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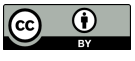
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The Influence of Soaking and Sprouting on the Physicochemical Characteristics of Tigernut Tubers (*Cyperus esculentus* L.)

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Abstract: The influence of soaking and germination on the physicochemical characteristics of tigernut tubers (*Cyperus esculentus* L.) are investigated. Tubers (Ø > 1 cm) were soaked in an ascorbic acid solution (0.1%) for 48 h at 40 °C before undergoing germination for 6 days. The textural profile, the capacity and germination energy and the biochemical composition of these tubers were determined. The germination energy varied from 76.18 to 79.48% for the quantities of solution of 24 and 48 mL, respectively. The textural profile of the tubers varied depending on the type of treatment. Germination resulted in a considerable reduction in amylose content from 14.15% for the native tigernut tuber to 9.98% for the sprouted one. This treatment also increased the protein, ascorbic acid and ash contents, which ranged from 7.54 to 8.82 g/100 g DM, 250 to 275.39 mg/100 g DM and 2.60 to 3.84 g/100 g DM, respectively. The starch content of the tubers following germination remained high, which could come up against the pasteurization of the milk from these tubers.

Keywords: *Cyperus esculentus*; soaking; germination; vegetable milks; tigernut

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1. Introduction

The interest of consumers for vegetable milks is growing in many countries as they are free of cholesterol and lactose, but rich in dietary fiber [1]. *Cyperus esculentus*, also called tigernut, belongs to the Cyperacea family and is widespread in southern of Europe, Asia and Africa. Tigernut tubers are used in Africa as a snack food, while in Spain they constitute the raw material of a vegetable drink called "horchata de chufa", which is a very popular during hot weather. In African nibbling practices on tigernut tubers, they undergo treatments, such as roasting and soaking, in order to soften them. The starch content (30–40%) of tigernut tubers constitutes one of the main production limits for vegetable milk as it can contain nearly 18% of this polysaccharide after its extraction and grinding [2–4]. As a result, tigernut milk (*horchata*) is not easily suitable for pasteurization, due to the risk of gelatinization of this starch [4,5]. So, *horchata* production requires either the removal of pasteurization, leading to a reduction in its microbial stability, or to the elimination of the starch by decantation before pasteurization, leading, this time, to a loss of its nutritional value [4,6,7]. In both cases, sugar is added to the drink to improve its flavor.

Various works, such as those of [4,8–10], have shown the interest of germination of tigernut tubers, especially in the production of malted flour. This operation leads to

the hydrolysis of the starch into free sugars, which can be used to reduce the rate of this polysaccharide in the tuber and thus make it possible to reduce its rate in the drink following its extraction. The germination process is characterized by a soaking step that allows rehydrating [11]. This soaking treatment can lead to nutrient losses [2,12].

The aim of this study is therefore to study the influence of soaking and sprouting on the physicochemical characteristics of tigernut tubers, for processing it into vegetable milk.

2. Materials and Methods

2.1. Samples Origin and Treatments

Large tigernut tubers (Ø > 1 cm) (Figure 1) were harvested from the locality of Guily, Department of Mayo-Tsanaga, Far North Region of Cameroon.



(**a**) (**b**)

Figure 1. Tigernut plant (**a**) and tubers (**b**).

The tubers were subjected to soaking treatments in an ascorbic acid aqueous solution (0.1% (*w*/*v*) during 48 h at 40 °C. The objective was to obtain their maximum turgidity before their sprouting, according to Djomdi et al. (2013) [2], Djomdi et al. (2020), Ejoh et al. (2006) and Djomdi et al. (2007) [13,14]. A part of the soaked tubers (ST) was subjected to germination (Germinated Tubers, GT). Untreated tubers or Native Tubers (NT) were used as controls.

The purpose of the pre-germination test was to check whether the tigernut tubers were likely to germinate. It is a test of the viability of tubers, generally carried out on cereals [15].

2.2. Germination Capacity and Energy and Tigernut Tuber Sprouting

The germination capacity was determined using the hydrogen peroxide method [15], modified by replacing the hydrogen peroxide solution with an ascorbic acid one. It involves determining the percentage of viable tubers in a sample using hydrogen peroxide and ascorbic acid solutions as an inhibitor of mold development and a growth catalyst, respectively.

Two batches of 200 tubers were soaked at 22–25 °C, in a solution of hydrogen peroxide of concentration 7.5 g/L or ascorbic acid 1 g/L for 2 days. Subsequently, the used solutions were replaced by a fresh solution and then the soaking was left for two more days. At the end of this soaking, the solution was poured, and the count of the non-germinated tubers was carried out.

The germination capacity is expressed by Equation (1) [15]:

$$GC = \frac{200 - n}{2} \quad (1)$$

where GC was the germination capacity (%) and n the number of non-germinated tubers.

The germination energy consists of determining the percentage of tubers that are likely to fully germinate if the sample is normally malted at the time of the test [15]. To determine this parameter, two filter papers were each placed at the bottom of a Petri dish into which

24 and 48 mL of distilled water are added. One hundred tubers were placed in each Petri dish. The boxes were covered and placed in wrapping paper to protect from the light. The tubers were incubated for 24, 48 and 72 h. The sprouted tubers were then counted. The germinative energy is given by Equation (2).

$$GE = 100 - n \quad (2)$$

where GE is the germinative energy (%) and n the number of non-germinated tubers.

Tigernut tubers were germinated according to the methods developed by Umerie et al. (1996) [9] and Garcia-Jimenez et al. (2004) [11]. For this purpose, the tubers soaked in the ascorbic acid solution for 48 h at 40 °C were germinated at 25 °C on jute bags protected from light for 6 days and sprayed with water twice a day (morning and evening). It should be noted that the treatment with ascorbic acid is also a treatment for destroying molds (*Dematophora necatrix*), germination-inhibiting agents [11]. At the end of germination, tubers were dried for 48 h at 40 °C in an oven and the rootlets removed manually.

2.3. Physicochemical and Functional Analysis

2.3.1. Textural Profile of Tigernut Tubers

The treated and untreated tigernut tubers constituted 3 batches: the first batch, consisting of NT, the second batch consisted of ST and the third batch consisted of germinated tubers (GT). They were analyzed with a texturometer (TA XT. Plus) consisting of a support and a spherical probe 59 mm in diameter to quantify the texture (TPA, Texture Profile Analysis).

2.3.2. Biochemical Assays

Alpha Amylase Activity

The alpha amylasic activity of the GT tubers was determined by a spectrophotometric assay (Ceralpha method K-GLUC, Megazyme, 2006). The assay consisted in quantifying the depolymerization reaction of p-nitrophenylmaltoheptaoside (PNPG 7) by α-amylase activity.

Twenty g of malted tigernut tubers were reduced to powder using the Dangoumeau type ball mill (Dangoumill 300, Lonjumeau, France) under liquid nitrogen and the powder obtained was sieved to 500 µm. One g of the powder was suspended in 50 mL of a buffer (buffer A) composed of malic acid (1 M), NaOH (70.0 g/L), $CaCl_2$ (4 mM) and NaN_3 (1 g/L) and adjusted at pH 5.4. The enzymatic extraction was carried out at room temperature for 20 min with stirring. The mixture was then filtered using Whatman N° 1 filter paper. One mL of the filtrate was diluted in 9 mL of a buffer B composed of maleic acid (0.1 M), $CaCl_2$ (2 mM) and NaN_3 (0.01%) adjusted at a pH of 6.5. The enzyme solution was used 2 h after its preparation.

Half mL of the substrate solution (PNPG 7), as the enzyme extract, were poured into the test tubes pre-incubated at 45 °C for 5 min. Half mL of enzyme extract was added to PNPG 7 and the mixture was incubated at 45 °C for 10 min. The reaction was stopped by adding 2.5 mL of ethanol (95% *v*/*v*) and mixing thoroughly with the vortex for 10 min. The tubes were left at room temperature for 10 min to be centrifuged at 1000× *g* for 10 min using the Universal 16 centrifuge (Hettich Zentrifugen Company, Tuttlingen, Germany). The supernatant was collected and the A510 was measured. A blank was prepared adding ethanol to the substrate solution before adding the enzyme extract. One unit (U) of amylase activity is defined as equivalent to the release of 1 µg of glucose per minute and per mL under the conditions of the tests.

The enzymatic activity is given by Equation (3):

$$\text{Enzymatic activity (EA, U/mL)} = \frac{\Delta D0 \times V_t \times 10^6}{\varepsilon x L x v_s} \quad (3)$$

where ε: PNPG7 in the reading solution = 17.8 × 10^3 L. mol. cm^{-1}; ΔD0 the difference of A between assay and control; Vt: Total volume extracted; vs: volume of the test; L: optical path (1 cm).

The protein, crude fiber and lipid contents quantifications were carried out by standard methods [16]. Total energy was quantified by the method of Edem et al. [17]. The dry matter and ash contents were determined by the UICPA assay [18]. The starch content was determined by the method of Mestres and Mestres [19]. Vitamin E levels were determined by the (NP)-HPLC method [20] and ascorbic acid by the AOAC method [21].

For amino acid analyses, 5 to 9 mg of each sample were placed in 2 mL vials containing norleucine and 0.45 mL of HCl (6 M). A vacuum was made in the vial using N_2; it was sealed and placed in an oven at 110 °C for 24 h for protein hydrolysis. After this hydrolysis, the acid was evaporated under vacuum. Twenty µL of the hydrolysate were mixed with 10 µL of ethanol, water, triethylamine (2 2:1) solution. The solution was evaporated and the residue was mixed with 20 µL phenylisothiocyanate in order to perform derivatization for 20 min at room temperature, using ethanol, water, and a triethylamine: phenylisothiocyanate (7:1:1:1) reagent [22]. The excess reagent was evaporated under vacuum at room temperature. The derivatized samples were dissolved in 0.1 mL of sodium acetate solution (0.14 M) and the pH was adjusted to 6.4. Twenty µL were injected into a Waters C18 column (3.9 × 150 mm; Waters, Milford, MA, USA). The derivatized amino acids were eluted in the column using increasingly concentrated acetonitrile, as described by Glew et al. [23].

For their specific quantifications, cysteine and methionine were first oxidized using performic acid (80% formic acid and 20% hydrogen peroxide, 9:1) for 18 h at room temperature. The performic acid was subsequently evaporated and the samples were hydrolyzed with HCl (6 M) as described above.

Tryptophan was determined separately. For this purpose, the samples were hydrolyzed for 18 h at 110 °C in polypropylene tubes with KOH (4.2 M) containing 1% thioglycol (*m*/*v*), according to Hugli and Moore [24]. After hydrolysis, the KOH was neutralized with 4.2 M perchloric acid and the pH of the mixture was adjusted to 3.0 with acetic acid. Twenty µL of the mixture was subjected to derivatization, as described above. The standard amino acid solution containing tryptophan was used for the quantification of amino acids. Tryptophan was analyzed using the C18 column (3.9 × 150 mm; Waters, Milford, MA, USA) under the conditions described by Buzzigoli et al. [25]. The use of this protocol and of the eluent was necessary since they make it possible to separate tryptophan from ornithine, which comes from the alkaline hydrolysis of arginine.

The egg white lysozyme sample served as the reference protein.

The amylose content of the starches from tigernut tubers was measured according to the method of Mc Grane et al. [26]. In a diluted aqueous iodo-iodide (I_2-IK) solution, native amylose forms a complex with iodine. The reaction corresponds to an inclusion of iodine in the form $(I_2)_nI^-$ in the helix channel of amylose. Iodide is essential for stabilizing iodine (I_2) sequences. The intensity of the color of the complex is a function of the amylose content. The first step in determining amyloiodosis was to defat the starch, which may contain residual lipids. A mass of 20 mg of powdered starch was introduced into a test tube. Five mL of 85% methanol was added, and the tube was incubated in a 60 °C water bath during 30 min. The solution obtained was then centrifuged at 2000 rpm for 30 min. The extraction was repeated (with 85% methanol at 60 °C in a water bath) three times, removing the supernatant each time. Pellets were introduced into a screw test tube fitted with a stopper. A total of 2 mL of 1 M NaOH and 4 mL of distilled water were added. The tube was capped and placed in a 95 °C water bath for 30 min under agitation to form the "S" solution ready for dosing. 0.1 mL of solution "S" was mixed with 5 mL of 1% trichloroacetic acid (TCA), 0.05 mL of 0.01 M of I_2-IK solution and 2 mL distilled water was added to it. The A_{620} was measured. A standard amylose solution at 0.2 mg/mL was used to make the standard range and the amylose content (AC) was calculated using Equation (4):

$$A_c = \frac{100 \times \mathrm{q} \times T_V \times 100}{\mathrm{V} \times 1000 \times (100 - \mathrm{Te}) \times \mathrm{m}} \quad (4)$$

where A_C was the amylose content in g/100 g of dry starch; q: the amount of amyloiodosis in mg in each test sample; T_V: the total volume in mL of the solution (S); V: the volume of solution S in each test tube (0.1 mL); m: the mass of the test sample in mg (20 mg); T_e: the residual water content of the starches.

2.4. Statistical Analysis

The variation in the data collected and the statistical significance of the effect of the treatments were analyzed by the analysis of variance. The comparisons of means were made by Duncan's test at probability thresholds 1 and 5%. Statistical data were analyzed using STATGRAPHICS Centurion XVI.I (2010) software and SigmaPlot 11.0 was used to plot the curves.

3. Results

3.1. Behavior of Tigernut Tubers at Germination

The capacity and the germinative energy translate a good aptitude for malting. Indeed, the value of the germinative capacity gives the percentage of viable vegetables [27]. Table 1 summarizes all of these parameters. Unlike cereals, the germination capacities of tigernut tubers in the presence of hydrogen peroxide and ascorbic acid were, respectively, 0 and 94.740%. According to Desogbo et al. [28], even if the germination capacity of sorghum in hydrogen peroxide is 99.66%, in the case of tigernut tubers, this compound inhibits germination. In contrast, ascorbic acid solutions promoted the germination process. The germination energy varies from 76.18 to 79.48% depending on the volumes of solution, respectively 24 and 48 mL. This indicates that the tigernut tubers were sprayed regularly to facilitate germination. These values are in the order of the standards set by EBC (European Brewery Convention) Analytica in 1998 [15] (70 to 100% for germination energy and 92 to 100% for germination capacity). As a result, tigernut tubers are likely to undergo germination in the manner of cereals (Figure 2).

Table 1. Germination characteristics of tigernut tubers.

Parameters	Values (%)
Water content	7.542 ± 0.483
Germination capacity (H_20_2, 7.5 g/L)	0
Germination capacity (ascorbic acid, 1 g/L)	94.740 ± 2.322
Germination energy (24 mL)	76.180 ± 0.541
Germination energy (48 mL)	79.482 ± 0.790



(**a**) (**b**)

Figure 2. Native tigernut tubers (**a**) and germinated for 6 days (**b**).

3.2. Textural Profile of Tigernut Tubers

The textural profile of the tubers varied according to treatments (Figure 3 and Table 2). Germination and soaking significantly influenced ($p < 0.05$) the linear distance (DL), which is 39,650.74, 49,500.63 and 62,207.53 mm, respectively, for NT, GT and ST. These treatments

also have significant effects on the number of peaks (NP) ($p < 0.01\%$), which is 2.29, 10.20 and 14.77, respectively, for NT, GT and ST. As regards the area (A) up to the first break of the peak ($p < 0.05$), there is no significant difference ($p < 0.05$) between ST and GT, but a significant difference with NT.

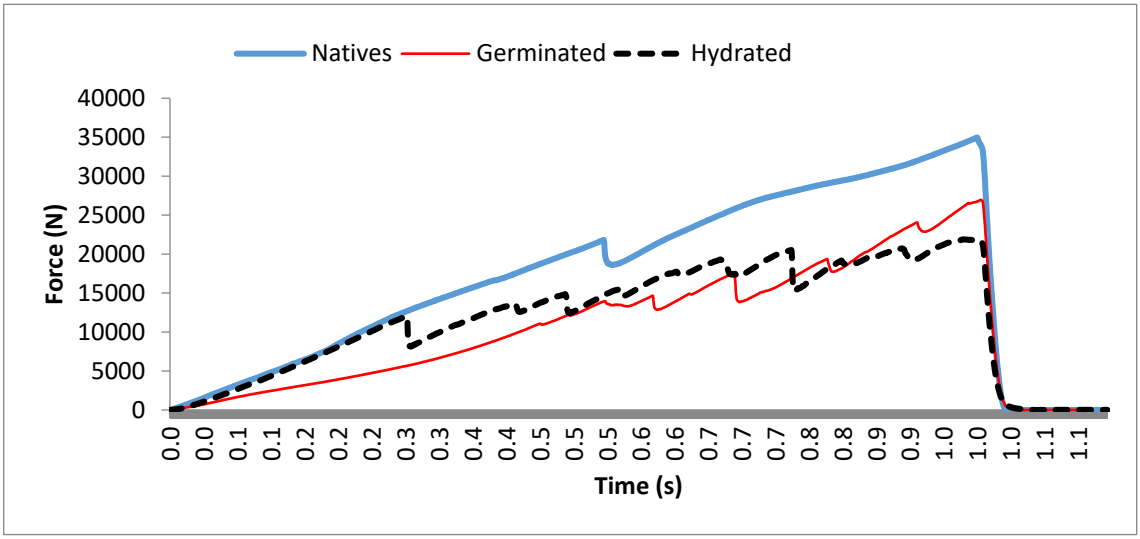


Figure 3. Evolution of the textural parameters of native (NT), soaked (Hydrated) (ST) and germinated (TG) tigernut tubers.

Table 2. Influence of soaking and germination treatments on the textural profile of tigernut tubers.

Processes	DL (mm)	NPm	A (mm^2)
NT	39,650.74 ^{a}	2.29 ^{a}	9751.61 ^{a}
GT	49,500.63 ^{b}	10.20 ^{b}	14,120.03 ^{b}
ST	62,207.53 ^{c}	14.77 ^{c}	16,731.64 ^{b}

All the values followed by the same letter in the same column are not significantly different at the probability threshold $p < 0.05$ for DL and A and $p < 0.01$ for NPm.NT: Native tubers; GT: Germinated tubers; ST: Soaked tubers.

3.3. Impact of Germination on the Biochemical Composition of Tigernut Tubers

Germination led to a considerable reduction in the starch content, which is transformed into reducing sugars because there is an increase of 66.66% in the concentration of these compounds in the sprouted tubers (Table 3). This observation has also been reported by Obeng-Koranteng et al. [29], who recorded an increase of 67.34% in the concentration of reducing sugars after malting the tigernut tubers for the preparation of syrup.

The structure of amylose and amylopectin plays a determining role in the final functionality of native starch and its derivatives: viscosity, shear strength, gelatinization, texture, solubility, adhesive power, gel stability, swelling at cold and downshift, depending on the amylose/amylopectin ratio [30].

The amylopectin contents of the tigernut tubers were 85.95% and 90.02%, whereas those of amylose were 14.15 and 9.98% for, respectively, NT and GT. These results are consistent with the 83.82% amylopectin and 16.18% amylose contents found by Jing et al. [31] for Chinese tigernut tubers, but in contradiction with those of Abdel-Kader and Michalinos [32]. Indeed, these authors found that the starch of tigernut tubers was mainly composed of amylopectin (99%), while amylose is found in trace amounts. These differences may also be dependent on the varietal origin or the agro-ecological production areas. According to these results, the amyloidosis profile of tigernut tubers is different from other tubers, such as yams (26%) and potatoes (21%), but is close to that of cassava (17%) [33,34]. In all cases, the relative low amylose content of tigernut starch is indicative of a low retrogradability of this starch. Germination significantly reduced the amylose content, from 14.15% for NT

to 9.98% for GT. Germination also increased the protein, ascorbic acid and ash contents, which ranged from 7.54 to 8.82 g/100 g DM, 250 to 275.39 mg/100 g DM and 2.60 to 3.84 g/100 g DM, respectively. The increase in protein and ash contents could be linked to the synthesis of enzymes, which are protein in nature and whose prosthetic groups can be metals. With regard to the increase in the ascorbic acid content, Yudkin [35] pointed out that the germination of cereal seeds and legumes were accompanied by an increase in ascorbic acid contents, which would justify, in the present case, the increase in the content of this element in the GT. On the other hand, this treatment resulted in a slight reduction in lipid contents. It could be explained by the fact that lipids are used to produce the energy necessary for the biochemical and physiological changes occurring in the tuber during germination [36]. The interest of this process was the stimulation or the biosynthesis of amylolytic enzymes. It appears from Table 3 that NT have only a slight amylase activity (3 U/mL), which increased during soaking. The highest activity was observed after germination of the tubers (60 U/mL) (Table 3). It turned out that, during this experiment, the amylase activity of the GT increased from 3 to 60 U/mL, respectively, for NT and GT, which justified the reduction in the starch content and the increase in the content of reduced sugars at the end of germination. These results are similar to those of Traore et al. [36], who highlighted that the native grains of sorghum, corn and millet had zero amylase activity, while the soaked and sprouted grains developed a certain amylase activity.

Table 3. Chemical composition (g/100 gDM) of sprouted and non-sprouted tigernut tubers.

Characteristics (g/100 g DM)	NT (Control)	ST	GT
Water content (%)	7.38 ± 0.14^{c}	57.34 ± 0.23^{a}	54.38 ± 0.54^{b}
Protein (g/100 g DM)	7.62 ± 0.11^{b}	7.42 ± 0.42^{b}	8.82 ± 1.31^{a}
Total carbohydrates (g/100 g DM)	49.92 ± 0.12^{a}	47.52 ± 1.84^{a}	48.93 ± 1.18^{a}
Reducing sugars (g/100 g DM)	20.12 ± 1.11^{c}	23.74 ± 1.74^{b}	33.36 ± 0.35^{a}
Starch (g/100 g DM)	26.14 ± 0.27^{a}	25.13 ± 0.10^{a}	16.63 ± 0.50^{b}
Amyloidosis (%)	14.15 ± 0.72^{a}	13.83 ± 1.21^{a}	9.98 ± 0.15^{b}
Amylopectin (%)	85.95 ± 0.73^{b}	86.17 ± 1.27^{b}	90.02 ± 0.13^{a}
Lipids (g/100 g DM)	25.56 ± 0.41^{a}	26.25 ± 0.53^{a}	24.15 ± 0.02^{b}
Fibers (g/100 g DM)	$15.56 \pm 0,12^{a}$	12.03 ± 0.94^{b}	15.72 ± 0.09^{a}
Ashes (g/100 g DM)	2.73 ± 0.31^{a}	1.84 ± 0.07^{b}	3.84 ± 0.18^{a}
Ascorbic acid (mg/100 g)	252 ± 0.39^{c}	328 ± 4.37^{a}	275.39 ± 3.41^{b}
Vitamin E (mg/100 g)	123 ± 0.18^{a}	118.79 ± 3.26^{b}	118.73 ± 0.55^{b}
Caloric value (kcal/100 g DM)	445	450	462
AA * (U/mL)	3 ± 1.69^{c}	15 ± 0.58^{b}	60 ± 3.72^{a}

The results that have the same letters in exponent on the same line are not significantly different (probability threshold $p \leq 0.05$) * AA: Amylase Activity; U (unit of amylase activity) is defined as equivalent to the release of 1 µg of glucose per minute and per mL. *NT*: *Native tubers*; *GT*: *Germinated tubers*; *ST*: *Soaked tubers*.

3.4. Influence of Germination on the Amino Acid Profile of Tigernut Tubers

The amino acid profiles of NT, GT and ST are presented in Table 4. All the amino acids present in NT are found in GT. The amino acid profile of GT and ST did not differ significantly from that of NT. However, germination increased the contents of tigernut tubers in certain essential amino acids, such as phenylalanine, threonine, valine and leucine. The same is true for certain non-essential amino acids, such as arginine, tyrosine, cysteine and aspartic acid. These observations have also been reported by Noort et al. [37] during their work on the germination of sorghum. These increases would be due to the synthesis of enzymes in these sprouted products. These results also shown that the content of essential amino acids in the samples of sprouted tigernut tubers is higher than that of the FAO/WHO presented in Table 4 [38], hence their chemical indices greater than 100. This suggests that

germination increases the capacity of *C. esculentus* tubers to meet the needs of these entities. In general, the chemical index of essential amino acids in GT is higher than that of NT and ST. These results corroborate the observations, which stipulate that germination could improve the nutritional quality of tigernut tubers and hence that of its milky extracts.

Table 4. Influence of germination on the banal and essential amino acid composition of tigernut tubers.

	Amino Acids	NT (Control)	ST	GT
Essential amino acids (mg/g DM)	His	0.98 ± 0.07^{a}	0.94 ± 0.16^{a}	1.04 ± 0.11^{a}
	Ile	1.32 ± 0.07^{a}	1.44 ± 0.22^{a}	1.49 ± 0.05^{a}
	Leu	2.41 ± 0.13^{b}	2.73 ± 0.48^{a}	2.88 ± 0.15^{a}
	Lys	2.37 ± 0.14^{a}	2.48 ± 0.43^{a}	2.40 ± 0.19^{a}
	Met	0.71 ± 0.01^{a}	0.57 ± 0.18^{a}	0.87 ± 0.03^{a}
	Phe	1.45 ± 0.06^{a}	1.63 ± 0.28^{a}	1.72 ± 0.08^{a}
	Thr	1.44 ± 0.17^{c}	1.99 ± 0.21^{b}	2.62 ± 0.23^{a}
	Trp	0.92 ± 0.04^{a}	0.85 ± 0.02^{b}	0.95 ± 0.02^{a}
	Val	1.90 ± 0.12^{b}	2.10 ± 0.25^{b}	2.88 ± 0.16^{a}
	Chemical index	**106**	**102**	**115**
Banal amino acids (mg/g DM)	Ala	2.30 ± 0.14^{a}	2.34 ± 0.31^{a}	2.37 ± 0.20^{a}
	Arg	6.89 ± 0.61^{a}	4.74 ± 0.41^{b}	7.26 ± 1.07^{a}
	Asp	4.27 ± 0.56^{a}	4.11 ± 0.85^{a}	4.63 ± 0.39^{a}
	Cys	0.75 ± 0.12^{a}	0.63 ± 0.04^{a}	0.88 ± 0.05^{a}
	Glu	7.37 ± 0.73^{a}	6.09 ± 1.02^{a}	7.55 ± 0.58^{a}
	Gly	1.54 ± 0.06^{b}	1.67 ± 0.31^{a}	1.76 ± 0.08^{a}
	Pro	1.78 ± 0.06^{a}	1.73 ± 0.34^{a}	1.84 ± 0.19^{a}
	Ser	1.88 ± 0.11^{a}	2.04 ± 0.43^{a}	1.92 ± 0.13^{a}
	Tyr	1.04 ± 0.07^{b}	1.12 ± 0.20^{b}	1.24 ± 0.18^{a}
	Total	$\mathbf{75.4 \pm 0.22^{b}}$	$\mathbf{76.2 \pm 0.15^{b}}$	$\mathbf{88.2 \pm 0.48^{a}}$

The results that have the same letters in exponent on the same line are not significantly different (probability threshold $p \leq 0.05$), *NT*: *Native tubers*; *GT*: *Germinated tubers*; *ST*: *Soaked tubers.*

4. Discussion

A tuber or seed needs light, water and oxygen to germinate. As seeds or tubers age, their ability to sprout declines even if stored under optimal conditions. This loss of production potential is an obstacle to agricultural production and to germplasm and species conservation. Understanding how this occurs and potentially preventing or slowing the loss of seed vigor is of great interest to plant researchers.

According to Jira-Anunkul and Pattanagul [39], reactive oxygen species (ROS), released during the normal metabolism of oxygen, are known to have significant roles in the process of tuber or seed sprouting. However, the ROS levels must be closely regulated in a relatively narrow range for germination to proceed. If ROS levels are too low seeds will never leave dormancy, and if they are too high, then the seeds will suffer excessive oxidative damage during seed imbibition and will be non-viable [40]. Research has shown that mitochondrial function and antioxidant scavenging systems are critical for maintaining the right balance of ROS.

Sridharan et al. [41] stipulate that the impact of ROS on the mitochondrial protein profile in elm seeds as they aged can be determined. Using a previously developed protocol to simulate deterioration due to aging, they examined a number of markers of oxidative stress and mitochondrial function in the presence of either MitoTEMPO (a mitochondria-specific ROS scavenger) or methyl violet (an inhibitor of electron transport that ultimately increases ROS production). Oxidative stress was assessed using a variety of techniques, including the determination of hydrogen peroxide levels, confocal microscopy with hydrogen peroxide and mitochondrial function staining, mitochondrial respiration assay, analysis of mitochondrial proteins, MDH activity and NADH oxidation assays, and immunoblotting for carbonylated proteins. The control elm seeds showed a typical response to the simulated accelerated aging protocol, with germination rates declining progressively

beginning on day 2 of treatment through to day 5 when viability was completely lost. The pretreatment of the seeds with MitoTEMPO significantly inhibited hydrogen peroxide production at the early stages of seed aging, protecting seed germination rates on day 2, but the effect was short-lived and germination rates declined on days 3–5. With methyl violet pretreatment, hydrogen peroxide levels were increased over control initially and accelerated the loss of vigor by approximately 1.5 days.

The study of Hasanuzzaman et al. [42] demonstrated that the increase in ROS during seed storage may ultimately be linked to a decrease in function of antioxidant enzymes, and once the imbalance forms, the excess ROS can cause damage through multiple different pathways. This finding means that to prevent the accumulation of excess ROS in long-term stored seeds is a promising area of research to extend the viability of and protect valuable stores. For this purpose, in our case, it could be that the concentration of hydrogen peroxide (germination capacity (H_2O_2, 7.5 g/L), 0%) is not ideal. It must be either low or high, which inhibited the germination of the tubers, which is not the case for ascorbic acid, where germination is at 94.740 $\pm$ 2.322% (Table 1).

Water uptake by tuber or seed as a function of physical and metabolic events occurring during germination and during early seedling growth impact the textural profile and chemical content.

Germination improves the nutritional quality of wheat [43]. The contents of nutrients and bioactive molecules depend on the germination conditions [44]. Table 3 shows that reducing sugar content increases significantly with germination. Jribi et al. [45] also observed this phenomenon. In fact, the germination of tigernut tubers leads to a degradation of starch and the release of simple α-amylase. On the other hand, this bioprocess allows an increase in the fiber content in germinated tubers, which is in accordance with the work of Singkhornart et al. [46].

The role of fibers in the prevention of food-related pathologies, such as diseases cardiovascular disease or type 2 diabetes, is clearly established. Fiber has interesting nutritional properties, such as the reduction in cholesterol, improving the adsorption of mineral salts and contributing to the control metabolic rate in people with type 2 diabetes [47].

Overall, this work shows that germination improves the nutritional quality of nutgrass tubers.

5. Conclusions

This work focused on the influence of soaking and germination treatment on the nutrient content of tigernut tubers grown in the savannas of Cameroon. It aimed also to investigate the impact of these treatments on the textural profile of tigernut tubers.

These treatments significantly influence the textural profile of the tubers. In fact, soaking and germination significantly impacted the linear distance (LD) ($p < 0.05$), the number of peaks (NP) ($p < 0.01$%) and the area (A) under the first break of the peak ($p < 0.05$). "Malting of tigernut tubers" led to a considerable reduction in the starch content. It significantly reduced ($p < 0.05$) the amylose content, which ranged from 14.15% for the native tigernut tubers to 9.98% for the sprouted tubers. This treatment also increased the protein, ascorbic acid and ash contents, which ranged from 7.54 to 8.82 g/100 g DM, 250 to 275.39 mg/100 g DM and 2.60 to 3.84 g/100 g DM, respectively.

The starch content of the tubers following germination (16.63 $\pm$ 0.50 g/100 g DM) remained high, which could come up against the pasteurization of the milk from these tubers. In this respect, this constraint could be removed by in situ hydrolysis tests of the starch of tigernut tubers in the production of this drink.

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