Nutritional Evaluation of Sorghum Flour (*Sorghum bicolor L. Moench)* During Processing of Injera

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Abstract—The present study was carried out to evaluate the nutritional value of sorghum flour during processing of injera (unleavened thick bread). The proximate composition of sorghum flour before and after fermentation and that of injera was determined. Compared to the raw flour and fermented one, injera had low protein (11.55%), ash (1.57%) and fat (2.40%) contents but high in fiber content. Moreover, injera was found to have significantly ($P \leq 0.05$) higher energy (389.08 Kcal/100g) compared to raw and fermented sorghum flour. Injera contained lower levels of anti-nutritional factors (polyphenols, phytate and tannins) compared to raw and fermented sorghum. Also it was found to be rich in Ca (4.75mg/100g), Fe (3.95 mg/100g), and Cu (0.7 mg/100g) compared to that of raw and fermented flour. Moreover, both the extractable minerals and protein digestibility were high for injera due to low amount of anti-nutrients. Injera was found to contain an appreciable amount of amino acids except arginine and tyrosine.

Keywords—Cooking, Fermentation, Malt, Protein fractions, Sorghum.

I. INTRODUCTION

SORGHUM is one of the cereals that constitute a major source of proteins, calories, minerals for millions of people in Africa and Asia. This cereal is mainly considered as subsistence crop because of its unique tolerance to drought and adaptation to dry tropical and subtropical ecosystems throughout the world. The crop is rich in minerals but with bioavailability vary from less than 1% for some forms of iron to greater than 90% for sodium and potassium. The reasons for this are varied and complex, since many factors interact to determine the ultimate bioavailability of a nutrient [1]. Like other grains, sorghum protein is generally low in the essential amino acids such as lysine and therionine [2]. Sorghum, like legume and oil seed meals has some limitations, due to the presence of antinutritional factors, such as trypsin and amylase inhibitors, phytic acid, and tannins. These compounds are known to interfere with protein, carbohydrates and mineral metabolism. Most varieties of sorghum have gained universal fame for production of fermented foods, because of the wide adaptability and low cost of production. Sudan seems to have

the greatest number of fermented sorghum products. There are about 30 such products that are basically different from one another [3]. Fermentation makes the foods easier to digest and the nutrients easier to assimilate and also it retains enzymes, vitamins, and other nutrients that are usually destroyed by food processing [3]. Fermentation has been used for several thousand years as an effective and low cost means to preserve the quality and safety of foods. Animal and plant tissues subjected to the action of microorganisms and/or enzymes which caused desirable biochemical changes and significant modification of food quality. Fermentation is an oldest known form of food biotechnology. Food fermentations is an important technique in the developing countries where the lack of resources limits the use of recent techniques such as vitamin enrichment of foods and the use of energy and capital intensive processes for food preservation. Injera is the undisputed national food of Ethiopians [4], [5]. It can be made from different cereals, including sorghum, tef, corn, finger millet and barley, although tef (*Eragrostis tef*) is the major cereal ingredient in Ethiopian injera. Umeta et al. [6] analyzed four types of injera and found that the moisture content of them ranged from 52.6 to 58.2g/100g. Zinc ranged from 0.63 to 0.86mg/100g, iron ranged from 6.9 to 10.2mg/100g, calcium ranged from 10.3 to 13.2mg/100g, phosphorus ranged from 96 to108mg/100g, phytate ranged from 70 to 82mg/100g and tannin ranged from 46.2 to 53.6mg/100g. Kebede and Menkir [7] reported that sorghum ranks second to tef in preference for making injera. This could be due to the relative brittleness and dryness of sorghum injera after storage [8]. This study aimed to evaluate the nutritional quality of injera as a prodct of fermented sorghum.

II. MATERIALS AND METHODS

A. Materials

Sorghum grain variety Tabat was obtained from the Agronomy Department, Faculty of Agriculture, University of Khartoum, Sudan. All chemicals and reagents used in this study are of technical grade.

B. Injera preparation

Sorghum grains were cleaned from impurities and broken seeds and then milled into fine flour using laboratory miller to pass a 0.4 mm screen and kept in polythene bags at 4°C till used. A starter was taken from previous fermented sorghum injera dough. It was clear, yellow liquid that accumulates on the surface of the contained. Sorghum flour was mixed with

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the starter and fermented for 72h. The water to flour ratio was 1:1.71. Baking of injera was done on hot plate (steam bake) in a thin layer on a covered clay griddle 2-3min according to the traditional method of Ethiopian.

C. Chemical composition

The chemical composition of the samples was determined according to the method described by the AOAC [9].

D. Determination of amino acids

The amino acids were determined according to the official methods of analysis [10]. 500 mg of pulverized sample was hydrolyzed with 5 ml (6NHCl) in an evacuated sealed tube for 24 hours at 110°C, after oxidation (H_2O_2/HCOOH, 24h, chilled) and without previous oxidation, the pH was adjusted to 2.2 with NaOH and filled to 100 ml with buffer (pH 2.2), about 2 ml was then filtrated (membrane filter). The liberated amino acids were separated by LKB Biochrom 4150 (Alpha) Automatic Amino Acid Analyzer based on Ion-exchange chromatography.

E. Total energy (calorific value)

Energy was calculated as described by Osborne and Voogt [11] using the Atwater factors: 1g of carbohydrates (C.) provides (4Kcalories), 1g of protein (P.) provides (4Kcalories) and 1g fat (f.) provides (9Kcalories).

c. (g) X 4: Kcal of carbohydrate
p. (g) X4: Kcal of protein
f. (g) X9: Kcal of fat.

F. Phytic acid determination

Phytic acid content was determined by the method described by Wheeler and Ferrel [12] using 2.0 g dried sample. A standard curve was prepared expressing the results as $Fe(NO_3)_3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming a 4:6 iron to phosphorus molar ratio.

G. Polyphenols determination

Total polyphenols was determined according to the Prussian blue spectrophotometric method [13] with a minor modification. Sixty milligrams of ground sample were shaken manually for 1 min in 3.0 ml methanol. The mixture was filtered. The filtrate was mixed with 50 ml distilled water and analyzed within an hour. About 3.0 ml of 0.1 M $FeCl_3$ in 0.1 M HCl was added to 1 ml filtrate, followed immediately by timed addition of 3.0 ml freshly prepared $K_3Fe(CN)_6$. The absorbance was monitored on a spectrophotometer (Pye Unicam SP6-550 UV, London, UK) at 720 nm after 10 min from the addition of 3.0 ml of 0.1 M $FeCl_3$ and 3.0 ml of 0.008 M $K_3Fe(CN)_6$. A standard curve was prepared expressing the result as tannic acid equivalents, that is, the amount of tannic acid (mg/100 g) that gives a color intensity equivalent to that given by polyphenols after correction for blank.

H. Determination of tannins

Tannin content of the samples was determined according to the modified Vanillin-HCl methanol method as described by Price, et al. [14]. The Vanillin-HCl reagent was prepared by mixing equal volumes of 8% concentrated HCl in methanol and 1% Vanillin in methanol. The solutions of the reagent were mixed just prior to use. About 0.2g of the ground sample was placed in a small conical flask. Then 10 ml of 1% concentrated HCl in methanol was added. The conical flask was capped and continuously shaken for 20 minutes and the content then centrifuged at 2500 rpm for 5 minutes. About 1.0 ml of the supernatant was pippetted into a test tube containing 5ml of Vanillin-HCl reagent. Absorbance at 450nm was read on spectrophotometer (corning, 259) after 20 minutes incubation at 30°C, a blank sample was carried out with each run of sample. A standard curve was prepared expressing the result as catechin equivalent, i.e. catechin (mg per ml) which gives color intensity equivalent to that given by tannin after correcting for blank. Tannin content was expressed as catechin equivalent as follows:

$$\text{Tannin (\%)} = \frac{C \times 10 \times 100}{200}$$

where:

C = Concentration corresponding to the optical density.
10 = Volume of the extract (ml).
200 = Sample weight (mg).

I. Total minerals determination

Minerals were extracted from the samples by the dry ashing method that described by Chapman and Pratt [15]. About 2.0 g of sample was acid-digested with diacid mixture (HNO_3:$HClO_4$, 5:1, v/v) in a digestion chamber. The digested samples were dissolved in double-distilled water and filtered (Whatman No. 42). The filtrate was made to 50 mL with double-distilled water and was used for determination of total calcium, phosphorus and iron. Calcium and magnesium were determined by a titration method. Iron zinc and copper were determined by atomic absorption spectrophotometer (Perkin-Elmer 2380). Phosphorus was determined spectrophotometerically by using molybdovanadate method.

J. HCl extractability of minerals

Minerals in the samples were extracted by the method described by Chauhan and Mahjan [16]. About 1.0 gm of the sample was shaken with 10 mL of 0.03 M HCl for 3 h at 37°C and then filtered. The clear extract obtained was oven-dried at 100 °C and then acid-digested. The amount of the extractable minerals was determined by the methods described above. HCl extractability (%) was determined as follows:

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractable in } 0.03\text{N HCl (mg/100g)}}{\text{Total minerals (mg/100g)}} \times 100$$

K. Determination of in vitro protein digestibility (IVPD)

The IVPD was carried out according to Saunders et al. [17] method. About 200 mg of sorghum sample was placed into a

50 ml centrifuge tube, 15 ml of 0.1M HCl containing 1.5 mg pepsin. The tube was incubated at 37°C for 3 h. After incubation the sample was treated with 10ml trichloroacetic acid and centrifuged at 50,000 g for 2 min at room temperature. Nitrogen in the supernatant was estimated using microkjeldhl method [9]. Digestibility was calculated using the formula:

$$\text{Protein digestibility (\%)} = \frac{\text{N in supernatan - Enzyme Nitrogen}}{\text{N in sample}} \times 100$$

L. Statistical analysis

Each determination was carried out on three separate samples and analyzed in triplicate on a dry weight basis, the figures were then averaged. Data were assessed by the analysis of variance [18]. Means comparison for treatments was made by using Duncan's multiple range test. Significance was accepted at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

A. Chemical composition and energy content of sorghum flour during processing

The chemical composition of sorghum flour before and after fermentation and that of injera is shown in Table 1. The dry matter was found to be 92.87%, 91.83% and 96.07% for raw flour, fermented dough and injera, respectively. The results obtained for sorghum flour lies within the range reported by Ibrahim et al. [19]. On the other hand, the value obtained for injera was higher than that reported earlier [20]. The ash content was found to be 1.75%, 1.65% and 1.57% for raw flour, fermented dough and injera, respectively. The data obtained showed that the ash content of the cultivar did not change during injera processing. The protein content was found to be 12.25%, 10.70% and 11.55% for the samples, respectively. The protein content obtained lies within the range reported for Tetron, Dabar and Faterita [21]. The protein content of injera was found to be higher than that reported by Gebrekidan and GebreHiwot [20]. The fat content of the flour before and after fermentation and that of injera was found to be 4.24%, 3.93% and 2.40%, respectively. The values obtained for fat content were within the range reported for Tetron, Dabar and Faterita [21].

TABLE I
PROXIMATE COMPOSITION (%) OF RAW FLOUR, FERMENTED DOUGH AND INJERA OF SORGHUM

Sample	Sorghum flour	Fermented	Injera
Dry matter	92.87[b](±1.91)	91.83[c](±1.90)	96.07[a](±0.27)
Ash	1.75[a](±0.04)	1.65[b](±0.11)	1.57[b](±0.02)
Protein	12.25[a](±0.00)	10.70[c](±0.39)	11.55[b](±0.4)
Fiber	1.71[a](±0.08)	1.82[a](±0.08)	1.95[a](±0.16)
Fat	4.24[a](±0.21)	3.93[a](±0.11)	2.40[b](±0.3)
Carbohydrate	74.68[b](±1.8)	75.36[b](±1.61)	80.16[a](±0.19)

Values are means (± SD). Means not sharing a common superscript letter in a column are significantly different at (p ≤ 0.05) as assessed by Duncan's multiple range tests.

The fiber content was found to be 1.71%, 1.82 and 1.95% for the samples, respectively. The values obtained were found

to be lower than those reported earlier [21]. Carbohydrate content was found to be 72.93%, 50.15% and 24.99% for sorghum flour, fermented dough and injera, respectively. The value obtained for injera was lower than that reported by Gebrekidan and GebreHiwot [20].

The total energy of sorghum flour before and after fermentation and that of injera is shown in Fig. 1. The total energy of raw flour, fermented dough and injera was found to be 385.88, 379.67 and 388.48 kcal/100g, respectively. It was clear that the energy level significantly decreased after fermentation of the flour but increased when the fermented dough was processed (injera).
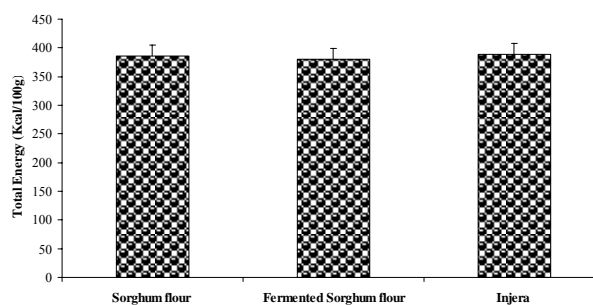

Fig. 1. Total energy (Kcal/100g) of raw flour, fermented dough and injera of sorghum. Error bars indicate the standard deviation of triplicate samples.

B. Antinutritional factors content of sorghum flour during processing

Total polyphenols, phytate and tannins contents are shown in Table 2. Polyphenols content was decreased during processing of the flour and reached minimum value (3.69 mg/100g) when the fermented dough was processed into injera. Fermentation and cooking were observed to reduce the antinutritional factors as reported by many researchers [19], [22]. Tannin content was slightly reduced after injera processing. However, phytate content was significantly reduced after fermentation of the flour and further reduction was observed after cooking of the fermented dough (injera). Fermentation was found to decrease the antinutritional factors content of sorghum with time as reported by El Khalifa et al. [23]. Moreover, it has been reported that injera processed from 2-3 days fermented dough was found to contain low level of phytate [24].

TABLE II
ANTINUTRITIONAL FACTORS CONTENT (MG/100G) OF RAW FLOUR, FERMENTED DOUGH AND INJERA OF SORGHUM

Sample	Polyphenols	Phytate	Tannin
Sorghum flour	8.10[a] (±1.45)	317.65[a] (±13.5)	0.18[a] (0±.06)
Fermented	6.64[b] (±0.23)	247.92[c] (±2.64)	0.18[a] (±0.01)
Injera	3.69[c] (±1.41)	286.70[b] (±4.25)	0.16[a] (±0.02)

Values are means (± SD). Means not sharing a common superscript letter in a column are significantly different at (p ≤ 0.05) as assessed by Duncan's multiple range tests.

C. In vitro protein digestibility of sorghum flour during processing

The in vitro protein digestibility (IVPD) of sorghum flour, fermented dough and injera is shown in Fig. 2. The IVPD was done using single enzyme (pepsin) digestion. Both fermentation and cooking (injera) improved the in vitro protein digestibility and was found to be 23% for the fermented dough and injera. Similar improvement was observed after fermentation of sorghum flour [23]. The improvement in the in vitro protein digestibility was likely to be due to the reduction in antinutritional factors as a result of fermentation and cooking as reported by Osman [25].
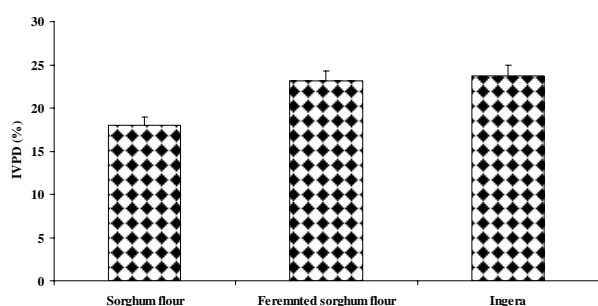

Fig. 2. *In vitro* protein digestibility (IVPD) of raw flour, fermented dough and injera of sorghum. Error bars indicate the standard deviation of triplicate samples.

D. Total and extractable minerals of sorghum flour during processing

Total and extractable minerals of sorghum flour during processing are shown in Table 3. Total calcium content was found to be 3.75mg/100g for sorghum flour, 4.99 mg/100g for the fermented dough and 4.75mg/100g for injera. The results obtained are lower than that recorded by Idris et al. [23]. Calcium extractability greatly improved when the flour was cooked into injera (78%) compared to the values obtained for the raw flour (56%) and that of the fermented dough (52%). Total phosphorous content was found to be 100.60mg/100g, 92.20mg/100g and 95.40mg/100g for sorghum flour, fermented dough and injera, respectively. The data obtained showed that phosphorus extractability was very low compared to all other minerals. However, injera gave a value (31%) double that of the raw flour (14%) and the fermented dough (24%). Iron content was found to be 2.24mg/100g, 3.64mg/100g and 3.95mg/100g for the samples, respectively. Iron extractability generally low but remained constant after cooking of the fermented dough. Magnesium content was found to be 75.02mg/100g, 75.13mg/100g and 30.06mg/100g for the raw flour, fermented dough and injera, respectively. Although injera contained low amount of magnesium but the extractability of it was high. The lower amount of magnesium may be due to the fact that divalent cations such as Mg may be present as mineral phytate chelates which may explain the lower availability of these minerals [26]. Zinc content was found to be 0.745mg/100g, 1.01mg/100g and 0.64mg/100g for raw flour, fermented dough and injera, respectively. Zinc was reported to be essential mineral adversely affected by phytate. Fermentation of injera reduced the zinc concentration [24]. Although injera contained lower amount of magnesium but with higher extractability (62%), total copper content was found to be 0.61mg/100g, 0.32mg/100g and 0.71mg/100g for the samples, respectively. Injera was found to contain higher amount (0.71mg/100g) of Cu as well as the extractable Cu (38%). Sorghum flour was found to have low minerals extractability and this may likely to be due to the presence of antinutritional factors. However, fermentation and/or cooking of the flour significantly ($P \leq 0.05$) reduced the level of such factors with a concomitant increase in minerals extractability.

E. Amino acid content of sorghum flour during processing

Amino acids content of sorghum flour, fermented dough and injera are shown in Table 4. As shown in Table 4 most of the essential amino acid content was increased when sorghum flour was cooked (injera). However, fermentation alone decreases the amino acid content. The increment in amino acid content after production of injera is likely to be due to concentration of the flour constituents as a result of heating.

IV. CONCLUSION

TABLE III
TOTAL (MG/100G) AND EXTRACTABLE (%) MINERALS OF RAW FLOUR, FERMENTED DOUGH AND INJERA OF SORGHUM

Minerals	Samples Sorghum flour	Fermented flour	Injera
Ca			
Total	3.75[b] (±0.39)	4.99[a] (±0.68)	4.75[a] (±0.20)
Extractable	56[c]	52[b]	78[a]
P			
Total	100.60[a] (±8.40)	92.20[c] (±3.75)	95.40[b] (±4.54)
Extractable	14[c]	24[b]	31[a]
Fe			
Total	2.24[b] (±0.40)	3.64[a] (±0.54)	3.95[a] (±0.15)
Extractable	34[a]	20[b]	34[a]
Mg			
Total	75.02[a] (±3.61)	75.13[a] (±4.24)	30.06[b] (± 0.82)
Extractable	20[c]	40[b]	50[a]
Zn			
Total	0.75[b] (±0.07)	1.01[a] (±0.02)	0.64[c] (±0.04)
Extractable	52[b]	34[c]	62[a]
Cu			
Total	0.61[a] (±0.09)	0.32[b] (±0.05)	0.71[a] (±0.01)
Extractable	34[b]	16[c]	38[a]

Values are means (± SD). Means not sharing a row superscript letter in a column are significantly different at p ≤ 0.05 as assessed by Duncan's multiple range tests.

Utilization of sorghum as food for human nutrition is constrained due to high level of antinutritional factors especially phytic acid, which can impair the bioavailability of trace element. Injera is a product of fermented and cooked sorghum flour. Fermentation was observed to alleviate the effect of the antinutritional factor and accordingly both mineral extractability and protein digestibility were improved as well as the amino acid content.

TABLE IV
AMINO ACIDS CONTENT (μG/100G) OF RAW FLOUR, FERMENTED
DOUGH AND INJERA OF SORGHUM

Amino acids	Raw flour	Fermented flour	Injera
Histidine	97.93	73.16	112.71
Isoleucine	187.5	142.1	198.86
Leucine	643.28	559.66	710.39
Lysine	115.8	76.75	127.59
Methionine	93.45	73.66	102.63
Phenylalanine	290.88	278.64	386.88
Threonine	149.44	129.15	187.55
Valine	243.05	232.24	328.09
Alanine	458.93	415.63	537.23
Arginine	176.24	124	164.18
Aspartic acid	311.81	255.56	353.15
Cystine	23.8	10.91	29.15
Glutamic	1264.05	1052.26	1537.6
Glycine	156.14	115.36	198.34
Proline	397.58	346.8	486.16
Serine	202.55	173.11	252.2
Tyrosine	138.05	93.18	106

Values are means (± SD). Means not sharing a row superscript letter in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range tests.

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