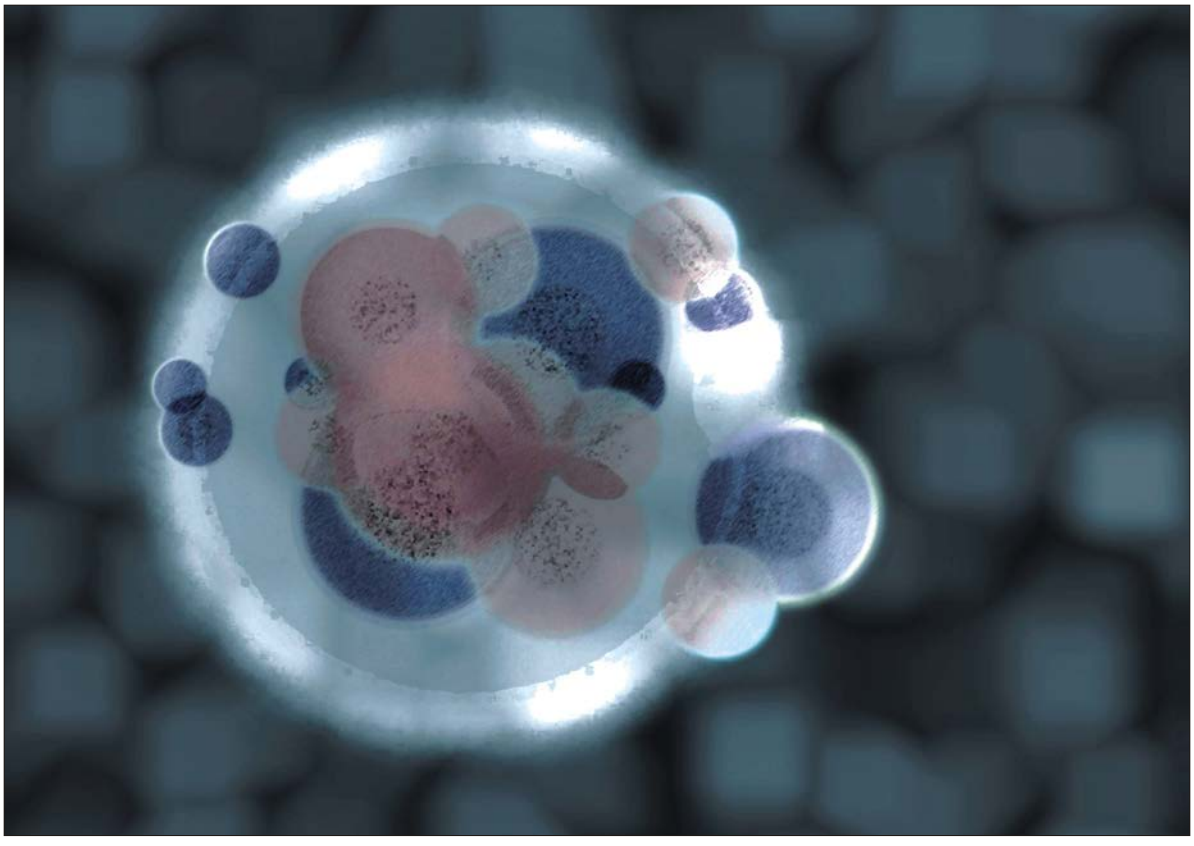

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Studies on Some Microorganisms Associated with Exposed Tigernut (*Cyperus esculentus* L.) Milk

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Abstract: Samples of tigernut milk were extracted from tubers of tigernut (*Cyperus esculentus*). The microorganisms isolated from the exposed samples included *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus flavus*, *A. niger*, *Fusarium solani*, *Saccharomyces cerevisiae*, *S. fubiligera* and *Candida pseudotropicalis*. The microbial isolated had the following percentage frequencies of occurrence, respectively: 13.04, 17.39, 4.35, 13.04, 13.04, 21.74, 13.04 and 4.35%. These microorganisms rendered the tigernut milk unpalatable and unsafe for consumption by the production of toxic metabolites. The unexposed samples had relatively lower load of microorganisms. The difference between the pH values of the exposed and unexposed milk was significant.

Key words: Microorganisms, *Cyperus esculentus*, tigernut milk, exposed milk samples, unexposed milk samples

INTRODUCTION

Tigernut (*Cyperus esculentus*) belongs to the family Cyperaccae (Uva *et al*., 1997). It is a tuber that grows freely and is consumed widely in Nigeria and in various other parts of West and East Africa (Abaejoh *et al*., 2006). As a food, tigernut can be eaten either unprepared or soaked in water. It is however fried and eaten mixed with roasted groundnuts (Abaejoh *et al*., 2006). Kofi (1990) reported that sweetened tigernut extract is curdled by boiling, kept in glass bottles or jars uncovered and sold in shops and markets in Ghana. According to Ojobe and Tempo (1983) the protein in tigernut is of high biological value considering the many essential amino acids that it contains.

Tigernut tubers contain myristic acid as the main saturated acid and oleic acid as the predominant unsaturated acid. Linoleic acid was present in the sample to the extent of 8.8-27.4% (Eteshola and Oraedu, 1996). Tigernut is also used as feed for livestock. Bamgbose *et al.* (1997) reported the effect of feeding tigernut meal on the performance of rabbits. Tigernut can be used to supplement maize and other foods or feeding stuff, which may be deficient in Iysine. This supplementation process can be useful in the preparation of protein concentrates for man and livestock, since tigernut can be grown fairly well on poor soils (Hayes, 1981).

The tigernut milk is rich in nutrients (Abaejoh *et al*., 2006). Despite high nutritive value of this milk, its production in Nigeria has been hampered due to the deteriorating effects of some microorganisms on the milk (Abaejoh *et al*., 2006). The present study is therefore aimed at carrying out a survey on the microorganisms associated with tigernut milk deterioration in Nigeria and factors that influence their spread on the milk.

MATERIALS AND METHODS

Extraction of tigernut milk: Healthy large brown varieties of tigernuts were collected from Jos, Plateau State, Nigeria in June 2006 and the survey conducted between July and August 2006. Two hundred gram of this were weighed out into a clean container. The tigernuts were thoroughly washed in three changes of sterile water. The washed tigernuts were then soaked in warm water (between 40-70°C) for 3 h and ground for 10 min to a fine paste with the aid of a clean blender (Abaejoh *et al*., 2006). Four parts of warm water was added to one part of the fine paste tigernut and stirred with the aid of a sterilized spoon. The liquid was then squeezed out with a piece of clean muslin cloth. The filtrate, which is tigernut milk was then simmered for 5 min in order to concentrate the resultant tigernut milk and to give the milk a form of pasteurization.

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Exposure of milk samples: A volume of 300 mL of tigernut milk was measured out into 3 separate sets of sterile conical flasks, each receiving equal quantity of the milk. The three sets of conical flasks containing the milk were labeled T_1, T_2 and T_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, 37 and 45°C, respectively for the isolation of mesophilic, thermotolerant and thermophilic fungi and other microorganisms, respectively.

Isolation of microbial species and measurement of pH: The culture plates were examined after 24 and 48 h 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 after a week for the development of new species of microorganisms.

The experiment was repeated with the other samples in the conical flasks (sample belonging to the same set of flasks (e.g., T_1, T_2 and T_3).

The unexposed milk samples were also plated out as controls, also in replicates. A volume of 10 mL of 0.013% (w/v) of streptomycin sulphate solution was added to 100 mL of Potato Dextrose Agar in order to suppress the growth of bacteria. Fungi colonies were subculture to obtain pure cultures and identification was done according to Domsch *et al.* (1980), Samson *et al.* (1984) and Rippon (1974). The bacterial colonies that developed on the culture plates were subcultured until pure cultures were obtained. The various bacterial colonies were examined under the microscope. The various bacterial isolates were also subjected to biochemical and physiological tests like sugar fermentation abilities and carbon assimilation tests. References were also made to stock cultures and different microbiology monographs in order to make proper identifications of the various microbial isolates. The pH-values of the unexposed milk samples were determined with the aid of a corning pH meter model 7.

RESULTS

The microbial species isolated and identified from the milk samples were *Bacillus subtilis*, *Staplylococcus aureus*, *Aspergillus flavus*, *A. niger*, *Fusarium solani*, *Saccharomyces cerevisiae*, *S. fubiligera* and *Candida pseudotropicalis*. Their frequencies of occurrence were 13.04, 17.39, 4.35, 13.04, 13.04, 21.74, 13.04 and 4.35%, respectively (Table 1). The biochemical characteristics of the microbial isolates are presented in Table 2, while Table 3 shows the bacterial load of both the unexposed and exposed samples of tigernut milk. The average pH-value of fresh milk samples was 6.8, while the change

Table 1: Frequency of occurrence of microbial isolates in the exposed and unexposed milk samples

	Site 1		Site 2		Site 3		Total		
	TM		TM		TM		TM		
Microbial isolates Time 48 h	E	U	E	U	E	U	E	U	Incidence (%)
Bacillus subtilis	+	+	-	-	-	+	1	2	13.04
Staphylococcus aureus	+	-	+	+	+	-	3	1	17.39
A. flavus	-	-	+	-	-	-	1	0	4.35
A. niger	-	+	+	-	+	-	2	1	13.04
Fusarium solani	-	-	-	+	+	+	1	2	13.04
Candida pseudotropicalis	-	-	-	-	-	+	0	1	4.35
Saccharomyces cerevisiae	+	-	+	+	+	+	3	2	21.74
S. fubiligera	+	+	+	-	-	-	2	1	13.04
Total	4	3	5	3	4	4	23		100.00

TM: Tigernut Milk; U: Unexposed sample; E: Exposed sample; 1: 33.3% occurrence; 2: 66.7% occurrence; 3: 100% occurrence; -: Absent, +: Present

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

	Types of tests used											
Bacterial isolates	Cell shape	Gram reaction	Presence of spores	Starch hydrolysis	Catalase	Methyl red	Glucose	Mannitol	Mortality	Sucrose	Coagulase	Probable identity of the isolates
1	R	+	-	-	+	-	A	+	+	+	NT	*Bacillus subtilis*
2	R	+	-	+	-	+	A	+	-	+	NT	*Lactobacillus* sp.
3	C	+	-	-	+	-	A	+	-	NT	+	*Staphylococcus auteus*

R: Rod; C: Cocci; A: Acid; NT: Not tested; +: Positive, -: Negative

Table 3: Bacterial (cfu mL^{-1}) of the tigernut milk samples

	Tigernut milk	
Microorganism	Exposed	Unexposed
Bacteria	1.5×10^3*	0.3×10^3*
	0.9×10^3*	0.1×10^3*
Average	1.2×10^3	0.2×10^3

Value represent average of duplicates

in the average pH-values of the milk samples following exposure for a period of 24 and 48 h were 6.2 and 5.8, respectively. The quality of the plant milk stored in the refrigerator was maintained.

DISCUSSION

The results from the survey have shown that the contamination of the plant milk by the microorganisms was due to the exposure of the milk samples to aerial environment. The percentage incidence of these microbial isolates varied: *Bacillus subtilis* (13.04%), *Staplylococcus aureus* (17.39%), *Aspergillus flavus* (4.35%), *A. niger* (13.04%), *Fusarium solani* (13.04%), *Saccharomyces cerevisiae* (21.74%), *S. fubiligera* (13.04%) and *Candida pseudotropicalis* (4.35%). These microbial isolates are well known and have been reported by other authors (Hayes, 1981). Some of these microorganisms are known to by of public health and economic significance, as some are capable of causing diseases. For instance, the bacterium *Bacillus subtilis* is known to cause food poisoning (William, 1998). *Aspergillus flavus* produces mycotoxins (Dienner and Davis, 1969). Mycotoxins are hazardous to human and animal health (WHO, 1979). *Apergillus niger* also produces aflatoxins (B1 B2, G1, G2) of which aflatoxin B1 is highly carcinogenic causing hepatoma (WHO, 1983). *Fusarium* species produce *Fusarium* toxins such as T. Z, Trichothecenes, Diacetoxyscirpenol, Nivalenol and Zearalenone, these cause skin diseased, gastroenteritis, rectal hemorrhage, vomiting and several other disease (Krogh, 1988).

All living things have minimum and maximum temperature for growth. Fungi are an exception (Dienner and Davis, 1969). This may account for high incidence of fungi in this survey. This is because most fungi will grow at temperature 5-35°C. These are the mesophilic species. There are those that thrive at 35°C and above and are said to be thermophilic. It therefore means, that fungi thrive well at a very wide temperature range, which gives room for their existence in the tigernut milk expose at 25, 37 and 45°C in this survey.

The incidence of the observed microbial isolates may be due to their ability to produce wide range of enzymes which include cellulases, amylases, lipases, proteases (Dienner and Davis, 1969). These enzymes hydrolyses complex carbohydrates, proteins and fats in to simple soluble forms, for easy assimilation.

Usually, most chemical reactions are retarded by low temperatures. Also, low temperature slows down or stops the growth and activities of most microorganisms in food. The lower the temperature, the slower the chemical reactions, enzyme actions and microbial growth (Kofi 1990). It is therefore recommended, that when tigernut milk is not to be served immediately, it should be 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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