Varnam and Sutherland, 1994). Soybeans have been included in diverse food sauce like Koji (Nester et al., 2004). That is also why it was referred to as the nature's perfect food as cow milk and human milk (Kon, 1972; IITA, 1989; Mita and Stemkrain, 1975).

Despite these arrays of benefits derivable from soymilk it can easily be a route for transmitting food borne Bacterial pathogens identified with food poisoning, gastroenteritis and enteric fever can be harbored in unhygienically prepared soymilk. Some potential pathogenic contaminants of dairy food have also been implicated in causing mycetoma in human (Cheesbrough, 2000). *Aspergillus flavus* is involved in allergic aspergillosis (pulmonary aspergillosis) and also produces aflatoxin that is highly carcinogenic (Prescott et al., 2005). This study reports the microbial quality, proximate and aflatoxin content of both branded and unbranded soymilk in Akwa Ibom state, Nigeria.

MATERIALS AND METHODS

Samples collection

Branded and unbranded powdered soymilks (fifteen packs each) were purchased at different market and stores in Uyo Metropolis and Ikot-Ekpene. The samples were taken to the laboratory for analysis.

Microbiological studies

Ten fold dilutions of 10 g each of the samples (randomly selected) were made to achieve dilution factor of 10-4 and 10-5. Exactly 0.1 ml of the diluents were pour plated in triplicate plates on Nutrient agar for total bacteria counts, MacConkey agar (Oxoid) for coliform count and Sabourad dextrose agar with chloramphenicol (250 mg/100 ml) for fungal counts. All plates were incubated for 48 h at 30°C except Sabouraud Dextrose agar that were incubated at 26°C for 6 days. Colonies were selected randomly, bacteria cultures were characterized and identified using various morphological and biochemical tests namely gram stain, spore stain, motility, catalase, coagulase, indole, MR –VP, urease, citrate, oxidase and sugar fermentation. Pure cultures of each isolate were obtained by streaking the specific colonies on suitable media and incubated appropriately; these were maintained in an agar slant in McCartney bottles. The identification of the microbial isolates was based on classification Scheme proposed by Buchanan and Gibbson (1974), Harrigan and McCance (1976), Collin and Lyne (1984) and works of CruickShank et al. (1975) and Cowan (1985). The identification was based essentially on morphological and biochemical reactions. The associated fungi were identified with reference to Frazier and Westholf (1998) and the work of Barnet and Hunter (1972).

Proximate analysis

The proximate composition was carried out according to the method of AOAC (1990). This includes determination of pH, moisture content, ash content, crude fat, crude fiber, crude protein and carbohydrate. The pH readings were taken according to the method of AOAC (1990). The amount of lactic acid in the samples was determined by the titration procedure of Spicher and Stephen (1982). Acid equivalent was the amount of NaOH consumed in 1 ml while each 1 N NaOH is equivalent to 90.08 mg of lactic acid. The analysis was carried with reference to earlier work of Duke and Atchley (1984).

Detection of aflatoxin in branded and unbranded powdered soymilk samples

Aflatoxins were extracted from the branded and unbranded powdered soymilk samples according to the method of Seitz and Mohr (1977). 10 g each of the branded and unbranded powdered soymilk samples were extracted with chloroform and concentrated. Thin layer chromatography of aflatoxin and samples extracted were performed on silica gel DG 254. Of the extracted sample 5, 10, and 15 ml were spotted on three different points on a ruled base line of the TLC plates. Also 5, 10 and 15 ml of the aflatoxin standard were spotted on another three points near the previous sample extract spotted points. The plates were developed first with diethyl ether and then with chloroform acetone (9:1v/v). Aflatoxins were identified on the basis of co-migration with aflatoxin standards (Fluka) and by their characteristic fluorescent color under long ultraviolet (UV) illumination were at 360nm. The fluorescent spots of aflatoxins were scraped off the TLC and eluted by chloroform: methanol (9:1, v:v). The solvent was evaporated under nitrogen to dryness and the residue was dissolve in methanol. The concentration of aflatoxins B1 and B2 in solution was determined by measuring its absorbance at 360 nm then calculated according to the method of Masri et al. (1969).

Confirmatory test for aflatoxin

Three different derivatives were prepared by treating portion of the isolated toxin on the aflatoxin standard with formic acid thionyl chloride, acetic-thionyl chloride and trifloroacetic acid. The test was then continued according to the method of Stolloff (1967).

RESULTS AND DISCUSSION

The total plate count ranged from 4.0 x 104 – 1.1 x 105 cfu/g and 2.4 x 104 – 7.2 x 104 cfu/g for branded and unbranded samples, respectively. Table 1 shows the microbial population of the branded and unbranded powdered soymilk samples. Coliform were not detected in the samples. The fungal count ranged from 2.1 x 104 – 4.9 x 104 cfu/g and 1.5 x104 – 2.6 x 104 cfu/g for branded and unbranded samples, respectively. The microbial population obtained in the samples was above the acceptable limit of 2.0 x 104cfu/g recommended for general bacterial count by the Soy Foods Association of America (SFAA). It was discovered that the microbial population of branded samples were higher than that of the unbranded samples which may be attributed to several factors, which include the initial contamination of the raw materials to the poor handling of finished products, the utensils and the sanitary condition of the processing environment. The total effects of such contaminating factors determine the quality of the powdered soymilk, its probable shelf life and the potential public health risk.

The morphological and biochemical characteristic of the bacteria and fungi isolates were shown in Tables 2 and 3 respectively. The bacteria isolated from the brand-

Table 1. Total count (cfu/g) of the microbial groups in branded and unbranded powdered soymilk samples.

Sample types	Sample code	Total aerobic count (cfu/g) x 10^4	Total fungal count (cfu/g) x10^4	Total coliform count (cfu/g) x10^4
Branded	$S_{1(15)}$	4.0	2.1	NG
	$S_{2(15)}$	8.7	4.5	NG
	$S_{3(15)}$	11	4.9	NG
Unbranded	$S_{4(15)}$	2.0	1.5	NG
	$S_{5(15)}$	4.4	1.9	NG
	$S_{6(15)}$	7.2	2.6	NG

Table 2. Microorganism associated with branded and unbranded powdered soymilk samples.

S/N	Microorganism	Branded powdered soymilk samples	Unbranded powdered soymilk samples
1	*Micrococcus* sp.	+	-
2	*Streptococcus* sp.	+	+
3	*Staphylococcus* sp.	+	-
4	*Pseudomonas* sp.	+	+
5	*Bacillus subtilis*	+	+
6	*Yesinia enterocolitica*	-	+
7	*Aspergillus niger*	-	+
8	*Aspergillus flavus*	+	+
9	*Penicillium citrium*	+	-
10	*Cladosporium* sp.	+	-
11	*Candida pseudotropicalis*	+	+
12	*Saccharomyces* sp.	-	+
13	*Saccharomyces cerevisae*	+	-

Table 3. pH and lactic acid content (mg) of branded and unbranded powdered soymilk samples.

Sample types	Sample code	pH	Acid equivalent (ml)	Lactic acid (mg)
Branded	$S_{1(15)}$	8.0	1.0	90.08
	$S_{2(15)}$	7.9	1.2	108.10
	$S_{3(15)}$	7.9	1.6	144.13
Unbranded	$S_{4(15)}$	8.0	0.9	81.07
104	$S_{5(15)}$	7.8	1.8	162.14
	$S_{6(15)}$	7.9	1.0	90.08

ed and unbranded samples are shown in Table 4 and their frequency of occurrence was shown in Figure 1. Streptococcus sp. and *Pseudomonas* sp. were detected from branded and unbranded samples. *Yersinia enterocolitica* were detected in unbranded samples. *Staphylococcus* and *Streptococcus* sp were possible contaminants from handlers. *Streptococcus* sp. has been implicated in human infections like pharyngities, scarlet fever and pneumonia. *Staphylococcus aureus*, a meso-phile have been implicated in food poisoning outbreak of some food material. Odunfa (1988) reported that *S. aureus* levels of 108 ml are considered potential hazardous to consumers. The presence of *S. aureus* is an indication of contamination by food handlers. 80% of them are being harbored by man as normal micro flora. *Yesinia* sp. are causative agents of illnesses like yersilosis in human. Bacillus sp. causes a toxin mediated diseases rather than infections (Bergdoll, 1981) such as diarrhea and emetic illness characterized by nausea and vomiting. The occurrences of Bacillus sp. can be said to be as a result of prevalence of their spores in the environment (Jay, 1978). Bacillus species are spore formers whose spores could survive high temperatures of processing. Bacillus has been isolated from non-alcoholic beverages (Osuntogun and Aboaba, 2004; Amusa et al., 2005). The organisms are present in most raw materials used in food manufacturing at concentration of 103/g or less. The infectious dose has been estimated to be 105/g (Adeleke and Odeola, 1997)

The presence of highly pathogenic bacterial isolates, like *Y. enterocolitica*, *Pseudomonas* sp. and *S. aureus* are organisms of public health concern. The presence of these microbes is an indication of possible contamination

Table 4. Proximate composition (%) of powdered soymilk samples.

Sample	Sample code	Crude protein	Fat	Fiber	Ash	Dry matter	Moisture
Branded	$S_{1(15)}$	16.68	7.36	2.34	4.34	87.18	12.82
	$S_{2(15)}$	16.89	7.39	2.26	4.29	87.24	12.76
	$S_{3(15)}$	17.74	6.67	2.05	5.15	86.95	13.05
Unbranded	$S_{4(15)}$	17.13	7.11	2.18	4.73	87.15	12.85
	$S_{5(15)}$	17.37	6.85	2.09	5.02	87.09	12.91
	$S_{6(15)}$	17.24	7.13	2.21	4.83	87.18	12.82

Table 5. The composition of aflatoxins produced is shown in the table below.

Sample	Sample code	B_1 (µ/kg)	B_2 (µ/kg)
Branded	$S_{1(15)}$	8.57	3.78
	$S_{2(15)}$	7.87	3.43
	$S_{3(15)}$	19.76	11.54
Unbranded	$S_{4(15)}$	11.67	6.54
	$S_{5(15)}$	16.74	9.79
	$S_{6(15)}$	4.58	2.57

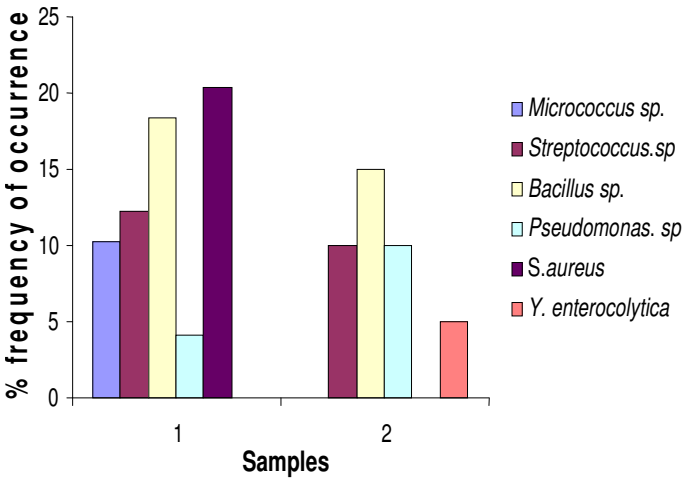

Figure 1. Frequency occurrence (%) of Bacteria associated with branded and unbranded soymilk samples. 1 – Branded soymilk samples, and 2 – Unbranded soymilk samples.

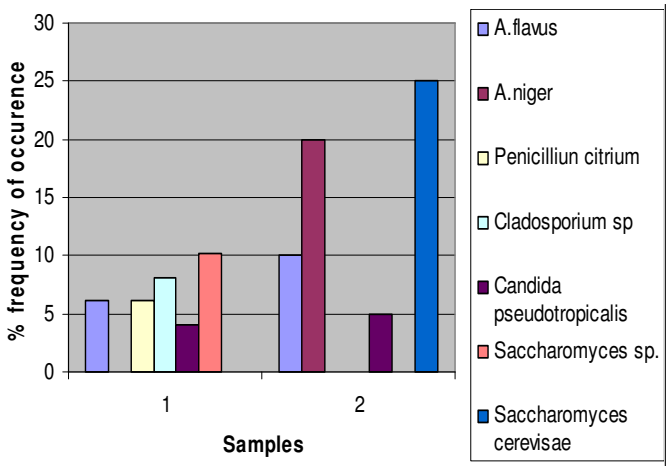

Figure 2. Frequency of occurrence (%) of fungi associated with branded and unbranded powdered soymilk samples. 1 – Branded soymilk samples, and 2 – Unbranded soymilk samples.

resulting from the use of well water, which is mostly used in local food processing industries are not free from microbial contamination (Potter, 1983). *S. aureus* known for production of heat stable enterotoxin (Stewart, 1974) and potentials for multiple antibiotic resistances when they get into the living tissue (Foster, 1996; Allen and Cowan, 1997; Okuma et al., 2002; Scott, 2002; Klein et al., 2007) makes the product of immense epidemiological danger. All the organisms isolated have health implication for man except *Micrococcus* sp., which have not been associated with human infections. The fungi associated with the branded and unbranded powdered soymilk samples were shown in Table 3 and their frequency of occurrence were shown in Figure 2. The fungal isolates were *A. flavus*, *Aspergillus niger*, *Cladosporium* sp. *Candida pseudotro-picalis* and *Penicillium citrium* for branded powdered soymilk and *A. flavus*, *A. niger* and *Candida pseudotropicalis* for unbranded powdered soymilk samples. The yeast isolates were *Saccharo-myces cerevisae* and *Saccharomyces* sp. *Aspergillus* spp. have also been implicated in causing mycetoma in human Cheesbrough, 2000). *A. flavus* is involved in allergic (aspergillosis (pulmonary aspergillo-sis) and also produces aflatoxin that is highly carcinogenic (Prescott et al., 2005).

The presence of species of Aspergillus could be attributed to the prevalence of their spores in the atmosphere. This organism was easily trapped during the post harvest processing and handling of soybean grains. Since most fungal spores are found in the air, the spores must have contaminated the grains during drying. The liberated spore can easily settle on food and ceilings of room and then germinate (Okhuoya and Ayanlola, 1986). Dongo and Ayodele (1997) have shown that Aspergillus occurred highest in the number of colonies identified from air spora of some localities. Ilodu and Iloh (2007) isolated and identified *A. flavus* and *A. niger* from sorrel drink.

The pH of branded and unbranded powdered soymilk

Table 6. Proximate Composition (%) of Powdered Soymilk Samples.

Sample	Sample Code	Crude Protein	Fat	Fiber	Ash	Dry Matter	Moisture
Branded	$S_{1(15)}$	16.68	7.36	2.34	4.34	87.18	12.82
	$S_{2(15)}$	16.89	7.39	2.26	4.29	87.24	12.76
	$S_{3(15)}$	17.74	6.67	2.05	5.15	86.95	13.05
Unbranded	$S_{4(15)}$	17.13	7.11	2.18	4.73	87.15	12.85
	$S_{5(15)}$	17.37	6.85	2.09	5.02	87.09	12.91
	$S_{6(15)}$	17.24	7.13	2.21	4.83	87.18	12.82

Table 7. The composition of aflatoxins produced is shown in the table below.

Sample	Sample Code	B_1 (µ/kg)	B_2 (µ/kg)
Branded	$S_{1(15)}$	8.57	3.78
	$S_{2(15)}$	7.87	3.43
	$S_{3(15)}$	19.76	11.54
Unbranded	$S_{4(15)}$	11.67	6.54
	$S_{5(15)}$	16.74	9.79
	$S_{6(15)}$	4.58	2.57

samples ranged from 7.8 – 8.0 and the percentage lactic acid content ranged from 90.08 – 162 mg (Table 5). This is in agreement with the work of Davies (1981). The pH ranges of the powdered soymilk samples encourage microbial growth hence the observed microbial populations.

The nutritional composition of the branded and un-branded powdered soymilk samples are shown in Table 6. The samples have a high nutritional value, it is an excellent food for man, and it also provides an excellent growth medium for microorganisms.

The concentration of aflatoxin B1 and B2 in the branded and unbranded powdered soymilk samples was shown in Table 7. It ranged from 4.58 - 19.76 µg/kg and 2.57 – 11.74 µg/kg respectively. Aflatoxin B1, secondary fungal metabolites has been reported to be responsible for several ailments in animals including man (Fennel et al., 1973). This aflatoxin is highly carcinogenic causing hepatoxin and has been associated with acute hepatitis in men (Butler and Barnes, 1968; Eaton and Groupman, 1994). *A. flavus* with its aflatoxin B1 and B2 has already been implicated in hepatoxin and cancer in mammal including man (Efiuviewevwere and Akoma, 1997). The presence of this organism even when there was no water of activity suggests the need to store powdered milk properly to avoid such contamination.

Conclusively, the quest for cheap source of protein has enhanced small scale production of vegetable protein products of which soymilk is an example. Soymilk consumption has encouraged small scale production of the product under household condition with little or no regard to quality control measures.

The unbranded product in this case had less microbial count than the branded and this further suggests contamination which might be due to breach in any of critical quality control points in the production of the branded sample. So, adequate monitoring and strict adherence to quality control measures and good manu-facturing practice during production as well as acceptable standard is needed during the production of soymilk since powdered soymilk is used as infant formula for neonate and as cow milk substitute for adult. Therefore, National Agency for Food and Drug Administration Control (NAFDAC) in Nigeria should develop microbiological and chemical standards for powdered soymilk in order to avoid the cumulative effects of microbial contaminants in the human systems.

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