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Microbiological Quality of *Cyperus esculentus* L. Products Sold in Ouagadougou and Bobo Dioulasso (Burkina Faso)

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Abstract

This study aimed to assess the microbiological quality of tiger nut (*Cyperus esculentus* L.) products sold in Ouagadougou and Bobo Dioulasso. The microbial load of tubers and tiger nut milk was determined using ISO 4833-1 (2003) standard (total mesophilic aerobic flora), NF ISO 7954 (1998) standard (yeasts and molds), and ISO 4832 (2006) standard (total coliforms, thermotolerant coliforms). The results showed that tiger nut tubers had an average microbial load between $4.86 \pm 7.03 \times 10^6$ UFC/g and $1.82 \pm 2.30 \times 10^7$ UFC/g for total mesophilic aerobic flora, from $4.34 \pm 10.6 \times 10^5$ UFC/g to $3.52 \pm 4.54 \times 10^6$ UFC/g for yeasts and molds, from $2.23 \pm 2.25 \times 10^5$ UFC/g to $1.41 \pm 2.16 \times 10^5$ UFC/g for total coliforms and from $1.83 \pm 2.03 \times 10^5$ UFC/g to $7.0 \pm 10.8 \times 10^4$ CFU/g for thermotolerant coliforms. For tiger nut milk samples, the average microbial load varied from $3.48 \pm 2.98 \times 10^6$ CFU/g to $2.80 \pm 5.69 \times 107$ CFU/g for total mesophilic aerobic flora, from $5.00 \pm 7.21 \times 10^4$ CFU/g to $1.88 \pm 3.31 \times 10^5$ CFU/g for yeasts and molds, from $4.58 \pm 10.4 \times 10^4$ CFU/g to $6.31 \pm 9.17 \times 10^5$ CFU/g for total coliforms and $7.00 \pm 7.00 \times 10^3$ CFU/g to $2.87 \pm 5.86 \times 10^5$ CFU/g for thermotolerant coliforms. This study revealed that the tubers and tiger nut milk sold in Ouagadougou and Bobo Dioulasso had a high microbial load which could lead to the degradation of these products and food poisoning for consumers.

Keywords

Cyperus esculentus L., Tiger Nut Tubers, Tiger Nut Milk, Microbiological Quality

1. Introduction

Yellow nutsedge (*Cyperus esculentus* L.) also known as tiger nut, is a perennial herbaceous sedge native to the eastern Mediterranean [1]. It was originally of concern along the eastern Mediterranean in southern Europe and North Africa. It has spread from its origin to all the continents of the world except Antarctica [1] [2]. In Africa, tiger nut is mostly cultivated in western countries including Burkina Faso, Côte d'Ivoire, Ghana, Mali, Niger, Nigeria, Senegal, and Togo where they are used primarily unprocessed as a side dish [3].

The nuts are highly appreciated for their health benefits and nutritious value due to their high content of fiber, proteins, and natural sugars. Indeed, Bado *et al.* [4] and Semdé *et al.* [5] showed that *Cyperus esculentus* L. tubers from Burkina Faso are rich in energy content (starch, fat, sugars, and protein), minerals (potassium, phosphorous, and Magnesium) and vitamins (vitamin E, vitamin C, and β-carotene).

Cyperus esculentus L. has been used medicinally in Mozambique, and the Chinese used the plant for stomachaches, as a stimulant, sedative, and tonic [6]. In North Africa, tubers are believed to be aphrodisiac and spermatogenic products. Preventing inflammation of the respiratory passages also has been attributed to the consumption of tubers [7]. Tiger nut "milk" has been reported to be used in the treatment of flatulence, indigestion, diarrhea, and dysentery [8], and its starch content presumably provides prebiotic properties for colon bacteria [9]. Nyarko *et al.* [10] reported that crude tiger nuts sold in retail in Cap Coast city in Ghana are associated with bacteria such as *Escherichia coli*, *Bacillus spp.*, *Enterococcus ssp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus ssp.*, and *Enterobacter cloacae*. Chukwu *et al.* [11] have isolated *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus terreus* in both dry and fresh tiger nuts and *Penicillium citrinum* and *Aspergillus fumigates* in dry nuts. Some of these molds are known to produce mycotoxins such as aflatoxins, ochratoxins, and fumonisins. Mycotoxins, particularly aflatoxins and ochratoxin A, lead to a significant threat to human health. Aflatoxins are potent carcinogens and, in association with the hepatitis B virus, are responsible for several thousands of human deaths per year, mostly in non-industrialized tropical countries [12].

Tiger nut is a minor crop in Burkina Faso, mainly grown in the regions of Hauts Bassins, Cascades, and Sud Ouest. Tiger nut products are sold on the edges of roads, in markets, and around schools. The tubers are usually sold in dry or rehydrated form, often displayed in not covered recipients at the mercy of dust and flies. Regarding tiger nut beverages, they are usually handmade products and packaged in reused packaging exposing the product to any kind of contamination. It was appropriate to analyze these products to assess their microbiological quality, the objective being to contribute to the protection of the population from food-borne diseases.

2. Material and Methods

2.1. Study Area

The study was conducted in two towns of Burkina Faso (**Figure 1**), Ouagadougou (region du Centre) and Bobo Dioulasso (region des Hauts Bassins).

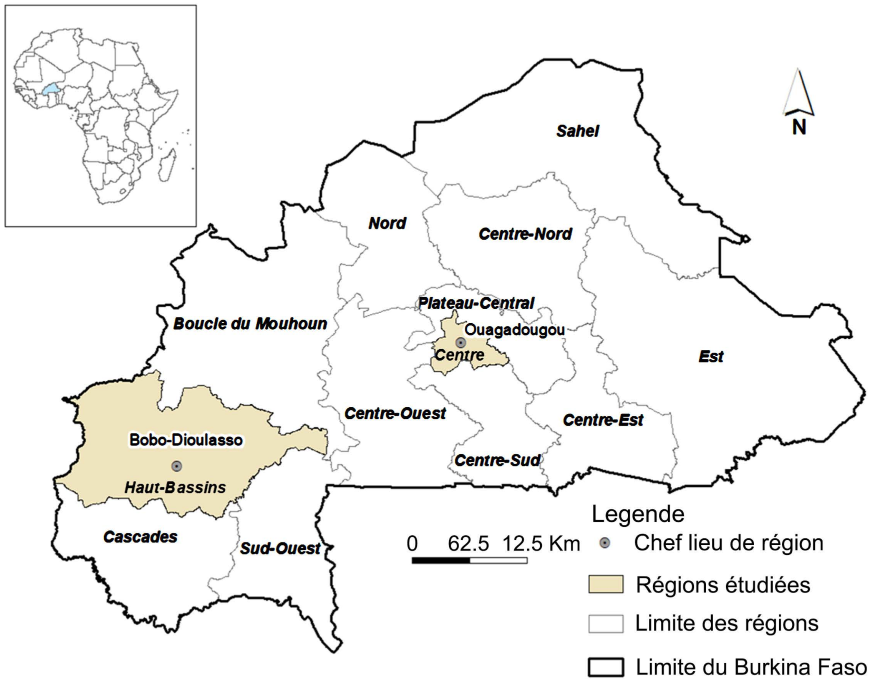

Figure 1. Study area.

2.2. Samples Collection

Tiger nut tubers and Tiger nut beverages were sampled aseptically at different retail sites in Ouagadougou and Bobo-Dioulasso. A total of 20 samples of tiger nut beverage and 12 samples of tiger nut tubers were collected.

2.3. Samples Treatment

Ten grams of each tiger nuts sample are collected aseptically in Stomacher bags and 90 ml of sterile saline solution (NaCl 9‰) are added and homogenized in a stomacher. For samples of tiger nut beverage, 10 ml of each sample are collected aseptically in a Stomacher bag and 90 ml of sterile saline solution (NaCl 9‰) are added and subjected to homogenization in a Stomacher. From these solutions, serial dilutions were made to obtain lower dilution solutions.

2.4. Sowing and Incubation

Culture and incubation conditions are given in Table 1.

2.5. Enumeration of Microorganisms

For enumeration, the plates of two successive dilutions were considered and the number of microorganisms per ml of tiger nut milk or per gram of tiger nut tubers was calculated by applying the Equation (1).

$$N = \frac{\sum C}{V \times \left(n_1 + n_2 \times 0.1\right) \times d} \quad (1)$$

N: Total number of microorganisms (CFU/ml)

Table 1. Culture and incubation conditions.

Microbiological parameters	Agar	Incubation	Methods
Total mesophilic aerobic flora	Plate count agar (PCA)	72 ± 2 h à 30˚C	ISO 4833-1, 2013
Yeasts and molds	Sabouraud chloramphenicol	3 - 5 days à 25˚C	NF ISO 7954, 1988
Total coliforms	Violet Red Bile Lactose (VRBL)	24 ± 2 h à 37˚C	ISO 4832, 2006
Thermo-tolerant coliforms	VRBL	24 ± 2 h à 44˚C	ISO 4832, 2006

$\sum C$: sum of colonies of Petri dishes of the two selected dilutions
V: volume of inoculum
d: dilution factor of the lowest dilution
n_1: Number of boxes of the lowest dilution
n_2: Number of boxes of the second dilution

2.6. Statistical Analysis

The data were processed in Microsoft Excel. The Means and standard deviations were also calculated using Microsoft Excel.

3. Results and Discussion

3.1. Results

The results of the microbiological analysis of tiger nuts samples are shown in Table 2. The total mesophilic load in dry tiger nuts is ranged between 1.5 × 10^5 CFU/g and 6.0 × 10^7 CFU/g with an average of 1.82 × 10^7 CFU/g. Total coliforms were found in all the dry tuber samples analyzed up to 9.5 × 10^2 and 4.8 × 10^5 with an average of 1.41 × 10^5 CFU/g. Thermo-tolerant coliforms have also been found in samples of dry tubers in the load of 5.8 × 10^2 CFU/g and 2.5 × 10^5 CFU/g with an average of 7.0 × 10^4 CFU/g. The yeasts and mold load of dry tubers ranged from 1.2 × 10^5 CFU/g to 1.2 × 10^7 CFU/g with an average of 3.52 × 10^6 CFU/g.

The rehydrated tiger nuts samples had a total mesophilic aerobic flora between 5.5 × 10^5 CFU/g and 1.9 × 10^7 CFU/g with an average of 4.86 × 10^6 CFU/g. Rehydrated tubers have a load of total coliforms varying between 4.6 × 10^3 CFU/g and 4.6 × 10^5 CFU/g with a load of thermotolerant coliforms of 3.1 × 10^3 CFU/g to 4.3 × 10^5 CFU/g. yeasts and molds were counted up to 5.9 × 10^1 CFU/g and 2.6 × 10^6 CFU/g with an average of 4.34 × 10^5 CFU/g.

Table 3 presents the results of the microbiological analysis of the samples of tiger nut milk. The samples of Ouagadougou had a microbial load ranging between 5.5 × 10^5 and 1.8 × 10^8 CFU/ml with total coliforms ranging between 7.9 × 10^2 and 2.5 × 10^6 CFU/ml, thermo-tolerant coliforms between 5.5 × 10^2 and 1.9 × 10^6 CFU/ml and yeasts and mold between 9.8 × 10^3 and 1.1 × 10^6 CFU/ml.

Table 2. Microbiological load of tiger nuts.

Samples	Total mesophilic aerobic flora (CFU/g)	Total coliforms (CFU/g)	Thermo-tolerant coliforms (UFC/g)	Yeasts and molds (CFU/g)
		Tiger nuts dry form		
1	2.0×10^7	3.5×10^5	**2.5×10^5**	3.4×10^6
2	2.1×10^6	6.8×10^3	4.2×10^3	7.0×10^5
3	**6.0×10^7**	**4.8×10^5**	1.6×10^5	**1.2×10^7**
4	1.9×10^6	1.3×10^3	6.7×10^2	3.2×10^5
5	2.5×10^7	6.4×10^3	4.4×10^3	4.6×10^6
6	**1.5×10^5**	**9.5×10^2**	**5.8×10^2**	**1.2×10^5**
Mean ± SD	**$1.82 \pm 2.30 \times 10^7$**	**$1.41 \pm 2.16 \times 10^5$**	**$7.0 \pm 10.8 \times 10^4$**	**$3.52 \pm 4.54 \times 10^6$**
		Tiger nuts rehydrated form		
1	**5.5×10^5**	**4.6×10^3**	**3.1×10^3**	3.1×10^3
2	3.8×10^6	2.6×10^4	1.3×10^4	**2.6×10^6**
3	**1.9×10^7**	2.6×10^4	2.1×10^4	1.0×10^3
4	1.2×10^6	**4.6×10^5**	4.2×10^5	1.1×10^2
5	1.7×10^6	4.4×10^5	**4.3×10^5**	**5.9×10^1**
6	2.9×10^6	3.8×10^5	2.1×10^5	2.1×10^2
Mean ± SD	**$4.86 \pm 7.03 \times 10^6$**	**$2.23 \pm 2.25 \times 10^5$**	**$1.83 \pm 2.03 \times 10^5$**	**$4.34 \pm 10.6 \times 10^5$**

Table 3. Microbiological load of tiger nut milk.

Samples	Total mesophilic aerobic flora (CFU/ml)	Total coliforms (CFU/ml)	Thermo-tolerant Coliforms (CFU/ml)	Yeasts and mold (CFU/ml)
		Ouagadougou		
1	7.1×10^6	1.5×10^5	7.8×10^4	8.0×10^4
2	1.7×10^7	**7.9×10^2**	**5.5×10^2**	3.9×10^4
3	**5.5×10^5**	2.0×10^4	1.3×10^4	**9.8×10^3**
4	6.5×10^7	2.1×10^6	**1.9×10^6**	**1.1×10^6**
5	2.6×10^6	6.9×10^4	5.8×10^4	2.9×10^4
6	8.6×10^5	6.9×10^4	1.1×10^4	1.4×10^4
7	3.7×10^6	6.8×10^4	6.6×10^4	6.2×10^4
8	**1.8×10^8**	**2.5×10^6**	4.7×10^5	4.7×10^5
9	1.3×10^6	1.3×10^6	2.7×10^5	2.3×10^4
10	1.8×10^6	3.3×10^4	6.7×10^3	5.7×10^4
Mean ± SD	**$2.80 \pm 5.69 \times 10^7$**	**$6.31 \pm 9.17 \times 10^5$**	**$2.87 \pm 5.86 \times 10^5$**	**$1.88 \pm 3.31 \times 10^5$**

Continued

		Bobo Dioulasso		
1	5.6×10^6	$\mathbf{3.4 \times 10^5}$	1.1×10^4	7.7×10^3
2	5.0×10^6	2.7×10^4	1.2×10^3	1.0×10^5
3	6.5×10^5	1.3×10^4	2.7×10^3	$\mathbf{1.7 \times 10^3}$
4	3.5×10^6	7.2×10^3	6.1×10^3	7.0×10^3
5	2.7×10^6	2.0×10^4	1.8×10^4	3.5×10^4
6	$\mathbf{8.9 \times 10^6}$	8.7×10^3	4.0×10^3	8.4×10^4
7	6.6×10^6	2.5×10^3	2.1×10^3	$\mathbf{2.3 \times 10^5}$
8	1.0×10^6	5.0×10^3	4.3×10^3	7.1×10^3
9	$\mathbf{1.5 \times 10^5}$	$\mathbf{1.6 \times 10^3}$	$\mathbf{5.7 \times 10^2}$	8.2×10^3
10	6.6×10^5	3.3×10^4	$\mathbf{2.0 \times 10^4}$	1.9×10^4
Mean ± SD	$\mathbf{3.48 \pm 2.98 \times 10^6}$	$\mathbf{4.58 \pm 10.4 \times 10^4}$	$\mathbf{7.00 \pm 7.00 \times 10^3}$	$\mathbf{5.00 \pm 7.21 \times 10^4}$

The microbial load of the tiger nut milk samples collected in Bobo Dioulasso varied from 1.5×10^5 to 8.9×10^6 CFU/ml, total coliforms from 1.6×10^3 to 3.4×10^5 CFU/ml, thermo-tolerant coliforms from 5.7×10^2 to 2×10^4 UFC/ml and yeasts and mold from 1.7×10^3 to 2.3×10^5 CFU/ml.

The average microbial loads of tiger nut milk samples from Ouagadougou were higher than those of samples from Bobo Dioulasso.

3.2. Discussion

The results of microbiological analysis of tiger nuts samples are similar to those found by Ayeh-Kumi *et al.* [13] and Nyarko *et al.* [10] who have also reported an important mesophilic aerobic flora and coliforms in tiger nuts sold in Ghana. However, the aerobic mesophilic flora and the total coliforms in tiger nuts from the present study are very high compared to those of Ayeh-Kumi *et al.* [13] and Nyarko *et al.* [10].

The presence of abundant microbial flora in the tubers and the tiger nut milk could be explained by the non-application of good manufacturing practices (GMP) by the processors. Poor post-harvest practices can be the source of microbial contamination of tiger nut tubers. During harvesting and drying the tubers are often in contact with the soil and can be contaminated by soil microorganisms. Thus, when good hygiene practices are not applied during the processing of tubers, these microorganisms can be found in the final product. The water used during processing can also be a source of microbial contamination.

The presence of a significant flora of coliforms may be due to fecal contamination or the sign of an uncontrolled sanitizing process. Certain coliforms are the cause of foodborne infections in the form of gastroenteritis and diarrhea. Yeasts and molds contaminate crops in the growing fields, during post-harvest treatments, or storage. They are usually the cause of food spoilage. However, certain molds (Aspergillus, Penicillium, Fusarium, etc.) produce toxins called mycotoxins (aflatoxins, ochratoxin, patulin, fumonisins, etc.) which can have various harmful effects on human health and farm animals (acute poisoning, deficiency immune system, cancer).

4. Conclusion

This study revealed that the tubers and tiger nut milk sold in Ouagadougou and Bobo Dioulasso have a high microbial load. This microbial load can be the cause of the deterioration of these products and of food poisoning for consumers. The application of good cultural practices and compliance with good post-harvest treatment practices by tiger nut producers could reduce the microbial load of tubers and processors will have access to good-quality raw materials. In addition, the application of good hygiene and manufacturing practices by the producers of tiger nut milk and the sellers of tiger nut tubers (dried and rehydrated) will enable them to provide the population with final products of good microbiological quality. Consumers can also wash and disinfect tiger nut tubers purchased from the market before consuming them to avoid foodborne illnesses.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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