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Effect of Supplementation and Processing on Amino Acids Composition and Score of Pearl Millet Flour

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Abstract

The present study was carried out to investigate the effect of supplementation of millet flour with fenugreek defatted seeds flour (FDSF) followed by processing on amino acids composition and score. All data were subjected to statistical analysis, each determination was carried out and analyzed in triplicate and figures were then averaged. The results showed that supplementation with 5, 10 and 15% FDSF increased the amino acids except alanine and glycine. The amino acids leucine, lysine, methionine, phenylalanine, aspartic acid, glutamic acid, tyrosine and proline of the flour supplemented with 10% FDSF were increased after 8h fermentation, while others were decreased, but after 16h fermentation all amino acids were decreased, except alanine and glycine. The amino acids lysine, methionine, glutamic acid, tyrosine and proline of 10% FDSF and 8h fermented dough were decreased after cooking, but the others were increased. The majority of the amino acids scores of supplemented flour were increased after cooking of fermented dough. The chemical scores of the essential amino acid of pearl millet were fluctuated after supplementation and processing.

Keywords

Supplementation, Amino Acid, Chemical Score, Cooking, Fermentation, Fenugreek Seed, Millet

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1. Introduction

Millet is an endogenous African cereal that, unlike wheat or rice, is well adapted to African semi–arid and sub-tropical agronomic conditions. It is known by a number of names: bulrush, cattail, or candle and millet (English), bajra or cumbu (India) duhum or dukhon (Arabic). The seeds range in colour from white to brown, blue, or almost purple. They are generally tear or ovoid shaped and smaller than those of sorghum (1). Pearl millet is an indispensable food for millions of peoples inhabiting the semi –arid tropics. It is used primarily for human food and remains a major source of calories and vital component of food security in the semi - arid areas in the developing world (2). The nutritional properties of pearl millet have received traditional cereal-based weaning foods as an acceptable more attention than those of the other common millets, protein supplement (3).Conversely, many proteins from vegetables are deficient in at least one indispensable amino acid. In developing

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countries, adequate protein intake is achieved mainly from vegetable proteins. By combing food stuff with different low concentrations of indispensable amino acids (e.g. maize with legumes), protein intake can be adequate provided enough vegetables are available (4). Proline is classified as an- imino acid, since its α-amine is a secondary amine, rather than a primary amine (5). Fenugreek seeds (*Trigonellafoenum-graecum* L.) is a legume commonly grown in many parts of the world for both culinary purposes and health benefits. The fenugreek seed is very bitter but does have interesting proximate composition. Protein content ranges between 23 and 43% of the seed, carbohydrate represents up to 58% , moisture make up about 10 – 13% of the seed , lipid represent 5 – 6 % and minerals make up less than 1% (6). FDSF contains several bioactive compounds, including proteins, protease inhibitors. A unique amino acid known as 4- hydroxy- isoleucine water–soluble dietary fiber, steroidal saponins, flavonoids, isoflavones, alkaloids, polyunsaturated oil, and phytic acid (7). Cooking reduced sulphur-containing amino acids and tryptophan in faba bean (8).The contents of all amino acids of uji, except aspartic acid, glycine, methionine, cystein, tyrosine and lysine, did not change significantly after fermentation (9). These amino acids increased after fermentation because lactic acid bacteria, the principle bacteria in uji, are fastidious in their amino acids requirements for growth and metabolism (9). Elfaki *et al*.(10) found that fermentation of sesame cake significantly increased sulphur containing amino acids and the essential branched chain amino acids leucine and isoleucine. This increase can probably be ascribed to the population in the fermentation of large quantities of lactic acid (10). This compound on oxidation to pyruvate will ready undergo transamination with other amino acids to form alanine. Cooking after fermentation decreased all amino acids of fermented uji (maize – finger millet blend) except histidine which increased slightly from 2.46 to 2.56g/16N (9). Non–enzymatic browning reactions were responsible for the slight declines in all amino acids contents of uji flour after cooking or extrusion, and these changes are accompanied by darkening of the extrudes (9). According to Osman, (2009) cooking after fermentation increased valine, cystein, lysine, arginine, glutamic acid and glycine of Sicklepod leaves (11). This study aimed to assess the effect of supplementation with DSF followed by processing on amino acids composition and scores of pearl millet flour.

2. Materials and Methods

The grains of pearl millet of green cultivar were obtained from the Department of Agronomy, Faculty of Agriculture University of Khartoum, Shambat, Sudan. The seeds were cleaned, freed from foreign seeds, broken and shrunken ones, then milled into fine flour using house blender and mortar to pass through 0.4 mm screen and then stored in polyethylene bags at 4°C for further analysis. Fenugreek seeds (*Trigonella foenum-graecum* L.) were brought from Omdurman local market, cleaned, freed from extraneous matter, and then milled in to fine flour using house blender and mortar to pass through a 0.4 mm screen and stored in polyethylene bags at 4°C for further analysis. All chemicals used in this study were of reagent grade. FDSF Supplements were added using Pearson square to increase nutritive value of millet flour by 5, 10 and 15%, respectively.

Cooking of the samples was performed by suspending the flour of the sample in distilled water in the ratio of 1:2 (flour : water, wt/v) and the slurry was shaken to avoid lumps while boiling in a water bath for 20 min. the viscous mass was spread out thinly in a dish and oven dried at 70°C. The dried flakes were milled into fine flour using house blender and mortar to pass through 0.4 mm screen and stored at 4°C for further analysis.

Natural fermentation of millet flour and composite flours was carried out by mixing flour with distilled water (1:2 w/v). Two hundred and fifty grams of each sample were mixed with 500 ml distilled water in 750 ml beaker and then incubated (Gallenkamp, England) at 37°C for periods 0, 8 and 16 h. After the incubation periods the samples were mixed using a glass rod and transferred to aluminum dishes (30 cm diameter), and dried in a hot air oven at 70°C for 3-4 hours. Dried samples were ground using house blender and mortar to pass a 0.4 mm screen and stored at 4°C for further analysis.

Slurry of fermented dough was cooked for 5 minutes and spread out thinly in dishes and oven dried at 70°C. The dried flakes were milled into fine flour by house blender and mortar to pass through 0.4 mm screen and stored at 4°C for further analysis.

Amino acid composition of samples was measured on hydrolysates using amino acids analyser (Sykam-S7130/ Germany) based on high performance liquid chromatography technique. Sample hydrolysates were prepared following the method of Moore and Stein (12). Two hundred milligrams of sample were taken in hydrolysis tube. Then 5ml6 N HCl were added to sample into tube, tightly closed and incubated at 110°C for 24 hours. After incubation period, the solution was filtered (Whatman No. 1) and 200ml of the filtrate were evaporated to dryness at 140°C for an hour. Each hydrolysate after dryness was diluted with one milliliter of 0.12 N,pH 2.2 citratebuffer (11.8 g trisodium citrate+ 6 g citric acid + 14ml biobiglycol + 12 ml 32% HCl +2.0 g phenolinoneliter), the same as the amino acid standards (amino acid standardsH, Pierce. Inc., Bockford).An amount of 150 µgL of sample hydrolyzate was injected in the cation separation column at

130℃. Ninhydrin solution(reaction reagent)and an eluent buffer (The buffer system contained solvent A, pH3.45, and solvent B, pH 10.85) were delivered simultaneously into a high temperature reactor coil (16 m length) with a flow rate of0.7mL/min. The buffer/ninhydrin mixture was heated in the reactor at 130℃ for two minutes to accelerate chemical reaction of amino acid with ninhydrin. The products of the reaction mixture were detected at wavelength of 570 nm and 440nm on a dual channel photometer. The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentages.

The essential amino acid (EAA) score was determined by employing the formula:

$$\text{EAA score}=\frac{\text{Grams of EAA in 100g test protein}\times 100}{\text{Grams of EAA in 100g FAo/WHO /UN (1985) refrence pattern}}$$

All data were subjected to statistical analysis, each determination was carried out and analyzed in triplicate and figures were then averaged. Data was assessed by the analysis of Variance (ANOVA) Gomez and Gomez (13). Duncan Multiple Range Test (DMRT) was used to separate means. Significance was accepted with at P≤ 0.05.

3. Results and Discussion

The amino acids contents of pearl millet protein, FDSF and flours supplemented with 5, 10 and 15% FDSF are shown in Table 1. FDSF had a high level of glutamic acid, leucine, arginine, alanine, aspartic acid, proline and isoleucine, with low values of methionine (106.59), tyrosine (358.59), serine (369.51) and glycine (432.51mg/100g). The amino acids histidine, isoleucine, leucine and lysine of FDSF were detected in higher values than recommended levels by FAO/WHO/UN (16), while methionine, phenylalanine and threonine were lower. All the essential amino acids of FDSF except, methionine were detected as higher than those of the recommended levels of FAO/WHO/UN (16). Supplementation with 5, 10 and 15% FDSF increased the levels of histidine, isoleucine, threonine, arginine and serine, while lysine, phenylalanine, aspartic acid, glutamic acid, alanine and proline were detected to be lower than that of the flour supplemented with 10 and 15% than that of flour supplemented with 5% FDSF, while leucine, methionine, and tyrosine of flour supplemented with 10% FDSF were lower and comparable to those of flour supplemented with 5% FDSF. The level of glycine of the flour supplemented with 15% was observed to be lower than that of the flour supplemented with 10% FDSF. Supplementation with 10 and 15% FDSF decreased part of amino acids when compared to those supplemented with 5% FDSF.

Table 1. Amino acids profiles (mg /1000g) of millet flour, defatted fenugreek seeds flour (FDSF) and supplements.

Amino acids	Millet flour	FDSF	Supplementation Levels (%)			Recommended levels (mg/1000g Protein)*
Essential Amino acids			5%	10%	15%	
Histidine	32.66	427.13	40.78	41.19	44.05	140
Isoleucine	242.61	1329.95	309.44	304.48	335.54	400
Leucine	407.01	1604.70	501.46	436.96	503.92	704
Lysine	10.96	946.08	19.55	17.90	18.78	544
Methionine	20.11	106.59	26.18	26.04	32.30	352
Phenylalanine	129.78	852.59	169.25	149.17	160.38	680
Threonine	37.56	553.99	49.65	52.41	56.81	400
Non-essentialAmino acids						
Aspartic acid	156.06	1479.26	200.36	161.66	197.19	
Glutamic acid	114.63	2356.18	194.61	106.46	124.14	
Alanine	703.88	1535.48	831.53	785.05	749.04	
Arginine	110.27	1586.83	168.31	232.52	249.27	
Glycine	7.69	432.51	9.24	10.81	10.29	
Serine	35.56	369.51	45.86	50.81	55.93	
Tyrosine	14.71	358.59	35.81	29.03	44.95	
Proline	812.68	1337.54	907.50	641.63	642.86	

*FAO/WHO/UN(16)reference pattern

The reason may be due to racemization process in which L amino acids were converted to D amino acids. The conversion is of nutritional importance because D amino acids are absorbed more slowly than the corresponding L form and even if digested and absorbed, most D isomers of essential amino acids are not utilized by humans (14). In addition, L – D, D – L, and D – D peptides bond introduced during the racemization process would resist attack by proteolytic enzymes, which function best with L – L bond (17). The result obtained was agreed with that reported by Awadelkareem (15) who found that, most of amino acids of sorghum flour supplemented with 12% soybean protein were detected to be lower than those of flour supplemented with 4 and 8% soybean protein. The isoleucine and leucine of millet flour and composite flours were higher than recommended levels of FAO/WHO/UN (16), while the other essential amino acids were lower.

Effect of processing methods on amino acids composition of millet flour supplemented with 10% FSP:

3.1. Cooking

As shown in Table 2, cooking increased the amino acids content of the flour supplemented with 10% FDSF except alanine and glycine which were slightly decreased. The amino acids alanine, proline, leucine, isoleucine, arginine, aspartic acid and glutamic acid were detected in high levels for both raw and cooked composite flour. The essential amino acids content of cooked 10% FDSF flour were lowers than that recommended by FAO/WHO/UN (16).

3.2. Fermentation

As shown in Table 2, fermentation of the flour supplemented with 10% FDSF for 8h increased leucine, lysine, methionine, phenylalanine, aspartic acid, glutamic acid, tyrosineand proline, while histidine, isoleucine, threonine, alanine, arginine, glycine and serine were decreased. The highest increase in amino acids after 8h fermentation was recorded for glutamic acid (70.06mg/100g) and proline (47.16mg/100g), while lysine, methionine, phenylalanine and aspartic acid were increased slightly except leucine and isoleucine. Fermentation for 16h decreased all amino acids except alanine and glycine which were increased from (707.99 to 709.86 mg/100g) and from (10.47 to 11.05mg/100g), respectively, while glycine; tyrosine and proline were detected to have high values than those of raw supplemented flour, while other ones were lower. The majority of the amino acids of fermented 10% composite flour were detected to have lower values than that recommended levels of FAO/WHO/UN (16).

Table 2. Effect of processing methods on amino acids profiles (mg/100g) of millet flour supplemented with 10% FDSF.

Amino acids	Composite flour Raw	Cooked	Fermentation period (hours)				Recommended levels (mg/100g protein)*
			8		16		
			Uncooked	cooked	Uncooked	cooked	
essential Amino acids							
Histidine	41.19	43.66	37.10	52.92	35.55	37.04	140
Isoleucine	304.48	322.66	301.79	335.59	293.99	275.67	400
Leucine	436.96	513.24	456.33	544.36	434.68	406.11	704
Lysine	17.90	22.88	19.75	19.60	14.68	14.82	544
Methionine	26.04	32.57	30.76	26.87	25.37	21.21	352
Phenylalanine	149.17	171.08	157.39	179.79	131.49	123.21	680
Threonine	52.41	54.45	47.53	46.99	42.17	47.79	400
Non-essential Amino acids							
Aspartic acids	161.66	179.26	169.29	181.34	148.88	153.17	
Glutamic acid	106.46	173.32	176.52	146.34	96.96	134.58	
Alanine	785.05	763.98	707.99	822.20	709.86	642.88	
Arginine	232.52	240.82	217.91	218.98	203.03	172.73	
Glycine	10.81	10.47	10.41	12.24	11.05	11.30	
Serine	50.81	55.62	44.57	60.64	43.84	47.72	
Tyrosine	29.03	39.06	46.75	22.68	36.37	28.48	
Proline	641.63	729.36	688.79	654.39	645.19	631.71	

*FAO/WHO/UN (16) reference pattern

Effect of cooking of fermented dough on amino acid composition of millet flour supplemented with 10% FDSF:

As shown in Table 2, cooking of 8h fermented dough increased histidine, isoleucine, leucine, phenylalanine, threonine, aspartic acids, alanine, arginine, glycine and serine, while lysine, methionine, glutamic acid, tyrosine and proline were decreased. Cooking of 16h fermented dough increased histidine, lysine, threonine, aspartic acid, glutamic acid, glycine and serine, while other amino acids were decreased. The amino acids after cooking of 8h and 16h fermented dough flour were lower than that of the FAO/WHO/UN (16) recommended levels despite the majority of them were increased after cooking of 8h fermented dough with highest values of lysine and methionine.

Amino acids scores of pearl millet, FDSF and composite flour with 5, 10 and 15% FDSF:

As shown in Table 3, the first and second limiting amino acids of FDSF were methionine (19.60%) and phenylalanine (81.91%), respectively, while that of millet flours supplemented with 5, 10 and 15% FDSF was lysine with values 10.29%, 10.85% and 10.66% for the supplements, respectively, while the second limiting amino acid was methionine 21.31%, 24.15% and 28.14% for the supplements, respectively.

Table 3. Amino acid scores of pearl millet flour, fenugreek defatted seeds flour (FDSF) and the supplements.

Essential Amino acids	Pearl millet	FDSF	Composite flours			Recommended levels (g/100g protein)
			5%	10%	15%	
Histidine	82.14	199.29	82.86	96.43	97.86	1.40
Isoleucine	213.75	217.25	220.5	249.75	260.00	4.00
Leucine	203.84	148.86	202.98	203.69	221.88	7.04
Lysine	7.17	113.60	10.29	10.85	10.66	5.44
Methionine	20.17	19.60	21.31	24.15	28.41	3.52
Phenylalanine	67.35	81.91	70.88	71.91	73.09	6.80
Threonine	33.00	90.50	35.25	43.00	44.00	4.00
Sum	627.42	871.01	644.07	699.78	735.9	

* FAO/ WHO/ UN (16) reference pattern

Effect of processing methods on essential amino acids scores of millet flour and supplements

Cooking:

As shown in Table 4, cooking increased the amino acids scores of leucine, lysine, methionine and phenylalanine of 10% FDSF supplements, while the score of other amino acids was decreased. The first and second limiting amino acids of cooked composite flour were lysine (12.5) and methionine (27.56).

Table 4. Effect of processing methods on essential amino acids scores of 10% FDSF supplement.

Amino acids	composite flour		Fermentation period (hours)				Recommended Levels(g/100g protein)
			8		16		
	Raw	Cooked	Uncooked	cooked	Uncooked	cooked	
Histidine	96.43	92.86	85.00	112.86	88.57	96.43	1.40
Isoleucine	249.75	240.50	241.75	251	255.75	250.75	4.00
Leucine	203.69	217.47	211.65	231.25	215.06	209.94	7.04
Lysine	10.85	12.5	11.58	10.29	9.38	9.93	5.44
Methionine	24.15	27.56	28.13	22.73	25.28	21.88	3.52
Phenylalanine	71.91	75.00	74.12	79.12	67.35	65.88	6.80
Threonine	43.00	40.50	38.00	48.50	36.75	43.25	4.00
Sum	699.78	706.23	690.23	755.75	698.14	698.06	

* FAO/ WHO/ UN (16) reference pattern

3.3. Fermentation

As shown in Table 4, the amino acids scores of leucine, methionine, glycine and phenylalanine were increased after 8h fermentation, but decreased after 16h fermentation except leucine which was increased from 211.65% to 215.06%. The chemical score of histidine (for children) was decreased after 8h Fermentation by 85%, but increased by 88.57% after 16h fermentation. The first and second limiting amino acids of 8 and 16h fermented 10% composite flour were lysine and methionine, respectively.

3.4. Cooking of Fermented Dough

The amino acids chemical scores of lysine and methionine were decreased after cooking of 8h fermented dough prepared by mixing 10% FDSF and scored 10.29 and 22.73, respectively, while after cooking of 16h fermented dough the chemical score of lysine was increased from 9.38 to 9.93. The first and second limiting amino acids after cooking of 8 and 16h fermented dough were lysine and methionine, respectively.

4. Conclusion

Supplementation of pearl millet with fenugreek defatted seeds flour (FDSF) increased the amino acids contents. This considered nutritionally desirable because it increase lysine content which cause improvement in the nutritional value of pearl millet. The amino acids content and score were fluctuated during processing of the flour and supplements.

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