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Effect of Pretreatments on the Chemical Composition, Antioxidant Activity and Techno-Functional Properties of Tigernut (*Cyperus esculentus*) Flour

[1]R. Zakka, [1]D.C. Alahira, [2]S.S. Choji, [3]B.S. Luka

[1]Department of Food Science and Technology, Federal University Wukari, Wukari, Taraba State, Nigeria
[2]Department of Biochemistry, Plateau State University Bokkos, Bokkos, Plateau State, Nigeria
[3]Department of Agricultural Engineering, Federal University Wukari, Wukari, Taraba State, Nigeria

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Corresponding author:
R. Zakka,
Department of Food Science and Technology, Federal University Wukari, Wukari, Taraba State, Nigeria

ABSTRACT

Objective: Tigernut flour was evaluated to determine the effect of germination, autoclaving, or a combination of both on its chemical composition.

Methodology: The proximate composition, phytochemical constituents, and antioxidant activity of pretreated tigernut flour were evaluated using AOAC method. In addition, the techno-functional and antinutrient properties of the pretreated flour were assessed. Pretreatment method with four treatment groups was used in this study. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

Results: Germination, autoclaving, and their combination as pretreatments enhanced the proximate composition, antioxidant activity, phytochemical content, and techno-functional properties of tigernut while reducing antinutrient levels. Although minor negative effects were observed, the overall benefits predominated. These pretreatment methods are therefore recommended to improve the nutritional quality of tigernut and promote its use in food formulations and product development.

Conclusion: It is concluded that pretreatment could increase the proximate composition, antioxidant, phytochemical properties and improve the techno-functional properties of tiger nut.

INTRODUCTION

The growing demand for nutrient-dense foods has intensified the need to optimize nutritional value and expand potential applications within the food and beverage industries, ultimately contributing to improved quality of life. Tigernut (*Cyperus esculentus*), also known as chufa, is classified as a functional ingredient, although it remains relatively neglected and underutilized[1]. Tiger nuts have shown promising potential as feed material, flou, plant-based milk, oil, and starch sources-due to their high levels of lipids, proteins, starch, fiber, vitamins, minerals and bioactive compounds[2-5].

Recent studies have demonstrated that the application of conventional processing techniques-including heat treatments, germination, and spontaneous fermentation-can enhance the quality of tigernut flour[6]. Pretreatment techniques reduce anti-nutritional factors present in tigernut, thereby improving nutrient absorption[7]. Such treatments increase the bioavailability of nutrients, particularly proteins and carbohydrates, as endogenous enzymes help break interactions between these macromolecules within the grain, providing greater nutritional benefits to consumers[8]. During germination, hydrolytic enzymes such as α-amylase, β-amylase, and proteases become activated, catalyzing the breakdown of starch and proteins and resulting in increased levels of oligosaccharides, simple sugars, and amino acids[9].

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Germination is considered an effective method for improving the nutritional, biochemical and sensory properties of foods. It reduces anti-nutritional factors and induces various metabolic reactions within the seed, leading to significant alterations in chemical composition compared with the raw grain[10]. Among these changes, amino acids play a vital role in supporting growth and metabolic processes during seed development[11]. According to Okoye and Ene[12], germination enhances the availability of bioactive compounds and decreases anti-nutrients in tiger nuts. These improvements are closely associated with the activation of endogenous enzymes, making germinated seeds nutritionally superior to their non-germinated counterparts[13]. Germination has also been shown to increase calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid contents in tigernut flour.

Hydrothermal processing involves the use of an aqueous medium within a sealed reaction vessel, where elevated temperature and pressure are generated either externally or through self-produced vapor. This process is typically conducted in an autoclave, which allows strict control of both temperature and pressure. Hydrothermal treatments offer several advantages in tigernut flour processing: Reduced energy requirements for drying and grinding, increased extraction yields, enhanced flour quality, shorter processing time and extended shelf life. Sheikh et al.[13] highlighted that hydrothermal treatments may also mitigate the toxic effects of anti-nutritional factors. Overall, hydrothermal processing can significantly improve both the quality and efficiency of tigernut flour production. Therefore, the objective of this study was to evaluate the effects of germination, autoclaving, and their combined application on the chemical composition of tigernut flour.

MATERIALS AND METHODS

Materials: Brown varieties of tigernuts were procured from the Old Market in Wukari, Taraba State. All reagents used in the experiments were of analytical grade.

Production of pretreated tigernut flour

Control flour preparation: Five hundred grams of dry tigernut tubers were washed with potable water and dried in an oven dryer (Binatone MO-4500, 148 Connaught Rd Central, Sheung Wan, Hong Kong) at 100°C for 30 min. The dried nuts were milled and passed through a 0.45 mm sieve. The resulting flour was packaged in polyethylene bags, placed in an airtight plastic container and stored at room temperature to serve as the control.

Germinated tigernut flour: A 500 g portion of tigernuts was steeped in water for 24 hrs, drained, and spread under a wet muslin cloth for germination at 37°C for 48 hrs. Water was sprinkled every 8 hrs to maintain moisture. After germination, the samples were dried at 100°C for 30 min, milled, sieved through a 0.45 mm mesh, packaged in airtight plastic containers, labelled, and stored in a freezer until analysis.

Autoclaved tigernut flour: Hydrothermal pretreatment was performed using an autoclave (Seradon LS-75HD, London, England) operated at 120°C for 60 min. For this treatment, 500 g of dried tigernuts were evenly arranged on a metal tray. The autoclave was preheated to 120°C with the top valve open to expel air. Once the target temperature was reached, the valve was closed and the timer activated. After completion of the heating period, the tubers were removed, dried at 100°C for 30 min, milled, sieved (0.45 mm), packaged, labelled and stored in a freezer until further use.

Germinated-autoclaved tigernut flour: Tigernuts were steeped in water overnight, drained and germinated under a wet muslin cloth at 37°C for 48 hrs with water applied every 8 hrs. Following germination, the samples were autoclaved at 120°C for 30 min, dried at 100°C for 30 min, milled, sieved (0.45 mm), packaged in airtight containers, labelled, and kept frozen until required.

Determination of proximate composition, phytochemicals and antioxidant activity: The proximate composition of the flour samples was determined according to AOAC methods[15]. Carbohydrate content was calculated by difference. Moisture content was assessed using a moisture analyzer (Searchtech Instruments, SH-10A) based on the loss-on-drying (LOD) principle.

Selected phytochemicals (total phenolic content, flavonoids, carotenoids, anthocyanins and lycopene) and antioxidant activities (2,2-diphenyl-1-picrylhydrazyl [DPPH] and ferric reducing antioxidant power [FRAP]) were also evaluated. Sample extracts were prepared using 80% methanol.

For Total Phenolic Content (TPC), 1 mL of sample extract was combined with 0.5 mL of Folin–Ciocalteu reagent (Sigma-Aldrich, USA) and 10 mL of 7.5% sodium carbonate (Junsei Chemicals, Japan) in a 25 mL test tube. The mixture was vortexed for 20 sec, brought to 25 mL with distilled water, and incubated in the dark for 2 hrs. Absorbance was measured at 750 nm and TPC was quantified using a gallic acid standard curve, expressed as µg/g[16].

Flavonoid content was determined using the aluminum chloride colorimetric method with quercetin (Sigma-Aldrich, USA) as the standard, and results were expressed as mg/g[17].

For carotenoid determination, an acetone-water mixture (4:1 v/v) was used as the extraction solvent. The absorbance (UV/AVIS spectrophotometer T60U, Leicestershire, UK) maxima was read at 470.0 nm. Contents of total carotenoids was calculated using the following equation:

$$\text{Total carotenoids } (\mu g/ml) = \frac{10000A470 - 2.27\ (\text{chl a}) - 81.4(\text{chl b})}{227}$$

Where
Chl a : Chlorophyll a
Chl b : Chlorophyll b

For the determination of anthocyanin content, 0.5 g of the sample was homogenized with 5 ml of acidic methanol (80:20:1 of methanol, water and HCl respectively) and centrifuged at 4000 rpm for 15 min. The absorbance of the methanolic extract of anthocyanin was measured at 657 and 530 nm. Total anthocyanin content was calculated using the following equation.

$$\text{Total anthocyannin } (A/g) = \frac{A_{657} - 0.3A_{530} \times V}{M}$$

Where:
V : Volume of extract
M : Mass of sample used

For the determination of lycopene content, one gram of tigernut flour was homogenized with 10 mL of an acetone hexane mixture (2:3) for 2 mm to uniform mass. Homogenates were centrifuged at 5000 rpm for 10 min at 20°C. The absorbance spectrum of each supernatant was measured and the absorption maxima was read at 453, 505, 645 and 663 nm (UV/VIS spectrophotometer T60U, Leicestershire, UK). Lycopene content was calculated using the equation below:

$$\text{Lycopene } (mg/100mL) = -0.0485A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$$

Where
A : Absorbance

For the determination of DPPH radical scavenging activity, 1 mL sample extract and 10 mL DPPH solution (Sigma-Aldrich, USA) were mixed and kept in a dark cupboard at 25°C for 30 min, and the absorbance reading was taken at 517 nm[4]. FRAP of samples was determined by mixing 0.1 mL of sample extract with 3 mL FRAP, prepared in 300 mM acetate buffer at pH 3.6- and 20-mM iron chloride. The mixture was incubated at 25°C for 5 min and absorbance reading was taken at 593 nm[4].

Determination of techno-functional properties and antinutrient composition of pretreated tigernut flour: The loose bulk density was determined by introducing 10 g of each sample into a 100 mL graduated cylinder without tapping and recording the volume while the packed bulk density was determined by tapping the graduated cylinder until a constant volume was observed. Water and oil absorption capacities (mL/g) were assessed by measuring the amount of water and oil absorbed by 1g of sample in 10 mL distilled water or oil, respectively[17]. For the measurement of the swelling capacity (g/g) of samples, 1 g of our was added into 10 mL of distilled water and the suspension was heated at 80°C for 30 min. Swelling capacity was calculated as the ratio of the weight of the gel formed to the initial weight of sample[18].

For the determination of alkaloid conent, 5 g of sample was dispersed in 50 ml of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand for 4 hrs before it was filtered. The filtrate was evaporated to one quarter of its original volume. Concentrated NH_4OH was added drop wise to precipitate the alkaloids. The precipitate was filtered off with weighed filter paper and washed with 1% NH_4OH solution in a test tube. The precipitate in the filter paper was dried in the oven at 60°C for 30 min and reweighed. Percentage of alkaloids was calculated as the ratio of alkaloid to sample weight multiplied by 100.

For the determination of phytic acid, 0.5 g of sample was extracted with 10ml 2.4% HCL for 1 hr at ambient temperature and centrifuged (3000 rpm) for 30 min. Wade reagent (0.03%) solution of FeCl3.6H2O containing 0.3% sulfosalicylic acid in water was added to 3 mL of the sample solution and the mixtures were centrifuged. The absorbance at 500 mm was measured using a UV-VIS spectrophotometer. The concentration of phytate was calculated using phytic acid standard curve and the results were expressed as mg/g.

Statistical analysis: All experiments were conducted in duplicate. The study employed a single-factor design in which pretreatment method served as the independent variable, comprising four treatment groups: Control (no treatment), germination, autoclaving and combined germination-autoclaving. Data obtained from the analyses were subjected to one-way analysis of variance (ANOVA), and mean separation was performed using Duncan's multiple range test. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS), version 20.0 (IBM Statistics, Armonk, NY, USA).

RESULTS AND DISCUSSION

Proximate composition, phytochemicals and antioxidant activity of pretreated tigernut flour: The proximate composition, phytochemical properties and antioxidant activities of raw and pretreated tigernut flours are presented in Table 1. Carbohydrate content increased significantly in the germinated flour by 5.73%. This increase may be attributed to the hydrolysis of starch into simple sugars such as glucose and fructose, or the enhanced activity of amylolytic enzymes that degrade complex carbohydrates

Table 1: The proximate composition, phytochemical properties and antioxidant activities of raw and pretreated tigernut flours

Parameters	Untreated flour	Germinated flour	Autoclaved flour	Germinated-autoclaved flour
Protein (%)	6.57±0.12[a]	3.77±0.12[c]	4.78±0.64[b]	6.83±0.25[a]
Fat (%)	10.70±0.71[a]	10.00±0.57[a]	11.10±0.42[a]	12.00±1.41[a]
Moisture (%)	4.70±0.14[b]	5.15±0.07[ab]	5.11±0.15[ab]	5.48±1.41[a]
Ash(%)	3.00±0.00[ab]	1.00±0.00[a]	3.25±1.06[a]	4.75±1.06[a]
CHO (%)	75.04±0.45[b]	79.34±0.45[a]	74.27±0.8[b]	72.70±2.33[b]
Energy (kcal/100g)	423.10±4.65[a]	422.40±7.37[a]	416.06±6.96[a]	421.91±5.82[a]
TPC (µg/g)	71.47±5.64[a]	17.64±0.64[b]	73.10±3.64[a]	14.30±0.21[b]
Flavonoid (mg/g)	0.85±0.01[a]	0.81±0.10[a]	0.90±0.03[a]	0.93±0.00[a]
Carotenoid (µg/mL)	2.94± 0.25[a]	2.45± 0.08[b]	3.19± 0.08[a]	3.14± 0.20[a]
Anthocyanin (A/g)	0.66±0.06[c]	5.44±0.42[a]	4.89±0.45[a]	0.04±0.12[b]
Lycopene (mg/100mL)	0.07±0.02[c]	0.22±0.05[a]	0.21±0.00[a]	0.14±0.01[b]
DPPH (%)	92.08±0.26[b]	93.33±0.46[a]	90.42±0.36[c]	90.21±0.36[c]
FRAP (mg/g)	0.24±0.02[c]	0.19±0.05[c]	0.31±0.04[b]	0.38±0.01[a]

The data are presented as means±standard deviation of duplicate scores. Mean within a row with different superscripts were slightly significantly different ($p<0.05$)

into more readily available forms. This finding contrasts with the report of Mane et al.[19], who observed a significant reduction in carbohydrate content during the germination of finger millet (73.28-72.43%).

Crude protein decreased significantly ($p<0.05$) from 6.57-3.77% and 4.78% in the germinated and autoclaved samples, respectively. The reduction in germinated flour may be linked to the activation of proteolytic enzymes that hydrolyze proteins into amino acids for seed metabolic activities. The decline observed during autoclaving is likely due to heat-induced protein denaturation, which alters structural configuration and functional properties. These observations partly align with the findings of Mabrouki et al.[20], who reported no significant changes in the protein content of autoclaved fenugreek seeds but noted an increase following germination (23.79-24.15%).

Fat content did not differ significantly ($p>0.05$) among the samples, with values ranging from 10.70-12.00%. This indicates that the pretreatments applied may not substantially affect lipid composition[21]. The trend observed here is inconsistent with the findings of Okoye and Ene[12], who reported a decrease in fat content following germination of tigernut flour but is consistent with the report of Ghaed et al.[22], who observed no significant differences between autoclaved and untreated fesenjan (12.06 and 12.26%, respectively).

Moisture content increased significantly ($p<0.05$) in the germinated, autoclaved, and germinated–autoclaved samples relative to the control, with values rising from 4.70-5.15%, 5.11 and 5.48%, respectively. This increase may be attributed to moisture absorption during germination and hydrothermal processing. Similar results were reported by Okoye and Ene1[12], who noted an increase in moisture content of germinated tigernut flour (8.69-8.85%). Ash content did not differ significantly ($p>0.05$), ranging from 1-4.75%, likely due to the stability of mineral constituents against processing-induced physical and chemical changes. Energy values also showed no significant variation (416.06-423.10 kcal/100 g).

Total Phenolic Content (TPC) decreased significantly ($p<0.05$) after germination and combined germination-autoclaving, from 71.47-17.64 mg/g and 14.30 mg/g, respectively. The reduction may be due to the activation of oxidative enzymes such as polyphenol oxidase and peroxidase, increased metabolic utilization of phenolics as energy substrates during germination, or oxidation induced by exposure to light and oxygen. In contrast, autoclaving alone increased TPC, possibly due to the thermal breakdown of complex phenolics into more extractable forms. These findings contradict the results of Tarzi et al.[23], who reported an increase in TPC in germinated chickpea (39.20-75.60 mg/kg), and Siah et al.[24], who observed an 83% reduction in TPC in autoclaved faba bean.

Flavonoid content showed no significant difference ($p>0.05$), ranging from 0.81-0.93 mg/g. Carotenoid content decreased significantly ($p<0.05$) in the germinated sample, likely due to enzymatic degradation or oxidation of carotenoid molecules during germination. This observation agrees with the report of Samaila et al.[25], who noted a reduction in carotenoid content of cowpea from 0.95-0.83 mg/100 g following germination.

Anthocyanin content increased significantly after germination and autoclaving individually but decreased significantly when both treatments were combined. This pattern is consistent with Verma et al.[26], who documented an increase in anthocyanin content in germinated wheat (189.3-271.1%), but contrasts with Le et al.[27], who reported a 92.94% reduction in green-kernel black bean following processing. Lycopene content increased significantly ($p<0.05$) in all pretreated samples, possibly due to enzymatic conversion of precursors such as phytoene and phytofluene or thermal isomerization. Lycopene is associated with reduced risks of prostate, lung, breast and gastrointestinal cancers.

DPPH radical scavenging activity differed significantly ($p<0.05$), ranging from 90.21-93.30%. Germinated flour exhibited the highest activity, potentially due to increased levels of bioactive compounds. The lowest activity was

Table 2: Techno-functional properties and antinutrient composition of pretreated tigernut flour

Parameters	Untreated flour	Germinated flour	Autoclaved flour	Germinated-autoclaved flour
Loose bulk (g)	24.50±0.71^{a}	26.00±1.41^{a}	23.50±0.71^{a}	25.50±0.71^{a}
Bulk density (g/ml)	0.17±0.00^{b}	0.21±0.01^{a}	0.18±0.01^{b}	0.20±0.00^{a}
WAC (mL/g)	2.25±0.07^{b}	2.80±0.21^{ab}	2.55±0.21^{ab}	3.50±0.71^{a}
OHC (mL/g)	1.50±0.14^{a}	1.70±0.14^{a}	1.40±0.57^{a}	1.60±0.28^{a}
Swelling power (g/g)	3.85±0.22^{a}	3.33±0.09^{a}	3.17±0.51^{a}	3.38±0.27^{a}
Alkaloids (%)	8.50±0.71^{a}	6.50±0.71^{b}	3.50±0.71^{c}	5.50±0.71^{b}
Phytates (mg/g)	1.02±0.02^{a}	0.88±0.00^{ab}	0.94±0.07^{ab}	0.80±0.10^{c}

The data are presented as means±standard deviation of duplicate scores. Mean within a row with different superscripts were slightly significantly different ($p<0.05$)

observed in the germinated-autoclaved sample, possibly reflecting degradation of antioxidant compounds during combined processing. This partially aligns with the findings of Le et al.[27], who reported decreases in DPPH activity of autoclaved and germinated green-kernel black bean flours (63.3 and 14.4%, respectively).

FRAP values also differed significantly ($p<0.05$), ranging from 0.19-0.38 mg/g. Germinated flour had the lowest FRAP value, likely due to utilization of antioxidant compounds during seed growth, whereas the germinated-autoclaved sample showed the highest value, possibly due to enhanced release of antioxidant constituents through combined processing. These results do not agree with Le et al.[27], who reported a 67.13% decrease in FRAP activity of autoclaved tigernut.

Techno-functional properties and antinutrient composition of pretreated tigernut flour: The techno-functional properties and antinutrient composition of pretreated tigernut flour are presented in Table 2. Loose bulk density ranged from 23.50-26.00 g/mL, with no significant differences among the samples. True bulk density varied between 0.17 and 0.21 g/mL, with the germinated flour exhibiting the highest value and the untreated sample the lowest. Differences in bulk density ($p>0.05$) may be attributed to starch gelatinization during steam treatment or increased water absorption during germination[28]. These findings do not align with those of Yenasew and Urga[29], who reported a reduction in bulk density of germinated finger millet (Axum species) from 0.89-0.73%.

Water Absorption Capacity (WAC) ranged from 2.25-3.50 mL/g, with significant differences ($p>0.05$) among samples. The germinated–autoclaved flour recorded the highest WAC, while the untreated flour had the lowest. The increased WAC may be due to disruption of cell wall integrity and increased porosity. These results do not correspond with Ukwuru[30], who reported decreased WAC in germinated soybean flour (1.53-0.70 mL/g) and increased WAC in autoclaved soybean flour (1.24-1.68 mL/g).

Oil Holding Capacity (OHC) ranged from 1.40-1.70 mL/g. The germinated flour had the highest OHC, while the autoclaved sample had the lowest. The increased OHC following germination may be associated with starch hydrolysis, as hydrolyzed starch granules tend to absorb more oil. Conversely, reduced OHC in autoclaved samples may result from decreased granule porosity. These findings are consistent with Yenasew and Urga[29], who reported an increase in OHC of germinated finger millet by up to 70%, but contrast with Obi and Okoye[31], who observed increased OHC in autoclaved *Mucuna flagellipes* seed flour (6.26-6.42 mg/g). Swelling power did not differ significantly across treatments, although autoclaved flour had the lowest mean value (3.17 g/g), while the untreated flour had the highest (3.85 g/g).

Significant differences ($p<0.05$) were observed in alkaloid content, which decreased across treatments. The reduction may be attributed to degradation, conversion to other compounds, or enzymatic breakdown of alkaloids. In autoclaved samples, high temperatures may promote alkaloid decomposition, destabilization, or disruption of biosynthetic enzymes. These observations partially agree with Salawu et al.[32], who reported decreased alkaloid content after autoclaving but increased levels following germination.

Phytate levels ranged from 0.80±0.10 to 1.02±0.02, with significant reductions observed in pretreated samples. The decrease in germinated samples may be attributed to phytase activation, which catalyzes phytate hydrolysis and thereby lowers phytic acid content. This trend aligns with findings by Azeke et al.[33], who reported significant reductions in phytate after 10 days of germination. Lower antinutrient levels such as tannins and phytates are known to enhance mineral bioavailability, thereby improving the nutritional quality of cereal-based foods[34]. Decreases in phytates after autoclaving may also result from heat-induced phytase activation and increased acidity[35]. Vadivel and Biesalski[36] similarly reported substantial reductions in phytic acid in legume grains following cooking and soaking. The reduced phytate content observed in germinated–autoclaved samples is likely due to the combined effects of both treatments.

CONCLUSION

The findings of this study demonstrate that pretreatment of tigernut through germination, autoclaving, or their combined application effectively enhances its proximate

composition, antioxidant capacity, phytochemical profile, and techno-functional properties, while simultaneously reducing antinutrient levels. Although, certain pretreatments may induce minor undesirable effects, the overall improvements markedly outweigh these limitations. Therefore, germination, autoclaving, and their combined use are recommended as suitable pretreatment strategies for improving the nutritional and functional quality of tigernut. Furthermore, pretreated tigernut flour shows considerable potential for incorporation into food formulations and new product development, thereby contributing to the valorization and reduced underutilization of this nutrient-rich crop.

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