



Effect of Traditional Processing Methods on the Nutritional Composition of Sorghum (*Sorghum bicolor* L. Moench) Flour

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Authors' contributions

This work was carried out in collaboration between both authors. Author AK designed the study. Author TT performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Sorghum is a drought-resistant crop grown widely in various parts of India. It has low protein content, highly deficient in essential amino acids lysine and tryptophan and high anti-nutritional content. Therefore, keeping in view the present study aimed to determine the effect of traditional processing methods such as fermentation, malting and roasting on the proximate composition, anti-nutritional factors, protein digestibility and lysine content of sorghum. The results showed that ash content increased from 1.73 to 1.89% during roasting. Fermentation increased crude protein content from 8.27 to 8.98% and *in vitro* protein digestibility from 13.62 to 69.63%. Malting decreased the crude fat content from 1.87 to 1.22% while it shows a significant increase in crude fiber content from 3.34 to 4.26% and carbohydrate content from 84.77 to 86.15%. Total phenol content reduced from 92.62 to 48.40 mg GAE/100 g, and tannin content reduced from 8.46 to 1.25 mg TAE/100 g during fermentation. The lysine content increased significantly during fermentation (1.88 g/16 g of N) and malting (1.94 g/16 g of N). Natural fermentation of sorghum flour found to have improved nutritional quality than other processing methods.

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1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench), a dryland crop, belongs to the family *Poaceae* and is ranked fifth in terms of total world production. In parts of Asia and Africa, people consume sorghum as a substantial source of energy, protein and minerals [1]. Sorghum is cultivated in various ecological conditions than other food crops [2]. FAO [3] denoted sorghum grain as "Lifesaver" and "Poor people crop." In developed countries, the utilization of sorghum for human consumption increased over the years by blending in composite flours [4]. India is the second-largest producer of sorghum next to the USA. The total sorghum production in India is about 5.35 MT [5]. In India, sorghum is grown in Kharif and Rabi seasons and used as a staple food in the state of Maharashtra, parts of Karnataka, Madhya Pradesh, TamilNadu, Gujarat and Andhra Pradesh. Sorghum consumed in different forms viz., flatbread, boiled grain, thick and thin porridges.

Sorghum protein content (10.20%) is lower compared to other major cereals such as wheat (13.2%), barley (11.60%), rye (11.36%) but higher than millets (9.48%) and oats (8.33%) [1,6,7]. However, the protein quality of sorghum is low due to the high deficiency of essential amino acid lysine as compared to other cereals. Lysine cannot be synthesized by our body, and it plays a vital role in the synthesis of protein and mineral absorption. Hence, it is necessary to increase the lysine content in food. The simple, cost-effective processing methods such as fermentation, malting and roasting can improve both the nutritional and functional properties of sorghum. Fermentation increases the mineral absorption [8] and decreases the anti-nutrients such as tannins and phytates, which binds with carbohydrates, proteins and minerals forming insoluble complexes which inhibit digestion. Tannin and phytate forms iron complexes in the intestine, results in low bioavailability [9]. Fermentation increases protein and starch digestibilities and amino acid contents, especially lysine, leucine, isoleucine and methionine significantly due to degradation of anti-nutrients [10].

In African countries, malted sorghum grain used for the production of alcoholic and non-alcoholic beverages [11]. Malting increases the availability of critical nutrients by breaking them into simpler

forms [12]. Malting increases the protein digestibilities, vitamin C content, mineral availability and increases the synthesis of lysine and tryptophan [13]. *In vitro* availability of iron and zinc in sorghum significantly improved from 8.02 to 16.67% and 8.87 to 18.30% respectively after germination [9]. Processing methods increase the nutrient bioavailability in the sorghum consuming population.

Roasting is an Indian food processing technique that uses dry heat but at a temperature lesser than parching. It increases the shelf life by reducing the water activity, decreases the rate of enzymatic activity and microbial activity [14]. Roasting of sorghum grains increases edibility, impart desirable sensory qualities, enhance palatability and reduces the anti-nutritional factors [15].

The current study will evaluate the effect of different processing methods viz., fermentation, malting, roasting on the nutritional composition (proximate composition, anti-nutritional factors, *in vitro* protein digestibility and lysine content) of sorghum flour.

2. MATERIALS AND METHODS

The present study was conducted at the Department of Foods and Nutrition, GBPUA&T, Pantnagar, Uttarakhand, India.

2.1 Physical Characteristics of Sorghum

Physical characteristics of sorghum grains affect the functional and sensory properties and also useful in processing. Thousand seed weight, thousand seed volume and bulk density of sorghum grains determined according to the method given by [16]. Hydration capacity was measured by the method of [17]. The Color of sorghum grains was noted by matching with color code of the Royal Horticultural Society color chart [18]. All samples were analyzed in triplicates.

2.2 Processing of Sorghum Samples

Sorghum (*Sorghum bicolor* (L.) Moench) variety CSV21F (dual purpose) procured from the Department of Plant Breeding and Genetics, GBPUA&T, Pantnagar, India. Sorghum grains were cleaned free from dust, dirt and other extraneous materials and pearled (Vivek Mandua

Thresher cum Pearler, Saharanpur, Uttar Pradesh). A part of cleaned sorghum grains used for malting and roasting. The remaining grains were ground into flour and used for natural fermentation.

2.2.1 Unprocessed raw sorghum flour (Control)

Pearled and cleaned sorghum grains were ground into flour using a mill (Atta Master, Navdeep Domestic Flour Mill, Gujarat, India) (particle size <0.25mm) and stored in a plastic hermetically sealed container at 4°C for future analysis. Unprocessed raw sorghum flour was used as control (C).

2.2.2 Fermented sorghum flour (FSF)

Natural fermentation of sorghum flour done according to the method of [19]. Sorghum flour mixed with distilled water in the ratio 1:2 (w/v) and then incubated at 37°C for 24 hours. After incubation, fermented flour was then mixed, transferred to aluminum trays and dried in an air oven at 70°C for 4 hours before being milled into flour, as was done with the unprocessed sorghum flour.

2.2.3 Malted sorghum flour (MSF)

Malting is done by soaking the grains in distilled water for 12 hours (1:2 w/v). Soaked grains were drained, germinated by spreading, covered using jute bags and kept in the dark for 48 hours. After germination, sorghum grains dried in an air oven at 65°C for 6 hours before being milled and stored as mentioned above [20].

2.2.4 Roasted sorghum flour (RSF)

A hundred grams of cleaned and sorted raw sorghum grains roasted using the hot plate for 2-3 mins with continuous stirring. The roasted seeds were cooled and milled, followed by storage as above [21].

2.3 Estimation of Proximate Composition

Total moisture content determined by the air oven method [22]. Total ash content done using the direct method, according to [22]. Crude fat estimated by direct method given in [23] using the SOCS plus assembly, Crude fiber determined according to [22], Crude protein determined by Micro-Kjeldahl method and Total Nitrogen calculated using the formula $N\% \times 6.25$

[22]. Total and available carbohydrates derived by the difference method given by [24]. Physiological energy value calculated using the formula given by [25].

2.4 Estimation of Tannin and Total Phenol Content

Estimation of Tannin content in sorghum flour samples carried out using the Folin-Denis spectrophotometric method, described by [26]. Total Phenol estimation analyzed using the Folin-Ciocalteu reagent given by [27].

2.5 Estimation of *In-vitro* Protein Digestibility

In-vitro protein digestibility of the sorghum flour samples determined according to the method of [28]. Percent digestibility calculated using the formula

$$\% \text{ digestibility} = \% \text{ N in digested sample} / \% \text{ N in sample} \times 100$$

2.6 Estimation of Amino Acid Lysine Content

Lysine content estimated, according to [29]. Defatted sorghum flour sample incubated overnight with papain at 65°C, then centrifuged at 3000 rpm for 5 minutes and 0.5 mL of copper phosphate suspension and 0.5 mL of amino acid mixture solution was added. A 0.1 mL of 2-chloro-3,5-dinitropyridine solution was added to the supernatant, mixed well and shaken for 2 hours. Five mL of 1.2 N HCL was added and mixed. The solution was extracted three times with ethyl acetate using separating funnel. The absorbance of the aqueous layer was read at 390 nm against reagent blank. Blank was prepared with 5 mL of papain solution alone.

$$\text{Lysine content} = \text{Lysine value from the graph in } \mu\text{g} \times 0.16 / \% \text{ N in the sample.}$$

2.7 Statistical Analysis

Four different samples were analyzed in triplicates, and values averaged. Data obtained from different processing methods were statistically analyzed using one-way ANOVA in a completely randomized design. To find out the difference between means Duncan multiple range test (DMRT) was used at a significance level of 5% ($p \leq 0.05$). SPSS software (version

22.0) for Windows was used for statistical analysis (IBM Inc., New York, USA).

3. RESULTS AND DISCUSSION

3.1 Physical Characteristics of Sorghum Grains

Thousand seed weight of sorghum grains used was 24.96 g. [30] also reported the weight of thousand sorghum grains as 25.1 g. The seed volume of sorghum grains was 27.83 ml. The results are in accordance with [31] who mentioned it as 29 ml. Bulk density observed as 0.899 g/ml. Kigozi et al. [32] indicated sorghum has a bulk density of 0.832 g/ml. Hydration capacity denotes grains ability to absorb water. The hydration capacity value was 10.97%. The pericarp color of sorghum grains matched with the color code '161A' of the greyed-yellow group the RHS color chart. The data on the physical characteristics of sorghum grains is shown in Table 1.

3.2 Effect of Processing Methods on Proximate Composition of Sorghum Flours

Proximate composition values of control, fermented, malted and roasted sorghum flour are given in Table 2.

The highest moisture content (11.88%) was present in control, and the lowest (5.90%) observed in fermented sorghum flour. The moisture content of all processed sorghum flours was significantly different ($p \leq 0.05$). The decrease in the moisture content of fermented and malted sorghum flour may be due to drying in the oven after processing.

The roasted sorghum flour (1.89%) had significantly higher ($p \leq 0.05$) ash content than other processing methods, i.e., control (1.73%), fermented (1.48%) and malted (1.44%) sorghum flours. Ash content of malted and fermented sorghum flours did not differ significantly ($p > 0.05$) with each other. During malting, the utilization of minerals for the growth of germ may be the reason for decreased ash content [19]. The results are in accordance with [33] who demonstrated increased ash content in pearl millet after roasting.

The protein content of sorghum flours ranged from 6.90 to 8.98%, and the values varied

significantly ($p \leq 0.05$) between different processing methods. The higher protein content in fermentation may be due to degradation of protein to peptides and amino acids by proteolytic activity of natural microorganisms present and also suggested that reduced protein content after sprouting may be due to loss of nitrogenous compounds during rinsing and soaking of seeds [34,35]. The decreased protein content in malting may be due to the utilization of protein for the growth and development of the embryo [19].

Table 1. Physical characteristics of sorghum grains

Physical characteristics	Values
Thousand seed weight (g)	24.96±0.19
Thousand seed volume (ml)	27.83 ± 2.02
Bulk density (g/ml)	0.899± 0.05
Hydration capacity (%)	10.97 ± 1.08

Values are expressed as mean ± S.D. of three independent observations

The highest crude fat content observed in control (1.87%) followed by fermented (1.52%), roasted (1.39%) and malted sorghum flours (1.22%). Crude fat content between the processing methods varied significantly ($p \leq 0.05$). During fermentation, the decrease in crude fat may be due to lipids broken down into free fatty acids by micro-organisms present [36]. Rumiyati et al. [37] suggested that a reduction in lipid content due to the oxidation of fatty acids to carbon dioxide and water to produce energy source for germination [38,39]. observed similar results of low-fat content after the germination process.

The crude fibre content of malted sorghum flour (4.26%) was higher than fermented (3.40%), roasted (3.33%) and control flour (3.34%). Control, fermented and roasted sorghum flour showed no significant difference ($p > 0.05$) between them in crude fibre content except malting which differed significantly. [37] suggested that fibre content increased during germination due to changes in polysaccharides present in the cell wall and increased cellular structure in plants while sprouting. A similar observation of increased crude fibre content after germination observed by the study of [40] in soybean. Chavan et al. [34] also reported increased crude fibre content in sorghum during natural fermentation.

Table 2. Proximate composition of differently processed sorghum flour (% DWB)

Nutrients	C	FSF	MSF	RSF
Moisture (%)	11.88 ± 0.28 ^a	5.90 ± 0.05 ^{be}	7.10 ± 0.33 ^{bd}	11.16 ± 0.20 ^{bc}
Ash (%)	1.73 ± 0.11 ^{bc}	1.48 ± 0.03 ^b	1.44 ± 0.03 ^b	1.89 ± 0.02 ^a
Crude protein (%)	8.27 ± 0.30 ^a	8.98 ± 0.28 ^a	6.90 ± 0.28 ^{bc}	8.04 ± 0.29 ^{ab}
Crude fat (%)	1.87 ± 0.05 ^a	1.52 ± 0.05 ^{bc}	1.22 ± 0.04 ^{be}	1.39 ± 0.06 ^{bd}
Crude fibre (%)	3.34 ± 0.04 ^b	3.40 ± 0.14 ^b	4.26 ± 0.07 ^a	3.33 ± 0.08 ^b
Carbohydrate (Exclude crude fibre)**(%)	84.77 ± 0.34 ^b	84.60 ± 0.32 ^{bc}	86.15 ± 0.33 ^a	85.33 ± 0.36 ^b
Physiological energy (Kcal/100 g)	389.01 ± 0.54 ^a	388.07 ± 0.65 ^{ab}	383.28 ± 0.65 ^{bc}	386.05 ± 2.20 ^b

All results expressed as mean ± standard deviation of three replicates, Mean values followed by different superscripts (a, b, c, d, e) in each row are significantly different from each other at 5% level of significance (p ≤ 0.05), C-Control (Unprocessed raw sorghum flour), FSF – Fermented Sorghum Flour, MSF – Malted Sorghum Flour, RSF – Roasted Sorghum Flour, ** Calculated by difference (= 100-crude protein-crude fat-ash-crude fiber)

Table 3. Total phenol, tannin and *in vitro* protein digestibility (IVPD) of differently processed sorghum flour

Types of sorghum flour	C	FSF	MSF	RSF
Total Phenol (mg GAE/100 g, DWB)	92.62 ± 0.6 ^a	48.40 ± 0.18 ^d	58.02 ± 0.69 ^c	68.44 ± 0.46 ^b
Tannin (mg TAE /100 g, DWB)	8.46 ± 0.47 ^a	1.25 ± 0.06 ^d	2.10 ± 0.15 ^c	3.51 ± 0.08 ^b
IVPD (% DWB)	13.62 ± 0.48 ^d	69.63 ± 2.83 ^a	48.86 ± 2.37 ^b	32.14 ± 3.61 ^c

All results expressed as mean ± standard deviation of three replicates, Mean values followed by different superscripts (a, b, c, d) in each row are significantly different from each other at 5 % level (p ≤ 0.05), C-Unprocessed sorghum flour, FSF – Fermented Sorghum Flour, MSF – Malted Sorghum Flour, RSF – Roasted Sorghum Flour

Table 4. Effect of processing methods on lysine content of sorghum flour

Processing methods	Lysine (g/16 g of N)
C	1.21 ± 0.10 ^b
FSF	1.88 ± 0.05 ^a
MSF	1.94 ± 0.09 ^a
RSF	1.23 ± 0.05 ^b

All results expressed as mean ± standard deviation of three replicates Mean values followed by different superscripts (a, b, c, d) in each row are significantly different from each other at 5 % level (p ≤ 0.05), C-Unprocessed sorghum flour, FSF – Fermented Sorghum Flour, MSF – Malted Sorghum Flour, RSF – Roasted Sorghum Flour

The total carbohydrate content of malted sorghum flour found to be 90.41%, which was significantly higher (p ≤ 0.05) than fermented (88.01%), roasted (88.67%) and control (88.13%) sorghum flour. Among the processing methods, the available carbohydrate content was highest in malted flour (86.15%) and lowest in fermented sorghum flour (84.60%). The processing method differed significantly among each other (p≤0.05) in available carbohydrate content. Ashworth and Draper [41] mentioned that the concentration of carbohydrates decreased due to the microorganism activity during fermentation. The results are similar to the findings of [42] who found that reduction in carbohydrate content from

80.87% to 79.66% during fermentation of maize grains.

Higher physiological energy observed in control (389.01 Kcal/100 g) and the lowest in malted (383.28 Kcal/100 g) sorghum flour. The remaining roasted and fermented sorghum flours showed a physiological energy value of 386.05 and 388.07 Kcal/100 g, respectively. Non-significant difference (p>0.05) observed between the energy values of control and fermented sorghum flour. Higher physiological energy value in fermented sorghum flour compared to other methods attributes to higher crude protein content. Nour et al. [19] observed a similar trend

of decrease in energy value after processing of sorghum flours.

3.3 Effect of Processing Methods on Anti-nutrients and *in vitro* Protein Digestibility (IVPD) of Sorghum Flour

Total phenol content of control (92.62 mg GAE/100 g), fermented (48.40 mg GAE/100 g), malted (58.02 mg GAE/100 g) and roasted sorghum flour (68.44 mg GAE/100 g). The total phenolic content differed significantly ($p \leq 0.05$) between the processing methods. Iwuoha and Aina [43] denoted that decreased total phenolic content during the malting process may be due to leaching of polyphenols into the soaking medium. The factors such as genotype, geographical location and growing system also attributed to phenolic content variation in sorghum.

Tannin content observed to be highest in control (8.46 mg TAE/100 g) when compared to the fermented, malted and roasted sorghum flours (1.25, 2.10 and 3.51 mg TAE/100 g), respectively. The tannin content of processing methods varied significantly ($p \leq 0.05$). Ikemefuna et al. [44] suggested that reduction in tannin content may be due to natural fermentation of flour broke down the tannin-enzyme and protein-tannin complexes due to which protein content also increased. The released free tannins leached out into the water [45] concluded a decrease in tannin content during malting due to the activity of enzymes associated with seeds. This claim was supported by [19], who reported that a reduction in tannin might be due to peroxidase enzyme activation. Gernah et al. [42] reported a significant decrease in tannin content from 2.62 g/100 g to 0.42 g/100 g during 24 hr fermentation.

In vitro protein digestibility was higher in fermented sorghum flour (69.63%) and malted, roasted and raw sorghum flour (48.86, 32.14 and 13.62% respectively). IVPD significantly varied ($p \leq 0.05$) between processing methods. The increase in protein digestibility during malting and fermentation may be due to anti-nutritional contents are catabolized, and seed proteins are metabolized. Devi et al. [46] reported sprouting of cowpea showed a significant increase in protein digestibility from 52.65% to 63.61%. Nour et al. [19] observed that protein digestibility increased from 5.12% in raw sorghum to 65.03% in fermented and 54.66% in malted sorghum.

3.4 Effect of Processing Method on Lysine Content of Sorghum Flour

Generally, lysine is the limiting amino acid in cereals. The values presented in Table 4. The lysine content found to be higher in malted sorghum flour (1.94 g/16 g of N) compared to control flour (1.21 g/16 g of N). Fermented and roasted sorghum flour has 1.88 and 1.23 g/16 g of N, respectively. Amount of lysine between fermented and malted sorghum flour not differed significantly ($p \leq 0.05$). Tongnual et al. [47] reported an increase in lysine content from 10 mg/100 g meal to 50 mg/100 g meal during corn fermentation for 24 hours. Asiedu et al. [48] reported during germination, lysine content increased upto 56% in sorghum. Chavan et al. [34] proposed that prolamins degraded into lower peptides and free amino acids supply the amino group, which are used through transamination to synthesize lysine. Due to reduced tannin content and higher protein digestibility percentage in the fermentation process, lysine availability may be higher in fermented sorghum flour compared to malted sorghum flour.

4. CONCLUSION

The results showed that in all processing methods, roasting improves ash content slightly and does not improve other nutritional content. Malting and fermentation have significantly lower anti-nutritional content compared to raw sorghum flour (Control). Natural fermentation of sorghum flour shows a significant increase in crude protein content and its digestibility. Hence essential amino acid lysine availability is higher in fermented sorghum flour than malted sorghum flour. Overall, the protein quality is better in fermented sorghum flour compared to all other processing methods. Thus, the natural fermentation of sorghum flour was superior in nutritional quality. Therefore, it can be useful in value-added food product development and as an ingredient in composite flour to avoid malnutrition. Further studies required to find out the effect of fermented sorghum flour in food product development.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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