Review	Article

# Contamination of Tigernut Tubers (*Cyperus esculentus*) and Tigernut-Derived Products of Economic Importance: A Review of Some Prevention Strategies

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## ABSTRACT

7 Tigernut is a popular plant because of its sweet tubers (Cyperus esculentus) which has numerous benefits. 8 Contamination of tigernut tubers which could be physical, microbial or chemical start in the field through post 9 harvest processes, handling, packaging, storage and retailing. Any form of contamination of tigernut tubers could 10 impact on overall quality and shelf life of tigernut-derived products. Although several studies had been carried out on the nutritional, antinutritional, physicochemical, sensory and microbiological quality of tigernut tubers as well as 11 12 numerous edible tigernut-derived products, it is important to review recent research findings on contamination of 13 tigernut tubers and popular tigernut-derived products in order to effectively implement prevention strategies. This 14 will promote food safety and food security especially in developing countries such as Nigeria.

15 Keywords: Contamination; tigernut tubers, tigernut-derived products; prevention strategies; economic importance.

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# 17 **1. INTRODUCTION**

Food contamination occurs when food gets in contact with potentially harmful microorganisms and/or substances. This could happen intentionally or unintentionally. As far back as 8,000 years ago, food contamination which affected some persons occurred. Since then till date, instances of food contamination are still occurring with severe casualties being recorded in some incidents [1]. Consumption of contaminated foods usually affect human health and in extreme cases leads to death. As human population is increasing globally, availability of non-contaminated foods at all times in sufficient quantity to guarantee 'food safety' and 'food security' is becoming more challenging [2, 3, 4].

Food safety as defined by Codex Alimentarius Commission (CAC) is the assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use [5]. Any unsafe food must not be placed on the market based on general food safety requirements stipulated by Regulation EC No. 178/2002 [6]. Food security is in existence among any group of persons when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life [7].

Nutrient rich diets are insufficient and unaffordable for many low income earners in developing countries like Nigeria [8]. Yet, there are many plants such as tigernut (*Cyperus esculentus*) that produce affordable tubers that are rich in nutrients but still considered as being underutilized. In order to balance population growth with agricultural productivity particularly in tropical and sub-tropical regions where many children are suffering from malnutrition, optimum utilization of tigernut tubers need to be promoted [9, 10, 11].

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Tigernut is popular because of the sweet tuber [12]. It is a reliable source of food in times of food scarcity [13].

37 Since tigernut tubers are readily available in the market almost throughout the year, it is usually processed into

38 different edible products. Maduka and Ire [14] reviewed the useful application of tigernut tubers in development of

many edible products. However, several factors predispose these products to contamination. Microbial assessment

40 of 'kunu-aya' which is a popular product of tigernut tubers in Nigeria revealed that the product had a high bacterial

41 count, total coliform count and salmonella-shigella count [15, 16]. Local traders often expose fresh, dried and

42 rehydrated tigernut tubers during retailing. This practice including activities in the field during harvesting and 43 storage of tigernut exposes the tubers to contamination. Three types of contamination of tigernut tubers could occur.

- They are physical, microbial and chemical contamination. Retailing contaminated tigernut tubers and/or edible tigernut-derived products poses a public health risk [17, 18].
- Therefore, this review article is aimed at categorizing contamination of tigernut tubers and tigernut-derived
  products, share recent information related to contamination of these products as well as prevention and control
  measures required to mitigate contamination of these edible products for economic gains.
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## 50 2. Contamination of tigernut tubers

51 The physicochemical properties of tigernut tubers as well as existing environmental condition where tigernut is 52 grown and the tubers stored could influence microbial contamination of the tubers. Chemical contamination of 53 tigernut tubers could occur in the field or during storage. Physical contamination of tigernut tubers is very common. 54 Therefore, contamination of tigernut tubers could be conveniently grouped as physical, chemical or microbial.

55 During harvesting of tigernut tubers, the tubers are usually gathered with foreign materials such as soil, insect or 56 damaged tubers from the field. The part of a growing plant where the edible part is located determines its level of 57 contamination. Generally, the edible part of a plant that develop in the soil as well as the ones that touch the soil 58 surface are more predisposed to contaminants than those located at the aerial parts of the plant. Therefore, tigernut 59 tuber is likely to be contaminated in the field because the tubers develop under the soil [19].

Microbial contamination of tigernut tubers can also occur during handling of the product by vendors. Several studies
had been carried out to ascertain the level of microbial contamination of ready-to-eat tigernut tubers retailed in
different localities [18, 19]. Table 1 and 2 shows identified parasites in tigernut tubers retailed in different markets.
Similarly, Figure 1 shows the total plate count of wash water of tigernut sampled from two states in Nigeria.

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65 Several pathogenic bacteria species have been isolated from exposed tigernut tubers. The presence of *Escherichia* 66 *coli*, *Streptococcus faecalis* and *Staphylococcus aureus* from exposed tubers is an indication that faecal 67 contamination occurred. This could be traced to irrigation water used in the tigernut farm. According to Chukwu et 68 al. [20], fungi associated with fresh tigernut tubers are *Aspergillus niger*, A. *flavus* and A. *terreus* while that of dried 69 tubers is *Penicillium citrinum* and A. *fumigates*. Table 3 shows the mean microbial loads of washed and unwashed 67 tigernut tubers. The parasite recovery at various washes of tigernuts from different markets is presented in Table 4.

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Parasites		Samp	ling site (Markets)		
	Marian (%)	Ikot Ishie (%)	Watt (%)	CRUTECH Gate	Total (%)
				(%)	
Ascaris		7			
<i>lumbicoides</i> (Ova)	2 (18.2)	3 (25.0)	6 (50.0)	4 (44.4)	15 (34.0)
Trichuris trichiura					
(Ova)	2 (18.2)	3 (25.0)	2 (16.7)	2 (22.2)	9 (20.5)
	The second secon				
Strongyloides					
oocysts	3 (27.3)	3 (25.0)	1 (8.3)	2 (22.2)	9 (20.5)
~ .					
Cyclospora	1 (26.2)	2 (25.0)	2 (25.0)	1 /11 1	11 (225)
cayetanensis	4 (36.3)	3 (25.0)	3 (25.0)	1 (11.1)	11 (225)
Total no. (%)	11 (25.0)	12 (27.3)	12 (27.3)	9 (20.4)	44
Source: Ogban and U	· /	(-, 13)		/ (=0)	••

#### 72 Table 1. Parasites recovered from tigernuts from markets in Calabar

75 Table 2. 76

Parasites Locations UTC area Shangrila area Madina area Airport Total no. (%) Strongyloides stercoralis larvae 9 9 (22.5) Ancylostoma 10 (25.0) *duodenale* ova 10 Cryptosporidium 12 (30.0) parvum oocysts 5 7 Cyclospora 3 2 9 (225) cayetanensis 4 40 (100) Total no. (%) 30 8 2 0 \*No parasites were found in the tiger nuts bought from market places. Source: Ayeh-Kumi et al. [22]. 140 120 100 CFU /ml 80 60 40 20 0 Bashiri (EK) Oja Oba (EK) Shasha (EK) Old Garage (AK) Oja Oba (AK) Location

Parasites identified on tigernuts from various locations in Accra

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- 81 Fig-1
- 82 Total plate count of wash water (CFU ml<sup>-1</sup>) of tigernut sampled in Ado Ekiti and Akure metropolis
- 83 EK = Ekiti; AK = Akure; Source: Akomolafe and Awe [19].
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96	Table 3.	Mean microbial loads <sup>*</sup> of the washed and unwashed experimental tiger nuts
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	Sample	В	acteria (cfu/g x 10 <sup>3</sup> ) at 37 °C, 2	24 h Fungi (cfu/g $x10^3$ ) at 26 °C, 48 h
	Dried (unwashed)	2.	3 <sup>a</sup>	15 <sup>a</sup>
	Dried (washed)	1	9 <sup>b</sup>	$9^{b}$
	Rehydrated (unwash	ed) 1	3 <sup>°</sup>	9 <sup>b</sup>
	Rehydrated (washed)	) 7.	$.0^{d}$	5°
98 99 100 101 102	Source: Ukpabi and	d Ukenye [18]	letter are not significantly difference of tiger nuts from the second se	
	Tigernuts (100 g)	Frequency	of wash (% parasite recovery)	Total (%)
		$\mathbf{W}_1$	W 2	W 3
	Dry nuts	14 (70.0)	5 (25.0)	1 (5.0) 20 (45.5)
	Fresh nuts	11 (45.8)	10 (41.6)	3 (12.5) 24 (54.5)
	Total (%)	25 (56.8)	15 (34.1)	4 (9.1) 44
103 104	N/B: $W_1 - W_3 = 1^{st}$ to Source: Ogban and U		$\langle X \rangle$	

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# 107 2.1 Physical contamination

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109 This could be considered as the commonest type of contamination of tigernut tubers. Physical contamination of 110 tigernut tubers which usually occur during harvesting of the tubers involves foreign materials such as stone, sand and animal droppings mixed with freshly harvested tigernut tubers. Other sources of contamination could be from 111 composted organic matter, insects and soil. Physical contamination from these sources is most likely to cause 112 113 microbial contamination of tigernut tubers. Chewing foreign material together with tigernut tubers can cause serious 114 discomfort to the teeth. Therefore, any foreign material gathered with tigernut tubers should carefully be removed before using the tubers to produce edible products. Although physical contamination of tigernut tubers could be 115 116 considered as being prevalent, the contaminants involved is easy to identify and remove [22]. Ability to design 117 suitable machinery and processing equipment for tigernut tubers based on engineering properties of the tubers could 118 significantly reduce physical contamination of the tubers [23, 24].

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#### 120 2.2 Chemical contamination

Tigernut tubers could be contaminated by chemical substances used in the field. Chemical residues in tigernut tubers could have long term effect in the human body if consumed in large quantity. Results obtained from investigating possible chemical contamination of tigernut tubers are scarce. However, Chukwu et al. [20] in their studies reported that tigernut tubers contain some quantity of lead. According to the result of their analysis, fresh tigernut tubers contain 0.36±0.0895 mg/100 g lead whereas dried tigernut tubers contain 2.05±0.112 mg/100 g lead. According to Al-Shaikh [25], lead is regarded as a major environmental pollutant which is implicated in industrial pollution. The 127 presence of lead in human body inhibits haemoglobin synthesis. Therefore, Chukwu et al. [20] advised people not to 128 eat too much quantity of tigernut tubers in order to reduce intake of large quantity of lead.

129 One interesting study by Al-Shaikh et al. [25] demonstrated that *Cyperus esculentus* extract had a regenerative effect

when administered on male rats which had their testicular histology destroyed by lead acetate. Disinfection of tigernut tubers using 1 % chlorine for a minimum of 30 minutes before using the tubers to produce 'horchata' could result in chemical contamination of the product if excess quantity of the germicide is used [26].

133 Possible contamination of tigernut tubers by mycotoxins which are secondary metabolites produced by toxigenic 134 species of Aspergillus is a health concern because tigernut tubers is commonly eaten as raw tubers without 135 subjecting the tubers to any treatment that will reduce the level of contamination [27]. Aspergillus flavus and A. 136 parasiticus is largely responsible for aflatoxin production which usually contaminate some stored food products. 137 Shamsuddeen and Aminu [27] reported the presence of aflatoxin in some samples of tigernut tubers evaluated which 138 range between 0.2 - 23.0 µg/Kg. Consumption of stored nuts, cereals and grains contaminated with aflatoxin above 139 allowable limit has serious health implications. Studies have shown that aflatoxin is hepatotoxic and has potential of 140 being hepatocarcinogenic in animals. In other words, aflatoxin could contaminate stored tigernut tubers as well as 141 tigernut-milk [27, 28]. International commission on microbiological specifications for food (ICSMF) recommend 142 that maximum acceptable limit of aflatoxin in foods is  $1 \times 10^5$  cfu/g [27]. According to CODEX alimentarius, 143 maximum level of aflatoxins allowable in an edible product must not exceed 4 µg/Kg. A study to evaluate presence 144 of aflatoxins in 'horchata' which is a popular tigernut-milk drink consumed in Spain reported that 4.5 % of the 145 samples were contaminated. In a similar study, 10-120 µg/Kg of aflatoxins was reported in 35 % tigernut samples 146 collected from different locations in Nigeria [28]. Shamsuddeen and Aminu [27] recommended that proper storage 147 condition of raw tigernut tubers will help reduce fungi proliferation and quantity of aflatoxins that could 148 contaminate the tubers [27].

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# 150 2.3 Microbial contamination

151 Microbial contamination of tigernut tubers easily occurs and often undetected before the tubers are consumed. The 152 source of microbial contamination could be from infected field workers, contaminated soil, irrigation water, wash 153 tanks, harvesting equipment, fecal materials and transport vehicles. Growth of microorganisms in foods is usually 154 influenced by factors such as product temperature, product-to-headspace, gas volume ratio, initial microbial loads 155 and type of flora, packaging, barrier properties, storage condition and biochemical composition of the food [6]. The 156 water used to apply fungicides and insecticides is a source of microbial contamination of tigernut tubers [19]. 157 According to Okechukwu et al. [29], the use of water polluted with faecal matter to wash knives, polyethene bags 158 and trays could also be a source of microbial contamination of tigernut tubers. Microbial contamination of ready-to-159 eat-tigernut tubers is largely attributed to unhygienic practices of vendors and inappropriate storage conditions.

160 Chewing fresh or dried tigernut tubers contaminated with pathogenic microorganisms increases the risk of ingesting mycotoxins such as aflatoxins, ochratoxins and fumonisins [30]. Microorganisms isolated from exposed tigernut 161 162 tubers are Bacillus subtilis, Staphylococcus aureus, Aspergillus flavus, A. niger, Fusarium solani, Saccharomyces 163 cerevisiae, S. fibuligera and Candida pseudotropicalis. Isolation of Proteus vulgaris from exposed tigernut tubers 164 has a serious health implication in the sense that the bacteria demonstrated some level of multiple drug resistance 165 [22]. An assessment of wholesomeness of tigernut tubers imported into Nigeria as snack food was carried out by Ukpabi and Ukenye [18]. Similarly, Ayeh-Kumi et al. [22] also carried out a survey of pathogens associated with 166 167 exposed tigernut tubers sold in a Ghanaian city. Findings from the study showed that four different parasites and five 168 bacteria genera were present on tigernut tubers being retailed in the markets. The presence of these pathogens in 169 tigernut tubers sold in the market is a threat to public health [22].

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# 1712.3.1Microbial contamination of tigernut-derived products172

173 Microbial contamination of tigernut-derived products usually occurs as a result of unhygienic processing, handling, 174 transportation and exposure of these products during retailing [18]. Microbial contamination of tigernut-derived 175 products could be traced to microbial contamination of tigernut tubers used for production. A study carried out by 176 Arranz et al. [28] identified aflatoxin B<sub>1</sub> from samples of 'horchata' sold in Southern Europe. This is an indication 177 that the products were contaminated with fungi that produce mycotoxins. The bacterial load of tigernut juice sold in 178 many localities in Nigeria is quite high [15]. In Spain where tigernut beverages have been commercialized, a 179 comparative study between microbiological quality of commercial and home-made tigernut beverage was 180 determined. Findings from that study revealed that total viable count of all commercially prepared tigernut 181 beverages were below the detection limit. However, Enterobacteriaceae (3.41-5.47 log CFU/ml), Escherichia coli 182 (2.69 log CFU/ml), Bacillus spp. (1.79-2.47 log CFU/ml), yeasts (2.69-4.47 log CFU/ml) and moulds (3.63-4.47 log CFU/ml) were present in home-made tigernut beverages. These values did not exceed legislated levels [31]. In a 183 184 related study, Onovo and Ogaraku [32] identified microorganisms associated with exposed and unexposed tigernutmilk. Results from their study revealed that frequency of occurrence of Bacillus subtilis, Staphylococcus aureus, 185 186 Aspergillus flavus, A. niger, Fusarium solani, Saccharomyces cerevisiae, S. fubiligera and Candida pseudotropicalis 187 isolated and identified from tigernut-milk samples are 13.04, 17.39, 4.35, 13.04, 13.04, 21.74, 13.04 and 4.35 %, respectively. On average, they reported that bacterial load in exposed and unexposed tigernut-milk samples are 1.2 x 188  $10^3$  cfu mL<sup>-1</sup> and 0.2 x  $10^3$  cfu mL<sup>-1</sup>, respectively. The presence of these microorganisms in tigernut-milk is 189 190 considered a threat to public health because they are capable of producing toxic metabolites which can cause ill 191 health in humans. Table 5 shows the microbiological load of tigernut-milk containing different concentration of 192 chemical preservatives during ambient temperature storage for twelve days. Similarly, Table 6 shows the microbial 193 load of tigernut drinks that contain natural preservatives stored at ambient and refrigeration temperature for eight 194 days.

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#### 196 Table 5. Microbiological loads of tigernut-milk during ambient storage

Past. Tigernut milk +0.08 % sodium benzoate Past. Tigernut milk + 0.04 % sodium benzoate Past. Tigernut milk + 0.02 % sodium azide Past. Tigernut milk without preservatives Day seven Past. Tigernut milk +0.08 % sodium benzoate Past. Tigernut milk + 0.04 % sodium benzoate Past. Tigernut milk + 0.02 % sodium azide Past. Tigernut-milk + 0.02 % sodium azide Past. Tigernut-milk + 0.02 % sodium azide Past. Tigernut milk +0.08 % sodium benzoate Past. Tigernut milk +0.04 % sodium benzoate Past. Tigernut milk +0.04 % sodium benzoate Past. Tigernut milk +0.04 % sodium benzoate	Microbial co	unt (cfu/ml) $^{1,2,3,4}$
	Total viable count	Fungal count
Day one		-
Past. Tigernut milk +0.08 % sodium benzoate	$1.33 \times 10^4 \pm 3.33 \times 10^{2b}$	$1.93 \ge 10^2 \pm 2.3 \ge 10^{1a}$
Past. Tigernut milk + 0.04 % sodium benzoate &	$2.26 \times 10^4 \pm 8.71 \times 10^{2b}$	$1.96 \text{ x } 10^2 \pm 2.6 \text{ x } 10^{1a}$
0.01 % sodium azide		
Past. Tigernut-milk + 0.02 % sodium azide 🥂	$7.6 \ge 10^1 \pm 8.0 \ge 10^{0c}$	$1.3 \times 10^{1} \pm 3 \times 10^{0b}$
Past. Tigernut milk without preservatives	$2.03 \text{ x } 10^{b} \pm 2.40 \text{ x } 10^{4a}$	$2.3 \times 10^3 \pm 1.85 \times 10^{2a}$
Day sayan		
-	$5.66 \ge 10^{b} \pm 1.45 \ge 10^{ba}$	$4.00 \ge 10^2 \pm 1.15 \ge 10^{2D}$
	$6.70 \times 10^4 \pm 7.23 \times 10^{3a}$	$4.66 \times 10^{2} \pm 8.8 \times 10^{16}$
	$0.70 \times 10 \pm 7.23 \times 10$	4.00 X 10 ±0.0 X 10
	$1.3 \times 10^{1} \pm 3.00 \times 10^{UD}$	$1.3 \text{ x } 10^1 \pm 3 \text{ x } 10^{\text{Uc}}$
	$4.33 \times 10^{6} \pm 8.81 \times 10^{5a}$	$7.86 \times 10^{6} \pm 6.10 \times 10^{6a}$
and regeneration provide the		
Day twelve		
Past. Tigernut milk +0.08 % sodium benzoate	Too numerous to count	$9.5 \ge 10^3 \pm 4.2 \ge 10^{2b}$
Past. Tigernut milk + 0.04 % sodium benzoate &	Too numerous to count	$9.2 \times 10^4 \pm 2.5 \times 10^{2C}$
0.01 % sodium azide		
Past. Tigernut-milk + 0.02 % sodium azide	$3.1 \ge 10^1 \pm 1.2 \ge 10^D$	$2.1 \ge 10^{1} \pm 1.5 \ge 10^{ud}$
Past. Tigernut milk without preservatives	Too numerous to count	$1.2 \text{ x} 1.210^5 \pm 2.7 \text{ x} 10^{3a}$

<sup>1</sup>Each value is the mean ± Standard error of triplicate determinations

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<sup>&</sup>lt;sup>2</sup>Different letters within the column subset are significantly different (p<0.05)

<sup>&</sup>lt;sup>3</sup>Bacterial isolates:- *Bacillus* spp., *Lactobacillus* spp., *Pediococcus* spp.

<sup>&</sup>lt;sup>4</sup>Fungal isolates:- *Aspergillus niger*, *A. flavus*, *A. ochraceus* 

<sup>202</sup> Source: Akoma et al. [33]

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# Table 6. The effect of storage time (day) on the microbial loads of tigernut drinks<br/>treated with natural tropical preservativesAnalysesStorageStorageTigernut+Tigernut+Tigernut

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Anaryses	period (day)	condition	extract (control)	garlic	ginger	citric acid	ginger + garlic
Total viable	0		< 1	< 1	< 1	< 1	< 1
count log <sub>10</sub> cfu/ml	4	28 °C	$1.7\pm0.022^{a}$	1.22±0.044 <sup>b</sup>	1.62±0.058 <sup>a</sup>	1.00±0.051 <sup>b</sup>	1.11±0.115 <sup>bc</sup>
10810010/111	8		4.68±0.017 <sup>a</sup>	4.28±0.012 <sup>bc</sup>	4.35±0.040 <sup>b</sup>	2.57±0.009 <sup>d</sup>	4.25±0.026 <sup>bc</sup>
Total viable	0		< 1	< 1	< 1	<1	< 1
count log <sub>10</sub> cfu/ml	4	4 °C	1.40±0.009	< 1	1.37±0.033	<1	1.09±0.018
10810010/111	8		2.48±0.039 <sup>a</sup>	1.10±0.015 <sup>b</sup>	2.51±0.049 <sup>a</sup>	1.05±0.005 <sup>b</sup>	1.12±0.023 <sup>b</sup>
Fungal count	0		<1	<1	<1	<1	<1
log <sub>10</sub> cfu/ml	4	28 °C	1.53±0.027 <sup>a</sup>	1.34±0.031 <sup>b</sup>	1.58±0.015 <sup>a</sup>	1.03±0.014 <sup>c</sup>	1.38±0.016 <sup>b</sup>
	8		2.52±0.012 <sup>a</sup>	2.34±0.031 <sup>c</sup>	2.58±0.012 <sup>a</sup>	2.31±0.021 <sup>c</sup>	2.40±0.009 <sup>b</sup>
Fungal count	0		<1	<1	<1	<1	<1
log <sub>10</sub> cfu/ml	4	4 °C	1.25±0.029	<1	1.25±0.012	<1	< 1
	8		2.71±0.018 <sup>a</sup>	2.16±0.012 <sup>c</sup>	2.56±0.021 <sup>b</sup>	1.09±0.009 <sup>d</sup>	$1.06 \pm 0.008^{d}$

213 <sup>1</sup>Each data is the mean + standard error of 3 determinations

214 <sup>2</sup>Different letters within the same row subset are significantly different at p<0.05;

**215**  $^{3}$  < 1 = no growth

<sup>4</sup>Each data is the mean + standard error of 12 member taste panelist determinations using 5-point hedonic scale where 1 = very bad, 2 = bad, 3 = neither good nor bad, 4 = good, 5=very good

218 <sup>5</sup>Different letters within the same row which are significantly different at p<0.05

- 219 Source: Nwobosi et al. [16]
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- 221 222
- 223 3.0 Reduction in level of contamination of tigernut tubers and tigernut-derived products

It is advisable that contamination of tigernut tubers and tigernut-derived products is reduced to a safe level or completely eliminated before the product is consumed. Two methods - removal of foreign materials mixed with tigernut tubers and washing the tubers with potable water were adopted by Akoma et al. [33] before using the tubers to produce tigernut-milk drink. The following subsections highlight the processes recommended for reduction in level of contamination in tigernut tubers and tigernut-derived products.

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230 3.1 Hand-picking of foreign materials

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232 In order to remove foreign contaminants gathered with tigernut tubers, hand-picking of good tubers or careful 233 removal of all foreign materials is recommended. The unwanted materials mixed with tigernut tubers are usually 234 stones, sand, metal objects, plant remains, other grains planted in the field, animal droppings and damaged nuts. 235 Different parts of the tigernut plant such as roots, small sticks, empty and broken nuts as well as the tubers damaged 236 by parasites and insects could also be gathered alongside good tubers. Removal of these physical contaminants is 237 usually done manually and the process is laborious. Recently, an automated system such as sieving systems and wet 238 separating is employed by industries that use tigernut tubers for different purposes [25]. Before tigernut tuber is processed into tigernut-milk, it is necessary to hand-pick foreign materials gathered with the tubers and also remove 239 240 all bad/cracked tubers. Otherwise, the taste and quality of tigernut-milk might be affected [34]. It is interesting to 241 note that dehulling of tigernut tubers can significantly reduce microbial load of the tubers [35].

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#### 3.2 Use of extracts, antimicrobials, water and other solutions

246 Before raw tigernut tuber is chewed in its raw form, thorough washing of the tubers with potable water is 247 recommended because it significantly reduces microbial load of tigernut tubers. In an attempt to reduce microbial 248 contamination of stored tigernut tubers, Djomdi et al. [35] compared the effectiveness of dehulling and soaking 249 tigernut tubers in Ca (OH)2, 'kanwa' and vitamin C solution at 20 °C, 40 °C and 60 °C in reduction of microbial load 250 in the tubers. The study revealed that soaking dehulled tigernut tubers in vitamin C proved most effective than other 251 solutions. Other solutions such as phosphate buffered water and phosphate buffered water plus 1 % sodium 252 hypochlorite have also been tested and found effective in reducing microbial load of tigernut tubers to a safe level fit 253 for human consumption [36]. The practice of eating raw tigernut tubers stored for a long period without any 254 treatment to reduce the level of aflatoxins in the tubers is not advisable because climatic condition in Nigeria favours 255 fungal growth that result in mycotoxin contamination of stored food products. An interesting study by Omoniyi et al. 256 [37] demonstrated that tigernut samples contaminated with aflatoxin B1 was reduced by 75 % and 67 % when the 257 samples were treated with 50 % and 100 % (v/v) orange juice extract, respectively. Similarly, aflatoxin G2 in the 258 tigernut samples was reduced by 75 % using 100 % (v/v) orange juice. Findings from that study showed that 259 distilled water performed better in reducing the level of aflatoxins B2 and G1 than orange juice extract.

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# 261 3.3 Use of packaging materials262

263 Vendors usually use polyethylene bags to package fresh or dried tigernut tubers basically to make retailing of the 264 commodity easier. Polyethylene bag is the most widely used and cheapest means of packaging in food and beverage industries [18]. Akomolafe and Awe [18] did a study to determine the effectiveness of using packaging materials 265 266 such as polyethylene bags to reduce the level of microbial contamination of ready-to-eat fruits and vegetables 267 including tigernut tubers retailed in some states in South Western Nigeria. Their findings was that tigernut tubers 268 that had been washed, disinfected and packed inside polyethylene bags was prevented from being re-contaminated 269 during retailing. Results from the study revealed that tigernut tubers at the wholesale points were heavily 270 contaminated compared with the samples from retail points. Lower microbial load of tigernut tubers sampled at the retail points was attributed to washing of the tubers with water by retailers. However, Staphylococcus spp. was 271 isolated from all the samples of tigernut tubers packaged with polyethylene bags from the retail points. 272

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#### 275 3.4 Industrial processing

276 Tigernut-derived products could either be home-made or subjected to industrial processing. Microbial assessment of 277 home-made tigernut beverage which is not usually subjected to thermal methods of preservation revealed that it had 278 higher microbial contamination than the ones produced by beverage industries that practice strict good 279 manufacturing practices (GMPs) which also involves thermal methods of treatment. Some beverage industries that 280 produce tigernut-milk drink expose the drink to gamma radiation to guarantee the wholesomeness of the drink. The 281 effect of exposing tigernut-milk drink to gamma radiation in terms of its physicochemical, functional and sensory 282 qualities was reported by Ukpabi and Ukenye [18]. The tigernut-milk drink subjected to gamma irradiation can as 283 well be pasteurized to further reduce microbial load in the drink [38]. Implementation of Hazard Analysis and 284 Critical Control Points (HACCP) plan and GMPs is required to drastically reduce microbial contamination of homemade tigernut beverages. This will improve its bacteriological quality to be like that of commercially-made tigernut
beverages sold in Valencia Spain [31].

### 287 4. Preservation of tigernut tubers

288 Preservation of tigernut tubers is aimed at slowing down undesirable changes that occur in the tubers as a result of 289 activities of spoilage microorganisms and chemical reactions. Different storage conditions are employed in 290 preserving fresh tigernut tubers. The use of polyethylene bags by street vendors to retail and store tigernut tuber is a 291 common practice in developing countries like Nigeria. Polyethylene bags have different thickness which could 292 impact on shelf life of tigernut tubers. Akomolafe and Awe [19] reported that tigernut tubers left unpacked for 10 293 days at ambient temperature (25±1 °C) were better than the ones sealed inside polyethylene bags which experienced 294 disease severity between 3.00 and 4.25. This result shows that preserving tigernut tubers inside polyethylene bags is 295 not conducive for storage but beneficial in reducing recontamination of the tubers during retailing. Tigernut tubers 296 sealed for 10 days inside polyethylene bag that has a thickness of 1 µm experienced a significant colour change 297 compared with that of 7 µm thickness which experienced no colour change.

Storage of tigernut tubers in a refrigerator or freezer maintained at a constant temperature will prevent the tubers from changing its original colour as well as slow down microbial growth on the tubers which usually result in spoilage. Air tight container filled with tigernut tubers kept at ambient temperature  $(28\pm2 \text{ °C})$  is a suitable storage condition. The use of some artificial preservatives ensures that the unique qualities of tigernut tubers are maintained during storage. Shriveled and wrinkled tigernut tubers that are properly dried can store for a long time without undergoing spoilage. Tigernut tubers soaked inside water changed daily can remain fresh up to 10 days [39].

After fresh tigernut tuber is properly dried, the taste becomes better appreciated by many consumers [40]. Properly dried tigernut tubers can be preserved up to a year or more with minimal risk of being attacked by spoilage microorganisms [39]. The process of reducing moisture content of fresh tigernut tubers is a necessary step that ensures activity of spoilage microorganisms in the tubers are drastically reduced during storage. Therefore, dried tigernut tuber is easier to store than fresh tigernut tubers.

Bulk storage of tigernut tubers under anaerobic warm storage condition is not advisable because it encourages microorganisms that naturally occur on tigernut tubers to rapidly ferment the tubers. Under that condition, chemical spoilage products predominantly mycotoxins is released. However, if the environment where tigernut tubers is stored is well aerated and fumigated every 6 weeks against insects or bugs which cause damage to the tubers, it can be stored up to two years [39].

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# 315 5. Preservation of tigernut-derived products

316 Chemical preservatives such as sodium azide has proven to be effective in extending the shelf life of tigernut 317 derived products such as tigernut-milk and still maintain acceptable sensory characteristics of the product [33]. Sá id 318 et al. [41] evaluated the combined effect of different processing treatments and preservation methods on the sensory 319 characteristics and microbial quality of tigernut beverage. Addition of natural preservative to pasteurized tigernut-320 milk which was stored at refrigeration temperature proved an effective method to extend shelf life of freshly 321 prepared tigernut-milk up to one week [15, 41]. Pasteurization and addition of preservatives reduces microbial load 322 in tigernut-milk which can extend the product shelf life by few days [33]. However, during packaging of the 323 product, recontamination might occur. This might reduce the product shelf life. According to Codex Alimentarius 324 Commission, the acceptance level of microbes in a milk product should be less than  $2.0 \times 10^5$  cfu/ml [41].

Tigernut-soya milk extract and yoghurt-like products are popular tigernut-derived products that can be stored at room  $(28\pm2 \ ^{\circ}C)$  and refrigeration temperature  $(4\pm2 \ ^{\circ}C)$ . However, *Bacillus* spp., *Saccharomyces* spp., *Staphylococcus aureus*, *Penicillium* spp., *Aspergillus flavus*, *Mucor* spp. and *Rhizopus* spp. can survive both storage conditions. Notwithstanding addition of preservatives to tigernut-milk, Nwobosi et al. [15] demonstrated that the products stored at  $28\pm2 \ ^{\circ}C$  had a higher microbial count than similar products stored at  $4\pm2 \ ^{\circ}C$ . Table 7 and 8 shows the total heterotrophic bacterial count of tigernut-soy milk extract stored at  $4 \ ^{\circ}C$  and  $28 \pm 2 \ ^{\circ}C$ , respectively. Similarly, Table 9 and 10 shows the total heterotrophic fungal count of tigernut-soy milk extract stored at  $4 \ ^{\circ}C$  and

- 332  $28 \pm 2$  °C, respectively. The microorganisms isolated from tigernut-soy milk extract (YSME) during storage at both 333 storage conditions are depicted in Table 11.
- 334

335	Table 7.	Total heterotrophic bacterial count in cfu/ml of various tigernut-soy milk extract
336		(TSME) under storage at 4 $^{\circ}$ C

Sample		Storage period (day)		
	0	7	14	
A <sub>R</sub>	NG	NG	NG	-
B <sub>R</sub>	NG	NG	NG	
C <sub>R</sub>	NG	$1.21 \times 10^2$	$1.74 \times 10^2$	
$D_R$	$1.4 \text{ x } 10^2$	$1.76 \ge 10^2$	$3.6 \times 10^2$	
E <sub>R</sub>	$2.4 \text{ x } 10^2$	$2.6 \times 10^2$	$5.8 \times 10^2$	
F <sub>R</sub>	$2.62 \ge 10^2$	$2.8 \times 10^2$	$7.2 \times 10^2$	

NG=No growth, Subscript R = Refrigerated; A= 100 % Tigernut milk + 0 % soymilk; B= 90 % Tigernut milk +10 % soymilk; C= 80 % Tigernut milk + 20 % soymilk; D = 70 % Tigernut milk + 30 % soymilk; E=60 % Tigernut

340 milk + 40 % soymilk; F= 50 % Tigernut milk + 50 % soymilk

341 Source: Udeozor and Awonorin [34].

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345

Table 8. Total heterotrophic bacterial count in cfu/ml of various tigernut-soy milk extract (TSME) under storage at 28±2 °C

Sample		Storage period (day)	
	0	7	14
A <sub>R</sub>	$1.0 \ge 10^2$	$6.1 \times 10^2$	$1.31 \times 10^5$
B <sub>R</sub>	$2.1 \times 10^2$	9.6 x $10^2$	$1.69 \ge 10^5$
C <sub>R</sub>	$4.9 \times 10^2$	$1.28 \times 10^2$	$1.72 \times 10^5$
D <sub>R</sub>	$6.8 \times 10^2$	$4.9 \times 10^2$	$2.01 \times 10^5$
E <sub>R</sub>	$2.8 \times 10^2$	$8.4 \times 10^2$	$2.12 \times 10^5$
F <sub>R</sub>	$3.04 \times 10^3$	$2.51 \times 10^2$	$2.35 \times 10^5$

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NG=No growth, Subscript R = Refrigerated; A= 100 % Tigernut milk + 0 % soymilk; B= 90 % Tigernut milk +10 % soymilk; C= 80 % Tigernut milk + 20 % soymilk; D = 70 % Tigernut milk + 30 % soymilk; E=60 % Tigernut milk + 40 % soymilk; F= 50 % Tigernut milk + 50 % soymilk

350 Source: Udeozor and Awonorin [34].

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- 353 354
- Table 9. Total heterotrophic fungal count in cfu/ml of various tigernut-soy milk extract (TSME) under storage at 4 °C
- 355 356

Sample	Storage period (d	lay)		
	0	7	14	
A <sub>R</sub>	$7.8 \times 10^2$	$9.6 \times 10^2$	$9.8 \times 10^2$	
B <sub>R</sub>	$7.7 \times 10^2$	$8.3 \times 10^2$	$9.2 \times 10^2$	
C <sub>R</sub>	$6.9 \times 10^2$	$8.7 \times 10^2$	$8.92 \times 10^2$	
$D_R$	$3.17 \times 10^2$	$5.9 \times 10^2$	$6.3 \times 10^2$	
E <sub>R</sub>	$1.48 \ge 10^2$	$1.68 \ge 10^2$	$1.71 \text{ x } 10^2$	
F <sub>R</sub>	$1.21 \times 10^2$	$1.81 \times 10^2$	$1.92 \ge 10^2$	

357 NG=No growth, Subscript A = Ambient-stored; A= 100 % Tigernut milk + 0 % soymilk; B= 90 % Tigernut milk 358 +10 % soymilk; C= 80 % Tigernut milk + 20 % soymilk; D = 70 % Tigernut milk + 30 % soymilk; E=60 %

10% soymink; C= 80\% figernut mink + 20\% soymink; D = 70\% figernut mink 359 Tigernut milk + 40% soymilk; F= 50% Tigernut milk + 50% soymilk

360 Source: Udeozor and Awonorin [34].

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- 362
- Table 10. Total heterotrophic fungal count in cfu/ml of various tigernut-soy milk extract (TSME) under storage at 28±2 °C
- 365

Sample		Storage period (day)		
	0	7	14	
A <sub>A</sub>	9.9 x 10 <sup>2</sup>	$1.16 \times 10^3$	$2.01 \times 10^4$	
B <sub>A</sub>	$9.2 \ge 10^2$	$9.6 \times 10^3$	$1.21 \times 10^4$	
$C_A$	$7.2 \ge 10^2$	$8.1 \times 10^2$	1.96 x 10 <sup>4</sup>	
$D_A$	$4.2 \ge 10^2$	$1.26 \times 10^3$	$2.41 \times 10^4$	
$\mathbf{E}_{\mathbf{A}}$	$2.6 \ge 10^2$	$1.01 \times 10^3$	$1.16 \ge 10^4$	
F <sub>A</sub>	$1.61 \ge 10^2$	$2.7 \times 10^2$	2.69 x 10 <sup>4</sup>	

366NG=No growth, Subscript R = Refrigerated; A= 100 % Tigernut milk + 0 % soymilk; B= 90 % Tigernut milk +10367% soymilk; C= 80 % Tigernut milk + 20 % soymilk; D = 70 % Tigernut milk + 30 % soymilk; E=60 % Tigernut

368 milk + 40 % soymilk; F= 50 % Tigernut milk + 50 % soymilk

369 Source: Udeozor and Awonorin [34].

370

#### 371 Table 11. Distribution of microflora isolated from tigernut-soy milk extract (YSME) during storage

			<pre></pre>	AL A				
Microorganism	Storage condition		V					
		A	В	C	D	Е	F	
Bacillus subtilis	Refrigerated	+	+	+	+	+	+	
	Ambient-stored	+	+	+	+	+	+	
Bacillus cereus	Refrigerated	_	-	-	-	-	-	
	Ambient-stored	+	+	-	+	+	+	
Staphylococcus aureus	Refrigerated	-	-	-	-	+	+	
	Ambient-stored	+	-	+	+	+	+	
Penicillium notatum	Refrigerated	_	_	_	+	-	-	
	Ambient-stored	-	-	+	-	-	+	
Aspergillus flavus	Refrigerated	-	+	+	-	-	-	
	Ambient-stored	-	-	+	-	-	-	
Rhizopus spp.	Refrigerated	+	+	+	+	+	-	
	Ambient-stored	+	+	+	+	-	-	
Saccharomyces spp.	Refrigerated	+	+	+	+	+	+	

	Ambient-stored	+	+	+	+	+	+
Mucor spp.	Refrigerated	+	+	+	-	+	+
	Ambient-stored	+	+	+	+	+	+

372 A = 100 % Tigernut milk + 0 % soymilk; B = 90 % Tigernut milk + 10 % soymilk; C = 80 % Tigernut milk + 20 %

soymilk; D = 70 % Tigernut milk + 30 % soymilk; E=60 % Tigernut milk + 40 % soymilk; F= 50 % Tigernut milk +
50 % soymilk. Source: Udeozor and Awonorin [34]

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6. Economic importance of tigernut and tigernut-derived products

378 The economic value of tigernut is majorly a function of the numerous edible and non-edible products obtained from 379 tigernut tubers. Spain is well known for production of tigernut-milk popularly known as 'horchata de chufa'. 380 Consumption of tigernut-derived products especially tigernut-milk has also gained popularity in many European 381 countries [42]. The 'horchata' industry in Spain has considerable economic importance. Yearly, approximately 40-382 50 million liters of 'horchata' are manufactured in Spain according to an industrial production survey. In the retail 383 market, this represents about 60 million euros [43]. Due to high demand of tigernut tubers, Spain import tigernut 384 tubers from other countries. Nigeria, Niger, Burkina Faso, Benin, Mali and Ghana export tigernut tubers [28]. 385 Annual tigernut production in Spain is approximately 3.3 million euros [43]. In Nigeria, Asogwa et al. [44] did a 386 study to identify entrepreneurial skills that will enable women farmers in Benue State Nigeria to process tigernut 387 tubers into milk as a strategy to fight poverty.

388

Cyperus esculentus being listed among top 20 worst weeds which usually result in loss in crop yield up to 50 % should not limit its utilization for economic benefits [45]. Since tigernut is an agricultural byproduct considered as being underutilized, Agbabiaka et al. [46] were able to show that raw and fermented tigernut discard meals have prospects of being incorporated into animal feedstuff because of its nutritional composition. This will translate to economic gain rather than posing a challenge to environmental cleanliness which has cost implication.

394

As a result of lactose intolerance which has become a major health concern to many individuals, the demand for lactose-free products which largely involves utilization of tigernut tubers has been on the increase. Globally, glutenfree retail market was \$ 1.7 billion in 2011. The value increased to \$ 3.5 billion in 2016. It is expected to hit \$ 4.7 billion in 2020. Therefore, there is great opportunity for farmers and industrialists to increase their income by massive cultivation and large scale processing of tigernut tubers into useful products both for local consumption and export [47].

401

# 402 CONCLUSION

403 Contamination of tigernut tubers could either be physical, chemical, microbial or a combination of any of them. 404 Consumption of contaminated tigernut tubers and utilization of the tubers to produce edible products will invariably 405 result in contaminated tigernut-derived products if no further treatment to reduce or completely eliminate the 406 contaminant is applied. Based on available information, there has not been any reported casualty as a result of 407 consumption of contaminated tigernut tubers or tigernut-derived products. In order to maximize the potential of 408 tigernut tubers and guarantee safe tigernut-derived products, it is important to prevent tigernut tubers and tigernut-409 derived products from any form of contamination.

410

# 411 **COMPETING INTERESTS**

- 412
- 413 The authors have declared that no competing interests exist.
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