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RESEARCH ARTICLE

Evaluation of Microbial and Nutritional Values of Commercially Packaged Soymilk Sold in Akure, Nigeria

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Abstract

The sale of soymilk is quite popular in Akure but there is no related information on its microbial safety and nutritional status.

Objectives: To determine the microbial and nutritional values of selected commercially packaged soymilk sold in Akure, Nigeria.

Materials and methods: The branded samples were coded HL, GD, VT and SF while a soymilk locally produced (unbranded) served as control. Microbial counts and isolation was carried out using pour plate technique. Microbial count was carried out with a digital colony counter (Gallenkamp model). Morphological and biochemical characteristics were used in the identification of bacteria isolates while fungal isolates were identified based on their cultural morphology and microscopy.

Results: Results showed that the bacterial counts ($x10^1$ cfu/ml) of branded samples which ranged from 0.00 ± 0.00 in VT to 0.80 ± 0.70 in GD were not significantly different ($p > 0.05$) from each other but significantly different (< 0.05) from the unbranded samples (20.00 ± 0.40). However, there was no fungi growth in all the branded samples but the unbranded recorded fungi count of 18.00 ± 0.08 x 10^1 sfu/ml. Similarly, there was no coliform growth in both the branded and unbranded samples. The probable bacterial isolates in both branded and unbranded samples were *Lactobacillus* sp and *Bacillus* sp while the only fungi isolate was *Rhizopus* sp in the unbranded.

Conclusion: Remarkably, absence of coliforms in both the branded and unbranded samples and the microbial load obtained which did not exceed the acceptable limit (3×10^4 cfu/ml) for pasteurized milk suggests good manufacturing practices in the production of the soymilk samples. Consequently, industries producing soymilk and soymilk vendors should follow suit in ensuring that their products are consistently produced hygienically with regards to quality control measures to avoid microbial contaminants.

Keywords

Soymilk, Branded, Unbranded, Microbial, Nutritional, Public health

Introduction

Soyabean (*Glycine max*) is incorporated into so many food formulation for both children and adults to enhance nutritional value of foods [1], in preparations such as "dawadawa", allele, moi-moi, akara, soy-ogi and most recently as soymilk [2] which is a high protein, iron-rich milky liquid produced from pressing ground, cooked soybeans [3]. The milk which is a white or creamy emulsion resembles cow milk in both appearance and consistency [4].

The diets of people in many developing countries lack animal sources of proteins such as milk which are expensive and out of reach for low income families. Hence, soymilk which is a cheap source of protein is therefore used to supplement such diets. Soybeans and products derived from them have served as an important source of protein in the diet of millions of oriental people for nearly 5,000 years [5]. In fact, the increasing popularity of soymilk is credited to health benefits and being a good alternative to animal protein [6]. Besides, it is a good nutrient for vegetarian diet [7].

Nevertheless, soymilk can also serve as a source for transmitting food borne infections. 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 [1]. Bacterial pathogens identified with food poisoning, gas-

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troenteritis and enteric fever can be harbored in soymilk that was not hygienically prepared [4]. Hence, this study was carried out to assess microbial and nutritional status of selected commercially packaged soymilk sold in Akure, Nigeria.

Materials and Methods

Source and collection of sample

Four selected commercially branded soymilk samples were obtained from super markets and stores in Akure metropolis, Ondo state, Nigeria. The branded samples were designated HL, GD, VT and SF while a soymilk locally produced (unbranded) served as control. They were then taken to the Department of Biology laboratory, Federal University of Technology, Akure, Nigeria and preserved at 4 °C for microbial and proximate analyses.

Counting and isolation of microorganisms

Pour-plate technique was used for microbial counts and isolation. One ml of each soymilk sample was serially diluted to $x10^1$ dilution factor and 1 ml of the dilution factor was then pipetted aseptically into a sterilized Petri-dish. This was followed by the pouring of already prepared sterilized molten nutrient agar and potato dextrose agar media for bacteria and fungi counts respectively. The Petri dish was later incubated at 37 °C for 24 h (bacteria) and 28 ± 2 °C for 72 h (fungi) after being allowed to solidify. After 24 h, bacteria count was carried out with a digital colony counter (Gallenkamp model, UK) and recorded as colony forming units per ml (cfu/ml). Similarly, after 72 h, fungi count was equally carried out with a digital colony counter and recorded as spore forming units per ml (sfu/ml). Observable isolate was then sub cultured severally to obtain pure culture. The same procedure was repeated for coliform count and isolation except that the agar used was eosin methylene blue agar and incubation was done for 24 h at 37 °C.

Identification of bacterial isolates

Cultural characteristics of discrete colonies such as color, shape, elevation, pigmentation, opacity, nature of edges of the colonies and biochemical tests were used in the identification of bacteria isolates.

Identification of fungal Isolates

Fungal isolates were identified according to their cultural morphology and microscopy.

pH determination

This was done by inserting the electrode of a digital pH meter directly into the soymilk samples and reading was taken.

Proximate analysis of soy milk

The method of Pearson [8] was used to determine the moisture content of the soymilk samples. The weight of an empty aluminium dish can (W0) was determined before the sample was introduced into it. About 20 ml of the soymilk sample was measured out and further weighed (W1) 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 (W2) measured. Percentage of moisture content was determined as follows:

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

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

Also, standard methods according to AOAC [9] were used to determine protein, ash, fat, fibre and carbohydrate contents of the soymilk samples. Crude protein of the soymilk sample was determined using Kjeldahl method. For digestion at high temperature, 10 ml of concentrated sulfuric acid and 1.1 g digestion mixture were added to 0.5 g of the soymilk sample in digestion tube. Then the digestion tubes were set in digestion chamber fixing at 420 °C for 45 minutes. Thereafter, the digestion tubes were allowed to cool and 5 ml of sodium thio-sulphate ($Na_2S_2O_3$, 33%) and 30 ml sodium hydroxide (NaOH) solution was added to the digestion tube. Then the distilled extraction was collected with 25 ml of Boric acid (4%) and titrated with standard hydrochloric acid (0.2N). The nitrogen values obtained was converted into percentage of crude protein by multiplying with a factor of 6.25.

Ash content was determined by heating 1 ml of the soymilk sample inside a weighed crucible in a muffle furnace at 550 °C for 5 h. Crude lipid (fat) was determined by extracting 3 mls of each soymilk sample with analytical grade acetone using Soxhlet method. Extraction was allowed to continue by heating at 70 °C for 3 h. For crude fibre determination, 1 ml of the soymilk sample was defatted, followed by successive treatment with boiling solutions of H_2SO_4 and KOH, then, the residue was filtered, washed, dried and weighed after which it was ashed in a muffle furnace at 550 °C where the loss in weight after ashing is the crude fibre content. Amount of carbohydrate was determined by difference:

100- (% protein + ash + fat + fibre)

Statistical analysis

All experiment was conducted in triplicates. Microbial counts and proximate values were subjected to analysis of variance (ANOVA) using SPSS, version 16.0 and where significant, means were separated by Tukey-posthoc test at p = 0.05.

Results

The result of the microbial counts of the soymilk samples is presented in Table 1. Bacterial counts ($x10^1$ cfu/ml) of the branded samples were not significantly different ($p > 0.05$) from one another. The least count (0.00 ± 0.00) occurred in VT while the highest count (0.80 ± 0.70) occurred in GD. However, these counts were significantly different ($p < 0.05$) from the unbranded samples (20.00 ± 0.40). Meanwhile, there was no fungi

Table 1: Microbial counts of soymilk samples.

Soymilk samples	Sample code	Bacterial count $x10^1$ cfu/ml	Fungi count $x10^1$ sfu/ml	Coliform count $x10^1$ cfu/ml
Branded	HL	0.50 ± 0.70**a**	0.00 ± 0.00**a**	0.00 ± 0.00**a**
	GD	0.80 ± 0.70**a**	0.00 ± 0.00**a**	0.00 ± 0.00**a**
	VT	0.00 ± 0.00**a**	0.00 ± 0.00**a**	0.00 ± 0.00**a**
	SF	0.50 ± 0.70**a**	0.00 ± 0.00**a**	0.00 ± 0.00**a**
Unbranded	LOCAL	20.00 ± 0.40**b**	18.00 ± 0.08**b**	0.00 ± 0.00**a**

Each values is a mean of triplicate determination ± SE and means having the same letter in the column are not significantly different ($p > 0.05$) by Tukey test at $\alpha = 0.05$.

Note: HL, GD, VT and SF = codes for branded soymilk samples.

Table 2: Identities of bacterial isolates found associated with the soymilk samples.

Parameters	Morphological and Biochenmical Characteristics	
Colour of colony	Yellowish	Whitish
Elevation of colony	Raised	Flat
Edge of colony	Entire	Crenated edge
Texture of colony	Smooth	Smooth
Size of colony	Medium	Large
Gram's reaction	**+**	+
Shape of colony	Circular	Circular
Shape of cells	Rod	Rod
Catalase	+	-
Coagulase	-	-
Glucose	-	+
Galactose	-	+
Sucrose	-	+
Lactose	-	+
Likely Organisms	*Lactobacillus* sp	*Bacillus* sp

Key:
+ = Positive
- = Negative

Table 3: Occurrence of microorganisms in soymilk samples.

Soymilk samples	Sample code	Bacterial isolates		Fungi isolate
		***Lactobacillus* sp**	***Bacillus* sp**	***Rhizopus* sp**
Branded	HL	+	+	-
	GD	+	+	-
	VT	+	+	-
	SF	+	+	-
Unbranded	LOCAL	+	+	+

HL, GD, VT and SF = codes for branded Soymilk samples.

Note:
+ = Positive or present
- = Negative or absent

growth in all the branded samples but the unbranded recorded fungi count (18. 0.00 ± 0.08 x 10^1 sfu/ml) that was also significantly different ($p < 0.05$). Interestingly, there was no coliform growth in all the samples.

Various bacteria isolates from both the branded and unbranded samples are presented in Table 2 and were *Lactobacillus* and *Bacillus* while *Rhizopus* was the only fungal isolate from unbranded samples (Table 3).

The result of the pH values is shown in Table 4 and ranged between 7.2 in VT to 7.9 in the unbranded while Table 5 showed the proximate compositions of the soymilk samples. The moisture contents (%) of all the branded and the unbranded samples were not significantly different ($p < 0.05$) and ranged from 77.90 ± 0.73 in VT to 79. 00 ± 0.00 in the unbranded while protein contents (%) of the branded (except GD) and the unbranded samples were also not significantly different ($p < 0.05$) and ranged from 2.01 ± 0.10 in GD to 3.07 ± 0.73 in HL. Similarly, fat content (%) of the branded (aside GD and SF) and the unbranded were not significantly different ($p < 0.05$) and ranged from 1.76 ± 0.73 in SF to 3.00 ± 0.00 in VT. Meanwhile, the fibre content (%) for both branded and unbranded samples was 0.00 ± 0.00. Ash content (%) in both branded and unbranded samples were also not significantly different ($p < 0.05$ and ranged from 0.29 ± 0.01 in HL to 0.38 ± 0.08 in SF. In the same vein, there was no significant difference in the carbohydrate contents of both the branded (except VT and SF) when compared with the unbranded samples and the values ranged from 5.83 ± 0.88 in GD to 11.67 ± 0.33 in VT.

Table 4: pH of soymilk samples.

Soymilk samples	Sample code	pH
Branded	HL	7.3
	GD	7.5
	VT	7.2
	SF	7.3
Unbranded	LOCAL	7.9

Note: HL, GD, VT and SF = codes for branded soymilk samples.

Discussion

From the results, it was observed that both the bacterial and fungal loads in the unbranded samples were higher than the branded. This observation was supported by the work of Adeleke, et al. [10] that branded soymilk samples purchased from selected markets in Ibadan had lower microbial counts than the unbranded samples. Although the observation contradicted the work of Adebayo-Tayo, et al. [4] who reported higher microbial population in branded samples than the unbranded samples. The contradiction unarguably may be as a result of the fact that Adebayo-Tayo, et al. [4] work on powdered soymilk samples while this work investigated already prepared liquid soymilk samples. Again, the observed microbial load in this study must have been influenced by pH as buttressed by Adeleke, et al. [10] that pH favours bacteria growth.

Meanwhile, absence of coliform growth observed in this work was consistent with the work of Adebayo-Tayo, et al. [4] who equally detected no coliforms in their soy milk samples. The various bacteria isolates from both the branded and unbranded were *Lactobacillus* and *Bacillus* while the only fungal isolate from the unbranded samples was *Rhizopus*. This observation contradicts the earlier works of Soomro [11] 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 [12]**.** Similarly, none of these bacterial isolates was identified in soymilk on sale in Makurdi as reported by Liamngee, et al. [13].

Nevertheless, Ozoh and Umeakwu [14] in their own work identified *Bacillus* sp and *Rhizopus*. In the same vein, Mbajiuka, et al. [15] also reported similar isolates of *Bacillus cereus* and *Lactobacillus* species in soymilk. The occurrences of *Bacillus* sp. can be said to be as a result of prevalence of their spores in the environment. *Bacillus* species are spore formers whose spores could survive high temperatures of processing. *Bacillus* has equally been isolated from non-alcoholic beverages [16,17]. Likewise, the presence of *Rhizopus* sp could not

Table 5: Proximate compositions of soymilk samples.

Soymilk Samples	Sample code	Moisture content (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	CHO (%)
Branded	HL	78.01 ± 0.07**a**	3.07 ± 0.73**b**	2.63 ± 0.88**b**	0.00 ± 0.00**a**	0.29 ± 0.01**a**	5.93 ± 0.67**a**
	GD	78.00 ± 0.00**a**	2.01 ± 0.10**a**	1.78 ± 0.88**a**	0.00 ± 0.00**a**	0.30 ± 0.11**a**	5.83 ± 0.88**a**
	VT	77.90 ± 0.73**a**	3.00 ± 0.11**b**	3.00 ± 0.00**c**	0.00 ± 0.00**a**	0.30 ± 0.05**a**	11.67 ± 0.33**c**
	SF	78.00 ± 0.00**a**	2.73 ± 0.67**b**	1.76 ± 0.88**a**	0.00 ± 0.00**a**	0.35 ± 0.08**a**	7.06 ± 0.67**b**
Unbranded	LOCAL	79.00 ± 0.00**a**	3.00 ± 0.00**b**	2.73 ± 0.67b**c**	0.00 ± 0.00**a**	0.30 ± 0.00**a**	5.93 ± 0.67**a**

Each value is a mean of triplicate determination ± SE and means having the same letter in the column are not significantly different ($p > 0.05$) by Tukey test at $\alpha = 0.05$.

Note: HL, GD, VT and SF = codes for branded Soymilk samples.

but be connected with contamination from the environment. This observation was supported by the report of Arotupin, et al. [18] that environmental contamination was responsible for the presence of *Aspergillus niger, Aspergillus flavus* and *Rhizopus* sp. since their spores are common contaminants.

Remarkably, the microbial population obtained in both branded and unbranded samples in this work was below the acceptable limit of 2.0×10^4 cfu/g recommended for general bacterial count by the Soy Foods Association of America (SFAA) and did not also exceed the acceptable limit for pasteurized milk (3×10^4 cfu/ml). Besides, the absence of *E. coli* in this work suggests non faecal contamination of the soymilk samples and consequently renders the liquid drink fit for consumption because a large number of microorganisms such as mesophilic aerobic bacteria, coliforms and fungi, are known to be responsible for the spoilage of soymilk, producing undesirable changes [19,20].

Proximate results showed that all the soymilk samples had high moisture contents and this is because soymilk is an emulsion containing high water and water soluble proteins, carbohydrate and oil droplets [3]. Although the protein values observed in this work was lower than 7.78-10.47% reported by Ozoh and Umeakwu [14] in ready to drink soy milk sold in Onitsa while the fat content was higher than 1.44% reported by Ozoh and Umeakwu [14], also in ready to drink soy milk sold in Onitsa. Meanwhile, the fibre content obtained in this work was the same as the one earlier obtained by Onuorah, et al. [21]**.** The ash content falls within the range of values (0.27-0.57%) for ash contents of ready to drink soymilk and soymilk yoghurt sold in Onitsha as earlier reported by Ozoh and Umeakwu [14]. Similarly, the carbohydrate values observed in this work were within the range of values (3.09-13.39%) earlier reported by Liamngee, et al. [13] for carbohydrate contents of soyabean milk sold at different locations in Makurdi metropolis. This has further showed that soymilk is highly nutritional and in agreement with Adebayo-Tayo [4] that soymilk samples have a high nutritional value, excellent food for man, and also provides an excellent growth medium for microorganisms. In fact, the increase in the consumption rate of soybean milk cannot but be connected to its high protein content which has encouraged low scale production of the soymilk under house hold condition with little or no regard to the quality control measures [22].

Conclusion

The result of the study revealed that the soymilk samples had no fungi count and lesser bacterial count below the acceptable limit as established by regulatory agency. Consequently, industries producing soymilk and other soymilk vendors should follow suit in ensuring that their products are consistently produced hygienically with regards to quality control measures to avoid microbial contaminants. Interestingly, absence of coliforms in both branded and unbranded samples was another index of good manufacturing practice in the production of the soymilk samples.

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Competing Interests

The authors declare no conflict of interest.

Author's Contributions

Oluwole Olakunle Oladele and Prudent Oseyomon Ofure designed the research concept, performed data analysis and interpretation, reviewed and approved the final version of the article.

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