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Studies on some microorganisms associated with exposed Soyabean milk

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ABSTRACT

Samples of soyabean milk were extracted from the seeds of soyabeans (*Glycine max*). The microorganisms isolated from the exposed samples included *Bacillus subtilis, B. cereus, Lactobacillus spp, Staphyloccus aureus, Mortierella wolfii, Candida pseudotropicalis, Saccharomyces cerevisiae* and *S. fubiliger*. These microorganisms rendered the soyabean milk unpalatable and unsafe for consumption by the production of toxic metabolites. The unexposed samples had relatively lower load of microorganisms. There was significant difference in the pH values of the exposed samples as compared to the unexposed samples.

Key words: Microorganisms, Soybean (*Glycine max*).

INTRODUCTION

The soybean (*Glycine max* (L) Merrill) belongs to the family Fabaceae (Norman, 1963). It is a crop of world wide economic importance that has a higher percentage of protein than many other foodstuffs and the protein is of higher biological value. The seeds are also a rich source of calcium, iron and vitamins especially of the B-Complex type (Oyenuga, 1968). Researchers have reported that soybean has high protein containing an average of between 40-43% by weight. Soybean milk contains antioxidants. These compounds protect cells from damage that is caused by free radicals. These free radicals are believed to be responsible for many cancers and premature ageing. This is due to antioxidant effects of genistein, one active component of soybeans (Binghan, 1996). The fat content and fatty acid compostion vary among soybean varieties (Ezeagu, *et al.,* 1996).

One economic way of preventing undernourishment in many people, especially in developing countries is to consume protein in the form of soybean milk. Popularization of the excellent nutritional quantities of home consumption of soybean milk had had little success because of the unpleasant beany or nutty taste produced regardless of the mode of preparation of the milk. Soaking and boiling (blanching), during soybean preparation inactivate the enzyme linpoxygenase that produces the volatile ethyl vinyl ketone responsible for the undesirable flavour (Ferrier, 1975). Although, in recent years, in almost all the continents of the world, more suitable methods of preparation of soybean milk that are void of the beany, nutty or unpleasant flavour have been developed.

Microorganisms differ greatly according to their abilities to decompose different compounds and to synthesize compounds from simple inorganic elements. The plant milks are rich in nutrients and therefore represent an ideal growth environment for a variety of microorganisms (Hayes, 1981).

In view of the remarkable increase in the number of people that consume soybean milk in the world in recent years, it became necessary to survey the microorganisms associated with exposed soybean milk.

MATERIALS AND METHODS

Soybean milk extraction

The seeds of soybean (*Glycine max*) were collected in Jos, Plateau State, Nigeria. 200g of soybeans were soaked in clean water for 6 hours. The soybeans were boiled for 20 minutes. The steamed beans were ground in a grinder. 5 parts of boiling water was added to one part of the ground soybeans and stirred with the aid of a sterilized spoon. The liquid was simmered till a thick white milk was obtained. All the liquid (milk) was then squeezed out with a piece of clean muslin cloth into a measuring jar to give a smooth textured milk.

Exposure of milk sample

A volume of 300ml of soybean milk was measured out into 3 separate sets of sterilized conical flasks, each of the sterile conical flasks receiving equal quantity of the milk (i.e each set contains 3 conical flasks). The three sets of conical flasks containing the milk were labeled S_1, S_2 and S_3. The samples were then exposed at three different sites: Botany laboratory, Botany laboratory preparatory room and Biochemistry laboratory of the University of Jos, Nigeria.

The exposed milk samples (S_1, S_2 and S_3) were plated out on Potato Dextrose Agar (PDA) and Nutrient Agar (NA). The plates were divided into 3 batches. The first, second and third batches were incubated at 25°C, 37°C and 45°C respectively for the isolation of mesophilic, thermotorelant and thermophilic fungi and other microorganisms respectively.

Isolation of microbial species and measurement of pH

The culture plates were examined after 24 and 48 hours for the presence of bacteria and yeast. The plates were examined after 4-7 days for the presence of fungi. All the culture plates were re-examined a week later for the development of new species of microorganisms. The experiment was repeated with the other conical flask samples belonging to the same set of flasks (i.e S_1, S_2 & S_3).

Table - 1 : Frequency of occurrence of microbial isolates in experimental exposed and unexposed milk samples at 25°C

S. No.	Microbial Isolates Time-48 Hours	Site I SM		Site II SM		Site III SM		Total SM	
		E	U	E	U	E	U	E	U
1.	*Bacillus subtilis*	+	+	+	+	+	+	3	3
2.	*Bacillus cereus*	+	+	-	+	-	-	1	2
3.	*Lactobacillus sp*	+	+	+	+	+	+	3	3
4.	*Staphylococcus aureus*	+	-	-	-	-	-	1	0
5.	*Aspergillus flavus*	+	+	-	-	-	-	1	1
6.	*A. niger*	-	-	-	+	-	-	0	1
7.	*Fusarium solani*	+	-	-	-	-	-	1	0
8.	*Mortierella wolfii*	+	-	+	+	+	-	3	1
9.	*Candida pseudotropalis*	+	-	-	+	+	-	2	1
10.	*Saccharomyces cerevisiae*	+	+	+	+	+	+	3	3
11.	*S. fubiliger*	+	-	+	-	-	-	2	0
	Total	10	5	5	7	5	3	35	

* SM - Soybean milk + - Present Total* - - Absent
3 - 100% occurrence E- Exposed Sample
2 - 66.7% occurrence U- Unexposed Sample
1 - 33.3%occurence

The non-exposed milk samples were also plated as controls. A volume of 10ml of 0.013% (w/v) of streptomycin sulphate solution was added to 100ml of Potato Dextrose Agar in order to suppress the growth of bacteria. The colonies of microorganisms were subcultured several times until pure cultures were obtained. The microbial isolates were identified with the aid of the microscope and the use of suitable biochemical tests and with reference made to stock cultures in the Department of Botany, University of Jos. The pH values of the unexposed milk samples were determined with the aid of corning digital pH meter model 7.

RESULTS AND DISCUSSION

The method of the soybean preparation reduced the unpleasant beany flavour or taste to the bearest minimum. More water could be added to the milk if necessary. Some people fortify the soybean milk by addition of sugar (7g per 100g milk), calcium salt, sodium and potassium, phosphates, and all essential vitamins in the same concentrations as in cow's milk. It is also fortified with iron and vitamin D. The milk is homogenized and steamed for 30 minutes, cooled, filled in bottles and kept in refrigerator till distributed or served. The exposed milk samples showed higher microbial load than the unexposed milk samples (Tables 1 and 3).

The microbial isolates from the exposed milk samples included *Aspergilus flavus, A. niger, Fusarium solani and Mortierella wolfii* of fungi species, while the yeast isolated included *Saccharomyces cerevisiae, S. fubiligera and Candida pseudotropicalis.* The bacteria species included *Bacillus subtilis, B. cereus, Lactobacillus* spp. and *Staphylococcus aureus.*

Table 1 shows the frequency of occurrence of the exposed and unexposed milk samples at 25°C. The average pH values of the fresh milk samples was 6.3, while the change in the average pH values of the milk samples following exposure at room temperature (i.e 25°C) for a period of 48hours were 5.5 and 4.2 for 24 hours and 48 hours respectively. The quality of the plant milk stored in the refrigerator was maintained. Also, there was no change in the pH of samples of plant milk stored in the refrigerator. Table 2 shows the biochemical tests

Table - 2: Biochemical tests used in the identification of bacteria isolates

Bacterial isolates	Cell shape	Gram reacton	Presence of spores	Types of tests used: Starch hydrolysis	Catalase	Methyl red	Glucose	Mannitol	Mortality	Sucrose	Coagulase	Probable identity of the isolates
I	R	+	+	-	+	-	A	-	-	+	NT	*B. cereus*
II	R	+	-	-	+	-	A	+	+	+	NT	*B. subtilis*
III	R	+	+	+	-	+	A	+	-	+	NT	*Lactobacillus spp*
IV	C	+	-	-	+	-	A	+	-	NT	+	*S. aureus*

+ Positive C - Cocci S - Soybean Milk
- Negative A - Acid
R Rod NT - Not Tested

Table - 3: Bacterial (cfu/ml) of the Soybean milk samples

	Soybean milk	
	Exposed	**Unexposed**
	2.4 x 10 ^{3}*	0.4 x 10^{3}*
Bacteria	3.5 x 10^{3}*	0.6 x 10^{3}*
Average	4.2 x 10^{3}	0.5 x 10^{3}

* Figures represent average of duplicates

used in the identification of bacteria isolates. Table 3 shows the bacterial load of both the exposed and unexposed samples of the soybean milk.

The microbial isolates in the soybean milk samples may have stemmed from the exposure of the milk samples to the aerial environment. If there is no proper storage after preparation, the soybean milk could be contaminated by microorganisms. It is possible for these microorganisms to increase to undesirable levels thereby rendering the milk unpalatable by the production of toxic metabolites. Some of these microorganisms are known to be of public health significance as some are capable of causing diseases. For instance, the bacteria *Bacillus subtilis* which is known to cause food poisoning, *Aspergillus flavus* and *Fusarium solani* produce mycotoxins (Dienner and Davis, 1969).

Low temperature is used to retard chemical reactions and to slow down or stop growth and activity of most microorganisms in food. The lower the temperature, the slower will be the chemical reactions, enzyme actions and microbial growth. Therefore, when soybean milk is stored at low temperature (i.e in the refrigerator), the growth of microorganisms that cause spoilage is prevented and the quality of the milk is maintained.

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