FERMENTATIVE-MODIFICATION OF SORGHUM AND MILLET BIOPOLYMERS: IMPACT ON FUNCTIONAL PROPERTIES, PROXIMATE COMPOSITION, AMINO ACIDS AND PROTEIN QUALITY OF MODIFIED FLOURS

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Abstract: Fermentation is being used in the preparation of cereal-based foods to impart changes that can influence the character of the final product, but the processes of are tedious, unhygienic and labor intensive. Scientific improvements can facilitate product standardization, specification, safety and availability. Fermentative modification of the sorghum and millet grains biopolymer was achieved by natural fermentation at 34 ^{o}C for 36hours, with the fermentation water being changed every 12 hours. The grains were decanted and dried in a solar dryer at 41 ^{o}C, ground and sieved through a 300 µm sieve, while laboratory analyses carried out by standard methods. The functional properties, proximate composition, amino acids profile and protein quality indices of the flours were determined using standard methods. The process led to reduction in functional properties and proximate composition but *carbohydrate* and *energy values* were not affected while protein contents were increased by 7.5 % for sorghum and 9.7 % for millet. Reduction of 11.5 % and 17.2 % in *leucine and phenylalanine* contents respectively were recorded in sorghum, but in millet the values increased by 15.8 % and 6.6 % respectively. There were 2.9 % and 20.6 % increase in *lysine* and *tryptophan* contents in sorghum and a decrease of 13.5 % and 7.7 % respectively in millet. There were increases in *histidine, isoleucine, methionine, threonine, and valine.*

Keywords: functional properties, amino acids, protein quality

1. INTRODUCTION

Sorghum and millet rank as the fifth and sixth most important cereal grains in the world and will remain important in the arid and semi-arid regions of India and sub-Saharan Africa where other crops tend to fail due to inadequate rainfall and poor soil conditions. Sorghum is a principal source of energy, protein and minerals including trace components like zinc and iron in the diets of large populations from India and Africa [1]. Millet is particularly important for its high level of metabolizable energy and protein contents, a balanced amino acid profile, and a rich source of dietary fiber and lipids, in addition to the fact that both sorghum and millet foods are gluten free and have low glycemic index. However, these benefits tend to be limited by high levels of anti-nutritional factors like phytic acid, polyphenols and tannins that readily forms complexes with monovalent and multivalent cations of

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potassium, calcium, iron, zinc, magnesium and others, thereby reducing their bioavailability and creating a deficit in their absorption [2]. Modification of the cereal biopolymer by different methods such as germination and fermentation are reported to significantly mitigate the anti-nutritional activities of such inhibitors by increasing the grain protein and carbohydrate digestibility and also improving the bioavailability of the trace elements especially phosphorus and calcium. Ojha et al. [3] reported that germination of sorghum reduced phytate, tannin, and oxalate by 40 %, 16.12 % and 49.1 %, respectively, whereas fermentation of sorghum flour reduced the three anti-nutrients by 77 %, 96.7 % and 67.85 %, respectively. However, in addition to the anti-nutrients reduction benefits, native starches are often modified to develop specific properties such as solubility, texture, adhesion and tolerance to the heating temperatures used in industrial processes [4, 5]. Several methods have been developed to produce modified starches with a variety of characteristics and applications. All these techniques alter the starch biopolymer, making it highly flexible and changing its physicochemical properties and structural attributes to increase its value for food and non-food applications [6].

In Nigeria, fermentation is extensively used traditionally in the preparation of many different recipes where the biopolymer modification imparts several benefits from preservative effects to nutritional enhancement and improved functionality. Nkama [7] reported that traditional foods made from sorghum and millets fall within the following classes: steam cooked and stiff porridges (such as *tuwo*, *burabusko*, *ndaleyi* and *fura*), fermented baked batter and pancakes (like *masa*, *sinasin*), non-alcoholic beverages and thin porridges (including *kunun-zaki* and *kunun-gyada*) fermented thin porridges (*akamu*), alcoholic beverages (*pito*, *burukutu*). In such processes, fresh grains or flour is mixed with water and allowed to ferment before cooking. However, such processes are not only tedious and time consuming, but they are generally unhygienic and labor intensive even though the processes impart positive attributes that give the products their specific characters. Readily available shelf-stable fermentation-modified flours, that can give similar attributes while reducing the preparation time of the products and also improve on the general hygiene of the processes, can revolutionize the traditional fermented food industry by increasing food safety and facilitating industrialization drive. The aim of this work therefore is to assess the impact of fermentative modification of sorghum and millet grains biopolymer on the nutritional composition and functional properties of the flours with the view to assisting in product/process standardization and general increase in fermented food availability and safety.

2. EXPERIMENTAL SETUP

2.1. Preparation of control samples

Sorghum (Masakwa Variety) and Millet (Sosat C88) for the whole work were obtained from Lake Chad Research Institute, Maiduguri, Nigeria. Ten kilograms of each sample grain were used for this study. Each batch was first tempered with water using a quantity of 3-4 % (v/w) followed by decortication of the grains in commercial dehulling machine (previously cleaned), where the germs and hulls of the grains were removed. The decorticated grains were aspirated manually to remove adhering hulls and then ground into flour using a Laboratory Hammer mill (Armfield). The sample flour thus obtained was sieved using a standard sieve with 300 μm aperture and then kept in airtight polythene bags until needed.

2.2. Preparation of fermented samples

Natural fermentation was carried out at the prevailing ambient temperature (34±1 ºC) for 36 hours for both sorghum and millet. Fermentation water was being discarded after every 12 hours to avoid undesirable microbial succession and the development of putrid odor. The fermented grains were decanted and then spread on a canvass material for drying. The dried grains were then ground into flour using a Laboratory Hammer mill. The fermented flour thus obtained was sieved through a standard sieve with 300μm aperture and then kept in airtight polythene bags until needed.

2.3. Determination of functional properties

Determination of Water Absorption Capacity (WAC): Water absorption capacity was determined using the method of Sathe and Salunkhe [8] with slight modifications. Ten (10) milliliters of distilled water were added to 1.0 g of the sample in a beaker. The suspension was stirred using a glass stirrer for 5 min. The suspension obtained was thereafter centrifuged at 3555 rpm for 30 minutes and the supernatant measured in a 10 mL graduated cylinder. The density of water was taken as 1.0 $g.cm^{-3}$. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant.

Determination of Least Gelling Concentration (LGC): The least gelling concentration was determined by the method of Sathe *et al.* [9]. Test tubes containing suspensions of 2, 4, 6, 8 up to 20 % (w/v) flour in 5 mL distilled water were heated for one 1h in boiling water, followed by cooling in ice and further cooling for 2 h at 4 ºC. The least gelling concentration was the one at which the sample did not fall down or slip when the test tube was inverted.

Determination of Gelatinization Temperature (GT): GT was determined according to the method described by Shinde [10]. One 1 gram of flour sample was weighed accurately in triplicate and transferred to 20 mL screw capped tubes. Ten (10) milliliters of water was added to each sample. The samples were heated slowly in a water bath until they formed a solid gel. At complete gel formation, the respective temperature was measured and taken as gelatinization temperature.

Determination of Swelling Capacity (SC): This was determined with the method described by Leach et al. [11] with modification for small samples. One gram of the flour sample was mixed with 10 mL distilled water in a centrifuge tube and heated at 80 °C for 30 min. This was continually shaken during the heating period. After heating, the suspension was centrifuged at 1000×g for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as equation (1):

$$Swelling\ power = weight\ of\ the\ paste / weight\ of\ dry\ flour \quad (1)$$

Determination of Bulk Density (BD): This was carried out using the procedure of Narayana and Narasinga [12]. A specified quantity of the flour sample was transferred into an already weighed measuring cylinder (W_1) and gently tapped to eliminate spaces between the flour. The level was noted to be the volume of the sample and then weighed (W_2). The study was conducted in triplicate and Bulk Density obtained as follows equation (2):

$$Bulk\ density\ (g.cm^{-3}) = W_2 - W_1 / Vol.\ of\ Sample \quad (2)$$

2.4. Proximate analyses

Moisture Content: Moisture content of samples was determined by hot air oven drying method as recommended by AOAC [13].

Crude Protein: The Kjeldahl digestion method was used to estimate the nitrogen in the sample which was then multiplied by the nitrogen conversion factor 6.25 to obtain the percentage of protein [13].

Fat Content: This was carried out according to AOAC [13]. Diethyl ether at 50 ºC was used for the extraction under reflux for 5h using Soxhlet apparatus.

Total Ash: The ash content in the sample was determined by incineration with the furnace at 550 ºC [13] after a period of 31-32 h a white ash was removed and placed in a desiccator for 1 h and then weighed calculated by equation (3):

$$Ash\ content\ (\%) = (Weight\ of\ Ash / Weight\ of\ sample) \times 100\% \quad (3)$$

Carbohydrate: Total carbohydrate percentage was calculated by difference [13]. The sum of Percentage Moisture, Fat, Protein and Ash was subtracted from 100 and the balance was recorded as percentage of Carbohydrate.

Energy Value (kcal.): The sample calorific value was estimated in kcal/g by multiplying the percentages of crude protein, crude fat and carbohydrate with the recommended factors as proposed by Martin and Coolidge [14].

2.5. Assessment of protein quality

Determination of Amino Acids: Amino acids were profiled by the Isocratic HPLC - 2 methods [13]. Samples were solubilized, centrifuged and filtered through a 0.22 µm membrane. The filtrate was then used for the experiment. Standard solutions of the amino acids (both essential and non-essential) were prepared and serially diluted to give 25 pmol of each amino acid derivative. Chromatographic separation of samples was carried on a Buck scientific BLC10/11 - model HPLC equipped with UV 338nm detector.

The Essential Amino Acid Index [EAAI]: was calculated using the method of Labuda et al. [15] according to the following equation (4):

$$EAAI = \sqrt[9]{\frac{[Lys\ x\ Thr\ x\ Val\ x\ Met\ x\ Ile\ x\ Leu\ x\ Phe\ x\ His\ x\ Trp]_a}{[Lys\ x\ Thr\ x\ Val\ x\ Met\ x\ Ile\ x\ Leu\ x\ Phe\ x\ His\ x\ Trp]_b}} \quad (4)$$

where: *[lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine and methionine] a* in test sample and *[lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cystine]b* the same amino acids content in standard protein [%] [egg or casein] respectively.

Protein Efficiency Ratio (PER): Protein Efficiency Ratio of the samples were calculated according to the equations developed by Alsmeyer et al. [16, 17] as used by Ogunmodimu et al. [18]:

$$PER = 0.06320\ [X_{10}] - 0.1539 \quad (5)$$

where: $X_{10} = Thr + Val + Met + Ile + Leu + Phe + Lys + His + Arg + Tyr$

Biological Value (BV): Biological Values were computed according to the methods of Mune-Mune et al. [19] as a function of EAAI.

$$BV = 1.09\ (EAA\ Index) - 11.7 \quad (6)$$

Nutritional Index (NI): The nutritional index of the food samples was calculated using the formula below as described by Crisan and Sands [20].

$$Nutritional\ Index(\%) = \frac{EAAI\ x\ \%\ Protein}{100} \quad (7)$$

TEAA + His + Arg/ TAA: This value expresses the percentage abundance of EAA including histidine and arginine relative to all the amino acids available in the test material *(TEAA – total essential amino acid, TAA – total amino acids)*.

TSAA (Meth + Cys g/100g): This is the total sum of all the sulfur containing amino acids in the sample *(TSAA – total sulfur amino acids)*.

TAEAA (Phe + Tyr g/100g): This expresses the total sum of the aromatic indispensable amino acids in the test material *(TArAA – total aromatic essential amino acids)*.

Amino Acid Score: The amino acid score was calculated using the ratio of a gram of amino acid in the food to the amount of the corresponding amino acid in the reference diet multiplied by 100.

$$Amino\ Acid\ Score\ (\%) = \frac{Value\ of\ EAA\ in\ Food\ Sample\ (g/100g\ Protein)}{Reference\ Value\ of\ the\ Essential\ Amino\ Acid}\ x\ 100 \quad (8)$$

2.6. Statistical analysis

The results were analyzed by 3-way ANOVA and mean separation carried out by the Tukey-Kramer HSD (Honestly Significant Difference) test using MATLAB statistical software [MATLAB 7.12.0 (R2011a)].

3. RESULTS AND DISCUSSION

3.1. Functional properties

Table 1 shows the results of the functional properties of the raw and fermentation-modified sorghum and millet flour as determined in this work.

These include *water absorption capacity* (WAC), *least gelling concentration* (LGC), *gelatinization temperature* (GT), *swelling capacity* (SC) and *bulk density* (BD). All the functional properties were lowered by fermentation to different extents.

It was observed from the results that initially the WAC and GT of unmodified millet flour were significantly higher than that of sorghum probably due to inherent variations in physico-chemical properties and the structure of grains depending on their genetic background and the environmental conditions in which the grains were grown [21]. However, after fermentative modification, both values were significantly reduced by different percentages. The

fermentation-induced decrease in *water absorption capacities* of the flour of both grains may be due to degradation of starch and soluble sugars by the intrinsic grain enzymes and enzymes of the fermenting media [22, 23].

Table 1. Functional properties of raw and modified flours.

Property	Sorghum flours			Millet flours		
	Raw	Modified	%Change	Raw	Modified	% Change
WAC (%)	189.7 ± 2.1^{bd}	170.5 ± 2.0^{bf}	10.1	192.3 ± 1.8^{cd}	172.3 ± 2.1^{cf}	10.4
LGC (%)	8.3 ± 0.8^{ad}	8.2 ± 1.15^{ad}	1.2	9.7 ± 0.8^{ad}	8.3 ± 0.8^{ad}	14.4
GT (ºC)	62 ± 1.7^{bd}	61.0 ± 1.73^{bf}	1.6	64.3 ± 1.2^{ad}	61 ± 1.7^{af}	5.1
SC (%)	15.7 ± 0.6^{ad}	14.0 ± 1.0^{af}	10.8	15.7 ± 0.3^{ad}	14.2 ± 0.29^{af}	9.6
BD (kg/m^3)	0.53 ± 0.02^{ad}	0.44 ± 0.01^{af}	17	0.52 ± 0.02^{ad}	0.44 ± 0.01^{af}	15.4

Mean values in the same row with different superscript differ significantly ($P < 0.05$)

It was also observed that the *least gelling concentrations* of the flours were slightly reduced (1.2 for sorghum and -14.4 for millet) but the reduction was not statistically significant at $P< 0.05$. This may be due to an increase in hydrogen bonding capacity and hydrophobic associations by Van der Waals attractions in the grains biopolymer as a result of fermentative degradation. Gel formation entails three-dimensional network of connected polymer molecules like starch granules and protein matrix joined together by hydrogen or covalent bonding which entraps aqueous solution of low-molecular-weight solutes and portions of the polymer chains [24]. Fermentative disruption of the starch granules and subsequent solubilization of organic molecules might have increased the three-dimensional network forming ability of the polymer molecules resulting in the observed slight drop in the concentration of the flours necessary for gel formation. The lowest flour concentration at which starch gelatinization takes place is the *least gelling concentration* which is related to the nature of starch granules, their sizes and densities; and the *LGC* may vary from one starch source to another.

Gelatinization Temperature (GT) of the flours, as shown in Table 1, indicated that millet flours have higher GT compared to sorghum, probably because corneous endosperms tend to exhibit higher GT than floury endosperm [25]. However, fermentation process significantly decreased the GT of the flours probably because of the breakdown of glycosidic bonds during the fermentation process [26]. Also, since the fermentation process is carried out at the prevailing ambient temperature (34±1 ºC), starch *tempering* did not occur to warrant increase in GT, as tempering is known to increase the gelatinization temperature due to the reorganization of the structure of the granule [27]. The GT values recorded in this work compared favorably with those reported by Chandra and Samsher [28], but with slight disparities. Such differences may be brought about by differences in the agronomic histories of the grains and probably also due to difference in the precision of measurement procedures used. It was observed from the results that the *swelling capacities (SC)* of the flours of the two grains did not vary significantly from each other, but fermentation significantly lowered the SC of both flours compared to the control. This may be because the grains have initially absorbed some water during fermentation unlike the control samples [12]. The extent to which a particular starch sample swells under given set of conditions is given by the *Swelling Capacity* (SC) of the starch which is an important parameter in the process specification and material balance.

Bulk Density (BD) of food materials gives a measure of the amount of matter contained in the sample. Like the case of SC, there is no significant difference ($P<0.05$) between the BD of sorghum and millet flours, but fermentation did significantly decrease their BDs. As fermentation is reported to cause starch degradation and the extraction of soluble substances that were discarded with the fermentation medium, the relative availability of matter in a given volume of fermented grain is reduced drastically which led to the observed drop in the BD of the fermented flour samples used in this work. Values of starch density recorded in literature ranges from 1.4 g/cm^2 to 1.6 g/cm^2 [26].

These functional properties collectively determine the physical and textural quality of the end products produced from the flours and their reduction by fermentative modification will influence the physical properties of food products produced therefrom.

3.2. Proximate composition and energy value

Table 2 shows the proximate composition of the raw and fermentation-modified flours of the sorghum and millet grain samples.

Significant differences were observed in the *moisture contents* of the flours of the two grains which were also significantly influenced by fermentation process. This may be due to reduction in their *bulk densities* which invariably increased the voidage between particles and facilitated faster energy and mass transfer during the drying process. Moisture values of 10.82%, 10.53 % were reported in literature [29, 30].

Table 2. Proximate composition.

Property	Sorghum flour			Millet flour		
	Raw	Modified	% Change	Raw	Modified	% Change
Moisture (%)	11.0 ± 1.0^{bdg}	9.3 ± 0.5^{bfg}	15.5	10.6 ± 0.5^{cdg}	11.0 ± 1.0^{cfg}	3.8
Ash (%)	1.5 ± 0.5^{bdg}	0.7 ± 0.2^{beg}	53.3	$1.1 \pm 0.2_{cbdg}$	0.6 ± 0.1^{cbeg}	45.5
Fat (%)	1.5 ± 0.2^{adg}	1.1 ± 0.1^{aeg}	26.7	2.0 ± 0.2^{adg}	1.0 ± 0.3^{aeg}	50
Protein (%)	12.0 ± 1.0^{bdfg}	12.9 ± 0.7^{bfdg}	7.5	11.3 ± 1.2^{bdfg}	12.4 ± 1.0^{bfdg}	9.7
Carbohydrate (%)	74.0 ± 2.3^{bdg}	76.1 ± 0.6^{bdg}	2.8	75.0 ± 1.0^{bdg}	76.1 ± 0.6^{bdg}	1.5
Energy value (kcal)	305.7 ± 5.1^{bdg}	311.9 ± 0.5^{bdg}	2.0	312.0 ± 2.0^{cbdg}	306.3 ± 5.6^{cbdg}	1.8

Mean values in the same row with different superscript differ significantly ($P < 0.05$)

The *ash content* of sorghum flour is significantly ($P < 0.05$) higher than that of millet due to specie differences. However, for both the flours fermentative-modification has significantly reduced their *ash contents* compared to the control samples by about 50% due to loss of solubilization and loss with fermentation media [24]. On the other hand, no significant difference was observed in the *total fat contents* of the flours of the two grains at $p < 0.05$, but fermentation has significantly reduced their fat content values relative to the control samples probably due to enzymatic de-esterification of the polyglycerol esters [31] as part of fermentative modification. It was also observed that while there is no significant difference ($P<0.05$) between the protein contents of the unmodified flours, fermentative-modification has significantly increased the protein contents of the two flours at $p < 0.05$ by 7.5 % for sorghum and 9.7 % for millet due the proteinaceous metabolites produced by the fermentation agents during the process [23, 32]. The results of proximate composition revealed that neither specie difference nor fermentative-modification significantly affected the *percentage carbohydrate* of the two flours, although slight increases of 2.8 % and 1.5 % for sorghum and millet respectively were observed. This may be due the solubilization and loss of other non-carbohydrate components via the fermentation by soaking grains in water which manifested as percentage increase in the carbohydrate but the actual molecules might remain the same albeit in modified form. As the *energy value* is obtained by multiplying the proximate values with the recommended factors, variations in the energy values of the samples follow logically from the variations in the proximate values as they are affected by the processes. Thus, higher energy values are recorded for samples with high fat and/or carbohydrate contents. In this way, fermentation has significantly affected the energy values of the flours.

3.3. Essential amino acids profile

Table 3 shows the results of indispensable amino acids determined in this work. Of the nine amino acids, unmodified millet flour exhibited higher values in seven of the amino acids excluding *leucine* and *lysine* where no significant difference ($P<0.05$) is observed between the unmodified flours of the two grains. This may be due to specie difference and differences in agronomic histories. However, fermentative-modification has variously affected the amino acids of the flours as shown in Table 3.

Table 3. Indispensable Amino Acids of raw and modified sorghum/millet flours (g/100g Protein).

Property	Sorghum Flours			Millet Flours		
	Raw	Modified	% Change	Raw	Modified	% Change
Histidine	0.72 ± 0.014^{bd}	0.88 ± 0.000^{bd}	22.2	1.05 ± 0.014^{cd}	1.115 ± 0.007^{cd}	6.2
Isoleucine	0.96 ± 0.014^{bd}	1.77 ± 0.000^{bd}	84.4	2.115 ± 0.007^{cd}	2.415 ± 0.007^{cd}	14.2
Leucine	1.865 ± 0.021^{ad}	1.65 ± 0.014^{ad}	11.5	2.345 ± 0.007^{ad}	2.715 ± 0.007^{ad}	15.8
Lysine	2.045 ± 0.007^{bd}	2.105 ± 0.007^{bd}	2.9	2.035 ± 0.021^{bd}	1.76 ± 0.014^{bd}	13.5
Methionine	1.245 ± 0.007^{bd}	1.34 ± 0.014^{bd}	7.6	3.415 ± 0.007^{cd}	3.59 ± 0.000^{cd}	5.1
Phenylalanine	1.045 ± 0.007^{ad}	0.865 ± 0.007^{ad}	17.2	1.525 ± 0.007^{bd}	1.625 ± 0.007^{bd}	6.6
Threonine	1.055 ± 0.007^{bd}	1.325 ± 0.007^{bd}	25.6	1.515 ± 0.007^{cd}	1.69 ± 0.014^{cd}	11.6
Tryptophan	0.875 ± 0.007^{bd}	1.055 ± 0.007^{bd}	20.6	2.01 ± 0.014^{cd}	1.855 ± 0.007^{cd}	7.7
Valine	0.35 ± 0.014^{bd}	0.39 ± 0.014^{bd}	11.4	1.05 ± 0.014^{ad}	1.22 ± 0.014^{ad}	16.2

TEAA	10.16	11.38	12.0	17.06	17.99	5.5

Mean values in the same row with different superscript differ significantly ($P < 0.05$)

It was observed that fermentative-modification of sorghum grain led to 11.5 % and 17.2 % reduction in *leucine and phenylalanine* contents of the sorghum flour respectively, but curiously the values for millet were instead increased by 15.8 % and 6.6 % respectively. This observation is baffling considering the similarity of the *leucine* and *phenylalanine* contents of the unmodified sorghum and millets flours. This may be due to the inherent compositional and structural differences in the biopolymer of the two grains. Sorghum was reported to ferment more slowly than other species [21] because of its higher proportion of peripheral endosperm which is extremely dense, hard and resistant to water penetration and digestion [26]. On the other hand, results of amino acids profiling revealed that *lysine* and *tryptophan* exhibited the opposite of *leucine* and *phenylalanine* where fermentative-modification of sorghum biopolymer led to a 2.9 % and 20.6 % increase in *lysine* and *tryptophan* contents of the flour respectively, while bringing about a 13.5 % and 7.7 % reduction in the contents of the two amino acids in millet flour respectively. This may also be due to specie differences and disparities in the fermentation rates and pathways of the two grains.

It was also observed that unmodified millet flour recorded significantly higher values of five amino acids including *histidine, isoleucine, methionine, threonine, and valine*; and interestingly, fermentative modification further increased their values in the flour of both grains by various percentages as shown in Table 3. This may be as a result of the production of bioactive peptides along with flavor compounds and vitamins during fermentative degradation of cereal biopolymer [33].

3.4. Protein quality indices

Amino Acid Score (AAS): the amino acid score also known as the chemical score of each EAA is presented in Table 4. With the exception of *leucine* and *lysine*, the AAS values of all the essential amino acids including *histidine, isoleucine, methionine, phenylalanine, threonine, tryptophan* and *valine* are significantly higher in raw millet flour compared to raw sorghum flour due to specie and genetic differences.

Table 4. Percentage Amino Acid Scores (AAS) of raw and modified sorghum/millet flours.

	Sorghum Flour			Millet Four		
Property	Raw	Modified	% Change	Raw	Modified	% Change
Histidine	32.73^{bdg}	40.0^{bdg}	22.2	47.73^{cdg}	50.68^{cdg}	6.2
Isoleucine	17.78^{bdg}	32.78^{bdg}	84.4	39.17^{cdg}	44.72^{cdg}	12.2
Leucine	21.69^{adg}	19.19^{adg}	11.5	27.27^{adg}	31.57^{adg}	15.8
Lysine	29.21^{bdg}	30.07^{bdg}	2.9	29.07^{bdg}	25.14^{bdg}	13.5
Methionine	13.39^{bdg}	14.41^{bdg}	7.6	36.72^{cdg}	38.6^{cdg}	5.1
Phenylalanine	22.23^{adg}	18.4^{adg}	17.2	32.45^{bdg}	34.57^{bdg}	6.5
Threonine	22.45^{bdg}	28.19^{bdg}	25.6	32.23^{cdg}	35.96^{cdg}	11.6
Tryptophan	18.62^{bdg}	22.45^{bdg}	20.6	42.77^{cdg}	39.47^{cdg}	7.7
Valine	5.30^{bdg}	5.91^{bdg}	11.5	15.90^{adg}	18.48^{adg}	16.2

Mean values in the same row with different superscript differ significantly ($P < 0.05$)

However, fermentative-modification of the grains induced different changes in the AAS of different amino acids. For *leucine* and *phenylalanine*, modification increased their value in millet by 15.8 % and 6.5 % respectively but decreased their value in sorghum by 11.5 % and 17.2 % respectively. In the case of *lysine* and *tryptophan* however, the opposite is true where fermentative-modification increased their values in sorghum by different percentages while the process led to a decrease in their values for millet by 13.5 % and 7.7 % respectively. For *histidine*, *isoleucine*, *methionine*, and *valine* however, the process led to appreciable increase in their values in both the grains.

Other protein Quality Indices: other protein quality indicators estimated in this work are shown in Table 5 which include *Essential Amino Acid Index* (*EAAI*), *Protein Efficiency Ratio (PER)*, *Biological Value (BV)*, *Nutritional Index (NI)* and *Total Sulfur-Containing Amino Acids (TSAA)*. In their review of protein quality, Boye, et al. [32] compiled some useful data from various sources that provide a list of protein quality indices for various food groups and food blends including the cereals maize, sorghum and millet for both raw and fermented samples. The results obtained in this work compare well with the Boye, et al. [32] data.

All the protein quality indices estimated in this work are found to be significantly higher in raw millet flour compared to sorghum. However, fermentative-modification tends to favor sorghum more in terms of percentage increase in protein quality relative to millet. For example, although the *essential amino acids index* and *biological values* of raw millet flour (32.34 % and 23.55 % respectively) are far greater than that of raw sorghum (18.14 % and 8.37 % respectively), fermentative modification of the two grains led to a 13.4 % and 32.0 % increase in the *EAAI* and *BV* of sorghum respectively while those of millet were increased only by 5.6 % and 8.3 % respectively. Similar pattern is observable in the rest of the indices recorded in Table 5. The improvement of PER during fermentation was attributed to better availability of AA and greater digestibility of the proteins in the substrates [32], but difference in the effects of fermentative-modification between the two grains may be due the inherent differences in the grains morphology and their chemical composition/configuration which might have resulted in remarkable difference in their metabolic pathways during fermentation; with each pathway favoring more production of certain metabolites over others [34]. In their review of twenty years of evaluating protein quality, Boye, et al. [32] reported various works that indicate that the micro-organisms used in fermentation synthesize enzymes which hydrolyze food constituents and contribute to the development of products with desirable organoleptic properties, and also contribute to the decrease of anti-nutritional factors which help in improving nutritional quality of the food. PER improved from 1.6 to 2.3, Net Protein Ratio (NPR) improved from 2.7 to 3 and Protein Digestibility Corrected Amino Acid Score (PDCAAS) improved from 73 % to 92 % [35]. These increments in the protein quality indices of sorghum and millet by fermentative modification are important in rural nutrition especially in areas where protein consumption is limited by poverty, armed conflicts or other social factors.

Table 5. Calculated protein quality of samples.

	Sorghum Flour			Millet Four		
Quality Index	Raw	Modified	% Change	Raw	Modified	% Change
EAAI (%)	18.41^{bd}	20.87^{bd}	13.4	32.34^{cd}	34.14^{cd}	5.6
PER (g/100g)	0.53^{bd}	0.62^{bd}	17.0	0.91^{cd}	1.00^{cd}	9.9
BV (%)	8.37^{bd}	11.05^{bd}	32.0	23.55^{cd}	25.51^{cd}	8.3
Nutritional Index	2.21^{bd}	2.69^{bf}	21.7	3.65^{cd}	4.23^{cd}	15.9
TSAA: (Met+Cys) (g/100g)	1.93^{bd}	2.13^{bf}	10.4	4.26^{cd}	4.49^{cd}	5.4
TAEAA:(Phe+Tyr) (g/100g)	1.99^{bd}	2.19^{bf}	10.1	2.59^{cd}	2.97^{cd}	14.7

Mean values in the same row with different superscript differ significantly ($P < 0.05$)

4. CONCLUSIONS

The fermentative modification induced decrease in *water absorption capacities, least gelling concentrations, gelatinization temperature, swelling capacities* and *bulk densities* of the flours. The process also significantly reduced the *ash* and *fat contents*, of both flours but significantly increased their protein contents by 7.5 % for sorghum and 9.7 % for millet. Sorghum grain recorded 11.5 % and 17.2 % reduction in *leucine and phenylalanine* respectively, but the values for millet were instead increased by 15.8 % and 6.6 % respectively. The process led a 2.9 % and 20.6 % increase in *lysine* and *tryptophan* in sorghum, while lowering those of millet flour by 13.5 % and 7.7 % respectively. The process increased the values of *leucine* and *phenylalanine* in millet but their values decreased in sorghum. The process brought 13.4 % and 32.0 % increase in the *EAAI* and *BV* of sorghum respectively while those of millet were increased only by 5.6 % and 8.3 %. Fermentative modification of sorghum and millet biopolymer for food applications can impart beneficial changes both in physical/engineering parameters relevant in processing and in nutritional attributes of the final products. It is thus a viable option for sorghum and millet flours processing at industrial levels.

REFERENCES

[1] FAO, http://www.fao.org/3/T0818E/T0818E0J.HTM, (Accessed 12.05.2006).
[2] Boncompagni, E., Orozco-Arroyo, G., Cominelli, E., Gangashetty, P. I., Grando, S., Kwaku Zu, T. T., Daminati, M. G., Nielsen, E., Sparvolis, F., Antinutritional factors in pearl millet grains: Phytate and goitrogens content variability and molecular characterization of genes involved in their pathways, PLoS ONE, vol. 13, no. 6, 2018, p. e0198394.
[3] Ojha, P., Adhikari, R., Karki, R., Mishra, A., Subedi, U., Karki. T. B., Malting and fermentation effects on antinutritional components and functional characteristics of sorghum flour, Food Science and Nutrition, vol. 6, no. 1, 2018, p. 47–53.

[4] Singh, J., Kaur, L., McCarthy, O. J., Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications - A review, Food Hydrocolloids, vol. 21, no. 1, 2007, p. 1-22.
[5] Sweedman, M. C., Tizzotti, M. J., Schäfer, C., Gilbert, R. G., Structure and physico-chemical properties of octenyl succinic anhydride modified starches: A review, Carbohydrate Polymers, vol. 92, no.1, 2013, p. 905-920.
[6] López, O. V., Zaritzky, N. E., García, M. A., Physicochemical characterization of chemically modified corn starches related to rheological behavior, retrogradation and film forming capacity, Journal of Food Engineering, vol. 100, no. 1, 2010, p. 160-168.
[7] Nkama, I. Traditional food preparations of pearl millet in Nigeria. In: Pearl millet in Nigeria Agriculture: Production, utilization and research priorities. Proc. of the pre-season national coordination and planning meeting of NCRP for pearl millet (A. M. Emechebe; M. C. Ikwelle; O. Ajayi; M. A. Kano and A. B. Anaso (Eds) LCRI Maiduguri, 1998, p. 179 – 208.
[8] Sathe, S. K., Salunkhe, D. K., Functional properties of great northern beans (*Phaseolus vulgaris*) proteins: emulsion, foaming, viscosity and gelation properties, Journal of Food Science, vol. 46, no. 1, 1981, p. 71-81
[9] Sathe, S. K., Deshpande, S. S., Salunkhe, D. K., Functional properties of black gram (*Phaseolus mango* L) proteins, Lebensmittel-Wissenschaft und -Technologie, vol. 16, 1981, p. 69-74.
[10] Shinde, B. G., Isolation and characteristics of starch horse grain, Unpublished M.Sc,Thesis, Mahatma Phule Krishi Vidyapeeth, Rahuri, India, 2001, 16-17.
[11] Leach, H.W., McCowen, L.D., Schoch, T.J., Structure of the starch granules. I. Swelling and solubility patterns of various starches, Cereal Chemistry, vol. 36, no. 6, 1959, p. 534-544.
[12] Narayana, K., Narsinga, R. S. M., Functional properties of raw and heat processed winged bean (Psophocarpus tetragonolobus) flour, Journal of Food Science, vol. 47, no. 5, 1982, p. 1534-1538.
[13] AOAC, Association of Official Analytical Chemists, 18th Edition, Official Methods of Analyses, Washington D.C., 2000.
[14] Martin, E. A., Coolidge, A. A., Nutrition in action, 4th edition, Holt, Rinehart and Wilson, New York, 1978.
[15] Ijarotimi, O. S., Keshinro, O. O., Determination of amino acid, fatty acid, mineral, functional and choking properties of germinated and fermented popcorn (Zea mays everta) flour, European Journal of Food Research and Review, vol. 1, no. 2, 2011, p. 102-122.
[16] Ijarotimi, O. S., Influence of germination and fermentation on chemical composition, protein quality and physical properties of wheat flour (Triticum aestivum), Journal of Cereals and Oil Seeds, vol. 3, no. 3, 2012, p. 35-47.
[17] Alsmeyer, R. H., Cunningham, A. E., Happich, M. L., Equations predict PER from amino acid analysis, Food Technology, vol. 28, no. 7, 1974, p. 34 – 40.
[18] Ogunmodimu, O. O., Ijarotimi, O. S., Fagbemi, T. N., Evaluation of nutritional properties of high protein-fiber based snacks formulated from wheat, soybean concentrate and cassava fiber, Sky Journal of Food Science, vol. 4, no. 3, 2015, p. 030 – 040.
[19] Mune, M. A., Minka, S. R., Mbome, I. L., Etoa, F. X., Nutritional potential of Bambara bean protein concentrate, Pakistan Journal of Nutrition, vol. 10, no. 2, 2011, p. 112-119
[20] Crisan, E.V., Sands, A., Nutritional value, tEds. Chang, S.T Hayer, W.A, Biology and Cultivation of Edible Mushrooms, Academic Press, New York, 1978, p. 137-142.
[21] Opatpatanakit, Y., Kellaway, R. C., Lean, I. J., Annison, G., Kirby, A., Microbial fermentation of cereal grains in vitro, Australian Journal of Agricultural Research, vol. 45, no. 6, 1994, p. 1247-1263.
[22] Adebowale, Y. A., Adeyemi, I. A., Oshodi, A. A., Functional and physicochemical properties of flours of six Mucuna species, African Journal of Biotechnology, vol. 4, no. 12, 2005 p. 1461 – 1468.
[23] Chavan, J. K., Kadam, S. S., Beuchat, L. R., Nutritional Improvement of cereals by fermentation, Food Science and Nutrition, vol. 28, no. 5, 1989, p. 349-400.
[24] Bemiller, J. N., Whistler, R. L., Fennema, O. R., Food Chemistry (Third edition), Marcel Dekker, Inc. 270 Madison Avenue, New York, 1996, p. 184-185.
[25] Cagampang, G. B., Kirleis, A. W., Properties of starches isolated from floury and corneous endosperm, Starch/Stärke, vol. 37, no. 8, 1985, p. 253-257.
[26] Rooney, L. W., Pflugfelder, R. L., Factors affecting starch digestibility with special emphasis on sorghum and corn, Journal of Animal Science, vol. 63, no. 5, 1986, p. 1607-1623.
[27] Belitz, H.-D., Grosch, W., Schieberle, P., Food chemistry (4th revised and extended edition), Springer-Verlag Berlin Heidelberg, 2009, p. 316 – 321.
[28] Chandra, S. S., Assessment of functional properties of different flours, African Journal of Agricultural Research, vol. 8, no. 38, 2013, p. 4849-4852.

[29] Adeoti, O. A., Elutilo, O. O., Babalola, J. O., Jimoh, K. O., Azeez, L. A., Rafiu, K. A., Proximate, mineral, amino acid and fatty acid compositions of maize tuwo-cirina forda flour blends, Greener Journal of Biological Sciences, vol. 3, no. 4, 2013, p. 165-171.
[30] Adenekan, M. K., Oguntoyinbo, S. I., Odunmbaku, L. A., Nupo, S. S., Effect of Sprouted Pigeon pea (cajanus cajan) on proximate composition and sensory value of tuwo, Journal of Sciences and Multidisciplinary Research, vol. 6, no. 2, 2014, p. 17-21.
[31] Whitaker, J. R., Enzymes, Fennema, O. R., Ed. Food chemistry, 3rd, Marcel Dekker, Inc. 270 Madison Avenue, New York, 1996, p. 499 – 504.
[32] Boye, J., Wijesinha-Bettoni, R., Burlingame, B., Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method, British Journal of Nutrition, vol. 108, 2012, p. S183–S211.
[33] Ross, R. P., Morgan, S., Hill, C., Preservation and fermentation: past, present and future, International Journal of Food Microbiology, vol. 79, 2002, p. 3-16.
[34] Cuevas-Rodrıguez, E. O., Verdugo-Montoya, N. M., Angulo-Bejarano, P. I., Nutritional properties of tempeh flour from quality protein maize (Zea mays L.), LWT - Food Science Technology, vol. 39, 2006, p. 1072–1079.
[35] Angulo-Bejarano, P. I., Verdugo-Montoya, N. M., Cuevas-Rodriguez, E. O., Tempeh flour from chickpea (Cicer arietinum L.) nutritional and physicochemical properties, Food Chemistry, vol. 106, 2008, p. 106–112.