

Effect of Alkaline Cooking on Proximate, Phenolics and Antioxidant activity of Foxtail Millet (*Setaria italica*)

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Abstract: Effect of alkaline cooking on proximate, phenolics and antioxidant activities of foxtail millet was investigated. Grains were cooked at 95°C for 30 minutes with different concentrations of alkaline solution (0.25, 0.5, 0.75, 1 % Calcium oxide). During alkaline cooking of foxtail millet, the fat content reduced maximum by 22.5 %; while protein, ash and carbohydrate content increased by a maximum of 41.2, 27.4 and 5.9% respectively. Alkaline cooking of foxtail millet showed reduction of free, bound and total polyphenols by 51.2, 22.3 and 36.6 % respectively. Total flavonoids and flavonol content also significantly ($p < 0.05$) reduced during alkaline cooking. Anti-oxidant properties estimated by ABTS, DPPH radical scavenging activity and total anti-oxidant activity of foxtail millet showed significantly ($p < 0.05$) low activity due to alkaline cooking. The results are useful to produce alkaline cooked foxtail millet flour which can be used as raw material for healthy food formulations.

Key words: Foxtail millet • Alkaline cooking • Antioxidant activity • Polyphenols • Flavonoids

INTRODUCTION

Foxtail millet (*Setaria italica*) also called Italian millet, is one of the most important food crops of the semiarid tropics. It has the ability to grow in adverse heat and drought conditions and forms a major part of food in many developing countries. It is usually milled to remove the husk, cooked or puffed for use as breakfast meal and specialty foods. Decorticated foxtail millet resulted in 10.2% protein, 77 % starch, 1.7% ash, 29 mg% calcium and 210 mg% phosphorus. These millets were used to prepare the popped, flaked and extrusion cooked products [1]. However, because the grains are coarse, the edible portion of this millet is only about 79%. It is an indication of the presence of a high amount of fiber. Foxtail millet flour is reported to be superior to wheat flour with respect to carotene, tocopherol and anti-oxidant activity [2]. The fiber is associated with polyphenols and phytate, which reduce the availability of proteins and important minerals especially iron and zinc. Hence foxtail millets need to be dehulled prior to use as food. Phytochemicals present in foxtail millet are responsible for the anti-oxidant, anti-microbial and anti-carcinogenic effects as well as other potential health benefits. In addition to this, foxtail

millet is an important sources of cereal fibre which is associated with a lower risk of type-2 diabetes mellitus (DM) and improved insulin sensitivity. It is reported that foxtail millet is helpful for managing DM [3]. The removal of anti-nutritional factors by simple processing techniques such as dehulling, soaking and alkaline cooking provides an opportunity to utilize the millet for nutrient rich food formulation.

Alkaline cooking refers to the process in which the grains are soaked and cooked in an alkaline solution, usually lime water at or near the mixture's boiling point and hulled. It also refers removal of pericarp from grains like sorghum; non-glutinous grains etc [4]. After cooking, the grains are steeped in the cooking liquid. During cooking and soaking, number of biochemical changes take place in the grain such as in solubilization of plant cell wall components including hemicelluloses and pectin [5]. As a result starch swells and gelatinizes, releasing some starches into the steeping solution [6]. The kernels soften and their pericarp (hull) loosens [7]. The grain hydrates and absorbs calcium from the cooking solution. This process is employed using both traditional and industrial methods, in the production of tortillas, tamales, chips, hominy and many other items.

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Grain subjected to alkaline cooking process has several benefits for food preparation. Grains become soft, nutritive value increases, flavor and aroma improves and mycotoxins are reduced [8]. These benefits make alkaline cooking a crucial preliminary step for further processing of coarse grains into food preparation. Several studies were reported on alkaline treatment of other coarse cereals [9,7]. However report on alkali processing of foxtail millet is rather scanty, despite of its enormous potential in development of nutraceuticals rich health foods. Hence, the objective of the present work was to investigate the effect of alkali cooking at different concentrations (0.25, 0.5, 0.75 and 1% CaO) on proximate composition, polyphenolic contents (Phenolic compounds, Flavonoids, Flavonol) and antioxidant activities (ABTS, DPPH radical scavenging activity and total anti-oxidant activity) of foxtail millet.

MATERIALS AND METHODS

Materials: Foxtail millet was procured from local market, cleaned and used for the study. Ferulic acid, epi-catechin, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other reagents and solvents used were of analytical or HPLC grade.

Methods

Sample Preparation: Foxtail millet was soaked and cooked in alkaline solution (1:3) at different concentrations (0.25, 0.5, 0.75 and 1% CaO). The grains were thoroughly washed with water and drained. The grains were then dried in hot-air-oven at a temperature of 60 ± 2 °C for 2 h, pulverized in a plate mill to pass through a 250 μ m sieve, packed and stored at a temperature of 4°C ± 2 until use. Control sample was prepared by pulverizing the foxtail millet (unprocessed) in a similar way and was analyzed for comparison.

Proximate Composition: The control and alkaline treated foxtail millet samples were analyzed for proximate composition by standard American Association of Cereal Chemists [10] method.

Extracts Preparation: About 1 g of control and alkaline treated foxtail millet flour fractions were extracted for polyphenol estimation and antioxidant activity successively with methanol and 1% Hcl in methanol (100 ml) for 1 h in a shaker at ambient temperature (approximately 28°C). The extraction was repeated with

the residue and the extracts were pooled and centrifuged at 3000 rpm for 6 min. The supernatant was evaporated in a rotary flash evaporator and stored at 20°C till use [11]. Methanolic extracts were used for the anti-oxidant studies.

Phenolic Compounds Determination: The phenolic compounds of the control and alkaline treated foxtail millet flour were determined by Folin-Ciocalteu reagent [11]. The reaction was initiated by mixing 0.2 ml of appropriate diluted sample extracts, 0.8 ml of freshly prepared diluted Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The final mixture was diluted to 7 ml with deionized water. The mixture was kept in the dark at ambient temperature for 2 h. The absorbance was measured at 765 nm, using a UV-Vis spectrophotometer (UV-1700 Pharma Spec, Hitachi and Milton Keynes). Total phenolic compounds were expressed as mg of gallic acid equivalents (GAEq) per gram of foxtail millet. Polyphenol value of methanolic extract was reported as free, acid methanol as bound and both added to get total polyphenol.

Total Flavonoids Estimation: Flavonoid content was estimated by the method described by Zhishen *et al.* [12]. 0.1 ml of aliquot from acidic methanolic extract was taken and volume was made up to 5 ml with distilled water. At 0 time, 5 % NaNO₂ (0.3 ml) was added, after 5 min, 10 % AlCl₃ (0.6 ml) and at the 6th min, 1 (M) NaOH (2 ml) solution was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Standard curve was prepared using known concentration of epi-catechin. The results were expressed as mg epi-catechin equivalents (Epi CAT Eq)/100 g of sample (dry basis).

Total Flavonol Estimation: Total flavonol estimation was carried out according to method described by Yermakov *et al.* [13]. 0.05 ml of aliquot of extract was taken and volume was made up to 1 ml with methanol. Then 0.5 ml of vanillin (1% in methanol) and 0.5 ml 25% H₂SO₄ (in methanol) were added successively. The tubes were cyclomixed and allowed to react for 15 minutes at ambient temperature. The absorbance was read at 500 nm in a UV-vis spectrophotometer (UV-1700 Pharma Spec, Hitachi and Milton Keynes) against blank. Standard curve was prepared using known concentration of epi-catechin. The results were expressed as mg epi-catechin equivalent (Epi CAT Eq)/100g of sample (dry basis).

DPPH⁺ Radical Scavenging Activity: The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH⁺) radical was used to measure the free radical scavenging capacity of the methanolic extracts as described by Goupy *et al.* [14] with some modifications. The reaction mixture consisted of 500µl of diluted sample and 500µl of a freshly made DPPH methanolic solution (0.05 mg/ml) and was prepared in 1.5 ml micro centrifuge tubes. After vortexing, the tubes were left in the dark for 30 min at room temperature. The absorbance was then measured against methanol at 515 nm using a UV-vis spectrophotometer (UV-1700 Pharma Spec, Hitachi and Milton Keynes). Inhibition (%) was calculated against reagent blank and the results were expressed as mg epi-catechin equivalent (Epi CAT Eq)/100 g of sample (dry basis).

ABTS⁺ Scavenging Activity: The ABTS^{•+} radical cation, was generated by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to remain in the dark at room temperature for 12-16 h before use. The effect of each extract on ABTS^{•+} radical was estimated according to the procedure described by Sanchez-Moreno *et al.* [15]. Aliquot of methanol (0.1 mL) solutions containing different Epicatechin standard concentrations was added to 3.9 mL of ABTS^{•+} (80 µm) in methanol and absorbance read at 658 nm using a UV-vis spectrophotometer (UV-1700 Pharma Spec, Hitachi and Milton Keynes). % Inhibition was calculated against reagent blank and the results were expressed as mg epi-catechin equivalent (Epi CAT Eq)/100 g of sample (dry basis).

Total Anti-oxidant Activity: According to the method adapted by Prieto *et al.* [16], 1.23 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added to 20 µl of the extract and the contents were incubated at 90°C for 90 min, cooled to ambient temperature and the absorbance was measured at 695 nm. The antioxidant capacity was expressed as ferulic acid (mg / 100 g of sample) equivalent.

Statistical Analysis: All experiments were performed in triplicate and data was presented as mean ± standard deviation (SD). All statistical analyses were done using Microsoft Excel 2007, data assessed for significant difference employing t-test and considered to be statistically significant at $p < 0.05$

RESULTS AND DISCUSSION

Proximate Composition: Table 1 presents the data on proximate composition of control and alkaline cooked foxtail millet (dry weight basis). Foxtail millet was found to be a good source of protein (5.1%) as well as ash (1.71%). During alkaline cooking of foxtail millet, protein and ash content increased by a maximum of 41.2 and 27.4 % respectively. These results were in accordance with the data reported by Gomez *et al.* [17] for alkaline cooking of corn. Similar results were also found during alkaline cooked maize to tortillas [18]. This may be due to leaching of fiber during cooking and soaking and subsequent washing in water, whereas increase in ash content may be due to the addition of calcium oxide. A marginal increase in total carbohydrate was also observed. A maximum loss of 22.5 % fat content resulted in the processed samples.

Total Polyphenolic Content: Total phenolic contents expressed as mg of gallic acid equivalent/100g of sample, are shown in Table 2. Control foxtail millet showed a free, bound and total polyphenolic content of 37.1, 21.0 and 57.9 mg GAEq/100g respectively. Alkaline cooking of foxtail millet showed a significant ($p < 0.05$) loss of free, bound and total phenolics when compared to that of control foxtail millet. The free, bound and total polyphenolic fractions were reduced maximum by 51.2, 22.3 and 36.6 %, respectively. Moreover, these losses were in direct proportion with the concentration of alkaline solution. Alkaline cooking of white and blue corn products also showed a similar pattern. The loss of phenolic fractions during alkaline cooking may be due to the loss of thermo labile components.

Table 1: Proximate composition of control and alkaline cooked foxtail millet

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Total Carbohydrate (%)
Control	8.1 ± 0.3	5.1 ± 0.4	7.1 ± 0.2	1.71 ± 0.2	78.2 ± 0.2
0.25% alkali	3.4 ± 0.2	7.2 ± 0.2	7.3 ± 0.2	1.98 ± 0.2	81.0 ± 0.2
0.5% alkali	6.5 ± 0.4	6.1 ± 0.1	6.1 ± 0.3	2.08 ± 0.3	79.9 ± 0.5
0.75% alkali	2.5 ± 0.1	7.1 ± 0.3	6.3 ± 0.4	2.14 ± 0.4	82.8 ± 0.4
1% alkali	4.4 ± 0.1	6.2 ± 0.3	5.5 ± 0.1	2.18 ± 0.1	82.3 ± 0.3

Results are mean of three independent determinations

Table 2: Antioxidant components in control and alkaline cooked foxtail millet extracts

Samples	Free Polyphenols (mg GAEq./g) ^A	Bound polyphenols (mg /g) ^A	Total polyphenols (mg./g) ^A	Total Flavonoids (mg /100g) ^B	Total Flavonol (mg /100g) ^C
Control	37.1 ± 0.4 ^a	21.0 ± 0.1 ^a	57.9 ± 1.3 ^a	21.7 ± 0.1 ^a	42.7 ± 0.9 ^a
0.25% alkali	20.4 ± 1.8 ^b	20.8 ± 1.0 ^b	40.9 ± 1.2 ^b	12.23 ± 0.1 ^b	25.1 ± 0.1 ^b
0.5% alkali	19.9 ± 1.8 ^c	19.1 ± 0.7 ^c	37.2 ± 0.8 ^c	8.7 ± 0.2 ^c	19.3 ± 0.5 ^c
0.75% alkali	18.8 ± 0.1 ^d	17.9 ± 1.0 ^d	36.8 ± 0.5 ^d	7.71 ± 0.2 ^d	18.1 ± 0.1 ^d
1% alkali	18.1 ± 1.0 ^e	16.3 ± 1.1 ^e	36.7 ± 1.4 ^e	7.36 ± 0.1 ^e	16.4 ± 0.1 ^e

Results are mean of three determinations, Values within columns a, b, c, d, e is significantly different at ($p < 0.05$). ^A mg gallic acid equivalents (GAEq)/g of sample, dry basis, ^B mg Epi-Catechin equivalents (Epi CAT Eq)/100g of sample, dry basis, ^C mg Epi-Catechin equivalents (Epi CAT Eq)/100g of sample, dry basis

Table 3: Effect of alkaline cooking of foxtail millet on anti-oxidant activity

Samples	DPPH (mg /100g) ^A	ABTS (mg /100g) ^B	TAC (mg /100g) ^C
Control	223.4 ± 2.4 ^a	169.2 ± 9 ^a	27.0 ± 3 ^a
0.25% alkali	116.2 ± 9 ^b	87.7 ± 5 ^b	13.0 ± 1 ^b
0.5% alkali	88.6 ± 8 ^c	64.7 ± 5 ^c	11.0 ± 1 ^c
0.75% alkali	82.7 ± 6 ^d	59.5 ± 6 ^d	10.2 ± 1 ^d
1% alkali	67.6 ± 6 ^e	55.7 ± 5 ^e	8.82 ± 0.1 ^e

Results are mean of three determinations, Values within columns a, b, c, d, e is significantly different at ($p < 0.05$). ^A mg Epi-Catechin equivalents (Epi CAT Eq)/100g of sample, dry basis, ^B mg Epi-Catechin equivalents (Epi CAT Eq)/100g of sample, dry basis, ^C mg ferulic acid equivalents (FAEq)/100g of sample, dry basis

Free and esterified phenolics that constitute the major portion of soluble fraction of phenolics are more vulnerable to thermal loss. The reduction of polyphenols can also be due to the physical losses of the pericarp, where polyphenols are largely concentrated and also due to leaching out in the soaking medium and destruction during cooking [9]. Polyphenols are also considered antinutrients owing to their ability to interact and bind with protein. This interaction renders structural and functional characteristics changes of the protein and makes them less soluble. Polyphenols also have the capability to interact with divalent cations such as Fe^{2+} , Zn^{2+} , Cu^{2+} , etc. Ultimately, they influence *in vitro* protein digestibility. Increase in protein digestibility is preferred as a positive characteristics of processed foods, as it is directly related to their bioavailability [19]. It can be reported from our study that reduction of polyphenols during alkaline cooking would improve the protein digestibility of alkaline cooked foxtail millet and thus improve its bioavailability.

Total Flavonoids Content: The total flavonoids content of control and alkaline cooked foxtail millet are presented in Table 2. Control foxtail millet contained 21.7 mg epi-catechin Eq/100g of total flavonoids and this was decreased by a maximum of 66.1 % during alkali treatment. The flavonoids content decreased with increase in the concentration of alkaline solution. This could be due to the breakdown of flavonoids during alkali treatment and

heating, since flavonoids are heat susceptible phenolic compounds [20]. It has been reported that, different heat treatment resulted in drastic reductions in flavonoid content. This could be due to the flavonoid breakdown during heating and extraction of glycosides by the steam [21].

Total Flavonol Content: A significant decrease in total flavonol content of alkaline cooked foxtail millet was observed (Table 2). This decrease may be due to the changes in chemical composition and also degradation of flavonol due to distribution and composition of individual phenolic components in seed coat and cotyledon. It is reported that, thermal processing of pinto, black beans and alkaline cooked sorghum significantly reduces the flavanol content [22, 23]. These significant losses might be attributed to those water-soluble components that were leached into cooking and soaking water during thermal processing and the breakdown of flavonols during alkaline cooking.

DPPH⁺ free Radical Scavenging Activity (FRSA): DPPH free radical scavenging activity (FRSA) was presented in Table 3. FRSA of control millet was 223.4 mg Epi.CAT Eq/100g and this decreased significantly during alkaline treatment. This may be due to the loss of thermo-labile components during alkaline cooking. Scavenging activity increased with the increasing of alkali concentration.

The decrease in scavenging activity may be due to the reduction of total flavonoids, total flavonol and total phenolic content. The results suggest that the phenolics were the main antioxidant compounds in foxtail millet. Thermal processing of buckwheat, purple wheat bran and alkaline cooked corn also are reported to have lower scavenging activity [20, 24]. Alkaline cooking has a critical role in reducing the antioxidant activity. Cooking process can increase or decrease the antioxidant activity of foods depending on the nature and molecular structure of the antioxidant compound.

ABTS⁺ Scavenging Activity: The ABTS⁺ Scavenging activity of control and alkaline cooked foxtail millet in terms of epicatechin equivalent was presented in table 3. ABTS⁺ Scavenging activity of control foxtail millet was significantly ($P < 0.05$) higher (169.2 mg Epi.CAT Eq: /100g) than the alkaline cooked foxtail millet. Alkaline cooked millet showed a reduction in scavenging activity due to the loss of antioxidant compounds. This reduction is correlating with the reduction of total polyphenolic content. This may be due to the cooking, soaking and draining of cooking water [25]. There was a correlation observed between total phenolic contents and ABTS scavenging activity, indicating the role of phenolic compounds in inhibiting free radicals and radical cations. The results suggest that phenolic compounds in the millet may be able to inhibit the excessive formation of free radicals in the human body. High molecular weight phenolics have higher ability to quench free radicals (ABTS +) and their effectiveness depends on the molecular weight, the number of aromatic rings and the nature of hydroxyl group's substitution [26].

Total Anti-Oxidant Activity: Total anti-oxidant activity of control and alkaline cooked foxtail millet was represented in table 3. Control foxtail millet showed a total anti-oxidant activity of 27mg FAEq:/100g and this was reduced during alkali cooking. Moreover total antioxidant activity decreased with increase in alkaline concentration. Losses in total anti-oxidant activity of yam flours, coffee brews were also observed during thermal treatment [27]. These are attributed to thermal degradation of phenolic and other bioactive compounds, presence of degradative enzymes and loss of antioxidant enzymes. Chandrasekara *et al.* [28] also reported that cooked grains of millets had a lower antioxidant capacity compared to their uncooked counterparts, thus suggesting that thermal processing may lower the antioxidants present in the grains.

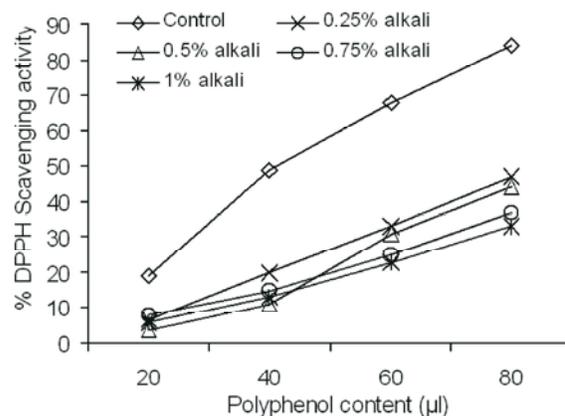


Fig. 1: Correlations between DPPH⁺ scavenging activity and polyphenol content

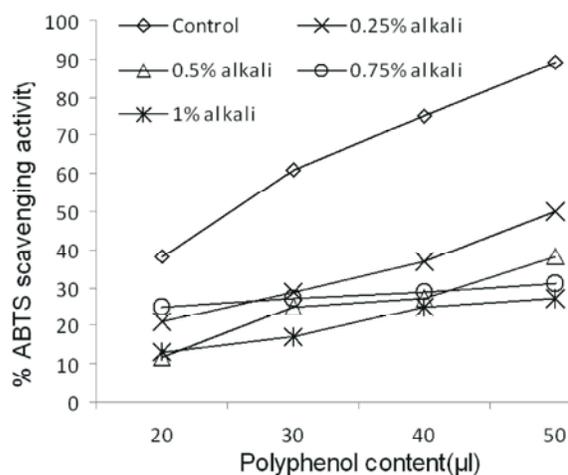


Fig. 2: Correlations between ABTS⁺ Scavenging activity and polyphenol content

Correlation Between Antioxidant Activity and Phenolic Content:

The effect of the concentration of antioxidant compounds on the DPPH radical as well as ABTS radical scavenging potentials increased with increasing concentration of extract, indicating the concentration dependency of the polyphenols for the antioxidant activity. The free radical scavenging activity in terms of percentage inhibition was shown in Figure 1 and 2. The *in vitro* antioxidant capacity of the millet polyphenols is mainly due to the presence of hydroxy-cinnamic and benzoic acid derivatives, as well as other flavonoids and such other compounds. Increase in the radical scavenging activity depends on the structure and substitution pattern of hydroxyl group present in polyphenols and also the presence of C2-C3 double bond which will configure keto-arrangement for electron delocalization [29]. It was observed that at any given concentration methanolic

extracts of control millet showed higher antioxidant power than alkali treated millet. This property is associated with the presence of reductones that are reported to be terminators of free radical chain reaction. These observations reveal the potent antioxidant activity of foxtail millet polyphenols. Hence, the information may be useful to understand the health benefits of the millet.

CONCLUSIONS

Alkaline cooked foxtail millet flour exhibited higher protein, ash, carbohydrate with lower fat content compared to the unprocessed (Control) flour. The extracts of alkali treated millet flour showed a significant decrease in antinutrients such as phenolic acids, flavonoids and flavonol. ABTS, DPPH radical Scavenging activity and total anti-oxidant activity of alkali treated flour showed lower activity compared to the control. The alkaline cooked dehulled foxtail millet flour would provide a suitable raw material for development of millet based diversified health food formulations.

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