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Evaluation of Microbiology Quality of Some Soybean Milk Products Consumed in Nigeria

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Abstract: The research is aimed at evaluation of microbiological quality of soybean milk highly consumed by the public in Nigeria. To verify the hygenicity, purity and safety of the Soybean consumed by the public in Nigeria. Ten samples of soybean milk locally prepared by different manufacturers were used for the study. The microbial load and identity of the microorganisms present were determined using the streak plate technique of isolation. Bacterial and fungal viable counts, biochemical characters of fungi present, the starch hydrolysis test and microscopic characters of the isolates were done using standard techniques. The microbial population detected in terms of number and types of organisms reflected poor hygienic standard of production, constituting a public health hazard among the populace. The products were found to contain pathogenic microorganisms like *Staphylococcus aureus, E. coli,* some fungi (*Candida* species) were also found in some of the Soybean products. The implication of this study is that the quality of some commercial soybean milk preparations marketed in Nigeria needs to be critically scrutinized to avoid transmission of infections to patients through them. Also it is very necessary that producers should be enlightened about Good Manufacturing Practices (GMP) as this will ensure products free of pathogenic microorganisms.

Key words: Soybean milk, microbiological content, public, *Staphylococcus aureus*, *E. coli, Candida* species

INTRODUCTION

Physical stability of pharmaceutical products may affect patient acceptance as well as diminish efficacy of the active ingredients (Chukwu, 2002). Microbial stability of pharmaceutical products affects patient acceptance of the product, because many drug products can support microbial growth when contaminated by bacteria, mould, yeast etc since most of them are pathogenic (Vincent, 2005). Control of microbial growth and spoilage of product is achieved by restricting and controlling microorganisms from contaminating the product through good manufacture and handling practice (Chukwu, 2002).

Soybean milk is one of the food preparations produced from the activity of micro organisms (Adegoke *et al*., 2002). Soybeans are an excellent source of protein both in quality and quantity. Approximately 35-40% of the total dry matter content of the whole soybeans is protein whereas the cowpeas contain only 23 to 24% protein. Soybeans contribute approximately 20% fat to the diet (Ayo *et al*., 2011). The fat from the soybean is unsaturated type unlike saturated fats from animal origin and hence is good for heart disease patients (Adegoke *et al*., 2002). Other than the high protein content, it also has good amount of calories and fat. Soya bean contains 43 g of protein per 100 g which is the highest among the pulses. It also contains 19.5 g of fat, 21 g carbohydrate and provides 432 kcal/100 g (Shurtlef and Agagi, 2000). The protein of soybean contains all the essential amino acids in adequate amount except methionine and cystine. It is one of the best vegetarian food items as far as protein content is concerned, it is a good source of riboflavin (Adegoke *et al*., 2002). Soybean contains a factor that inhibits the action of the digestive enzyme trypsin and this factor can be destroyed by heat (Farinde *et al*., 2010). Soybean should be cooked well for digestion and absorption. Studies have shown that in type II hypercholesterolemia, patients already on low lipid, low cholesterol diets, eight weeks substitution of animal protein by soybean protein reduced plasma cholesterol by 23 to 25% (Adegoke *et al*., 2000).

Pathogenic microorganisms are microorganisms capable of causing disease, although they represent only a small part in the total microbial world; they receive much

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attention because they represent a threat to the human or animal health and to agriculture (Onuorah *et al*., 2007). Pathogenic microorganisms can cause disease of plague dimensions with serious economic and environmental consequences (Twizeyimana *et al*., 2009). Pathogenicity represents a form of versatility and specialization that enables certain microorganisms to replicate within a specific host (infectivity) and such hosts show a sign of disease or eventually die (Rex *et al*., 2001).

The outcome of the infection is dependent on the properties (virulence, invasiveness, toxicity or allergic effects) of the organism but also upon the host's immune status (Vincent, 2005). Pathogens fall into two basic types: primary pathogens that cause disease among at least a portion of normal individuals and opportunistic pathogens that cause disease only in individuals who are compromised in either their innate or humoral immune defences (Vincent, 2005). Pathogenic bacteria include, *Salmonella* spp, *Clostridium botulism, Staphylococcus* and *Shigella* spp.

MATERIALS AND METHODS

Brands of soybean milk preparations used: The soybeans preparations employed in this study were produced locally in Nsukka and purchased from Nsukka central Market and were fresh supplies from the local manufacturers. A total of 10 brands were used in the study. Below is the tabular representation of the various soybean milk preparations used.

S/N	1	2	3	4	5	6	7	8	9	10
Sample	A	B	C	D	E	F	G	H	I	J

Reagents: Crystal violet (gentian violet) 0.5% in water (M and B England). Basic safranin (Merck, Germany), Alcohol (BDH, England), Iodine reagent (BDH, England). Immersion oil (CVI, Nigeria Ltd.), Malachite green (M and 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., UK), Maltose powder (Oxoid Ltd., U.K), Glucose powder (Oxoid Ltd., UK), Sucrose powder (Oxoid Ltd., UK), Galactose powder (Oxoid Ltd., UK), 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 Kenol laboratories, Nsukka.

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: The streak plate technique of isolation was used. The method was effectively used to detect the cultural characteristics of the various organisms isolated.

A sterile inoculating loop was used to collect a loop -full of sample from the 10 fold dilution of each sample and the suspension was spread over a small area of strokes. The streaking with the sterile loop was repeated three times to obtain a Zig-zag stroke which was finally made from the final series of strokes to the middle of the plate.

The petri-dish was inverted and incubated at 37°C for 24 h. This procedure was repeated for each of the 10 samples from each soybean milk brand. In situations where more than one type of organisms were found growing on the same plate, each of the organisms was isolated and subjected to micro gram staining.

The following standard biochemical tests were also carried out to characterize the isolated microorganisms: Carbohydrate Fermentation Test, Citrate Utilization Test, Motility Test, Methyl Red Test, Voges Proskauer Test, Nitrate Reduction, Gelatin Hydrolysis, Coagulase Test and Starch Hydrolysis Test, all these tests were carried out according to established standard (Onuorah *et al*., 2007).

RESULTS

Bacterial and fungal viable counts: From the result in the Table 1, it is very clear that the various soybeans milk samples contained large amounts bacteria mL^{-1} of the preparation. It could also be seen that samples A and B contain fungal cells while other samples do not. The table also shows that the bacterial counts were generally more than the fungal counts.

The general bacterial count obtained for every sample analyzed exceeded the acceptable limit for both pasteurized (3×10^4 CFU mL^{-1} and less than 10 coliforms) and ultrahigh temperature treated milk (Rex *et al*., 2001).

Table 2 indicated biochemical characters of fungi present.

Different species of the genes *Candida* were present in samples A and B containing yeasts. *Candida guilliermondii* were dominant and found in sample B. they fermented all sugars except maltose and lactose. The colonies are flat, soft with entire edge and off white shade.

The other *Candida species* found in sample A_{II} was *Candida albicans* with raised colonies, smooth and soft with creamy white or light yellow colour and an entire edge.

Table 1: Microbial viable counts of the various soybean milk brands

Sample	A	B	C	D	E	F	G	H	I	J
Av. Bacterial cells (CFU mL^{-1})	6.6×10^{7}	6.2×10^{7}	9.2×10^{7}	1.58×10^{8}	2.40×10^{7}	1.40×10^{8}	1.61×10^{8}	8.5×10^{7}	6.13×10^{7}	5.66×10^{7}
Av. Fungal cells (CFU mL^{-1})	1.32×10^{2}	1.25×10^{2}	-	-	-	-	-	-	-	-

The values obtained for the microbial counts were a mean of eight counts

Table 2: Biochemical characters of fungi present

Sample Code	Glucose	Maltose	Sucrose	Lactose	Galactose	Inference
A_I	G	O	O	O	O	*Candida krusei*
A_{II}	G	G	O	O	G/W	*Candida albicans*
B_I	G	O	G/W	O	G/W	*Candida guilliermondii*
B_{II}	G	O	G/W	O	G/W	*Candida guilliermondii*

Key G: Growth, meaning that the organism fermented the sugar, O: Sugar not fermented, G/W: The organism ferments this sugar, but the growth is weak

Table 3: Cultural characteristics of organisms isolated from the soybean milk sample

Sample Code	Shape	Edge	Opacity	Colour	Texture
A_I	Circular	Entire	Transparent	Creamy white	Butyrous
A_{II}	Circular	Entire	Transparent	Creamy white	Butyrous
A_{III}	Circular	Entire	Transparent	Creamy white	Butyrous
B_I	Circular	Entire	Opaque	Golden yellow	Butyrous
B_{II}	Circular	Irregular	Opaque	Creamy white	Granular
C_I	Circular	Entire	Transparent	Creamy white	Butyrous
C_{II}	Circular	Entire	Transparent	Creamy white	Butyrous
C_{III}	Circular	Entire	Transparent	Creamy white	Butyrous
D_I	Circular	Entire	Opaque	Golden yellow	Butyrous
D_{II}	Circular	Entire	Opaque	Golden yellow	Butyrous
D_{III}	Circular	Irregular	Opaque	Creamy white	Granular
E_I	Circular	Mucoid	Transparent	Creamy white	Smooth
E_{II}	Circular	Mucoid	Transparent	Creamy white	Smooth
E_{III}	Circular	Entire	Transparent	Creamy white	Butyrous
F_I	Circular	Mucoid	Transparent	Creamy white	Butyrous
F_{II}	Circular	Entire	Transparent	Creamy white	Smooth
G_I	Circular	Entire	Transparent	Creamy white	Butyrous
G_{II}	Circular	Entire	Transparent	Creamy white	Butyrous
H_I	Circular	Entire	Transparent	Creamy white	Butyrous
H_{II}	Circular	Entire	Transparent	Creamy white	Butyrous
I_I	Circular	Irregular	Opaque	Creamy white	Granular
I_{II}	Circular	Mucoid	Transparent	Creamy white	Smooth
I_{III}	Circular	Entire	Transparent	Creamy white	Butyrous
J_I	Circular	Irregular	Opaque	Creamy white	Granular
J_{II}	Circular	Irregular	Opaque	Creamy white	Granular

Key I: First isolate present in a mixture of organisms in a particular preparation, ii: Second isolate present in a mixture of organisms in a particular preparation, iii: Third isolate present in a mixture of organisms in a particular preparation

Table 4: Biochemical characters of Gram positive bacteria present

Sample code	Nitrate reduction	Glucose fermentation	Mannitol fermentation	Citrate utilization	Starch	Inference
B_I	+	A	-	-	-	*Staphylococcus aureus*
B_{II}	+	A	A	+	+	*Bacillus subtilis*
D_I	+	+	+	-		*Staphylococcus aureus*
D_{II}	+	A	-	-	-	*Staphylococcus aureus*
D_{III}	+	AG	AG	-	-	*Bacillus cereus*
I_I	+	A	-	+	-	*Bacillus subtilis*
J_I	+	A	A	+	+	*Bacillus subtilis*
J_{II}	+	A	-	+	-	*Bacillus cereus*

Key A: Acid produced from either mannitol or glucose, Ag: Acid and gas produced, +: Positive, -: Negative

Sample A_I contained *Candida krusei* with flat colonies (Twizeyimana *et al*., 2009).

Cultural characterization: From the 10 fold dilution of the various soybeans milk preparation inoculated on sterile nutrient agar plates and evenly spread, various cultures of the organisms were observed. The observed organisms and their characters are characteristic of the organisms are shown in Table 3.

There is a presence of more than one type of organism in some of the preparations. Odour characteristics of *Staphylococcus* were observed in Sample B and D. No. greenish colour was observed suggesting the absence of *Pseudomonas species*. Creamy white colour and circular shape are characteristics of some *Candida* species in yeast form (Onuorah *et al*., 2007).

Table 4 shows that samples B, D, I and J contained some Gram positives.

Sample B contained two different types of organisms *Staphylococcus aureus* and *Bacillus subtilis*. *Staphylococcus aureus* has the ability to reduce nitrates to nitrites; produce acid in a variety of carbohydrate, ferment mannitol and is coagulase negative. *Bacillus subtilis* on the other hand produces acid from nitrates, hydrolyzed starch and produces acid from mannitol.

Sample D had two different species of *Staphylococcus* (*aureus* and *epidermidis*) and a species of *Bacillus polymixia*. The two species of were differentiated by the ability of *Staphylococcus aureus* which is coagulase positive to ferment mannitol while *Staphylococcus epidermidis* is coagulasae negative and does not ferment mannitol. Sample I had *Bacillus cereus* that has the ability to liquefy gelatin rapidly, does not produce acid from mannitol and produces nitrite from nitrates (Rex *et al*., 2001).

Starch hydrolysis test: The ability of the organisms to utilize starch as a source of carbohydrate as determined by the starch hydrolysis test is presented in Table 5.

From the result obtained, only isolates from sample B (B_{II}), Sample I (I_I) and Sample J (J_I) were able to hydrolyze starch detected by the presence of a clear zone around the point where the organism was inoculated. In others, there were no clear zones. This was due to the presence of unhydrolyzed starch showing that the organism did not use starch as a source of carbohydrate. The organism in samples mentioned above was found to be *Bacillus subtilis*. This is because the organism has ability to ferment or hydrolyze starch.

Table 5: Result of the starch hydrolysis test

Sample NO	B_I	B^{II}	D_I	D_{II}	D_{III}	I_I	I_{II}	I_{III}	J_I	J_{II}
Starch hydrolysis	-	+	-	-	-	-	-	-	+	-

Microscopy test: From the observations made from the colonies of the organisms on the solid media, some of the soybean milk preparation showed growth of one type of organisms.

The result of the microscopic characters of the various isolates is shown in Table 6. The result shows presence of Gram negative and Gram positive bacteria. The organisms were basically rods, singly dispersed, clustered and few in chains together with some oval shaped organisms which were yeasts indicated by their possession of buds (Rex *et al*., 2001).

Clusters of cocci were also observed indicating the presence of *Staphylococcus* spp.

Biochemical tests: From the Table 7, it can be observed that majority of the soybean milk samples were contaminated by the Gram negative bacteria of the family Enterobacteriaceae. Each sample has different contaminants. Sample H and I and the same contaminants *E. coli* and *Enterobacter* spp. but different species of Enterobacteria Sample H contained species *aerogenes* while sample I contained species cloacae. They differ by the ability of the species aerogenes to ferment glycerol while cloacae does not ferment glycerol . Also samples E_I, E_{II} and F contained *Klebsiella pneumoniae*.

Table 6: Microscopic Characters of the Isolates

Sample code	Shape	Color	Arrangement	Gram character	Presence of spores
A_I	Short rods	Red	Singly dispersed	-ve	-ve
A_{II}	Short rods	Red	Singly dispersed	-ve	-ve
A_{III}	Short rods	Red	Singly dispersed	-ve	-ve
B_I	Cocci	Purple	Clusters	+ve	-ve
B_{II}	Rods	Purple	Chains	+ve	+ve
C_I	Short rods	Red	Singly dispersed	-ve	-ve
C_{II}	Short rods	Red	Singly dispersed	-ve	-ve
C_{III}	Short rods	Red	Singly dispersed	-ve	-ve
D_I	Cocci	Purple	Clusters	+ve	-ve
D_{II}	Cocci	Purple	Clusters	+ve	-ve
D_{III}	Rod	Purple	Chain	+ve	+ve
E_I	Rod	Red	Singly	-ve	-ve
E_{II}	Rod	Red	Singly	-ve	-ve
E_{III}	Rod	Red	Singly	-ve	-ve
F_I	Short rods	Red	Singly	-ve	-ve
F_{II}	Rod	Red	Transparent	-ve	-ve
G_I	Short rods	Red	Transparent	-ve	-ve
G_{II}	Short rods	Red	Transparent	-ve	-ve
H_I	Short rods	Red	Transparent	-ve	-ve
H_{II}	Short rods	Red	Transparent	-ve	-ve
I_I	Rod	Purple	Opaque	+ve	+ve
I_{II}	Rod	Purple	Transparent	-ve	-ve
I_{III}	Rod	Red	Transparent	-ve	-ve
J_I	Rod	Purple	Opaque	+ve	+ve
J_{II}	Rod	Purple	Opaque	+ve	+ve

Table 7: Biochemical characters of Gram-negative bacteria present

Sample No.	Lactose fermentation	Motility	Citrate utilization	Methyl red	V.P	Inference
A_I	+ve	+ve	-ve	+ve	-ve	*E. coli*
A_{II}	+ve	+ve	-ve	+ve	-ve	*E. coli*
A_{III}	+ve	+ve	-ve	-ve	+ve	*Enterobacteraerogenes*
C_I	+	+	+	-	-	*Enterobacteraerogenes*
C_{II}	+	+	-	+	-	*E. coli*
C_{III}	+	+	+	-	-	*Enterobacteraerogenes*
E_I	+	-	+	-	-	*Klebsiella pneumoniae*
E_{II}	+	-	+	-	+	*Klebsiella pneumonia*
E_{III}	+	+	-	-	+	*Enterobacteraerogenes*
F_I	+	-	+	+	-	*Klebsiella pneumonia*
F_{II}	+	+	-	+	-	*E. coli*
G_I	+	+	-	+	-	*E. coli*
G_{II}	+	+	+	-	-	*Enterobacteraerogenes*
H_I	+	+	-	-	+	*Enterobacteraerogenes*
H_{II}	+	+	-	+	-	*E. coli*
I_I	+	-	+	-	+	*Klebsiella pneumonia*
I_{II}	+	+	+	-	-	*Enterobacteraerogenes*

DISCUSSION

The attendant increase in the rate of soybean milk consumption due to its high protein content has encouraged low scale production of the milk under household condition with little or no regard to quality control measures (Chukwu, 2002).

All the soybean milk samples used in the study contained one form of living microorganism or the other. Each sample contained different contaminants. The ubiquity in the hawking of locally produced soybean milk, packaged in different forms was considered a public health concern (Rex *et al*., 2001).

The following Microorganisms were detected *E. coli* and other faecal coliform like *Klebsiella pneumoniae, Enterobacter aerogenes* in the soybean milk samples tested. *Staphylococcus aureus* is known to be pathogenic while *Staphylococcus epidermidis* is less pathogenic. Considering the notoriety of the resistance of *Staphylococcus aureus* to methicillin, other penicillins and cephalosporins, its detection regularly in the soybean milk sample analysed, poses a serious health hazard to the consumers.

Large population of the *Candida* species could result to infection. The consumption of large amount of the *Candida* species in some of these products could change the normal flora which may lead to Candidiasis-vaginal thrush and Candidiasis of the colon. The large population of fungi in these products from the counts observed confirms that the soybeans sample products considered for this research were of low microbial quality and may pose serious health hazard to consumers.

It is concluded from this research that most of the soybean products marketed in Nigeria needs to pass through NAFDAC registration in order to ascertain and improve on the microbiological quality of the products.

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