



Effect of Natural Fermentation Period on Nutritional, Anti Nutritional, I Total Phenols, Flavonoids and Antioxidant Contents of Finger Millet Flour

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Authors' contributions

This work was carried out in collaboration among all authors. Authors VFA and JAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AOA and IOG managed the analysis of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study determined the changes in the chemical and nutritional composition of naturally fermented finger millet studied at ambient temperature (28±2°C) for 72 h.

Study Designs: Finger millet seeds were cleaned and fermented (72 h; 28±2°C). Samples were taken at 24 h interval and dried at 50°C for 48 h.

Methodology: The fermented finger millet samples were analyzed for microbial, biochemical changes, chemical, proximate and mineral composition.

Results: Biochemical changes showed a drop in pH from 6.74 to 6.04 while titratable acidity (lactic acid equivalent) increased from 0.04 to 0.62% after 72 h. The moisture, protein, ash, fat, fibre and carbohydrate were in the ranges of 7.08-9.449%; 5.31-7.274%; 1.10-3.392%, 1.296-2.47%, 1.154-2.46% and 77.44-81.58%, respectively. Significant increase were observed in the mineral composition with phosphorus, potassium, calcium, sodium, and iron identified in the fermented finger millet flour in the ranges of 93.5-176 mg/100 g; 171-247.5 mg/100 g; 87.04-196.5 mg/100 g; 1.30-3.075 mg/100 g and 5.28-11.95 mg/100 g, respectively. Tannin, oxalate, phytate and trypsin

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were in the ranges of 1.537 to 3.23 mg/100 g; 0.875 to 1.59 mg/100 g; 0.195 to 0.85 mg/100 g. and 2.731 to 6.23 mg/100 g, respectively. The total phenols and total flavonoids ranged between 11.605-40.29 mg/ 100 g and 63.36 -172.872 mg/100g while the 2,2-diphenyl-1-picrylhydrazyl (DPPH) of the flour samples ranged between 28.109 and 68.238 mg/ml. Microorganisms identified were *Bacillus cereus*, *Lactobacillus Plantarum*, *Lactobacillus casei* and *Lactobacillus brevis*. This study shows that fermentation decreased the anti-nutrients, increased the proximate and minerals contents and also improved the anti-oxidative properties of finger millet flour.

Keywords: Finger millet; fermentation; anti-nutrients; antioxidants.

1. INTRODUCTION

Finger millet (*Eleusine coracana*) commonly known as *ragi* and *mandua* in India is one of the minor cereals that is grown extensively in various regions of India and Africa [1,2]. The grains contain about 5–8% protein, 1–2% ether extractives, 65–75% carbohydrates, 15–20% dietary fiber and 2.5–3.5% minerals [3,4]. Finger millet is considered as one of the most nutritious cereals with highest content of calcium (344 mg/100g) and potassium (408 mg/100g) [5,6]. It has nutraceutical properties and it is also recognized for its health beneficial effects, such as antidiabetic, antitumorigenic, anti-diarrheal, anti inflammatory, atherosclerogenic effects, antioxidant and antimicrobial properties [2]. Finger millet is referred to as poor man's food due to its ability to be stored safely for many years without infestation by insects and pests [7,8,9].

Finger millet is an important cereal due to its excellent storage properties and the nutritive value, which is higher than that of rice and equal to that of wheat [10,8]. It is a good source of micronutrients (calcium, iron, phosphorus, zinc and potassium) and essential amino acids (valine, methionine, isoleucine, threonine and tryptophan) which are essential for human body [11,12,13]. It is gluten-free, has low Glycemic Index and. Millet is used as whole flour mostly for traditional food preparation and can be consumed after processing in form of noodles, biscuit, muffins, vermicelli, pasta and bread [14,15,16].

Bioavailability of some of these nutrients in finger millet is limited by the presence of anti-nutrients such as tannins, phytates, and oxalates [17,12,18]. Processing methods such as boiling, soaking, roasting, germination and fermentation have been reported to reduce the anti-nutrient content of finger millet and thus enhance the biological availability of the nutrients [19,20]. Fermentation is one of the oldest methods widely

used in processing of cereals such as sorghum and millets. During fermentation, flour undergoes major biochemical reactions such as starch hydrolysis, sugar transformation and softening which have been reported to improve nutritional quality of cereal grain, reduce the anti-nutrients and increase bioavailability of micro nutrients [21,22].

The microbial population and biochemical changes during the fermentation of finger millet have been reported [23]. Effects of fermentation of finger millet on the primary nutrients have also been reported [24]. However, there is scanty information on the effects of fermentation on the nutrients, anti-nutrients and antioxidative properties of finger millet. This study, therefore, determined the effect of natural fermentation on the biochemical changes, proximate, minerals, anti-nutrients, phenolic, flavonoids and antioxidant properties of finger millet.

2. MATERIALS

Finger millet (*Eleusine coracana*) was obtained from Jos, Plateau State and identified at Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Research Farm Unit.

2.1 Production of Fermented Finger Millet Flour

About 2500 grams of grains were sorted out by removing the debris, stones and adhering substances. The cleaned seeds were shared into five different portions of 500 g each. Each of the portion was steeped in water (1:5 w/v) and allowed to ferment at 28±2 °C for maximum of 72 h. The fermented grains were harvested after 24 h, washed, dried at 50 °C and milled into fine flour (300 µm). The flours were packaged in zip lock bags until further uses

2.2 Analyses

Lactic acid bacteria were enumerated and identified using the method described by [25].

Ten grams of each of the fermented sample was homogenized with 90 ml of 0.85% (w/v) saline and serially diluted. One hundred micro liter of the sample suspension was spread on MRS agar media after plating, the sample plates were incubated anaerobically at 37°C for 48 h. Colonies were selected and purified by re-streaking to obtain purified strains. The pH of each of the fermented sample was measured using a pH meter (Jenway Model). The titratable acidity (lactic acid, %) was determined by measuring the amount of 0.1 N NaOH necessary to adjust to pH 8.3. Microbial isolates were carried out as described by [23]. The fermented flour samples were analyzed for moisture, ash, crude fibre, protein (N*6.25), crude fat and the carbohydrate determined by difference according to the method described by [26]. The minerals present in the fermented flour samples were quantified using the dry-ash techniques according to (AOAC, 2010). The tannin content of the flour samples was determined using Folin-Ciocalteu method as described by [27]. Phytate content was determined using rapid colourimetry method as described by [28]. The oxalate content of the samples was determined using titration method as described by [29]. Total Phenolic Content (TPC) of the extracts was determined using spectrophotometry as described by [30]. Aluminium chloride colourimetric method was used for flavonoids determination [31]. The DPPH scavenging activity was carried out by the method of [32]. The results of the experiment were subjected to analysis of variance (ANOVA) and the means separated with the use of Duncan's multiple range test.

3. RESULTS AND DISCUSSION

3.1 Changes in pH and Titratable Acidity of Finger Millet and Microbial Isolates

The prominent microorganisms identified were *Bacillus cereus*, *Lactobacillus Plantarum*, *Lactobacillus casei* and *Lactobacillus brevis*. The pH decreased from 7 to 6.04 with increase in the hour of fermentation while titratable acidity increased from 0.04 to 0.3 with increase in the hour of fermentation as shown in Fig 1. This report is in line with the report of other researchers [33,34,35, and 36] that the pH decreased while titratable acidity increased with as fermentation period increased.

3.2 Proximate Composition of Finger Millet

The proximate composition of the fermented flour samples is as shown in Table 1. The moisture content ranged from 7.08% to 9.449% with increase in moisture content as the fermentation hours increased. There was increase in the moisture content with increase in fermentation time. The protein content of the fermented finger millet also followed the same trend as the hour of fermentation rose up. This is in line with previous reports of other researchers, [33] reported a rise in protein content of finger millet as the fermentation hour rose up. Similar reports were recorded for pearl millet by other researchers [37,38,39,40,22] while some others reported increase in some cereals during fermentation [41,42,43] reported improvement in amino acid balance as well as the sensory quality and nutritional value of the cereal grains during fermentation. Increase in protein content could be attributed to the loss of dry matter, mainly carbohydrates or due to the action of extracellular enzymes produced by the fermenting microorganisms [44]. The increase may also be partly as a result of degradation of complex protein by microorganism thereby releasing peptides and amino acids [45].

The ash content of the fermented finger millet flour ranged from 1.10 9% to 3.392% with gradual increase with increase in fermentation time. The result is in line with other reports that minerals increased with fermentation period [39,45]. Minerals from plant sources have very low bioavailability because they are found complexed with non-digestible material such as cell wall polysaccharides [46,47]. Fermentation was also reported to increase bioavailability of calcium, phosphorous, and iron likely due to degradation of oxalates and phytates that complex with minerals thereby reducing their bioavailability [48].

There was a decrease in the lipid content of the finger millet flour from 9.47 to 7.296. This is in conformity with the findings of [24] who reported about 42.9% reduction in the total fat content in fermented finger millet, [40] also reported a decrease in fat content of pearl millet during fermentation. The low lipid content observed in the fermented sample could help in improving the shelf life of fermented finger millet flour by decreasing the chances of rancidity and it may also contribute to the low energy value of the samples. The carbohydrate content of the fermented finger millet flour ranged between

71.435% and 74.59%. There was gradual decrease in the carbohydrates with increase in fermentation time. This is in line with reports of other researchers who had worked on other millets such as [49] and [21] who worked on pearl millet and [24] who reported on foxtail millet. This observation is in conformity with previous reports that carbohydrates are a major carbon source for fermenting microbes [50]. The fiber content values of the sampled finger millet flour were between 0.893% and 2.154%.

3.3 Mineral Composition of Finger Millet Flour

The mineral contents of the fermented finger millet are presented in Table 2. The results indicated increase in the mineral contents with increase in the fermentation time. This is in line with other researchers that fermentation increases the bioavailability of minerals in cereals [51].

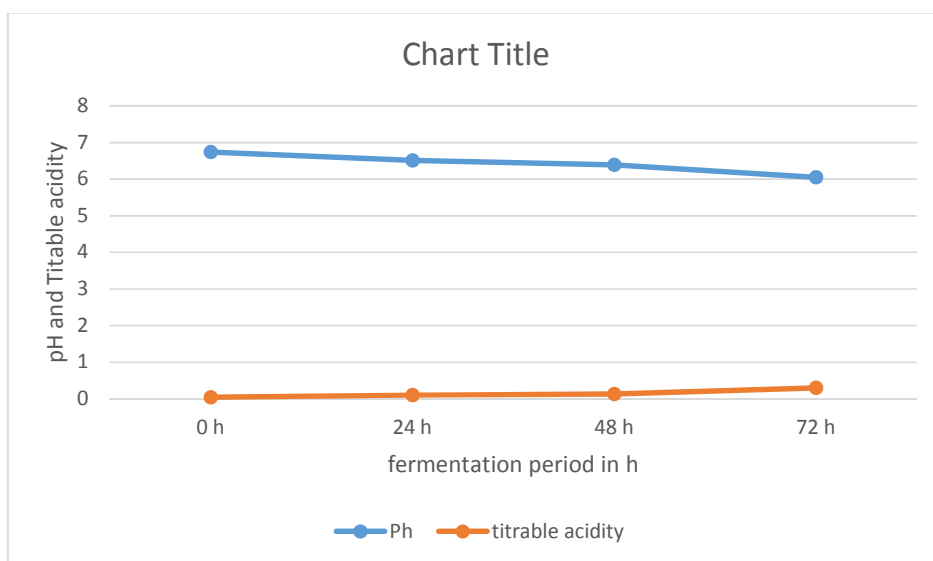


Fig. 1. Changes in pH and total titratable acidity during the natural fermentation of finger millet

Table 1. Proximate composition of finger millet flour (%)

Samples	Moisture Content	Crude Protein	Ash	Crude Lipid	Crude Fibre	CHO
A	7.080±0.282 ^a	5.310±0.014 ^b	1.100±0.00 ^a	2.470±0.014 ^d	2.460±0.014 ^d	81.580±0.028 ^d
B	8.289±0.001 ^b	5.241±0.001 ^a	2.019±0.001 ^b	1.697±0.001 ^c	1.893±0.004 ^c	80.861±0.001 ^c
C	9.121±0.001 ^c	6.019±0.001 ^c	2.080±0.014 ^c	1.437±0.001 ^b	1.319±0.000 ^b	80.031±0.001 ^b
D	9.449±0.001 ^d	7.274±0.006 ^d	3.392±0.003 ^d	1.297±0.002 ^a	1.154±0.006 ^a	77.435±0.001 ^a

Values represent means of triplicate reading, follow by different lowercase letter

Means within the same row with different alphabets are significantly different (p≤0.05)

Sample A: Samples that were not fermented (Control); Sample B: Samples fermented for 24 h; Sample C: Samples fermented for 48 h; Sample D: Samples fermented for 72 h

Table 2. Mineral composition of fermented finger millet flour (mg/100 g)

Samples	P	K	Ca	Na	Fe
A	93.500±0.141 ^a	171.000±1.414 ^a	87.040±0.000 ^a	1.300±0.000 ^a	5.280±0.014 ^a
B	185.000±1.414 ^c	228.000±0.000 ^b	108.800±0.141 ^b	2.649±0.000 ^c	10.800±0.141 ^b
C	255.000±1.414 ^d	239.000±1.414 ^c	156.200±0.283 ^c	2.351±0.001 ^b	10.700±0.141 ^b
D	176.000±1.000 ^b	247.500±0.141 ^d	196.500±0.141 ^d	3.008±0.001 ^d	11.450±0.495 ^b

Values represent means of triplicate reading, follow by different lowercase letter

Means within the same row with different alphabets are significantly different (p≤0.05)

Sample A: Samples that were not fermented (Control); Sample B: Samples fermented for 24 h; Sample C: Samples fermented for 48 h; Sample D: Samples fermented for 72 h

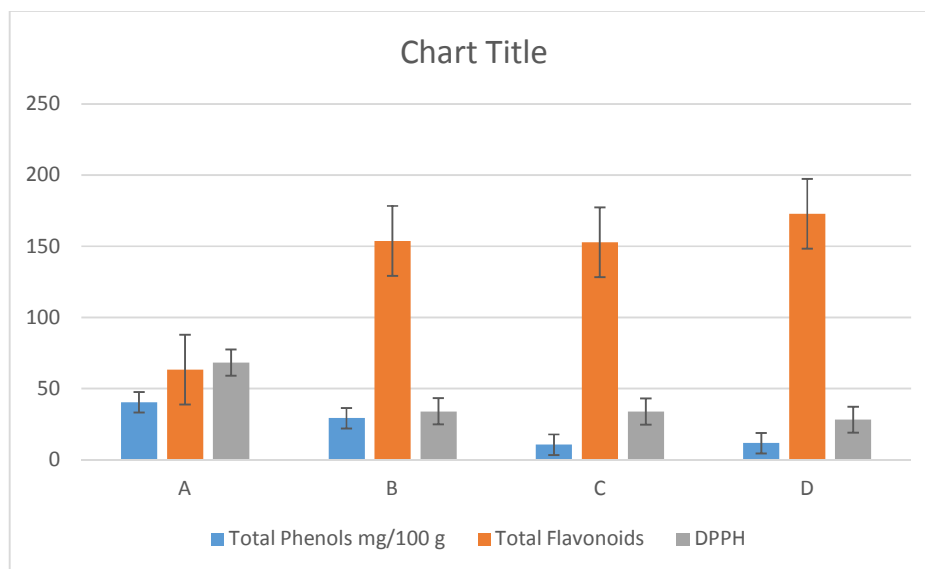
Table 3. Anti-nutritional factor of finger millet flour (mg/100g)

Samples	Oxalate	Trypsin	Phytate	Tannin
A	1.590±0.014 ^d	6.230±0.014 ^d	0.850±0.014 ^d	3.230±0.014 ^d
B	1.325±0.001 ^b	5.338±0.001 ^c	0.594±0.003 ^c	2.431±0.001 ^c
C	1.410±0.014 ^c	3.121±0.001 ^b	0.204±0.003 ^b	1.896±0.000 ^b
D	0.875±0.000 ^a	2.731±0.001 ^a	0.195±0.001 ^a	1.537±0.001 ^a

Values represent means of triplicate reading, follow by different lowercase letter

Means within the same row with different alphabets are significantly different ($p \leq 0.05$)

Sample A: Samples that were not fermented (Control); Sample B: Samples fermented for 24 h; Sample C: Samples fermented for 48 h; Sample D: Samples fermented for 72 h

**Fig. 2. Total phenols, total flavonoids and the antioxidant content of finger millet flour**

Sample A: Samples that were not fermented (Control); Sample B: Samples fermented for 24 h; Sample C: Samples fermented for 48 h; Sample D: Samples fermented for 72 h

3.4 Antinutritional composition of Finger Millet

The result of anti-nutritional contents of the fermented finger millet is as shown in Table 4. Finger millet has been reported to contain the highest amount of anti-nutrients among other millets. There was significant reduction ($p < 0.05$) in phytate and tannin content. Phytic acid has been reported as one of the antinutritional factors common in cereals responsible for binding minerals and thus making them not readily bioavailable (52, 42). The phytate in the finger millet decreased from 0.85 mg/100 g to 0.195 mg/100 g, the result is in line with other researchers who reported decrease in phytic acid with fermentation (52; Anthony and Chandra, 1998, Osman, 2011). The fermenting microorganisms are responsible for the cleavages of tannin-protein, tannic acid-starch and tannin-iron complexes thereby releasing

the free nutrients which improves the availability of these nutrients [52,53,54].

3.5 Antioxidant Properties of Finger Millet Flour

The total phenols, total flavonoids and the antioxidant content in the flour samples are as shown in Fig 2. The total phenols, total flavonoids and the antioxidant content were in the ranges of 11.605-40.29 mg/ 100 g, 63.36 - 172.872 mg/100g and 28.109 and 68.238 mg/ml, respectively. The total phenolic contents decreased with increase in fermentation time The effects of fermentation on phenolic compounds were reported to be a factor of grain types, microorganism species and fermentation conditions (temperature, pH, and time) [55,56]. The flavonoids content increased with increase in fermentation time, this agrees with findings of [40] that fermentation enhanced the flavonoids

content in fermented pearl millet food. Anti-oxidants are substances that neutralize the harmful free radicals in our bodies. Anti-oxidant act as free “radical scavengers” and hence prevent or slow the damage done by free radicals. Anti-oxidants are also used to prevent food quality meanly because the slow oxidative deterioration of lipids. Anti-oxidants function as reducing agents, ultimately removing free radical intermediates and preventing further oxidation [57].

4. CONCLUSION

This study has revealed that fermentation of finger millet can increase the protein, mineral content with significant reduction in the antinutrients thereby increasing the bioavailability of these nutrients. Fermentation also enhanced the polyphenols potentials of the crop.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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