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Microbiology Assessment of Soymilk Sold in Onitsha Metropolis

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Abstract: Microbiological assessment of Soymilk was carried out. The branded and unbranded soymilk samples were bought from six different sellers in Onitsha markets and aseptically taken to the laboratory for analysis. The analysis was carried out using standard microbiological, biochemical and mycological methods. The total viable count of the branded soymilk is lower (1.5×10^6 cfu/ml to 1.8×10^6 cfu/ml) than the counterpart unbranded soymilk sample (6.1×10^6 to 9.0×10^6 cfu/ml). Bacteria isolated include *Staphylococus aureus, Bacillus spp., Escherichia coli, Klebsiella spp., Salmonella spp., Pseudomonas spp.* and *Vibrio spp.,* while the fungi isolated are *Penicillium spp.* and *Aspergillus spp.* It is therefore concluded that good manufacturing and hygienic practices will go a long way in reducing the microbial load of the soymilk.

Key words: Branded Soymilk · Unbranded Soymilk · Bacteria and Fungi

INTRODUCTION

Soymilk, also called soya bean milk, soy juice and sometimes referred to as soy drink is a beverage made from soy beans, a leguminous seed nicknamed "the golden crop" or the "miracle seed" [1]. Soy milk is one of the food preparation from the activity of microorganisms [2].

Soya beans are excellent sources of protein both in quality and quantity about 35 - 40% dry matter, 20% fat, 23 – 24% protein [3]. It is one of the best vegetarian food items as far as protein content is concerned. It is a good source of riboflavin [4].

Soy beans contains a factor that inhibits the action of digestive enzyme, trypsin and this factor can be destroyed by heat.

A generalized method of processing soy milk include cleaning, soaking, boiling, cooling, dehulling, wet milling, sieving, reconstitution, pasteurization, packaging, cooling and refrigeration However, the local producers of soymilk do not undergo detailed processes and still produce and sell under unhygienic environment thereby exposing the soymilk to high levels of contamination by pathogenic and other spoilage organisms [1]. The poor manufacturing practices employed, contaminated raw materials and the quality of water used for the production of soymilk will surely pose its own dangers.

The most frightening thing is that soy milk is mostly consumed by nursery, primary and secondary school children. So, there is every need to ensure that it is produced under the most hygienic conditions so that it will not constitute health hazard to these consumers.

This study is therefore aimed at evaluating the microbiological quality of soy milk sold in Onitsha Metropolis.

MATERIALS AND METHODS

Sample Collection: A total of six samples of soymilk were bought from open markets in Onitsha Metropolis. The samples were taken to the laboratory in sterile containers and analyzed immediately. The samples were diluted using a tenfold serial dilution prior to inoculation on Nutrient agar, Macconkey agar, *Salmonella sligella* agar and Sabouraud Dextrose agar.

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Microbiological Characterisation of the Sample: Microbiological analysis of the samples included inoculation and enumeration using pour plate method and also the identification of microbiological contaminants as describe by FDA (2001). The plates were incubated at 37°c for 24 – 48 hours and those plates that showed discrete colories were counted. The total viable count was then obtained by using: Total Viable Count = N/V X D where N = Mean colony, V = volume plated and D = dilution and expressed as colony forming unit per mililitre (cfu/ml).

The organisms were sub-cultured to get pure cultures and the pure isolates were characterized using Gram staining and biochemical tests such as catalase, citrate, coagulase, oxidase, methyred, Niltrate, Indole, Voges Proskauer, Urease, hydrogen Sulphide Production, Motility and sugar fermentation. The sugars tested include Lactose, Maltose, glucose, sucrose, xylose, Manitol and Sorbitol.

The fungi isolates were also characterized by slide culture method as describe by Okafor (1999) and also on the basis of pigmentation and mycelia arrangement. The confirmations of these isolates (bacteria and fungi) were done with reference to standard bacteriological and mycological manuals.

The pH of the samples were also obtained using an electronic pH meter (Jenway, 2010).

RESULTS AND DISCUSSIONS

The result of the analysis shows high bacteria count in unbranded soy milk samples (C and D) than the branded samples (A and B) and that could be attributable to poor hygienic practices during processing probably, the bottles used in packaging may not be thoroughly sterilized before re use for subsequent production. The high bacteria count in the samples E to F is understandable because the nylon bags used to wrap the products may not even be washed before usage. Moreso, the nylon bags are easily punctured and then get contaminated. All these agree with the observations of [4].

The lower bacteria counts (1.5×10^6 to 1.8×10^6) in the branded bottle soy milk are understood because it undergoes pasteurization/sterilization process.

The Isolation of *Bacillus Spp., Staphylococcus spp., Klebsiella spp., Pseudomonas spp.* and *Salmonella spp.* and *E coli* and agrees with the findings of Ayo *et al.* (2004) and [3]. Akeen (2011), [4], also isolated *Staphylococcus spp., Shigella spp.* and *Salmonella spp.*

The Isolation of *Vibrio spp.* could be as a result of inpure water used in reconstitution and unsterilized equipment.

The Isolation of *Aspergillus spp.* and *Penicillium species* is still in line with the findings of [3].

The total viable counts and characteristics of bacteria and fungi isolates are shown in table 3.

Table 1: Total Viable Bacteria counts of soy milk samples

	Total Viable count cfu/ml on		
Sample	NA	MCA	SSA
A	1.8×10^6	NG	NG
B	1.5×10^6	4.2×10^5	NG
C	2.0×10^6	3.5×10^5	NG
D	2.1×10^6	2.0×10^5	NG
E	2.3×10^6	1.5×10^5	NG
F	9.0×10^6	5.0×10^6	2.0×10^6
G	8.0×10^6	4.2×10^6	1.5×10^6
H	7.5×10^6	4.5×10^6	1.8×10^6
I	6.5×10^6	4.6×10^6	2.1×10^6
J	7.2×10^6	3.9×10^6	2.2×10^6
K	8.6×10^6	3.5×10^6	3.8×10^6
L	9.5×10^6	4.4×10^6	2.8×10^6
M	8.1×10^6	3.8×10^6	3.2×10^6
N	7.8×10^6	5.3×10^6	3.3×10^6
O	6.8×10^6	5.4×10^6	3.6×10^6

KEY. NA = Nutrient Agar, MCA = Macconkey Agar,
SSA = Salmonella Shigella Agar, NG = No Growth,
Samples 1 – 5 branded soil milk, 6 – 10 unbranded, 11-15 = wrapped in nylon.

Table 2: Total Viable Fungal Count and pH in Soybean Samples

Sample	pH	Total Fungal count (cfu/ml)
A	6.9	NG
B	6.8	NG
C	6.5	NG
D	7.01	NG
E	7.02	NG
F	7.00	2.0×10^5
G	6.9	2.3×10^5
H	7.4	2.7×10^5
I	7.25	2.3×10^5
J	7.5	2.4×10^5
K	7.2	1.0×10^5
L	7.3	1.2×10^5
M	7.4	2.3×10^5
N	7.3	1.1×10^5
O	7.5	1.2×10^5

Table 3: Total Viable Counts and Characteristics of Fungi and Bacteria.

	Total Viable Count (cfu/ml)					
Sample	A	B	C	D	E	F
Bacteria	1.8×10^6	1.5×10^6	8.0×10^6	6.1×10^6	9.0×10^6	8.6×10^6
Fungi	NG	NG	0.1×10^5	0.2×10^5	1.2×10^5	1.1×10^5

Table 4: Show the Characteristics of Bacterial and Fungi Isolates

Bacteria Isolates	Colony Morphology	Microscopic Morphology	Gram reaction	Catalase Test	Citrate test	Oxidase Test	Methyred Test	Nitrate Test	Indole Test	Voges Prokauer	Urease	Coagulase Test
1	Yellow Colonies	Cocci in grap-like Cluster	+	+	-	-	+	+	-	+	+	A
2	Very large greenish colonies	Straight rod in single	+	+	+	+	-	V	-	+	-	-
3	Greyish colonies	Rod shaped	-	+	-	-	+	+	+	-	-	-
4	Mucoid greyish colonies	Rod shaped	-	+	+	-	-	+	-	+	+	-
5	Greyish white colonies	Rod shaped	-	+	+	-	+	+	-	-	-	-
6	Large oval colonies	Rod shaped	-	+	-	+	-	+	-	-	+	-
7	Round greenish colonies	Curved rod	-	+	+	+	+	+	+	+	+	-

Table 4: Continued

Bacteria Isolates	Colony Morphology	Microscopic Morphology	Motility Test	Lactose	Maltose	Glucose	Sucrose	Manitol	Probable Oganism
1	Yellow Colonies	Cocci in grap-like Cluster	G	A	A	A	A	A	*Staphylococcus aureus*
2	Very large greenish colonies	Straight rod in single	+	-	A G	A G	A G	A G	*Baccillus* spp
3	Greyish colonies	Rod shaped	+	A G	A G	A G	A G	A G	*Escherichia coli*
4	Mucoid greyish colonies	Rod shaped	-	A G	A	A G	A G	A G	*Klebsiella* spp
5	Greyish white colonies	Rod shaped	+	A G	A G	A G	A G	A G	*Salmonella* spp
6	Large oval colonies	Rod shaped	+	-	-	A	-	-	*Pseudomonas* spp
7	Round greenish colonies	Curved rod	+	-	A G	A	-	A	*Vibro* spp

KEY. + = Positive reaction, _ = Negative reaction, A = Acid production, G = Gas production, v = variable.

Fungi Isolate Identity	Colony Morphology	Microscopic Morphology	Probable Identity
1	Granular to wooly Colonies that have some shade of yellow	Long conidiophores	*Aspergillus Spp.*
2	Greenish white, Irregular Colonies	Septate hyphae, conidia arranged like mob-head	*Penicillium Spp.*

Key. NG = No significant growth, A and B = Branded Soymilk C and D = Unbranded soy milk (in used bottles), E and F = Soy milk wrapped in transparent nylon bags.

CONCLUSION

Control of microbial spoilage in soy milk can be achieved by restriction and controlling microorganisms from contaminating the product through good manufacturing and handling practices.

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