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RESEARCH ARTICLE

EFFECT OF PROCESSING METHODS ON CHEMICAL AND AMINO ACID COMPOSITION OF THE FLOURS OF TWO WINTER SORGHUM CULTIVARS

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Abstract: Grain sorghum is the leading cereal crop in the Sudan, grown in the summer season, and acts as the principal source of energy, protein, vitamins and minerals for the low-income population living in Sudan. To secure the sorghum grain availability throughout the year, farmers in a rural area of West and South Darfur developed two winter sorghum cultivars known as Abu Ragaba and Abu Kunjara. To date, studies on the nutritional quality of these winter sorghum cultivars are rare. Thus, in this research we examined the effect of fermentation and/or cooking on the chemical composition, amino acid content, and the scores of essential amino acids of the flour of two Sudanese winter season cultivars and one summer season cultivar locally known as Wad Ahmed. The results obtained showed that the cultivars differed significantly ($p \leq 0.05$) in nutrients contents. Abu Ragaba and Abu Kunjara had higher ash content (3.74 and 5.15 %, respectively) than Wad Ahmed (1.71%). Abu Kunjara had the highest protein content (19.37%) followed by Wad Ahmed (14.40%). Chemical composition of the cultivars gave inconsistent results after fermentation and cooking. Fermentation increased protein content while reducing the level of some amino acids due to the action of fermenting microorganisms. Cooking of raw and fermented flour had a minor effect on chemical composition. The starch content decreased after fermentation and increased after cooking of raw and fermented samples. Cooking of unfermented and fermented dough increased ($p \leq 0.05$) the amino acids content. Although cooking of both raw flour and fermented dough increased lysine score to 14.30, 26.60, and 34.20% of Wad Ahmed, Abu Ragaba, and Abu Kunjara, respectively, it remains the most limiting amino acid followed by sulphur amino acids. Overall, the results demonstrated that fermentation and cooking of winter sorghum grains could improve the nutritive quality of these grains.

Keywords: Amino acids, cooking, fermentation, winter sorghum

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RESEARCH ARTICLE

Introduction

In the last decades, there was increased demand for cereal grains mainly as the result of population growth worldwide. In the developing countries, most of the people rely primarily on cereal grains as the main food owing to their inadequate income and high costs of foods of animal origin (Sokrab *et al.*, 2014). Compared to maize and wheat, sorghum (*Sorghum bicolor* L. Moench) tolerates abiotic factors such as soil infertility and extreme temperature (El-Hag *et al.*, 2013). However, cultivation conditions could critically affect the amino acid composition, protein contents and the nutritional value of sorghum grains (Eppendorfer *et al.*, 1985). In Africa and India, sorghum-based foods represent the dominant source of proteins and calories for large numbers of poor people (Belton and Taylor, 2004). Besides being the main food in the developing countries, sorghum is also used as animal fodder and an industrial raw material for fuel and syrup production. In the Sudan, sorghum ranks number one in bulk ~~of~~ cereal with an annual production of 4.4 million tons (FAO, 2014), and the demand for sorghum gain is still rising because of the population explosion combined with the decline of individual's income. Most of the local sorghum cultivars (e.g. Wad Ahmed, Gadamalhamam, Dabar, Tabat) are summer season cultivars cultivated in both irrigated and rainfed agriculture during June–October (El-Hag *et al.*, 2013; Mohamed Nour *et al.*, 2010). The farmers in Kordofan and Darfur developed two new winter sorghum cultivars, locally recognized as Abu Ragaba and Abu Kunjara. These cultivars are grown by transplanting 30- to 35-day old seedlings to the field in early October and harvesting in late January to early February (Mohamed Nour *et al.*, 2010). These winter sorghum cultivars are cultivated in the moist soils of valleys of West and South Darfur states. In these valleys, cultivated plants use water preserved in moist soils until grain maturity and harvesting (Mohamed Nour *et al.*, 2010). Due to their high food security impacts, the nutritional quality of these winter sorghum cultivars was previously evaluated (Mohamed Nour *et al.*, 2010). The results indicated that the nutritional values of winter sorghum cultivars are comparable to that of the summer season sorghum. Like other summer sorghum grains, the availability of the nutrients in winter sorghum grains is reduced by antinutritional factors such as tannin and phytate polyphenols (El-Hag *et al.*, 2013; Mohamed Nour *et al.*, 2010). Abu Kunjara cultivar is high in tannin and phytate while Abu Ragaba contains moderate amounts of these antinutrients (El-Hag *et al.*, 2013). These antinutritional compounds are well-known to hinder the protein digestibility and mineral bioavailability of sorghum grain meals (Elkhalifa *et al.*, 2004; Taylor and Taylor, 2002). Therefore, reduction or exclusion of these undesirable components is crucial to improve the nutritional quality of sorghum-based foods. In this regard, different processing methods such as sprouting, fermentation and cooking were applied to improve the nutritional quality of winter sorghum grains (El-Hag *et al.*, 2013; Mohamed Nour *et al.*, 2010). Sprouting reduced the antinutritional factors and consequently enhanced the protein digestibility and mineral extractability of winter sorghum grains (Mohamed Nour *et al.*, 2010). In addition, fermentation and cooking of winter sorghum flours decreased the antinutritional factors with a concomitant increase in the HCl-extractability of minerals and *in vitro* protein digestibility (El-Hag *et al.*, 2013). Despite the vast information on the impact of fermentation and/or cooking on the chemical and amino acid composition of summer sorghum cultivars, research on the influence of such processing methods on the nutrient composition of Sudanese winter sorghum cultivars is not reported yet. Thus, the primary aim of this work was to examine the influence of fermentation and/or cooking on the chemical and amino acid composition of the flours of winter sorghum cultivars.

Materials and Methods

Materials

The grains of three sorghum cultivars namely Wad Ahmed (control; summer season), Abu Ragaba (winter sorghum) and Abu Kunjara (winter sorghums) were brought from Nyala Agricultural Research Station, Darfur, Sudan. One kilogram grain from each cultivar was cleaned from broken seeds and foreign matters and then milled into white flour (72% extraction rate) using Quadrumat Junior Mill (Brabender, GmbH & Co. KG, Duisburg, Germany).

The flour was sifted using 0.4 mm sieve and then divided into four parts (250 g each). One portion representing raw sample was stored at 4 ºC in clean polyethylene bags pending for analysis. The three other parts were fermented and/or cooked.

Fermentation

Samples were fermented according to the traditional method (lactic acid fermentation) practiced by the Sudanese housewives (El Tinay *et al.*, 1979). Briefly, approximately 500 g of the flour was mixed with 1 L sterile deionized water (1: 2, w/v) and then the starter culture obtained from previously fermented dough were added and mixed well with a glass rod. The fermentation was carried out for 14 h at room temperature (28-32 ^{o}C). After fermentation, the samples were dried in a hot air oven (Heraeus UT 5042, Germany) at 60 °C for 16 h. Dried samples were then ground in a morter and pestle to pass through a 0.4 mm sieve. The fermented flour was separated into two equal parts; one part was kept at 4 ºC for later analysis, and the other part was baked.

Cooking

Cooking of the flour samples was carried out as described by Arbab and El Tinay (1997). Briefly, about 250 g flour of raw and fermented samples were mixed with distilled water (1:10, w/v) and placed in a boiling water bath for about 20 min with continuous stirring to avoid lumps. The cooked samples were rapidly spread out on a thin sheet and then dehydrated. Thereafter, the dried sheets were milled into a fine powder and then saved in polyethylene bags at 4 ºC for further analysis.

Determination of the chemical composition

The ash, fat, total carbohydrates and total nitrogen (micro-Kjeldahl) of sorghum grain samples were determined following the official methods (AOAC, 2003). Moisture content was determined by drying the samples in the air oven drier (Heraeus UT 5042; Niedersachsen, Germany) at 105 ºC for overnight. Crude protein was calculated by multiplying total nitrogen with the conversion factor 6.25. Crude fibre content was estimated using the acid/alkali digestion method (Southgate, 1976). Carbohydrate contents were calculated by difference. The total energy was calculated on Atwater factors (Sukker, 1985), protein (4 kcal g^{-1}), oil (9 kcal g^{-1}) and carbohydrates (4 kcal g^{-1}).

Determination of starch content

A modified method of Faithful (1990) was applied to the determination of starch in the samples. A quantity of 100 mg defatted flour, in a beaker, were extracted with 10 mL ethanol (10% v/v) by continuous stirring using Toyo magnetic stirrer model AS-2 (Osaka, Japan) for 30 min to remove soluble carbohydrates. The mixture was centrifuged at 3000×g for 5 min, and the supernatant was decanted. The residue was washed thoroughly with 1 M H_2SO_4 solution and then centrifuged. Then 15 mL of 1 M H_2SO_4 was added to the clean residue, covered and heated in a boiling water bath for 45 min. Thereafter, the contents were quantitatively transferred to 100 mL volumetric flask and the volume completed to the mark. After settlement, 10 mL aliquot was taken and brought up to 100 mL in a volumetric flask. The glucose was quantified using the Dubois *et al.* (1956) method. About 10 mL of the sample was mixed with 4 mL of anthrone reagent (200 mg anthrone in 100 mL of ice-cold 95% H_2SO_4) and then boiled until the reaction was completed. The solution was then allowed to cool, and the absorbance of the green colour was measured at 630 nm using a spectrophotometer (Pye Unicam SP6-550 UV, London, UK). A blank was prepared following the above procedures without sample. Pure glucose was used to make a standard curve. The starch content was calculated by multiblying the glucose content by the factor 0.9.

Determination of amino acids composition

In order to hydrolyse the proteins the method of Moore and Stein (1963) was used. Briefly, 200 mg of sample was placed in the hydrolysis tube, and then 5 mL 6 N HCl was added and the mixture was incubated at 110 ºC for 24 h. Thereafter, the solution was filtered through Whatman No. 2. filtre paper and then 200 mL of the filtrate was evaporated to dryness for 1 h at 140 ºC. Dried hydrolysate was dissolved in 1 mL of 0.12 M sodium citrate buffer, pH 2.2. Amino acids composition was determined using amino acids analyzer (Sykam-S7130, Tokyo, Japan) based on high-performance liquid chromatography system. For the analysis, 150 µL aliquot of the sample hydrolysate was injected into a

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cation separation column at 130 ºC. The elution buffers (buffer A, pH 3.45 and buffer B, pH 10.85), and ninhydrin solution was concurrently delivered into a coil reactor at a flow rate of 0.7 mL min^{-1}. To accelerate the chemical reaction of amino acids with ninhydrin, the mixture of buffer and ninhydrin was heated at 130 ºC for 2 min. The reaction products were detected at 570 and 440 nm on a dual channel photometer. The amino acid values are expressed as g 100 g^{-1} protein.

Amino acid score

Essential amino acid (EAA) score was determined by applying the formula:

$$\text{EAA score (\%)} = \frac{\text{EAA (g) in 100 g of test protein}}{\text{EAA (g) in 100 g of FAO/WHO/UN reference pattern}} \times 100$$

Statistical analysis

The data of three independent experiments of each treatment were separately analyzed and the values were then averaged. Data were subjected to analysis of variance (Snedecor and Cochran, 1987), and Duncan's multiple range test was used to separate means. Significance was accepted at $p \leq 0.05$.

Results and discussion

Effect of processing methods on chemical composition and starch content of sorghum cultivars

Table 1 shows the results of the chemical composition and starch content of raw and processed sorghum cultivars [Wad Ahmed (summer cultivar), Abu Ragaba and Abu Kunjara (winter cultivars)]. The percentage of dry matter of raw sorghum cultivars Wad Ahmed, Abu Ragaba and Abu Kunjara was found to be 91.53(±0.15), 91.40(±0.17) and 92.47(±0.11), respectively. These values are comparable to the range reported by Ahmed (1993), but higher than the range stated by Arbab and El Tinay (1997). The dry matter content of fermented flour of Wad Ahmed, Abu Ragaba and Abu Kunjara was 90.55(±0.18), 90.90(±0.10) and 91.88(±0.13)%, respectively. Fermentation significantly ($p \leq 0.05$) reduced the dry matter content of the three cultivars. The results obtained are in agreement with those reported by Mohammed *et al.* (2011) who found that fermentation of sorghum flour significantly ($p \leq 0.05$) decreased the dry matter. The decrease in dry matter content of fermented sorghum flours in the current study could be because the respiratory and physiological activities of fermenting organisms consumed part of the meal nutrients, and thus causing a reduction in dry matter yields (Chavan and Kadam, 1989). There were slight changes in dry matter content of raw/cooked and fermented/cooked sorghum flour of the three cultivars.

The ash content was 1.71(±0.22), 3.74(±0.16) and 5.15(±0.22)% for the cultivars Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively. These values are comparable to the range reported by Hassan and El Tinay (1995). The ash content of the fermented sorghum flour was 1.61(±0.14), 3.56(±0.14) and 4.69(±0.14)% for the cultivars, respectively. The results showed that ash content of all cultivars decreased slightly after fermentation due to the leaching into soaking or cooking water (Kazanas and Fields, 1981). The results obtained are in agreement with Mohammed *et al.* (2011) who found a reduction in ash content because of the action of fermenting microorganisms. In addition, the cultivars showed slight changes in ash content after cooking of raw flour and fermented dough.

As shown in Table 1, the highest fat value (4.16±0.06 %) was observed for Abu Kunjara followed by Wad Ahmed (4.07±0.04 %) and Abu Ragaba (3.74±0.16 %). The fat content of Abu Ragaba showed a significant difference ($p \leq 0.05$) when compared to Wad Ahmed and Abu Kunjara cultivars, and there was no significant difference between the latter two cultivars. The fat content of the three cultivars was significantly ($p \leq 0.05$) decreased after fermentation to 2.47 (±0.16), 2.62 (±0.02) and 3.08 (±0.08)% for Wad Ahmed, Abu Ragaba, and Abu Kunjara, respectively.

The result obtained agreed with those of Mohammed *et al.* (2011) who found that fermentation of sorghum flour significantly ($p \leq 0.05$) decreased fat content. The cultivars showed a significant decline in fat content after cooking of both raw and fermented dough.

This could be attributed to the denaturing and hydrolysing effect of high cooking temperature on

fats and fatty acids, which might result in partial leaching of these constituents into cooking water.

The crude fibre values obtained for the Wad Ahmed, Abu Ragaba and Abu Kunjara cultivars were 3.39 (±0.11), 2.07 (±0.11) and 2.23 (±0.13) %, respectively. Crude fibre content of the three cultivars under investigation was higher than that reported by Zaparrat and Salgado (1994). There was a significant difference between the three cultivars; Wad Ahmed had higher crude fibre followed by Abu Kunjara and then Abu Ragaba. The fibre content of fermented sorghum flour was 3.75 (±0.11), 2.37 (±0.06) and 2.83 (±0.11) % for Wad Ahmed, Abu Ragaba, and Abu Kunjara, respectively. Elkhalifa *et al.* (2004) reported that the crude fibre content increased during sorghum fermentation.

The results also agreed with that obtained by Mohammed *et al.* (2011) who found an increase in fibre content because of fermentation. There was a significant decrease ($p \leq 0.05$) in crude fibre content when raw and fermented sorghum flour of the three cultivars was cooked.

The crude protein content of the three cultivars showed values of 14.40 (±0.17), 14.32 (±0.17) and 19.37 (±0.14) % for Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively. The result obtained was within the range reported by Subramanian *et al.* (1990). There was a high difference ($p \leq 0.05$) in protein content between Abu Kunjara and the other two cultivars. Such variation may be due to genotype and seed size (Belton and Taylor, 2004). The percentage of the protein content of the fermented sorghum flour was 14.61(±0.14), 14.57(±0.16) and 19.61(±0.10) for the cultivars, Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively. Upon fermentation, the crude protein content of sorghum flour was slightly increased.

The increment in protein content could be attributed to the action of extracellular enzymes formed by the fermenting microorganisms (Olagunju and Ifesan, 2013).

These enzymes hydrolyze and solublize the flour macromolecules such as starch, proteins, cell wall polysaccharides, tannins, and phytate and thus leading to the reduction in dry matter and an increase in proteins (Poutanen *et al.*, 2009). In addition, the multiplied cells of the fermenting microorganisms may also contribute to the increasment of protein content. In most cereal- based diet, protein is more limiting than carbohydrates.

Thus, any process that appears to increase its content, even at the expense of carbohydrates, may be nutritionally advantageous (Asiedu *et al.*, 1993). After fermentation, Abu Kunjara showed a significant difference ($p \leq 0.05$) in protein content compared to Wad Ahmed and Abu Ragaba. There was a slight decrease in crude protein content of raw/cooked and fermented/cooked sorghum flour of the three cultivars as shown in Table 1. This decrease may be attributed to partial removal of individual amino acids, along with other nitrogenous compounds on heating as reported by Clawson and Taylor (1993).

As shown in Table 1 carbohydrate content of Wad Ahmed, Abu Ragaba and Abu Kunjara was 76.19(±0.36), 75.76(±0.32) and 68.96(±0.26) %, respectively. There was an highly significant difference ($p \leq 0.05$) in carbohydrates content between Abu Kunjara and Wad Ahmed, while there was no significant difference between Wad Ahmed and Abu Ragaba cultivars. Carbohydrate content of fermented flour of Wad Ahmed, Abu Ragaba and Abu Kunjara was 77.18 (±0.22), 76.88 (±0.30) and 69.94 (±0.21) percentage, respectively. The results showed that total carbohydrate content was significantly ($p \leq 0.05$) increased after fermentation.

The increase in carbohydrate content of fermented dough could be due to the reduction of other constituents, since the percentage of carbohydrate was estimated by subtracting other constituents (moisture, ash, protein, and fat) from 100 %. Mohammed *et al.* (2011) reported a similar trend of carbohydrate reduction during fermentation. A significant ($p \leq 0.05$) increase in carbohydrates content (Table 1) of raw/cooked and fermented/cooked sorghum flour of the cultivars was observed.

The starch content of sorghum cultivars Wad Ahmed, Abu Ragaba and Abu Kunjara was 68.61(±0.44), 68.13(±0.33) and 59.65(±0.38) %, respectively (Table 1). The values were less than the range reported by Dendy (1995) and higher than the range reported by Torres *et al.* (1996).

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RESEARCH ARTICLE

Table 1. *Chemical composition (%) of raw and processed flours of sorghum cultivars*

Sorghum cultivar		**Chemical composition**						
	Treatment	Dry matter	Ash	Fat	Fibre	Protein	Carbohydrate	Starch
Wad Ahmed	Raw	91.53^{c}(±0.15)	1.71^{d}(±0.22)	4.07^{a}(±0.04)	3.39^{b}(±0.11)	14.40^{de}(±0.17)	76.19^{d}(±0.36)	68.61^{c}(±0.44)
	Fermented	90.55^{f}(±0.18)	1.61^{d}(±0.23)	2.47^{d}(±0.16)	3.75^{a}(±0.11)	14.61^{d}(±0.14)	77.18^{c}(±0.22)	68.16^{cd}(±0.17)
	Cooked	91.80^{b}(±0.10)	2.36^{cd}(±0.13)	3.83^{ab}(±0.12)	2.56^{d}(±0.17)	13.77^{g}(±0.49)	77.55^{c}(±0.40)	69.47^{ab}(±0.27)
	Fermented/cooked	91.22^{d}(±0.16)	2.19^{d}(±0.11)	1.42^{f}(±0.01)	2.36^{e}(±0.17)	13.88^{fg}(±0.16)	79.63^{a}(±0.11)	68.82^{bc}(±0.17)
Abu Ragaba (Winter white)	Raw	91.40^{cd}(±0.17)	3.74^{b}(±0.16)	3.74^{b}(±0.16)	2.07^{fg}(±0.11)	14.32^{def}(±0.17)	75.76^{d}(±0.32)	68.13^{cd}(±0.33)
	Fermented	90.90^{e}(±0.10)	3.56^{b}(±0.17)	2.62^{de}(±0.02)	2.37e(±0.06)	14.57^{d}(±0.16)	76.88^{c}(±0.30)	67.72^{d}(±0.03)
	Cooked	91.87^{b}(±0.15)	2.23^{d}(±0.06)	3.40^{c}(±0.16)	1.91^{gh}(±0.06)	13.75^{g}(±0.26)	77.21^{c}(±0.76)	69.77^{a}(±1.04)
	Fermented /cooked	91.87^{b}(±0.06)	2.55^{cd}(±0.13)	1.89^{e}(±0.06)	2.25^{ef}(±0.08)	14.00^{efg}(±0.04)	78.51^{b}(±0.31)	68.83^{bc}(±0.27)
Abu Kunjara (Winter red)	Raw	92.47^{a}(±0.11)	5.15^{a}(±0.22)	4.16^{a}(±0.06)	2.23^{ef}(±0.13)	19.37^{ab}(±0.14)	68.96^{h}(±0.26)	59.65^{fg}(±0.38)
	Fermented	91.88^{b}(±0.13)	4.69^{a}(±0.11)	3.08^{cd}(±0.08)	2.83^{c}(±0.11)	19.61^{a}(±0.10)	69.94^{g}(±0.21)	59.02^{g}(±0.14)
	Cooked	92.47^{a}(±0.04)	3.63^{b}(±1.67)	3.62^{bc}(±0.16)	2.02^{g}(±0.07)	19.06^{bc}(±0.46)	71.54^{f}(±0.73)	60.16^{ef}(±0.44)
	Fermented /cooked	91.93^{b}(±0.12)	3.18^{bc}(±0.12)	3.03^{cd}(±0.03)	1.81^{h}(±0.06)	18.88^{c}(±0.26)	73.01^{e}(±0.25)	60.53^{e}(±0.49)
$Lsd_{0.05}$		0.2197**	0.851**	0.1533**	0.1846**	0.4196**	0.6741**	0.7209**
SE		0.07528	0.2915	0.0598	0.6325	0.1438	0.2309	0.247

Mean values (±S.D) bearing different superscript letters within columns are significantly different at $P \leq 0.05$.

The starch content of fermented flour of Wad Ahmed, Abu Ragaba, and Abu Kunjara was 68.16(±0.17), 67.72(±0.03) and 59.02(±0.14)%, respectively. There was an appreciable decrease in starch content when sorghum flour was fermented. Elkhalifa *et al.* (2004) reported a similar trend of starch content reduction during the fermentation of kisra, a naturally lactic acid bacteria- and yeast-fermented sorghum thin pancake-like flatbread produced in Sudan. This decline is due to degradation of grain components, mainly starch and soluble sugars, by both intrinsic grain enzymes and enzymes of fermenting microbes. There was a significant ($p \leq 0.05$) increase in starch content of raw/cooked and fermented/cooked sorghum flour of the cultivars. This indicates that cooking caused an increase in starch content of raw and fermented sorghum flour, which may be due to the loss of soluble solids during cooking that would increase the concentration of starch.

Effect of processing methods on amino acid composition and score of sorghum cultivars

Amino acids content of raw and processed grains of sorghum cultivars is shown in Table 2. Sorghum cultivars were found to be rich in glutamic acid, proline, leucine, alanine, valine and aspartic acid and poor in cysteine, lysine, methionine, tyrosine, and threonine contents. Lysine content of the three cultivars was 0.13, 0.06 and 1.32 g 100 g^{-1} protein for Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively, while leucine content was 3.08, 3.41 and 8.12 g 100 g^{-1} protein, respectively. Leucine was the most abundant essential amino acid, while lysine was the most limiting essential amino acid for all cultivars.

These results are in agreement with ones reported by Brudevold and Southern (1994) who investigated the variation in amino acids among sorghum varieties and reported that amino acids content varied considerably. The results also showed a higher amount of glutamic acid compared to other amino acids. The reason could be that the reading for glutamic acid results from both glutamic acid and glutamine. The glutamic acid plus glutamine was the most abundant amino acid in all varieties followed by leucine. Appreciable amounts of aspartic acid, proline, phenylalanine and valine were also detected while tyrosine, cysteine and lysine were very little. The results agreed with Murty and Renard (2001) who reported that sorghum protein is lower in the essential amino acids such as lysine and threonine.

Serna-Saldivar and Rooney (1995) reported that the lysine content of normal sorghum cultivars ranged from 0.70 to 3.90 g 100 g^{-1} protein, and of brown cultivars ranged from 2.00 to 2.40 g 100 g^{-1} protein.

Low lysine content in sorghum was attributed to the fact that lysine is present in much higher quantities in the glutelin protein fraction than prolamin fraction while most regular sorghum varieties have higher prolamin content. There was a significant difference ($p \leq 0.05$) in lysine content among the three cultivars. Abu Kunjara had the highest content followed by Wad Ahmed then Abu Ragaba. This result agreed with Serna-Saldivar and Rooney (1995) who found that brown sorghum cultivars had the highest lysine content.

As shown in Table 2, fermentation significantly ($p \leq 0.05$) decreased the content of the amino acids of Abu Ragaba and Abu Kunjara cultivars; while for Wad Ahmed cultivar; fermentation decreased all amino acids content except valine, isoleucine, arginine and proline. This could be due to the metabolic activity of fermenting microorganisms through which some amino acids might be utlized and the other might be produced. Lysine content after fermentation for Wad Ahmed, Abu Ragaba and Abu Kunjara was 0.03, 0.03 and 0.02 g 100 g^{-1} protein, respectively. The results obtained disagreed with those of Hamad *et al.* (1992) who found that the amount of lysine is not affected by fermentation. The differences in amino acid composition after fermentation in this study and other reports could be attributed to the fact that this spontaneous fermentation is carried out by a consortum of strains of LAB and yeasts, and under different environmental conditions such as the fermentation temperature and the variety of sorghum used (Hamad *et al.*, 1992).

For unkown reasons, cooking of the raw sorghum flour of all cultivars significantly ($p \leq 0.05$) increased the amino acid content. The lysine content was 0.78, 1.45 and 1.86 g 100 g^{-1} protein for Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively.

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RESEARCH ARTICLE

Table 2. *Amino acids composition (g 100 g^{-1} protein) of raw and processed flours of sorghum cultivars*

Amino acid	Sorghum cultivar												$Lsd_{0.05}$	SE±
	Wad Ahmed				Abu Ragaba (Winter white)				Abu Kunjara (Winter red)					
	Treatments*													
	Raw	Fermented	Raw/cooked	Fermented /cooked	Raw	Fermented	Raw/cooked	Fermented /cooked	Raw	Fermented	Raw/cooked	Fermented /cooked		
Aspartic acid	1.63^{de} (±0.07)	1.20^{ef} (±0.04)	4.06^{bc} (±0.61)	3.46^{c} (±0.11)	2.22^{d} (±0.07)	1.42^{e} (±0.05)	6.43^{a} (±1.05)	1.77^{de} (±0.07)	4.60^{b} (±1.01)	1.04^{f} (±0.02)	5.70^{ab} (±1.54)	2.61^{cd} (±0.28)	0.010*	0.0012
Threonine	0.43^{d} (±0.02)	0.26^{ef} (±0.06)	1.72^{c} (±0.06)	1.60^{c} (±0.05)	0.44^{d} (±0.05)	0.30^{de} (±0.02)	2.71^{b} (±1.06)	0.42^{d} (±0.01)	2.16^{bc} (±1.00)	0.23^{f} (±0.03)	3.13^{a} (±0.85)	1.02^{cd} (±0.09)	0.084*	0.0066
Serine	0.65^{c} (±0.03)	0.24^{e} (±0.02)	1.45^{c} (±0.07)	1.66^{bc} (±0.07)	0.58^{cd} (±0.07)	0.35^{de} (±0.03)	2.87^{a} (±1.04)	0.43^{d} (±0.01)	1.92^{b} (±2.27)	0.23^{ef} (±0.05)	2.88^{a} (±1.00)	0.09^{f} (±0.02)	0.073*	0.0029
Glutamic acid	3.56^{de} (±0.08)	1.53^{f} (±0.04)	15.46^{b} (±0.02)	8.83^{c} (±0.15)	3.59^{d} (±0.12)	1.73^{ef} (±0.06)	18.47^{ab} (±2.25)	2.22^{e} (±0.09)	16.24^{b} (±1.86)	1.22^{fg} (±0.02)	19.61^{a} (±2.48)	8.89^{cd} (±1.07)	0.011**	0.0035
Glycine	0.07^{cd} (±0.03)	0.03^{e} (±0.02)	0.39^{bc} (±0.03)	0.39^{bc} (±0.04)	0.11^{c} (±0.02)	0.06^{cd} (±0.03)	1.23^{ab} (±0.04)	0.05^{d} (±0.00)	1.04^{b} (±0.06)	0.03^{de} (±0.01)	1.61^{a} (±0.09)	0.08^{c} (±0.00)	0.041*	0.0024
Alanine	2.94^{de} (±0.07)	2.42^{ef} (±0.08)	6.99^{b} (±0.03)	4.04^{c} (±0.09)	3.29^{cd} (±0.15)	2.81^{e} (±0.13)	9.21^{a} (±1.16)	3.15^{d} (±1.04)	5.69^{bc} (±1.05)	2.08^{f} (±0.02)	7.63^{ab} (±1.03)	5.68^{bc} (±0.16)	0.067*	0.0067
Cystine	0.00^{d} (±0.00)	0.00^{d} (±0.00)	0.10^{bc} (±0.00)	0.00^{d} (±0.00)	0.00^{d} (±0.00)	0.00^{cd} (±0.00)	0.07^{c} (±0.02)	0.00^{d} (±0.00)	0.25^{ab} (±0.05)	0.00^{d} (±0.00)	0.32^{a} (±0.05)	0.17^{b} (±0.03)	0.018*	0.0051
Valine	1.65^{de} (±0.06)	1.87^{d} (±0.05)	3.79^{bc} (±0.03)	3.84^{bc} (±0.08)	2.64^{c} (±0.05)	2.02^{cd} (±0.15)	6.42^{a} (±1.52)	2.62^{c} (±1.04)	4.35^{b} (±1.01)	1.62^{de} (±0.08)	5.61^{ab} (±1.01)	2.69^{c} (±0.06)	0.021*	0.0029
Methionine	0.28^{d} (±0.02)	0.11^{e} (±0.07)	0.60^{bc} (±0.03)	0.57^{c} (±0.04)	0.15^{de} (±0.01)	0.10^{ef} (±0.02)	0.87^{ab} (±0.05)	0.09^{f} (±0.01)	0.67^{b} (±0.05)	0.06^{fg} (±0.01)	1.13^{a} (±0.01)	0.48^{cd} (±0.01)	0.055*	0.0041
Isolucine	1.08^{g} (±0.05)	1.26^{ef} (±0.07)	2.87^{bc} (±0.05)	2.37^{c} (±0.15)	1.81^{d} (±0.06)	1.38^{e} (±0.05)	4.07^{a} (±0.07)	1.74^{de} (±0.09)	3.13^{b} (±1.02)	1.08^{g} (±0.09)	4.12^{ab} (±1.00)	1.93^{cd} (±0.05)	0.038*	0.0087
Leucine	3.08^{e} (±0.09)	2.21^{ef} (±0.04)	8.47^{b} (±0.09)	4.61^{c} (±0.17)	3.41^{d} (±0.11)	2.63^{e} (±0.16)	11.43^{a} (±2.67)	3.20^{de} (±0.05)	8.17^{bc} (±2.03)	1.93^{g} (±0.07)	11.03^{ab} (±1.09)	6.25^{c} (±0.17)	0.043**	0.0034
Tyrosine	0.20^{de} (±0.01)	0.17^{e} (±0.05)	0.55^{bc} (±0.01)	0.37^{c} (±0.02)	0.27^{cd} (±0.05)	0.21^{d} (±0.02)	0.69^{bc} (±0.03)	0.15^{ef} (±0.01)	0.87^{ab} (±0.01)	0.17^{ef} (±0.02)	1.07^{a} (±0.03)	0.63^{b} (±0.05)	0.017*	0.0069
Phenylalanine	0.95^{de}	0.45^{fg}	3.13^{bc}	2.54^{c}	0.82^{ef}	0.83^{e}	4.86^{ab}	1.07^{d}	3.99^{b}	0.61^{f}	5.48^{a}	2.09^{cd}	0.012*	0.0042

	(±0.05)	(±0.02)	(±0.03)	(±0.05)	(±0.01)	(±0.03)	(±1.07)	(±0.08)	(±0.06)	(±0.04)	(±1.05)	(±0.14)		
Histidine	0.37^{d}	0.17^{f}	1.01^{c}	1.08^{bc}	0.33^{de}	0.22^{ef}	1.99^{ab}	0.29^{e}	1.58^{b}	0.15^{fg}	2.26^{a}	0.69^{cd}	0.066*	0.0033
	(±0.02)	(±0.01)	(±0.03)	(±0.05)	(±0.02)	(±0.04)	(±0.06)	(±0.01)	(±0.02)	(±0.02)	(±0.08)	(±0.01)		
Lysine	0.13^{d}	0.03^{ef}	0.78^{c}	0.81^{bc}	0.06^{de}	0.03^{ef}	1.45^{ab}	0.04^{e}	1.32^{b}	0.02^{ef}	1.86^{a}	0.22^{cd}	0.021*	0.0054
	(±0.01)	(±0.01)	(±0.02)	(±0.02)	(±0.00)	(±0.05)	(±0.05)	(±0.00)	(±0.01)	(±0.02)	(±0.05)	(±0.03)		
Ammonia	2.35^{ef}	2.29^{cd}	4.72^{b}	2.97^{d}	2.36^{e}	2.30^{f}	6.88^{a}	2.47^{de}	3.89^{bc}	1.94^{f}	5.81^{ab}	3.72^{c}	0.065*	0.0020
	(±0.02)	(±0.07)	(±0.05)	(±0.11)	(±0.16)	(±0.17)	(±1.04)	(±0.11)	(±0.03)	(±0.05)	(±1.13)	(±0.11)		
Arginine	0.08^{fg}	0.57^{ef}	1.98^{bc}	1.76^{c}	0.96^{d}	0.63^{e}	3.17^{ab}	0.74^{de}	2.93^{b}	0.51^{f}	4.03^{a}	1.44^{cd}	0.058*	0.0044
	(±0.02)	(±0.02)	(±0.05)	(±0.03)	(±0.05)	(±0.02)	(±1.08)	(±0.05)	(±0.04)	(±0.03)	(±1.12)	(±0.07)		
Proline	4.40^{ef}	5.13^{e}	6.22^{de}	7.89^{c}	8.06^{c}	7.32^{cd}	10.63^{a}	8.45^{bc}	8.92^{b}	4.22^{f}	10.04^{ab}	7.07^{d}	0.039*	0.0065
	(±0.10)	(±0.09)	(±0.03)	(±0.18)	(±1.07)	(±1.07)	(±2.77)	(±1.16)	(±0.08)	(±1.92)	(±1.77)	(±1.02)		

Mean ±S.D value(s) bearing different superscript letters within rows (for each amino acid) are significantly different ($P \leq 0.05$).

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However, cooking of fermented dough significantly ($p \leq 0.05$) decreased the amino acid content of Abu Ragaba and Abu Kunjara and increased the content of Wad Ahmed.

Although the reasons for the increment in amino acid content after cooking of both raw and fermented flour are largely unknown, this phenomina could likely be due to the reduction of moisture content during heating which might result in elevating the concentration of the flour constituents.

The essential amino acids chemical scores (EAACs) of raw and processed sorghum flours are shown in Table 3. The chemical scores were calculated based on a comparison with the reference pattern recommended by FAO/WHO/UN (1973) and Dendy (1995). The results showed that lysine chemical score for Wad Ahmed, Abu Ragaba and Abu Kunjara cultivars was 2.40, 1.03 and 24.20 %, leucine score was 34.70, 48.50 and 115.30 % and that of methionine plus cysteine was 8.10, 4.20 and 26.80 %, respectively. The result indicated that leucine is the most abundant amino acid for the three cultivars while lysine and methionine plus cystine (sulphur amino acids) are the first and second limiting amino acids compared to the FAO/WHO/UN (1973) reference pattern. This result agreed with that of Gassem and Osman (2003) who reported that sorghum proteins were rich in glutamic acid, leucine and alanine; lysine being the first limiting amino acid followed by sulphur containing amino acids.

Fermentation significantly ($p \leq 0.05$) decreased the essential amino acids scores of the three sorghum cultivars;except valine and isoleucine in Wad Ahmed cultivar (Table 3). Lysine chemical score of fermented flour was 0.55, 0.60 and 0.40% for Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively. There was no significant difference ($p \geq 0.05$) among all cultivars. Cooking of raw sorghum flour for all cultivars increased ($p \leq 0.05$) the essential amino acids scores. Cooking of fermented dough significantly ($p \leq 0.05$) decreased the essential amino acids scores of raw flour of Abu Ragaba and Abu Kunjara cultivars and increased the score of Wad Ahmed. Lysine (the most limiting amino acid) chemical score of fermented/cooked flour of Wad Ahmed, Abu Ragaba and Abu Kunjara was 14.90, 0.70 and 4.00 %, while for raw flour was 2.40, 1.03 and 24.20 %, respectively. The result showed that cooking of fermented flour significantly ($p \leq 0.05$) decreased the adverse effect of fermentation on chemical score.

Conclusion

Fermentation of the sorghum grain flour resulted in an increase in protein content and decrease in amino acid content. Cooking of the flour of sorghum grains led to an improvement in amino acid composition. The combination of cooking with fermentation alleviated the effect of fermentation on amino acids composition. The results indicated that fermentation and cooking of winter sorghum is a potential process to improve the nutritive value of winter sorghum grain.

Table 3. *Essential amino acid scores (%) of raw and processed flours sorghum cultivars and FAO/WHO/ UN Reference Protein (g 100 g^{-1} protein)*

Essential amino acid	Sorghum cultivar												$Lsd_{0.05}$	SE±	FAO/WHO/ UN pattern (1973) (g/100g)
	Wad Ahmed				Abu Ragaba (Winter white)				Abu Kunjara (Winter red)						
	Treatments*														
	Raw	Fermented	Cooked	Fermented /cooked	Raw	Fermented	Cooked	Fermented /cooked	Raw	Fermented	Cooked	Fermented /cooked			
Isoleucine	27.10^{f} (±2.03)	31.60^{e} (±3.25)	71.70^{b} (±5.67)	59.30^{c} (±5.14)	45.20^{d} (±2.23)	34.40^{e} (±6.94)	101.70^{a} (±9.08)	43.55^{d} (±6.41)	78.30^{b} (±9.41)	27.10^{f} (±2.09)	103.00^{a} (±9.99)	48.40^{d} (±5.69)	1.398*	0.0764	4.00
Leucine	34.70^{h} (±5.91)	31.40^{i} (±2.06)	120.30^{c} (±9.77)	65.50^{f} (±6.20)	48.50^{g} (±2.87)	37.30^{h} (±6.47)	162.40^{a} (±11.62)	45.50^{g} (±5.01)	115.30^{d} (±16.30)	27.30^{j} (±2·11)	156.70^{b} (±11.84)	88.80^{e} (±7.41)	1.277*	0.0392	7.04
Lysine	2.40^{f} (±0.07)	0.55^{h} (±0.01)	14.30^{d} (±2.36)	14.90^{d} (±2.29)	1.03^{g} (±0.05)	0.60^{h} (±0.01)	26.60^{b} (±2.54)	0.70^{h} (±0.01)	24.20^{c} (±2.74)	0.40^{h} (±0.01)	34.20^{a} (±2.06)	49.00^{e} (±0.08)	1.401*	0.0475	5.44
Threonine	10.80^{f} (±1.11)	6.50^{g} (±0.07)	43.00^{d} (±5.78)	40.00^{d} (±5.07)	11.00^{f} (±1.06)	7.50^{g} (±2.16)	67.80^{b} (±7.94)	10.60^{f} (±2.15)	54.10^{c} (±7.49)	5.70^{h} (±0.07)	78.10^{a} (±5.84)	25.60^{e} (±2.09)	1.309*	0.0302	4.00
Valine	33.20^{i} (±7.03)	37.60^{f} (±5.66)	76.50^{c} (±5.04)	77.20^{c} (±8.71)	53.30^{d} (±9.56)	40.70^{e} (±6.77)	129.40^{a} (±9.11)	52.80^{d} (±3.51)	87.80^{b} (±2.06)	32.60^{i} (±1.16)	113.10^{b} (±10.99)	54.20^{d} (±6.84)	1.571*	0.0799	4.96
Meth. + cystine	8.10^{f} (±1.63)	3.00^{j} (±0.05)	20.00^{c} (±1.63)	16.10^{e} (±4.69)	4.20^{i} (±0.07)	2.90^{k} (±0.08)	26.90^{b} (±5.74)	2.40^{k} (±0.07)	26.80^{b} (±0.08)	1.60^{l} (±0.04)	41.60^{a} (±4.69)	18.60^{d} (±3.45)	1.846*	0.0564	3.50
Phen. + tyrosine	18.80^{f} (±2.09)	10.30^{l} (±2.69)	60.50^{d} (±5.20)	47.90^{e} (±7.51)	18.04^{j} (±0.05)	17.10^{j} (±0.29)	91.30^{b} (±10.52)	20.20^{i} (±1.29)	79.90^{c} (±7.54)	12.60^{k} (±0.07)	107.70^{a} (±9.88)	44.70^{f} (±6.77)	1.764*	0.0213	6.08
Histidine (for children)	26.50^{f} (±5.58)	11.90^{l} (±2.44)	72.40^{d} (±3.39)	76.80^{c} (±7.77)	23.80^{i} (±2.29)	15.50^{k} (±0.18)	1.40^{m} (±0.06)	20.90^{j} (±2.22)	113.10^{b} (±11.07)	10.70^{l} (±2.28)	161.50^{a} (±16.94)	49.50^{e} (±8.46)	1.308*	0.0546	1.40*

Mean values (±S.D) bearing different superscript letters within rows (for each amino acid) are significantly different ($P \leq 0.05$).

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