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EFFECT OF SOAKING CONDITIONS ON CHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY, TOTAL PHENOLS, FLAVONOIDS AND ANTI-NUTRITIONAL CONTENTS OF FINGER MILLET

Abioye VF[1], Olatunde SJ[1]*, Ogunlakin GO[2] and OA Abioye[2]

Victoria Abioye

*Corresponding author email: sjolatunde@lautech.edu.ng

[1]Department of Food Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

[2]Department of Food Engineering, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

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ABSTRACT

Finger millet (*Eleusine coracana*) is a staple cereal grain in certain parts of the world with low income populations. It is a minor cereal grain grown extensively in some areas of Africa and consumed for its several health benefits. Finger millet is nutritionally dense as well as a gluten-free food crop that is stable on storage, but less utilized in tropical and semi-arid regions of the world due to the presence of anti-nutrients. This study investigated the effect of soaking as a pretreatment method on the chemical composition, anti-nutrients, phenolic content, flavonoids and antioxidant properties of finger millet. Finger millet seeds were soaked in water at different temperatures (30, 40 and 50 °C) and time intervals (0, 6, 12, 18 and 24 h). Samples were taken at 6 h intervals, washed, dried in the oven at 50 °C to a constant weight, milled into flour and sieved to obtain fine flour (100 µm). The flour samples were analyzed for proximate composition, anti-nutrients, phenolic content, flavonoids and antioxidant activity. All data obtained were subjected to analysis of variance. The moisture, protein, fat, ash, crude fibre, and carbohydrate ranged between 7.08-9.41, 4.16-9.47, 2.91-5.32, 0.82-2.48, 1.32-2.95, and 73.57-78.47%, respectively. Residual anti-nutrients in the flour ranged from 1.20-2.23, 0.38-0.75 mg/100g, 2.30-6.73%, and 0.39-1.48 mg/100 g for tannin, phytate, trypsin and oxalate, respectively. Phosphorus, sodium, calcium, potassium, and iron ranged from 24.3-98.8, 0.123-0.90, 142.24 - 192.16, 105-396, and 2.20-6.59 mg/100 g, respectively. Total phenolics ranged from 16.00 to 40.29 mg/100g. The flavonoid and antioxidant activity of the soaked finger millet flour increased with increased soaking time and temperature. This study has established that soaking can be used as a pretreatment method in reducing the anti-nutrient content, improving nutrients, enhancing the mineral content of the grains, and hence, maximize the potentials of finger millet for the production of value-added products.

Key words: Finger millet, proximate, polyphenols, minerals, anti-nutrients, pretreatment, antioxidant activity, flavonoids

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INTRODUCTION

Finger millet (*Eleucine coracana)* also known as *ragi* in India is a minor cereal consumed as a staple food in some parts of Africa and India [1]. It is a good source of micronutrients (calcium, iron, phosphorus, zinc, potassium) and essential amino acids (valine, methionine, isoleucine, threonine and tryptophan) which are useful for the human body [2]. Finger millet is also a very good source of phenolic compounds with small amounts of flavonoids [3]. It is a gluten-free grain with low-glycemic index with nutritional and nutraceutical advantages [4]. Finger millet is an important cereal due to its excellent storage properties and nutritive value, which is higher than that of rice and equal to that of wheat [5]. These nutritional benefits make finger millet a potential food crop for improving nutrition to attain food security and rural development but it is neglected and underutilized [6]. Finger millets among other millet grains contain higher amounts of anti-nutrients such as tannins and phytates, which makes some of the micronutrients less bio-accessible [7]. However, these anti-nutrients in finger millet grains can be removed by processing techniques such as germination, soaking, roasting, fermentation and dehulling [8].

Soaking is a common household process that improves the nutritional quality of millet grain flour and also decreases the levels of anti-nutrients present in cereals [9]. The soaking of grains reduces phytic acid content, which might depend on the species, pH conditions and duration of soaking. It has been reported that phytate content was significantly reduced during soaking of soybeans [10]. The effect of soaking on the hydrating characteristics of finger millet has been studied [10, 11]. The effect of soaking in combination with other processing methods such as germination, malting and popping has also been studied [11]. There is, however, scanty information on the effects of soaking as a pretreatment method on the nutritional, anti-nutritional, phytochemicals and antioxidant properties of finger millet. The objective of this study was to investigate the changes in chemical composition, antioxidant activity, total phenols, flavonoids and anti-nutritional content of finger millet under different soaking conditions.

MATERIALS AND METHODS

Finger millet grains (*Eleusine coracana*) were obtained from Jos, Plateau State, Nigeria. Plateau State is one of the major states growing finger millet in Nigeria and identified at LAUTECH research farm, Ogbomoso, Oyo State, Nigeria.

Production of soaked finger millet flour

Finger millet grains were cleaned to remove stones, dirt, shafts, and other foreign bodies that may affect the quality of the final product. Triplicate samples of 300 g finger millet seeds were soaked in 1500 mL distilled water (1: 5 w/v) for 6, 12, 18, and 24 h. To study the effect of temperature, finger millet grains were soaked at 30, 40 and 50 $^{\circ}$C for about 24 h, to imitate household treatments. Soaked seeds were drained and dried at 50 $^{\circ}$C in an air conventional oven until constant weight was obtained, then milled, sieved at 300 µm and sealed in High Density Poly Ethylene (HDPE) bags for further analyses.

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ANALYSES

Determinations of proximate composition and mineral contents

The proximate composition of the soaked and un-soaked finger millet flour was determined using standard methods of the Association of Official Analytical Chemists [12]. Moisture content was determined by drying the flour samples in a hot air oven at 103 °C to a constant weight; protein was determined by micro-Kjeldahl method using 6.25 as the conversion factor for total nitrogen to protein; ether extract was determined by Soxhlet extraction method using petroleum spirit while the ash content was determined using a muffle furnace. The samples were burned-off to remove the organic materials at 600 °C for 6 h to a constant weight while the carbohydrate was determined by difference. Minerals were analyzed using the dry-ash techniques according to AOAC method [12] using atomic absorption spectrophotometry (AA Analyse Perkin Nerma).

ANTIOXIDATIVE AND ANTINUTRIENT PROPERTIES

Total phenolic contents

The total phenol content of the extract was determined as described by Singleton *et al.* [17]. Dilutions of the extracts were oxidized with 2.5 mL 10% Folin-Ciocalteu's reagent (v/v) and neutralized with 2.0 mL 7.5% $NaCO_3$. The reaction mixture was then incubated for 40 min at 45°C and the absorbance was measured at 765 nm in the spectrophotometer. Gallic acid was used as a standard and the total phenol content was subsequently calculated as gallic acid equivalents.

Total flavonoid content

Total flavonoid content in the flour samples was determined by the colourimetric method as described by Ayoub *et al*. [18]. About 0.075 mL of 5% $NaNO_2$ was mixed with 0.5 mL of the sample (1 mg/mL) and allowed to stand for 6 min. after which, 0.15 mL of a 10% $AlCl_3$ solution was added and the mixture left at ambient temperature (28±2 °C) for 5 min. Then, 0.5 mL of NaOH (1 M) was added, and the volume was made up to 2.5 mL with distilled water. The absorbance was measured at 510 nm using a spectrophotometer, against the blank containing the extraction solvent instead of the sample. Total flavonoid content was calculated using a standard calibration of Catechin solution and expressed as micrograms of Catechin Equivalent (CE) per gram of dry extract.

Antioxidative property

The effect of the extracts on the content of 1, 1-diphenyl-2-picrylhydrazyl (DPPH·) radicals was estimated using the method described by Sedej *et al*. [19]. About 90 mM of DPPH was diluted with 95% methanol to obtain 100 mL. An aliquot (1.0 mL) of the DPPH· solution (90 mM) was diluted in 2.9 mL methanol, and 0.1 ml of the extracts at various concentrations (0.1, 0.5, 1.0 and 2.0 mg/mL) was added. The mixture was shaken vigorously and left to stand for 60 mins in the dark and the absorbance was measured at 517 nm against the blank (without extract) with the spectrophotometer.

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Tannin content

The tannin contents were determined using Folin Denis Reagent as described by Makkar [13]. Aliquots of the tannin-containing extract (initially: 0.05, 0.2 and 0.5 mL) were pipetted in test tubes, made up to 1.00 mL with distilled water and 2.5 mL of sodium carbonate reagent was added. The test tubes were shaken and the absorbance read at 725 nm after 40 min. The amount of total phenols was calculated as tannic acid equivalent from the standard curve.

Phytate content

Phytate content was determined as described by Pearson [14]. About 0.5 g of the sample was weighed into a 500 ml flat bottomed flask and placed in a shaker. It was extracted with 100 ml of 2.4 % HCL for an hour at 25 °C, decanted and filtered. Five millilitres of the filtrate were measured and diluted to 25 ml with distilled water. Fifteen millilitres of 0.1 M sodium chloride were added to 10 ml of the diluted sample and filtered through Whatman No.1 filter paper to elute inorganic phosphorus. Fifteen milliliters of 0.7 M sodium chloride was also added to elute phytate and the absorbance was read at 520nm.

Oxalate content

The oxalate content was determined as described by Andrade *et al*. [15] using the volumetric analysis method. One gram of the sample was weighed into a 100 ml conical flask and 75 ml of 3 molequi/L H_2SO_4 was added, stirred continuously with a magnetic stirrer for 1 h. It was then filtered using Whatman No.1 filter paper to obtain the filtrate. About 25 ml of the filtrate was taken and titrated while hot (90 ^oC) against 0.1 molequi/L KMnO4 solution until a faint pink colour persisted for at least 30 s. The oxalate content was estimated using this calculation:

$$1\text{ml } 0.1\text{N permanganate} = 0.006303\text{ mg oxalate} \quad \text{Eqn 1}$$

Trypsin inhibitor

Trypsin inhibitor was estimated using the method described by Tanteeratarm and Weingartner [16]. About 1.0 g of the flour sample was extracted with 50 mL of 0.01 N NaOH and the extract was measured into duplicate test tubes in these portions (0, 0.6, 1.0, 1.4 and 1.8 mL) and adjusted to 2.0 mL with water. Two milliliters of trypsin solution was put in each test tube, agitated and heated in a water bath to 37 °C. Five milliliters of BAPA (Benzoyl-DL arginine-p-nitroanilide) solution (previously heated to 37 °C) were added and all tubes were replaced in the agitating water bath. After ten minutes, the reaction was stopped with 1.0 mL of acetic acid solution. Each solution was filtered through Whatman paper and measured at 410 nm on a spectrophotometer. The blank was prepared by mixing 2.0 mL of sample extract and 5.0 mL of BAPA solution heated at 37 °C for ten minutes with addition of 1.0 Ml of acetic acid solution. This was followed by the addition of 2.0 mL of the trypsin solution.

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RESULTS AND DISCUSSION

The proximate composition of the finger millet soaked under different temperatures and times is as shown in Table 1. The moisture content of the flour samples ranged from 7.08-9.41% with a general increase in the soaking hours. Higher moisture contents with soaking have been reported by researchers [21, 22]. The protein content ranged from 4.16 to 9.47% with the control sample having the highest and lowest value recorded with the sample soaked for 24 h at 50 °C. There was a general reduction in the protein content with higher temperature and longer period of soaking. This is in line with the report of Krishona *et al*. [22] that the protein concentration decreased with increase in the soaking hours. Protein content of finger millet has been reported to be in the range of 5.6 to 12.7% depending on the species [23]. The loss in protein content might be attributed to leaching of soluble nitrogen, minerals and other nutrients into the solution.

The effect of temperature could also be responsible for protein denaturation. The fat content ranged between 2.91 to 5.32%; the fat content decreased as soaking time increased indicating some leaching into the soaking water. The ash content of the samples soaked in water reduced with increase in soaking probably due to leaching of some minerals into the soaking water while higher mineral contents were recorded at a higher temperature of soaking. This could be attributed to reduction of the effect of anti-nutrients on the mineral contents which may lead to an increase in bioavailability of minerals. The crude fibre content ranged between 1.32-2.95%; there was no particular trend of decrease or increase with soaking condition and lower values were recorded. This may be due to the fact that after milling, the finger millet was sieved which could have removed some of crude fibre content of the flour. The carbohydrate content ranged between 73.57-78.47%.

The result of the anti-nutritional composition of soaked finger millet is as shown in Table 2. The oxalate content ranged between 0.39-1.48 mg/100; the result shows that there is a significant ($p<0.05$) difference among the samples with soaking time and temperature. Oxalate has been reported to have a significant effect on mineral level, which forms complexes with minerals such as calcium [25]. The soaking process will probably reduce this effect and thus increase mineral availability. There was also decrease in the trypsin content.

The tannin contents obtained ranged between 1.20 and 2.23 mg/100g, and reduced with increase in soaking time and temperature. This was about 30% decrease in the total tannin content, which is in line with report of Lestienne et al. [10]. The phytate content values ranged between 0.38-0.75 mg/100 g; there was about 37% reduction in phytate contents with increase in soaking time. This is in line with the report of Lestienne et al. [10] that soaking of small millet, maize and sorghum seeds at room temperature for 24 h decreases the phytic acid content by 50%. Phytic acid is the main phosphorus store in mature seeds with a strong binding capacity. Agte and Joshi [26] reported that for cereal processing such as soaking of cereal flour prior to heating can activate phytases and therefore favour zinc availability. Soaking has been reported to be the most effective method in decreasing phytic acid content [27]. This could be due to leaching away of the tannins with the soaking water since tannins are concentrated on the seed

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coats. The tannin content decreased with increase in the hour of soaking while the least value obtained was at soaking temperature of 50 °C for 24 h. Presence of tannins impact a bitter taste to finger millet, this indicates that with reduction in tannin content with the soaking process, there may be an improvement in the taste and availability of some nutrients.

The values obtained for the mineral contents of the soaked and unsoaked finger millet are as shown in Table 3. Phosphorus content of the flour samples ranged between 24.3 and 98.8 mg/100 g. There was an initial decrease in phosphorus content at 6-12 h of soaking while there was an increase with increase in the soaking time and temperature. The sodium content ranged between 0.123-0.90 mg/100 g with general reduction with increase in soaking time and temperature. The calcium content of the flour samples ranged between 142.24 and 192.16 mg/100 g and increase was observed with increase in the soaking time and temperature. The value obtained for the potassium contents of the flour samples ranged between 105-396 mg/100 g while the iron content ranged from 2.20-6.59 mg/100 g. The increase in the mineral content with increase in soaking time and temperature can be as a result of the breakdown on the anti-nutrients.

The effect of soaking of finger millet on total phenolic and flavonoid contents is as shown in Fig 1. The total phenolic content of the finger millet decreased from 40.29 to 16.00 mg/100 g with increase in soaking hour and the differences were significant (p<0.05). This result agrees with the previous findings that total phenols decrease with processing [28, 29]. The flavonoid content of the finger millet increased from 66.36 to 103.9 mg/100 g, although most of the differences were not significant (p<0.05). It was also reported that flavonoids are present in small quantities in finger millet [30]. The result obtained for the antioxidant activity of the soaked finger millet is as shown in Fig 2. This shows that finger millet contains some phytochemicals with some antioxidant activities.

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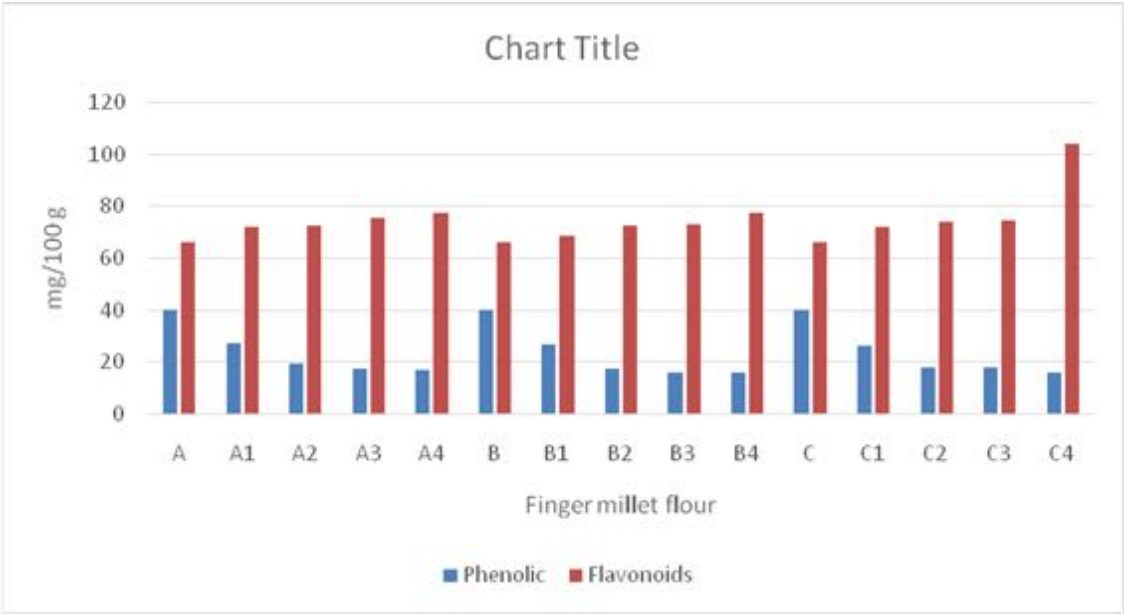

Figure 1: The phenolic and flavonoids content of finger millet

A: Control Sample (unsoaked)
A1: Samples soaked for 6 h @30^{0}C
A2: Samples soaked for 12 h @30^{0}C
A3: Samples soaked for 18 h @30^{0}C
A4: Samples soaked for 24 h @30^{0}C

B: Control Sample (unsoaked)
B1: Samples soaked for 6h @40^{0}C
B2: Samples soaked for 12hr @40^{0}C
B3: Samples soaked for 18hr @40^{0}C
B4: Samples soaked for 24hr @40^{0}C

C: Control Sample (unsoaked)
C1: Samples soaked for 6hr @50^{0}C
C2: Samples soaked for 12hr @50^{0}C
C3: Samples soaked for 18hr @50^{0}C
C4: Samples soaked for 24hr @50^{0}C

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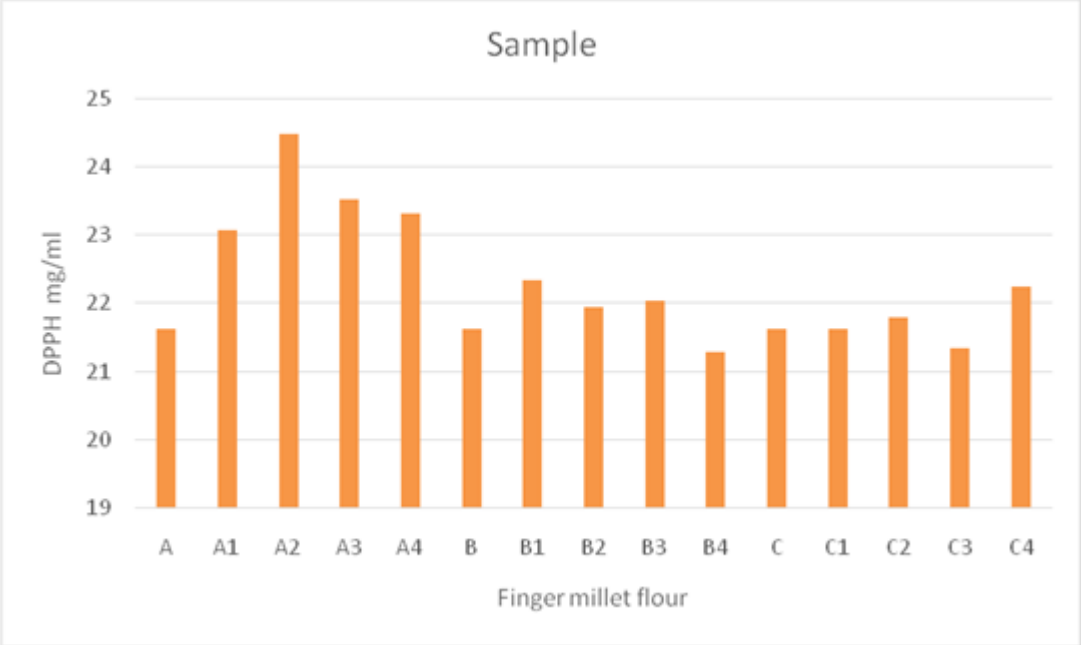

Figure 2: 1,1-diphenyl-2-picrylhydrazyl (DPPH) of finger millet flour samples

A: Control Sample (unsoaked)
A1: Samples soaked for 6 h @30^{0}C
A2: Samples soaked for 12 h @30^{0}C
A3: Samples soaked for 18 h @30^{0}C
A4: Samples soaked for 24 h @30^{0}C

B: Control Sample (unsoaked)
B1: Samples soaked for 6h @40^{0}C
B2: Samples soaked for 12hr @40^{0}C
B3: Samples soaked for 18hr @40^{0}C
B4: Samples soaked for 24hr @40^{0}C

C: Control Sample (unsoaked)
C1: Samples soaked for 6hr @50^{0}C
C2: Samples soaked for 12hr @50^{0}C
C3: Samples soaked for 18hr @50^{0}C
C4: Samples soaked for 24hr @50^{0}C

CONCLUSION

This study has shown that the soaking temperature and time has effects on proximate composition, anti-nutritional contents, total phenols, mineral availability, flavonoids and antioxidant properties of finger millet. The result indicated the effectiveness of soaking as a pre-processing method in reduction of the anti-nutrient content of finger millet, which is a limiting factor in the utilization of the crop with increased nutrient density in finger millet flour. The study also pointed to the presence of some phytochemicals and antioxidative properties in finger millet flour. Thus, soaking process could be used as a pretreatment method in the processing of finger millet for other probable uses.

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Table 1: Effect of soaking condition on proximate composition of finger millet flour (%)

Sample	Moisture	Protein	fat	Ash	CF	CHO
A	7.08±0.03^{g}	9.47±0.03^{a}	5.32±0.03^{a}	1.10±0.14^{e}	2.36±0.11^{de}	74.57±0.03^{h}
A1	7.92±0.01^{f}	9.20±0.28^{b}	4.61±0.01^{de}	1.15±0.04^{e}	2.79±0.01^{b}	74.51±0.01^{h}
A2	8.19±0.01^{e}	8.01±0.03^{d}	4.77±0.33^{cd}	1.64±0.07^{c}	2.74±0.03^{bc}	74.85±0.07^{g}
A3	8.73±0.04^{c}	6.26±0.03^{f}	4.54±0.04^{e}	1.91±0.01^{b}	1.77±0.03^{h}	76.79±0.07^{c}
A4	8.89±0.00^{c}	5.78±0.03^{g}	4.56±0.01^{e}	1.94±0.06^{b}	1.34±0.03^{i}	77.49±0.04^{b}
B	7.08±0.03^{g}	9.47±0.03^{a}	5.32±0.03^{a}	1.10±0.14^{e}	2.46±0.01^{d}	73.57±0.00^{j}
B1	7.83±0.04^{f}	8.59±0.03^{c}	5.28±0.06^{a}	1.35±0.01^{d}	2.65±0.03^{c}	74.30±0.28^{i}
B2	7.85±0.01^{f}	7.82±0.01^{e}	5.31±0.00^{a}	1.65±0.03^{c}	2.13±0.04^{g}	75.24±0.03^{f}
B3	9.30±0.28^{ab}	6.39±0.04^{f}	5.01±0.01^{b}	1.70±0.07^{c}	2.18±0.03^{g}	75.42±0.01^{e}
B4	9.41±0.01^{a}	5.13±0.03^{h}	4.87±0.00^{bc}	1.95±0.01^{b}	1.32±0.03^{i}	77.32±0.01^{b}
C	7.07±0.06^{g}	9.47±0.03^{a}	5.32±0.03^{a}	1.10±0.07^{e}	2.46±0.01^{d}	74.57±0.01^{h}
C1	8.52±0.01^{d}	8.50±0.07^{c}	5.25±0.03^{a}	0.82±0.01^{f}	2.29±0.01^{ef}	74.62±0.03^{h}
C2	9.24±0.03^{ab}	5.87±0.01^{g}	5.23±0.03^{a}	1.17±0.01^{e}	2.20±0.14^{f}	76.29±0.01^{d}
C3	9.13±0.01^{b}	5.76±0.03^{g}	4.66±0.01^{de}	2.05±0.03^{b}	2.95±0.01^{a}	75.45±0.01^{e}
C4	9.25±0.03^{ab}	4.16±0.01^{i}	2.91±0.03^{f}	2.48±0.04^{a}	2.73±0.03^{bc}	78.47±0.01^{a}

Mean values along the same column with different superscripts are significantly different (p <0.05)
CF: Crude Fibre, CHO: Carbohydrate

A: Control Sample (unsoaked)
A1: Sample soaked for 6 h @30^{0}C
A2 Sample soaked for 12 h @30^{0}C
A3: Sample soaked for 18 h @30^{0}C
A4: Sample soaked for 24 h @30^{0}C

B: Control sample (unsoaked)
B1: Sample soaked for 6h @40^{0}C
B2: Sample soaked for 12hr @40^{0}C
B3 Sample soaked for 18hr @40^{0}C
B4: Sample soaked for 24hr @40^{0}C

C: Control sample (unsoaked)
C1 Sample soaked for 6hr @50^{0}C
C2: Sample soaked for 12hr @50^{0}C
C3: Sample soaked for 18hr @50^{0}C
C4: Sample soaked for 24hr @50^{0}C

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Table 2: Effect of soaking condition on the anti-nutritional components of Finger millet flour (mg/100 g)

Sample	Oxalates mg/100g	Trypsin %	Phytate mg/100g	Tannin mg/ 100 g
A	1.48 ± 0.01^{g}	6.73 ±0.00^{a}	0.75 ± 0.00^{f}	2.23± 0.07^{gh}
A1	1.08 ± 0.04^{a}	5.08 ± 0.00^{a}	0. 71 ± 0.00^{f}	1.86 ± 0.03^{i}
A2	0.99± 0.03^{c}	4.63 ± 0.00^{a}	0.60 ± 0.01^{a}	1.48 ± 0.06^{efg}
A3	0.43 ± 0.01^{j}	4.08 ± 0.00^{a}	0.46 ±0.00^{b}	1.40 ± 0.03e^{f}
A4	0.40 ± 0.02^{f}	4.44 ± 0.00^{a}	0.44 ± 0.01^{g}	1.25 ± 0.09^{fgh}
B	1.48 ± 0.01^{g}	6.73 ±0.00^{a}	0.75 ± 0.00^{f}	2.23± 0.07^{gh}
B1	1.22 ± 0.03^{b}	6.71 ± 0.40^{a}	0.53± 0.01^{h}	1.89 ± 0.01^{b}
B2	0.88 ± 0.02^{d}	5.08 ± 0.00^{a}	0.46 ± 0.00^{c}	1.44 ± 0.02^{a}
B3	0.71 ± 0.01^{j}	4.68 ± 0.37^{ab}	0.41 ± 0.01^{f}	1.41 ± 0.01^{efgh}
B4	0.39 ± 0.02^{ef}	4.33 ± 0.05^{a}	0.38 ± 0.00^{i}	1.31 ± 0.01^{e}
C	1.48 ± 0.01^{g}	6.73 ±0.00^{a}	0.75 ± 0.00^{f}	2.23± 0.07^{gh}
C1	1.22 ± 0.01^{h}	5.09 ± 8.85^{b}	0.50 ± 0.00^{f}	1.83 ± 0.12^{b}
C2	0.82 ± 0.01^{ef}	4.85 ±0.00^{a}	0.48 ± 0.01^{e}	1.66 ± 0.05^{efg}
C3	0.53 ± 0.01^{b}	4.02 ± 0.00^{a}	0.45 ± 0.01^{d}	1.56 ± 0.12^{c}
C4	0.75 ± 0.01^{e}	2.30 ± 0.00^{a}	0.41 ±0.00^{h}	1.20 ± 0.05^{h}

Mean values along the same column with different superscripts are significantly different (p <0.05)

A: Control sample (Unsoaked)
A1: Sample soaked for 6 h @30^{0}C
A2 Samplesoaked for 12 h @30^{0}C
A3: Sample soaked for 18 h @30^{0}C
A4: Sample soaked for 24 h @30^{0}C
B: Control sample (Unsoaked)
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B2: Sample soaked for 12hr @40^{0}C
B3 Sample soaked for 18hr @40^{0}C
B4: Sample soaked for 24hr @40^{0}C
C: Control sample (Unsoaked)
C1 Sample soaked for 6hr @50^{0}C
C2: Sample soaked for 12hr @50^{0}C
C3: Sample soaked for 18hr @50^{0}C
C4: Sample soaked for 24hr @50^{0}C

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Table 3: Effect of soaking condition on the mineral composition of the flour samples (mg/100 g)

Sample	P	Na	Ca	K	Fe
A	98.80±0.03^{a}	0.31±0.03^{e}	142.24±0.03^{k}	196±1.41^{j}	4.89±0.03^{d}
A1	51.80±0.04^{d}	0.29±0.06^{e}	158.08±0.03^{i}	260±2.83^{h}	4.60±0.07^{f}
A2	35.70±0.21^{h}	0.26±0.01^{ef}	166.56±0.03^{h}	270±1.41^{g}	4.76±0.01^{e}
A3	28.40±0.07^{i}	0.23±0.00^{gh}	170.40±0.35^{g}	280±2.83^{f}	6.47±0.00^{b}
A4	24.30±0.28^{j}	0.23±0.00^{gh}	192.16±0.03^{a}	285±5.66^{f}	6.59±0.14^{a}
B	98.80±0.14^{a}	0.31±0.00^{e}	142.24±0.01^{k}	196±1.41^{j}	4.89±0.03^{d}
B1	46.30±0.14^{f}	0.54±0.00^{b}	178.87±0.08^{e}	216±3.54^{i}	2.20±0.07^{l}
B2	38.90±0.07^{g}	0.45±0.03^{c}	184.48±0.03^{d}	295±1.41^{e}	2.76±0.04^{j}
B3	60.20±0.07^{c}	0.38±0.00^{d}	187.04±0.01^{c}	330±5.66^{c}	2.81±0.03^{ij}
B4	93.50±0.28^{b}	0.17±0.00^{ghe}	190.88±0.00^{b}	335±2.82^{c}	5.28±0.04^{c}
C	98.80±0.07^{a}	0.31±0.00^{e}	142.24±0.03^{k}	396±0.00^{a}	4.89±0.03^{d}
C1	38.20±0.14^{g}	0.19±0.00^{gh}	143.46±0.01^{j}	105±2.82^{k}	2.57±0.03^{k}
C2	49.40±0.21^{e}	0.14±0.00^{hi}	143.52±0.03^{j}	300±4.24^{d}	2.87±0.06^{i}
C3	52.25±0.14^{d}	0.12±0.00^{i}	171.68±0.03^{f}	305±5.66^{de}	2.99±0.00^{h}
C4	98.00±2.83^{a}	0.90±0.07^{a}	192.16±0.00^{a}	370±7.07^{b}	3.09±0.04^{g++}

Samples
A: Control sample (Unsoaked)
A1: Sample soaked for 6 h @30^{0}C
A2 Samplesoaked for 12 h @30^{0}C
A3: Sample soaked for 18 h @30^{0}C
A4: Sample soaked for 24 h @30^{0}C
B: Control sample (Unsoaked)
B1: Sample soaked for 6h @40^{0}C
B2: Sample soaked for 12hr @40^{0}C
B3 Sample soaked for 18hr @40^{0}C
B4: Sample soaked for 24hr @40^{0}C
C: Control sample (Unsoaked)
C1 Sample soaked for 6hr @50^{0}C
C2: Sample soaked for 12hr @50^{0}C
C3: Sample soaked for 18hr @50^{0}C
C4: Sample soaked for 24hr @50^{0}C.

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