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Evaluation of microbial content of some soybean milk products consumed in Nigeria

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Abstract
Evaluating the microbiological content of soybean milk, highly consumed by the public is the aim of this research work. 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 standard techniques. The microbial population detected in terms of number and types 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* and *E. coli.* Some fungi (*Candida species*) were also found in few 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 Practice (GMP) as this will ensure products free of pathogenic microorganisms.

Keywords: Soybean milk; Microbial content; *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 (Ofoefule, 2002). Microbial stability of pharmaceutical products also affects patient acceptance 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 of microorganism through good manufacture and handling practice (Ofoefule, 2002).

Soybean milk is one of the food preparations produced from the activity of microorganisms (Adegoke *et al.*, 2002). Soybeans are an excellent source of protein both in quality and quantity. Approximately 35 - 40 percent 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 (Liu, 1997). 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

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bean contains 43 grams of protein per 100g, which is the highest among the peas. It also contains 19.5g of fat, 21g carbohydrate and provides 432 kcal per 100g (Shurtlef and Aogagi, 2000). The protein of soybean contains all the essential amino acids in adequate amount except methionine and cystine (Liu, 1997). Since it has majority of essential amino acids, 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 (Willikins *et al.*, 1967). 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 percent (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 attention because they represent a threat to the human or animal health and to agriculture (Ebim and Ofoefule, 1997). 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 (Odds *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 (Rex *et al.*, 2001). 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 (Rex *et al.*, 2001). Pathogenic bacteria include, *Salmonella* spp., *Clostridium botulism*, *Staphylococcus* and *Shigella* spp.

EXPERIMENTAL

Brands of soya bean milk preparations. 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 ten (10) brands were used in the study. Below is the tabular representation of the various soybean milk preparations used.

Culture media. The culture media used included 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 culture characterization. The streak plate technique of isolation was used. The method was effectively used to detect the culture 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 37^0C for 24 hours. This procedure was repeated for each of the 10 samples from each soybean milk brand. In situations where more than one type of organism was 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 (Rex *et al.*, 2001).

RESULTS

Bacterial and fungal viable counts. From the result in Table II, it is very clear that the various soya bean milk samples contained large amounts bacteria per ml 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 and less than 10 coliforms) and ultrahigh temperature treated milk (Odds *et al.*, 2001). Different species of the genes *Candida* were present in samples A and B containing yeasts. *Candida guilliermondi* 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. Sample A_I contained *Candida krusei* with flat colonies (Twizeyimana *et al.*, 2009).

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 IV.

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 (Ebim and Ofoefule, 1997).

Table V 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 (Odds *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 VI.

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.

Table I

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

Table II: Microbial viable counts of the various soybean milk brands.

Sample	A	B	C	D	E	F	G	H	I	J
Ave. bacterial cell (cfu/ml)	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
Ave. fungal cells(cfu/ml)	1.32×10^2	1.25×10^2	-	-	-	-	-	-	-	-

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

Table III: 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 guilerimondii*
B_{II}	G	O	G/W	O	G/W	*Candida guilerimondii*

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 IV: Culture characteristics of organisms isolated from the soyabean 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	Muciod	Transparent	Creamy White	Smooth
E_{II}	Circular	Muciod	Transparent	Creamy White	Smooth
E_{III}	Circular	Entire	Transparent	Creamy White	Butyrous
F_I	Circular	Muciod	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	Muciod	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 V: 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

Table VI: 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	-	+	-	-	-	-	-	-	+	-

Table VII: Microscopic Characters of the Isolates

Sample Code	Shape	Colour	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

The result shows presence of Gram negative and Gram positive bacteria. The organisms

Table VIII **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	*Enterobacter aerogenes*
C_I	+	+	+	-	-	*Enterobacter aerogenes*
C_{II}	+	+	-	+	-	*E. coli*
C_{III}	+	+	+	-	-	*Enterobacter aerogenes*
E_I	+	-	+	-	-	*Klebsiella pneumonia*
E_{II}	+	-	+	-	+	*Klebsiella pneumonia*
E_{III}	+	+	-	-	+	*Enterobacter aerogenes*
F_I	+	-	+	+	-	*Klebsiella pneumonia*
F_{II}	+	+	-	+	-	*E. coli*
G_I	+	+	-	+	-	*E. coli*
G_{II}	+	+	+	-	-	*Enterobacter aerogenes*
H_I	+	+	-	-	+	*Enterobacter aerogenes*
H_{II}	+	+	-	+	-	*E. coli*
I_I	+	-	+	-	+	*Klebsiella pneumonia*
I_{II}	+	+	+	-	-	*Enterobacter aerogenes*

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.

Microscopy test. From the observations made from the colonies of the organisms on the solid media, some of the soyabean milk preparation showed growth of one type of organisms. The result of the microscopic characters of the various isolates is shown in Table VII.

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, it can be observed that majority of the soyabean milk samples were contaminated by the Gram negative bacteria of the family enterobacteriaceace. 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 specieis aerogenes while sample I contained species cloacae. They differ by the ability of the species aerogenes to ferment glycerol while cloacae do not ferment glycerol [9]. Also samples E_I, E_{II} and F contained *Klebsiella pneumoniae.*

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 (Ebim and Ofoefule, 1997). 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 (Odds *et al.*, 2001).

The following Microorganisms were detected *E. coli* and other faecal coliform like *klebsiella pneunoniae, 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 (Warnock, 2001). The large population of fungi in these products from the counts observed confirms that the soybeans sample products considered for this research work 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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