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Supplementation and Fermentation of Sorghum Flour: Changes in Ash, Protein and Minerals contents.

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ABSTRACT

Background: Moringa leaves are rich in protein, vitamins and minerals complementing the nutrient profile of sorghum flour. Processing methods. Thus, fermented sorghum supplementation could improve insulin resistance, glucose tolerance and hyperglycemia by decreasing the circulating levels of detrimental metabolites indoxylsulfate, indolelactate, indole, indole 3-aldehyde, p-cresol, p-cresol sulfate, leucine, isoleucine, tyrosine, tryptophan, and phenylalanine .The effect of fermentation of sorghum flour (SF) supplemented with moringa leaves (ML) on ash, protein and minerals contents was investigated. **Methods**: Moringa leaves were added at different ratios (4, 8 and12%) to sorghum flour using person square. Ash, protein and minerals were analyzed and data were assessed bytheanalysis of variance (ANOVA). **Results**: The results revealed that supplementation with 4,8 and 12% ML increasedash , protein and minerals (Ca, K, Mg and Fe). Fermentation (12 hr.) of both sorghum flour (control) andsupplemented flour with moringa leaves increased protein and minerals contents. Whereas, ash content was insignificantly ($P < 0.05$) decreased due to the utilization of ash by fermentation microorganisms. **Conclusion**: Fermentation of sorghum flour enriched with moringa leaves improved protein and total minerals contents.

Keywords: Fermentation, Minerals, Moringa leaves, Sorghum, Supplementation

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INTRODUCTION

Sorghum grain is mainly used as a staple food in semi-arid regions of Africa, and to a much more limited extent in parts of India and Central America **(*Girard et al* ., 2018).** Sorghum is that the fifth most-produced cereal within the world and maybe a source of nutrients and bioactive compounds for the human diet. In developing countries, the high price of animal foods and limited income earned by majority people has resulted in their dependency on cereal grains as staple food. Also increase in human population in developing countries and short-falls in cereal production in several developed countries had resulted in increase in demand for cereals as foods, feed and industrial raw materials (**Sokrab *et al.,* 2012**. Flour from various cereals is one of the major raw materials used in the production of popular food products with high acceptability, good storage characteristics and cheap cost **(Mbata *et al*., 2009)** .After maize, sorghum ("Sorghum bicolor (L.) Moench") is considered the grain of choice in subsaharan Africa and other developing countries **Mabhaudhi *et al*. (2016),** where it is used as the main staple food by the inhabitants of the region leaves are rich in carbohydrates, lipids, proteins, minerals, salts, and vitamins **(Kashyap *et al*., 2022; Ahmed et al., 2023; Yang et al., 2023a).** Moringa oleifera is known to have antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic, cholesterol reducing, and hepatoprotective properties **(Khalid et al., 2023; Kashyap *et al*., 2022; Ntshambiwa *et al*., 2023).** The fungus causes both leaf spot **(anthracnose) and stalk rot (redrot).** The disease appears as small red coloured spots on both surfaces of the leaf. The centre of the spot is white in colour encircled by red, purple or brown margin. Moringa (*Moringa oleifera*) seeds have high protein content, which can be utilized to solving worldwide malnutrition or under nutrition problems by nutritionists and community health cautious persons **(Fahey, 2005).** The leaves contain also antinutritional substances such as tannins, saponins that limit their use **(Khalid et al., 2023; Kashyap *et al*., 2022).** Fortification and processing are some of the approaches that can also be used to solve such problems. Cheap and readily available plant proteins from legumes are large replacing animal protein as suitable source of high quality protein **(Annan and Plahar, 1995).** Fermented foods play an important role in providing food security, enhancing livelihoods, and improved nutrition and social well-being of the people **(Adesulu and Awojobi, 2014).** Fermentation leads to improved food preservation **(Ross et al., 2002),** food quality, and increase range of edible food products. An enhancement of the nutritive value by an increase level of essential nutrients or reduced levels of toxicants in food remains a benefit of fermentation process **(Evans et al., 2013).** The aim of this study was to investigate the effect of fermentation on ash, protein contents and minerals contents of sorghum flour supplemented with differences ratios by moringa leaves powder

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MATERIALS AND METHODS

Food materials

Sorghum grains locally known as Tabat was purchased from Omdurman local market, Sudan. The grains were cleaned and freed from foreign objects. Some of these grains were milled into flour (0.4 mm screen) and stored at 4 C0.

Moringa leaves obtained from the farm of Alsamrab , Khartoum North, Sudan. The leaves were dried under optimum condition, then milled into powder (0.4 mm screen) and stored at 4 C.

Supplementation

Moringa leaves were added using Pearson square to increase nutritive value of sorghum flour by 4, 8 and 12%. The number of samples of composite flour after supplementation were three samples.

Fermentation:

Natural fermentation of sorghum flour was carried out by mixing flour with distilled water (1:2 w/v). A natural lactic fermentation of ground grain sorghum produced significant increases ($P < 0.001$) in available lysine/leucine, isoleucine , and methionine. The protein quality, expressed as relative nutritive value, increased significantly ($P < 0.001$) as a result of fermentation. After the incubation period the sample was mixed using a glass rod and transferred to aluminum dishes

(30 cm diameter), and dried in a hot air oven at 70℃ for 3-4 hours. Dried sample was ground using house blender and mortar to pass through 0.4 mm screen and stored at 4℃ for further analysis.

Protein and ash content:

Crude protein (N x 6.25), ash content of the raw material (sorghum and composite flour) and fermented dough were determined according to AOAC (1995).

Determination of minerals contents

Minerals were determined in samples' extracts prepared by the dry-ashing method as described by Pearson (1981). Ferrous was determined according to the analytical method of atomic absorption spectroscopy (Perkin-Elmer 1100 V, Waltham, MA, USA). Phosphorus was determined by the ammonium molybdate ⁄ammonium vanadate method and Calcium by titration (Chapman & Pratt, 1982

Statistical analysis

Data was assessed by the analysis of Variance (ANOVA) Gomez, k and Gomez, A. (1984). Duncan Multiple Range Test (DMRT) was used to separate means. Significance was accepted with $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 . Protein, ash % and minerals contents (mg/100g) of sorghum flour and moringa leaves (ML)

Samples	Ash %	Protein %	Ca	K	Mg	Fe
Sorghum flour	1.83(±0.05)[a]	11.31(±0.03)[a]	1.85 (±0.02)[c]	182.75 (±0.01)[b]	51.52 (±0.02)[c]	1.42 (±0.01)[a]
Moringa leaves	11.97(±0.01)[b]	26.96 (±0.03)[c]	36.49(±0.05)[b]	436.63 (±0.03)[c]	235.53 (±0.00)[a]	9.18 (±0.03)[b]

*

Values are means (±SD), values not sharing a common superscript in a column are significantly ($p < 0.05$) different.

Ash, protein (%) and minerals (mg/100g) contents of sorghum flour (SF) and moringa leaves (ML) are presented in Table 1. Ash content of SF (1.83%) was significantly ($P<0.05$) lower than that of ML (11.97%) .The ash content of SF in this study was lower than that reported by Awadalkareem *et al* ., (2008) for sorghum cultivar (2.29%) . Ash content of ML (11.97%) was higher than that reported by Bolji *et al*., (2013) for moringa leaves (6.00 %).

The crude protein content (CP) of SF (11.31%) was significantly ($P<0.05$) lower compared to that of ML (26.96%). The CP of SF in this study was lower than that reported by Awadalkareem *et al* ., (2008) for sorghum cultivar (14.00%). The CP of ML obtained in this study was lower than that reported by Bolaji *et al* ., 2013 for moringa leaves (39.13 %). Minerals contents of SF (Ca, K, Mg and Fe mg/100g) were found to be 1.85 , 181.70, 51.52 and 1.42 mg/100g, respectively, which were lower than those reported Awadalkareem *et al* ., (2008) who reported that the values of Ca, K , and Fe contents of sorghum flour were 2.43, 225.23 and

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15.54mg /100g, respectively. Minerals contents of ML (Ca, K, Mg and Fe) were found to be 36.49 , 436.63 , 235.53 and 9.18mg/100g ,respectively .These values were lower than those obtained by Sodamade *et al* .,(2013) who gave 723.00 , 677.00 and 187.00 mg/100g for Ca, Mg and Fe , respectively for moringa leaves. The variation in minerals contents of ML and sorghum flour may be attributing to the genotypes and environmental conditions. Sorghum cereal and probiotic combination improves gut health in kidney disease patients.

Table 2. Effect of fermentation on protein and ash contents (%) of sorghum flour supplemented with different ratios of Moringa leaves (ML).

Supplementation Levels (%)	Fermentation time (12 hr)	Ash content (%)	Crude protein (%)
0	Raw	1.83^{c} (±0.05)	11.31^{h} (±0.01)
	Fermented	1.43^{c} (±0.01)	12.38^{f}(±0.4)
4	Raw	1.86^{c} (±0.03)	11.89^{g} (±0.08)
	Fermented	1.76^{c} (±0.04)	13.31^{d}(±0.07)
8	Raw	3.33^{b} (±0.69)	12.87^{e} (±0.09)
	Fermented	2.92^{b} (±0.16)	14.82^{b}(±0.06)
12	Raw	4.53^{a} (±0.07)	14.31^{c} (±0.07)
	Fermented	4.47^{a} (±0.13)	15.35^{a} (±0.07)

Values are means (±SD), values not sharing a common superscript in a column are significantly ($p < 0.05$) different.

Supplementation of SF with 4 % ML insignificantly ($P \geq 0.05$) increased the ash content from 1.83 % to 1.86% , while a significant($P < 0.05$) increase was observed in ash content of those supplemented with 8 and 12% ML (3.33 and 4.53%), respectively. This result was agreed with that reported by Nour *et al*. (2017) who reported that supplementation of millet flour with defatted moringa seeds flour (5, 10 and 15% DMF) were increased ash content of millet flour . Fermentation insignificantly ($P \geq 0.05$) decreased the ash content of SF and 4, 8 and 12% composite flour from 1.83 , 1.86 3.33 and 4.53 mg/100g to 1.43 , 1.76 , 2.92 and 4.47 mg/100g , respectively. Protein content of raw sorghum flour was found to be 11.31 %, supplementation of SF with 4, 8 and 12% ML significantly ($P < 0.05$) increased the protein content to 11.87 ,12.89 and 14.31%, respectively and this could be due to high CP content of ML. This finding agreed with that obtained by Nour *et al*., (2017) who reported that supplementation of millet flour with defatted moringa seeds flour increased the protein content of millet flour. Fermentation of raw sorghum flour (11.31mg/100g) and supplemented sorghum flour with 4, 8 and 12% ML (11.89, 12.37 and12.31mg/100g) significantly ($P < 0.05$) increased the protein content to 12.38, 13.32, 14.83 and 15.37, respectively. A similar trend was reported by Elhaj, (2009) who reported that fermentation for 36h significant

($P < 0.05$) increased the protein content of both sorghum flour and sorghum flour supplemented with 20% defatted ground nut flour. This increase in protein content during fermentation might be due to the synthesis of protein by microorganisms (El Hidai, 1978). Sorghum components, especially its protein is less digestible than other cereals for human and monogastric animals, because of its anti-nutritional factors such as tannins and Phytic acid.

Table 3. Effect of fermentation on minerals contents (mg/100g) of sorghum flour supplemented with different ratios with Moringa Leaves Powder (ML)

Supplementation level (%)	Fermented dough (12 hr	Ca	K	Mg	Fe
0	Raw	1.85^{d} (±0.02)	182.75^{a} (±0.01)	51.52^{a} (±0.02)	1.42^{a} (±0.01)
	Fermented	2.32^{d}(±04)	184.2^{a}(±1.84)	86.00^{b}(±.41)	2.06^{b}(±.01)
4	Raw	2.84^{c}(±01)	199.5^{b} (±3.27)	61.50^{c} (±2.12)	1.93^{b}(±.07)
	Fermented	3.42^{c}(±0.07)	211.8^{c} (±2.46)	120.00^{d} (±.48)	2.76^{f}(±.08)
8	Raw	3.86^{e}(±0.03)	211.89^{c}(±23. 33)	126.00^{e}(±.01)	2.63^{f}(±.05)
	Fermented	5.52^{b}(±0.43)	215.00^{d} (±2.12)	188.83^{f} (±0.75)	3.55^{d} (±.03
12	Raw	5.63^{b}(±0.44)	237.83^{e} (±2.46	263.50^{g} (±0.36)	2.94^{c}(±.05)
	Fermented	7.08^{a}(±0.35)	243.00^{f} (±8.73)	292.51^{h}(±46.01)	5.06^{e}(±.05)

Values are means (±SD), values not sharing a common superscript in a column are significantly ($p < 0.05$) different.

As shown Table 3. The minerals content (Ca , K , Mg and Fe) of SF were found to 1.85, 182.75 , 51.52 and 1.42 mg/100g , respectively which were lower than those reported by Awadalkareem *et al* ., (2008) who reported that the values of Ca, K , and Fe contents of sorghum flour were 2.43, 225.23 and 15.54mg /100g, respectively. Supplementation of SF with 4, 8 and 12% ML significantly ($P < 0.05$) increased Ca content from 1.85 to 2.84 , 3.86 and 5.63 mg/100g , k content from 182.75 to 199.5 211.89 and 237.83 mg/100g , Mg content from 51.52 to 61.50 , 126.00 and 263.50 and Fe content from 1.42 to 1.93 2.63 and 2.94 mg/100g , respectively . A serving of sorghum contains 48% of the recommended daily intake of fiber! Fiber is the ultimate body regulator, helping food stay its course through your digestive system. Adequate fiber intake also prevents constipation, diarrhea, bloating, cramping and other digestive issues. The improvement of minerals (Ca , K , Mg and Fe) after supplementation due to the high content of these minerals contents in moringa leaves. Fermentation of SF and supplemented flour with 4% ML insignificantly ($P < 0.05$) increased Ca content to 2.32 and 3.42mg/100g, while the fermentation of composite flours supplemented with 8 and 12 % ML significantly($P < 0.05$) increased the Ca content from 3.86 to 5.52 mg/100g for supplemented sorghum flour with 8% ML and from 5.63 to 7.08mg/100g for composite flour with 12% ML . K content of SF (182.75) insignificantly ($P < 0.05$) increased after fermentation to 184.2 mg/100g while a significant ($P < 0.05$) increase was observed after fermentation of

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SF supplemented with 4, 8 and 12% ML from 199.5 , 211.89 and 237.83 to 211.8, 215.00.00 and 243.00mg/100, respectively. Mg and Fe significantly ($P < 0.05$) were increased after fermentation for both raw sorghum and composite flour with ML.The major nutritional problems for sorghum growing on acid soils are toxicities of aluminium, iron, and manganese, and deficiencies of phosphorus, calcium, magnesium, molybdenum, and zinc A similar study was obtained by Nour *et al*., (2014), who reported that the supplementation of millet flour with Moringa Seeds cake and fermentation improved the total and extractable Fe. The improvement of minerals contents of fermented both SF and composite flour may be to the reduction of phytic acid content.

CONCLUSION

Supplementation of raw sorghum flour with moringa leaves improved nutritive value of sorghum flour. Fermentation for both raw and supplemented samples increased protein and minerals but decreased ash content .Further, sorghum contains anti-nutritional factors like tannin, cyanogenicglucoside, phytic acid, trypsin inhibitor, and oxalate due to these and other reasons, sorghum is categorized as of low nutritional value and a food for the poor,

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