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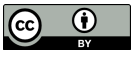
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Review

Nutritional and Microbiological Quality of Tiger Nut Tubers (*Cyperus esculentus*), Derived Plant-Based and Lactic Fermented Beverages

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Abstract: Tiger nut (*Cyperus esculentus*) is a tuber that can be consumed raw or processed into beverages. Its nutritional composition shows a high content of lipid and dietary fiber, close to those of nuts, and a high content of starch, like in other tubers. Tiger nuts also contain high levels of phosphorus, calcium, and phenolic compounds, which contribute to their antioxidant activity. From those characteristics, tiger nuts and derived beverages are particularly relevant to limit food insecurity in regions where the plant can grow. In Europe and United States, the tiger nut derived beverages are of high interest as alternatives to milk and for gluten-free diets. Fermentation or addition of probiotic cultures to tiger nut beverages has proven the ability of lactic acid bacteria to acidify the beverages. Preliminary sensory assays concluded that acceptable products are obtained. In the absence of pasteurization, the safety of tiger nut-based beverages is not warranted. In spite of fermentation, some foodborne pathogens or mycotoxigenic fungi have been observed in fermented beverages. Further studies are required to select a tailored bacterial cocktail which would effectively dominate endogenous flora, preserve bioactive compounds and result in a well-accepted beverage.

Keywords: tiger nuts; horchata; lactic fermentation; beverage; quality; product development

1. Introduction

Increased consumption of fruits and vegetables is recognized to protect against non-communicable diseases [1,2]. Health benefits of fruit and vegetable intake have been historically related to their high content in vitamins, minerals, and phytochemicals. They are also recommended as a source of dietary fibers. For this purpose, starchy roots and tubers have a considerable role for carbohydrate and dietary fiber intake [3]. This role is of great importance for gluten-free diets, which are characterized by insufficient dietary fiber uptake [4–6]. In addition, comparatively to dairy products, plant-based materials do not contain lactose or dairy allergens and exhibit low cholesterol content. Moreover, they benefit from a vegan-friendly status. Among plant-based alternatives to milk, the most popular are soya, almond- and rice-based beverages, but other substitutes, either directly obtained from traditional edible plant extraction, either fermented, are gaining interest as the market is rapidly expanding [6–8].

Lactic fermentation is a traditional and sustainable way to increase the shelf-life of food products as well as to change texture, flavor, and taste [9,10]. In addition, depending on the bacterial strains, a probiotic effect, defined as a health benefit on the host, conferred by living microorganisms administered in adequate amounts, can be expected [11].

Historically, lactic fermentation has been applied to a variety of raw materials, including milk, meat, fish, cereals, vegetables, and fruits [9,12–14]. Lactic fermentation of cereals, including maize, millet, barley, oats, rye, wheat, rice or sorghum, into beverages is ancestral and commonly used in Africa [7,15]. Lactic fermented beverages obtained from teas or vegetables or fruits, with high functional value, were also described [16,17]. The development of new lactic-fermented products is explored worldwide and is recognized as the most suitable way for increasing the daily consumption of fresh-like vegetables and fruits [7,10,16].

Tiger nut (*Cyperus esculentus*) is a tuber, mainly harvested in Spain, West Africa countries like Nigeria, Senegal, or Ghana, and also in South America, as in Chile [18,19]. From an economic point of view, tiger nut is described as an underutilized African crop with high potential for development [20]. Because of its ecological plasticity and its invasive capacity, this plant is considered as a weed or a crop depending on the context [18]. In West Africa, the tubers are often part of the diet as they are cheap, available all the year around, and with nutritional benefits [20]. Recent market changes show how innovative tiger nut based beverages are arising around Europe and this tuber is becoming popular in the US [21].

Nutritional composition of tiger nut tubers shows some unique features, between other tubers and nuts [19]. Starch content of tiger nut is closely related to that of cassava, whereas lipid and even more fiber contents resemble those found for almonds or pistachios. Its composition and associated health benefits offer to this tuber a huge potential for product development. Nowadays, tubers are consumed raw, roasted or after transformation into beverage or flour. The beverage is obtained after several washing steps to eliminate dusts and poor-quality tubers, soaking overnight the dry tubers in water, disinfection with chlorinated water, blending with water, pressing, and filtering (Figure 1).

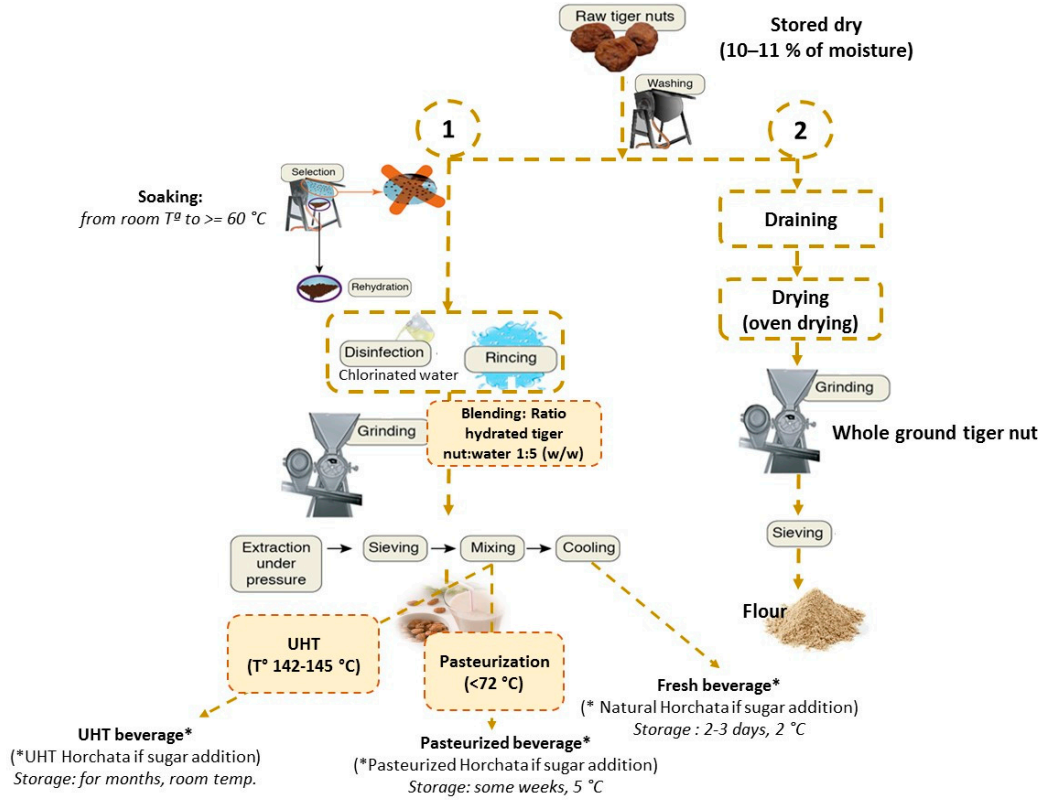


Figure 1. Flowchart showing industrial processing of tiger nuts (1) beverages and (2) flours.

The beverages obtained from this process are called improperly tiger nut milk [22,23] or juice [24]. In Spain, added with a minimum of 10% sugar, this is the very popular drink called "Horchata". Processing this product is regulated: a minimum of 12 Brix, 1.9% of starch, 2% of fats, total sugars expressed as sucrose of 10%, and a pH above 6.3 [25]. Horchata can be produced fresh, pasteurized, sterilized, concentrated or even in powder. When heat treatments are applied, an amylolytic step is required before applying a temperature above 75 °C to avoid starch gelatinization. Whole ground tiger nut and flour are also commercialized. Both the fermentation of tiger nut derived beverage or the addition of probiotic cultures have been evaluated in several studies, resulting in tiger nut yogurts [23,26–31]. Moreover, the "Horchata" production process results in several by-products, such as the drained-water, which is suitable for other applications [32].

This review examines the latest description of nutritional composition, together with microbiological quality, of tiger nuts and derived beverages. We provide a review of the impact of lactic fermentation on the quality of tiger nut beverages. From those data, we focus on the relevance of the consumption of fermented beverages and their potential for new product development.

2. Nutritional Characteristics and Bioactive Compounds of Tiger Nut

The energy value of tiger nut tuber ranges within 400–413.8 kcal/100 g [19,33]. The main components of tiger nut are carbohydrates, which represent 43.3 g/100g. Starch content, 29.9% in wet matter, is similar to that of cassava and about twice that of potato [19]. Dietary fiber content, of 8.81 g/100g, is much higher than in other tubers, which contain 0.66–2.55 g/100g, and in similar ranges than nuts. It is constituted of insoluble dietary fiber at 99.8%. Sucrose content, 13.03 g/100g, is also much higher than in other tubers, ranging from 0.31 to 4.77 g/100g.

Tiger nut contains 22% to 45% of fat in dry matter, depending on the origin of the tubers [34]. In wet matter, due to a higher moisture content of ca. 26%, lipid content is lower than in tree nuts.

The examination of the fat structure in seed oils obtained from tiger nut showed that neutral lipids, dominated by triacylglycerols constitute the bulk of lipids and represent 65.9% of total lipids, while glycolipids and phospholipids represent 5.6–6.9% and 1.4–3.1%, respectively. The structural feature is consistent with most vegetable oils, with monounsaturated acids predominantly found in greater amounts at the sn-2 position, and a lower prevalence of saturated fatty acids, located in the sn-1 and sn-3 positions [35,36]. The main fatty acids are oleic 56% to 85%, palmitic 10% to 20%, linoleic 8% to 12% and stearic 0.3% to 5.3% acids [19,34,37,38], while the minor acids were linolenic and palmitoleic [35]. Tiger nuts composition in monounsaturated fatty acids is in agreement to that found for olive oil, being the fatty acid profile used as a possible geographical authenticity marker. Tiger nut naturally contains a number of sterol components that are of a different composition than the components found in olive oil. β-sitosterol was found as the main compound (≈49–60 mg/100g), followed by stigmasterol, campesterol, α and β-tocopherols [39].

Protein content in tiger nut (5.04–6.67% wet matter) is higher than that found in other tubers, but lower than in nuts. For instance, the protein content of tubers ranges from 0.66% in sweet potato to 2.55% in yam, whereas pine nuts and peanuts contain 13.7% and 25.8% of proteins, respectively [19]. The predominant protein fraction (82–91%) corresponds to the water-soluble fraction of albumin and non-protein nitrogen. The analysis of these fractions by electrophoresis presented a high diversity in polypeptide molecular weights, highlighted at 20, 25, 37, 55, 75 and ∼106 kDa. The other solubilized fractions of globulins (1.11–3.96%), prolamins (0.91–3.45%) and glutelins (0.63–1.98%) presented a pattern with fewer different molecular weight polypeptides [40]. The amino acid profile shows, in decreasing order, aspartic acid, glutamic acid, leucine, alanine and arginine [37]. In addition to protein anabolism, L-Arginine is of particular interest as it is the main precursor of nitric oxide (NO), a non-adrenergic and non-cholinergic neurotransmitter involved in numerous physiological and signaling processes, with a strong vasodilator action. For instance, a dried defatted tiger nut tuber dietary supplementation of rats fed with L-NAME nitric oxide synthase inhibitor (Nω-nitro-L-arginine methyl ester hydrochloride) presented an effect on NO metabolism, preventing the reduction in

the production of nitric oxide synthesis markers in serum and penile tissue. It also prevented the increased activity of enzymes like acetylcholinesterase, arginase, and adenosine deaminase, leading to the inhibition of NO production whether on serum, on brain or on penile tissue [41].

Tiger nut tubers exhibit high calcium, and phosphorus mineral contents [33,34,37]. On the other hand, magnesium, manganese, iron, zinc and copper are also present, but at lower levels.

Apart from those main characteristics, phenolic and anti-nutrient compounds were analyzed [38,42,43] (Table 1). Phytates represented ≈21.4 mg/100g in raw tubers, oxalates ≈13.1 mg/100g, alkaloids ≈2.6 mg/100g and tannins ≈2.4 mg/100g. The content of all these families of compounds was decreased after soaking, with a more marked effect when soaking was performed at 60 °C/7h compared to room temperature 12h [42]. The highest decrease was observed for tannins (61%), followed by oxalates with (58%) and polyphenols (48%). The main discrepancies were observed with the data published by Chukwuma et al. (2010) [43], but for tannins, phytates and polyphenols, the contents were 100-fold below those observed in other tubers or in chickpeas [44–46]. The composition analysis shows that *trans*-ferulic acid, vanillic acid, vanillin and *trans*-cinnamic acid are the main phenolic compounds in tiger nut oils [38]. Moreover, the antioxidant activity of tiger nut polyphenols was investigated in comparison with flavonoids from a chemoprotective perspective. A dietary supplementation with 25% of whole tiger nut showed moderate renal and hepatoprotective properties against acrylamide-induced toxicity in rats [47].

Table 1. Phenolic profile of tiger nut.

Compound	Concentration	Ref.
Apigenin	7.91–50.58 mg GAE/100 g	[48]
Caffeic acid	1.07–15.25 mg GAE/100 g	[48]
	3.90–102.19 µg RE/kg	[49]
(Epi)Catechin	8.83×10^{-4} – 6.58 mg GAE/100 g	[48]
Cinnamic acid	0–40.66 µg RE/kg	[49]
Coumaric acid	0-6801.0 µg/g	[50]
	4.20×10^{-4} – 17.25 mg GAE/100 g	[48]
	0–126.76 µg RE/kg	[49]
Ferulic acid	3.5–2,284 µg/g	[50]
	33.79–58.38 mg GAE/100 g	[48]
	0–22.33 µg RE/kg	[49]
Diferulic acid	0.0–829.0 µg/g	[50]
Ferulic acid-4-*O*-glucoside	0–46.95 µg RE/kg	[49]
Gallic acid	3.95×10^{-3} – 1.74 mg GAE/100 g	[48]
Homovanillyl alcohol	0–4.54 µg RE/kg	[49]
p-Hydroxybenzaldehyde	0–16.47 mg GAE/100 g	[48]
	4.0–337.0 µg/g	[50]
p-Hydroxybenzoic acid	0–8.3 µg/g	[50]
	2.18–29.12 mg GAE/100 g	[48]
	2.52–67.70 µg RE/kg	[49]
Isohydroxymatairesinol	0–1331.45	[49]
Kaempferol	3.62–24.44 mg GAE/100 g	[48]
Luteolin	7.29–72.17 mg GAE/100 g	[48]
24-Methylcholestanol ferulate	0–45.40 µg RE/kg	[49]
Naringenin	2.38×10^{-3} – 16.16 mg GAE/100 g	[48]
Peonidin	0–7.81 µg RE/kg	[49]
Protocatechuic acid	0.61–0.79 mg GAE/100 g	[48]
Quercetin	3.76×10^{-3}-60.63 mg GAE/100 g	[48]
trans-Resveratrol-3-*O*-glucoside	0–25.68 µg RE/kg	[49]
Scopoletin	0–310.80 µg RE/kg	[49]
Sesamin	0-28.67 µg RE/kg	[49]
Sinapinic acid	8.53×10^{-1} – 20.97 mg GAE/100 g	[48]

Table 1. *Cont.*

Compound	Concentration	Ref.
Sinensetin	0–16.07 µg RE/kg	[49]
Syringic acid	4.58×10^{-4} – 4.12 mg GAE/100 g	[48]
Vanillic acid	3.0–25.3 µg/g	[50]
	5.88–15.20 mg GAE/100 g	[48]
	0–10.84 µg RE/kg	[49]
Vanillin	15.5–68.7 µg/g	[50]
ethyl Vanillin	0–25.38 µg RE/kg	[49]
4-Vinylphenol	0–1084.48 µg RE/kg	[49]

GAE: Gallic Acid Equivalent; RE: resveratrol Equivalent.

3. Nutritional Characteristics of Tiger Nut-Based Beverages

The main composition of tiger nut-based beverages, i.e., Horchata de Chufa and juices, is presented in Table 2.

Table 2. Proximate major nutritional content in tiger nut tubers and beverages.

Nutrient	Tiger Nut Tuber (g/100g) [a]	Horchata De Chufa (g/100g) [a]	Tiger Nut Beverage (g/100g)	
Total fat	24.49	3.09	1.26–1.59 [b]	1.88–2.27 [c]
SFA (% total fatty acid)	17.5			
MUFA (% total fatty acid)	72.9			
PUFA (% total fatty acid)	9.3			
Ratio *n*-6/*n*-3	22			
Proteins	5.04	0.91	2.34–2.51 [b]	0.47–0.54 [c]
Ash	1.7	0.25	0.31–0.39 [b]	0.16–0.18 [c]
Carbohydrates	43.3	*nd*	1.93–2.34 [b]	2.31–2.74 [c]
Total dietary fiber	8.91	1.03	0.23–0.31 [b]	0.53–0.65 [c]
Sucrose	13.03	>10		
Total energy (kcal/100g)	413.8	>71.45	28.42–33.71 [b]	28.04–33.55 [c]

[a] data from Sanchez-Zapata 2012 [19], Horchata de Chufa was prepared from tiger nut beverage and additives including more than 10% of sucrose; [b] Tiger nut beverage was obtained by soaking Nigerian tiger nut in water (1:3 *w*/*v*) for 10 h, then by blending drained nuts with water (1:5 *w*/*v*). The resulting homogenous slurry was filtered using a muslin cloth and the resultant filtrate was pasteurized at 67 °C for 30 min [24]; [c] To obtain tiger nut beverage, tiger nut from Ghana were first dried for two months at room temperature. The nuts were hydrated by soaking in a water bath for 24 h at 40 °C then grinded with water (1:4 *w*/*v*). Finer particles were obtained by dispersing the sample with ultra-turrax mixer at 13,000 rpm for 20 min and were then transferred into a pneumatic press using one volume of water, to be filtered through a 4 µm-pore-size filter membrane [51]; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; *nd:* not determined value.

As can be seen in the table, tiger nut beverage, which differed from that of "Horchata" in the fact that there is no sucrose addition, exhibits energy values ranging from 28.42 to 33.55 kcal/100 mL [19,42]. These values are close to those of skim cow milk (33 kcal/100 mL), and in the early window by comparison with soy-based "milk" alternatives, which range from 33 to 58 kcal/100 mL. The overall plant-based "milk" alternatives present a larger range (12–92 kcal/100 mL).

A protein content between 0.47–2.51% can be expected in tiger nut beverages according to the process of extraction [24,51]. Considering the plant-based "milk" alternatives, the highest overall protein content is found in soy-based beverages, ranging from 2.50 to 3.16% whether rice and almond-based "milk" alternatives exhibit the lowest protein content with 0.28% and 0.31–0.59%, respectively [52]. The tiger nut beverage concentration of essential amino acids is 14.27 g/100 g of proteins, which represents ≈28% of the total amino acid content. It can be observed that leucine is the limiting amino acid [24]. Although the tiger nut beverage amino acid scores are lower than the few existing values, namely for quinoa or soy, which have amino acid scores all above 100%, the essential amino acid composition is of interest and differs from some other plant-based beverage proteins.

Methionine and cysteine show a relative abundance in tiger nut whereas they are the limiting amino acids in pea, almond and soy proteins [52]. The calculation of tiger nut beverage amino acid score values [53] ranged from 29% for leucine to 87% for methionine plus cysteine for infants/pre-school children, and 31% to 102% respectively for adults.

The fat content of tiger nut tuber is far above those of beverages, and as expected from tuber composition, the lipid fraction is mainly composed of oleic, palmitic, and linoleic acids. Interestingly, the fractionation of compounds between beverage and residue fraction is dependent on the compound family, being approximately 4:1 for protein, lipid, salts, and soluble fiber, whereas it is 1:6 for insoluble fiber and 1:1 for carbohydrates [51].

Tiger nut beverage is an emulsion of oil droplets in an aqueous phase and contains starch granules and small solid particles [25]. Starch granules are related to product stability, in particular regarding color and texture. For that matter, a maximal pasteurization temperature is recommended to avoid starch gelatinization [54]. Milling conditions were shown to modulate colloidal stability of the beverage [51]. The stability of the system is ensured by proteins, located at the interphase oil-water. The addition of emulsifiers could be required for long-term storage of the beverage. For instance, the analysis of 87 samples, either fresh artisanal-made, or UHT-treated in industry, revealed that emulsifiers like citric acid esters of mono- or diglycerides, and monoacyl glycerol, are present into industrial tiger nut beverages. On the opposite, phosphatidic acid is present only in fresh beverage, and the fresh beverage content of biotin and of L-arginine is respectively 4-fold and 10-fold higher than in UHT beverage [55].

4. Products Obtained by Lactic Fermentation or Lactic Acid Bacteria Addition

Until now, lactic fermentation of tiger nut beverages has been poorly investigated compared to its multiple interesting applications (e.g., from a nutritional point of view and for new product development). Fermentation of non-dairy beverages has been mainly focused on vegetable juices [56], fruits juices [17,57–60], cereal-based beverages [7,59], but also walnut and cashew nut beverages [61,62]. Although the demand for plant-based milk substitutes is increasing, the unwillingness of the mainstream consumer to try unfamiliar foods that are perceived as unappealing may be a limiting factor. Some undesired flavors could efficiently be reduced by fermentation and the flavor could be more appreciated in presence of lactic acid [8]. Lactic acid bacteria not only modify sensory characteristics of the beverages, but also can produce additional micronutrients [63,64] or increase antioxidant properties [17], produce texturing compounds like exopolysaccharides [65,66], or limit undesirable microorganism development [67–69].

Besides lactic fermentation of foods and beverages, the addition of lactic acid bacteria cultures *per se* can bring numerous advantages by the production of compounds, like exopolysaccharides, which modify the texture [6]. The use of probiotic lactic acid bacteria to develop new functional beverages from fruits or vegetables is challenging, because the growth or survival of bacteria in such media has to be carefully examined [70–75]. But the interest is even more significant for food matrices that contain a high content of dietary fiber, and can serve as a prebiotic [71,75]. For developing countries, the selection of probiotic bacteria can be an issue to limit food insecurity [76].

The first study reporting tiger nut beverage fermentation compared the obtained product with cow milk yogurt [29]. Classical yogurt starters were used, i.e., *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, to ferment tiger nut beverage. As expected, the fermentation resulted into an acidified product, at a pH of 3.9, which was overall less appreciated by a small untrained panel than the classical yogurt. Ukwuru *et al.* (2008) could not detect any difference of general acceptability between fermented tiger nut beverage and cow milk [30], but the lower acceptability of yogurt-processed tiger nut beverage compared to classical cow milk yogurt was confirmed by Sanful et al. (2009) with a broader sensory panel [31]. At this stage, the production of a fermented product from a 1:1 mix of tiger nut and cow milks seems to be the best compromise for sensory attributes, but this product loses the advantages of a plant-based yogurt.

Tiger nut beverage fermentation performed with a *Lactobacillus plantarum* isolated from spontaneous fermentation demonstrated the ability of this strain to grow and to acidify the beverage [27]. Interestingly, an attempt to ferment tiger nut beverage with isolates from *Ogi*, a traditional fermented cereal porridge from Nigeria, resulted in a pH decrease up to 4.04–4.36 depending on isolated combinations [23]. In that study, four species (*Lb. plantarum*, *Lactobacillus acidophilus*, *St. thermophilus* and *Lactobacillus brevis*) were used, and three combinations of three isolates among those bacteria were used, resulting into comparable growth and acidification levels.

None of the previous studies reported a phase separation over fermentation. However, this could be expected from its composition, as the stability of this complex system depends on oil-water droplets, the protein emulsifying effect, and small solid particles. Acidification would modify protein surface properties. Moreover, in fermented non-dairy beverages, the role of exopolysaccharides, produced by bacteria, on texture and sensory quality has been demonstrated for a long time [77,78]. The use of exopolysaccharides producing lactic acid bacteria demonstrated their positive impact on the texture of fermented soy beverages [79] and on pureed carrots [80]. A similar approach would be of great interest for tiger nut beverages. Moreover, exopolysaccharides exert some beneficial health effects [81].

The role of proteins in beverage stability and the interest in protein fortification of food encouraged the formulation of fermented beverages from tiger nuts supplemented with soybean, sorghum, whey proteins, and caseinate [28,82]. Xanthan gum was also used for its thickening and stabilization effects [28]. A beverage containing sorghum, soybean, and tiger nut flours in ratio 5:3:2 and fermented with *Lb. plantarum* exhibited a decrease of counts of yeasts and molds and of enterobacteria below the detection limit over 24h of fermentation. This beverage contained 26.6% of proteins and 26.2% of carbohydrates on a wet base, and was positively evaluated by a sensory panel [82]. Dairy protein addition modified gel properties, leading to semi-solid, yogurt-like gels [28]. Gel stiffness and viscosity were higher and whey drainage was lower when sodium caseinate was used instead of whey proteins, hence opening a way for texture modulation by varying the protein type and content.

Compared to other lactic fermented beverages with a plant-origin, there are some features observed in the original nutritional composition of tiger nut beverages. The natural balance in good quality nutrients, likewise, unsaturated fat, minerals, and antioxidant, make tiger nut fermented beverages a potential candidate for diet complementation. The abundance of unsaturated fatty acids together with antioxidant compounds is an effective choice for lowering the dietary intake of saturated fatty acids and to limit oxidative stress. Oleic acid especially is suggested to reverse induced hyperlipidemia and to have a strong hepatoprotective effect on rats receiving a high fat/high cholesterol diet [83,84]. Depending on the process flow and the final protein content of tiger nut beverages, the determination of protein bioavailability is definitely required to evaluate the effect of lactic fermentation on these nutrients. Tiger nut beverage calcium content of 40 mg/100g is higher than other unfortified milk alternatives having drastically lower contents of calcium, ranging from 0 to 12 mg/100 g [52]. The calcium bioavailability in plant-based beverages is however uncertainly comparable with the 120 mg/100 g cow milk calcium content, because its natural association with caseins facilitates absorption and limits sedimentation. Whether fermentation improves this parameter, it is also worth further investigation.

Tiger nut composition showed the presence of numerous phenolic compounds. However, the impact of lactic fermentation on these compounds in tiger nut beverages has never been investigated.

5. Safety and Microbiological Quality

In a study evaluating the parasite presence of raw tubers collected from street vendors and market places in Ghana, several parasites were found; *Cryptosporidium parvum* being the most common [85]. Enterobacteria and *Staphylococcus* sp. were also detected. Moreover, several species of mycotoxin-producing molds, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Penicillium italicum*, were found in raw tiger nut tubers [27]. Mycotoxins were detected in both raw tiger nuts

and beverages [86,87]. Aflatoxins B_1 and G_2, beauvericin and ochratoxin A were the predominant mycotoxins detected in tubers, with respectively 14, 10, 11 and 8 samples upon 83 that exhibited toxin contents over the limits of quantification.

The pH of tiger nut beverage is in the range 6.3–6.8 [19], which is not limiting the growth of food-borne pathogens. On tiger nut beverage samples, two microbiological profiles were observed depending on the process applied, either industrial or home-made [88]. On industrial products, which were heat-treated, no foodborne pathogen could be detected, and total viable bacteria counts were below the detection limit. On the opposite, the home-made products exhibited total viable counts in the range of 3.6–6.5 log CFU/mL, and on certain samples, enterobacteria, *Bacillus* sp., *Escherichia coli* and yeast and molds were found. In another study [30], an increase of bacterial counts from 1.3 log CFU/mL to 3.5 log CFU/mL and 8.5 log CFU/mL, when stored at 4 °C or at 32 °C respectively, was observed after 6 days of storage. The samples stored at 32 °C showed spoilage signs after 6 days. The refrigerated sample remained drinkable over 14 days and the bacterial counts reached then 4.4 log CFU/mL.

In tiger nut beverage, ochratoxin A was detected in 32 samples from 238, but below quantification limit. Aflatoxins B1, B2 and G2 were detected in 12, 2 and 1 samples, respectively from 238, and below the maximal limits fixed by EU.

Fermentation generally results in an increase of safety thanks to acidification and to the production of bacteriostatic compounds such as organic acids, hydrogen peroxide or bacteriocins [9,89]. Lactic acid bacteria also can impair the growth of mycotoxin-producing fungi, inhibit the biosynthesis of mycotoxins or degrade the compounds [89–91]. Only preliminary studies have investigated safety or the microbiological quality of the fermented tiger nut beverages. Agbaje *et al.* (2015) [27] isolated and identified potential foodborne pathogens, such as *Staphylococcus aureus*, and mycotoxinogen fungi. Traditional fermented beverages hold their own microbial ecosystem, among those lactic acid bacteria are diverse and often associated with yeasts [7,59,92]. To date, contrarily to other fermented vegetables [93–96], there is no data on tiger nut beverage microbiota.

6. Conclusions and Future Research for Functional Beverages and Foods

The interest in the lactic fermentation of plant-based beverages emerged from the search of "substitutes" to milk, due to the allergenicity or vegan-based considerations. In developing countries, the objective to fulfill nutritional requirements of people affected by food insecurity is another leading reason for this type of research. Most of these studies highlighted the value of developing featured tastes and flavors, together with an increased shelf-life of perishable products. In line with that objective, the development of fermented products from tiger nut beverages is particularly relevant.

The requirement of starters isolated from a similar origin is pointed out as a key-factor for successful fermentation [97–99]. When developing new fermented beverages from plant-origin, the choice for fermentative bacteria selected from fermented plants should be recommended. Moreover, the selection of tailored microbial starters contributes to the safety of the fermented foods and beverages by two aspects: i) the safety of the starter itself, ensured by its GRAS status and not producing toxic compounds, and ii) its ability to decrease hazards in a given food or beverage [100]. Consequently, extensive research on lactic acid bacterial starters adapted to tiger nut beverage fermentation is still required.

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