



Impact of Fermentative Modification of Maize Grain Biopolymer on Functional Properties, Proximate Composition, Amino Acids Profile and Protein Quality of Maize Flour

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ABSTRACT

Fermentative modification of maize grain biopolymer led to a significant reduction in *Water Absorption Capacity (WAC)*, *Gelatinization Temperature (GT)*, *Swelling Capacity (SC)* and *Bulk Density (BD)* of the modified flour, but a 17.9% increase in *Least Gelling Concentration (LGC)* was observed due to a decrease in the “network-forming-units” of the modified maize biopolymer. The process also led to a significant reduction in the percentage ash and fat contents of the flours by -50% and -65% respectively. An increase in protein content by more than 42% was recorded and a slight reduction in total carbohydrates and energy value of -1% and -2% respectively were observed. Fermentative modification has improved the percentage values of all the amino acids in the modified maize flour leading to a general improvement in protein quality. *Nutritional Index (NI)* of the modified maize flour was improved by about 130% which is quite remarkable considering the relative ease and the cost effective way the process was achieved. The improvement in *Biological Value (BV)* was most remarkable as almost 3000% increase was recorded. *Protein Efficiency Ratio (PER)* was improved by 72.4%, while the *Essential Amino Acids Index (EAAI)* was improved by 61.4%. Of the essential amino acids, *tryptophan* recorded the highest percentage increase in chemical score (142.3%) as a result of fermentative modification. The increase in chemical scores of *methionine*, *leucine*, *histidine* and *isoleucine*, in that order, were 94%, 74.9%, 69.9%, and 66.6%. *Lysine* and *valine* recorded considerable increase of more than 50% in their chemical score while *phenylalanine* and *threonine* recorded the least increase with 8.7% and 4.5% respectively.

Keywords: Fermentative-modification, functional properties, amino acids profile, protein quality,

INTRODUCTION

Fermentation has, for long, been used in the preparation of cereal-based foods including stiff porridges like *tuwo*, thin gruels like *banga*, alcoholic and non-alcoholic beverages such as *pito* and *burkutu* in almost all parts of Africa and some Asian countries. Fermentation serves different purposes in different food applications ranging from preservation, nutritional improvement, taste/flavour enhancement and starch modification among others (FAO, 2012). Oladeji *et al.* (2018), working on the effects of fermentation on the physicochemical properties of maize flours concluded that fermentation improved the pasting profile and some physicochemical properties desirable in the production of weaning foods; and that the reduction effect of fermentation on some nutrient content of maize is more pronounced in

normal endosperm than quality protein maize. In all such processes, the changes that occur in the functional properties of the flours due to fermentative modification of the cereal biopolymer play vital roles in the characteristics of the final product. Recently, attention has been focused on the characterization of shelf stable cereal flours produced by different methods for traditional food uses especially *tuwo* preparation (Bolade, 2009), and even for non-food uses such as modified biopolymer for packaging applications (Alcázar-Alay and Meireles, 2015). In such attempts, modification of the cereal grain biopolymer by various methods presents a veritable option for the development of shelf-stable flours with improved functionality and

Received 13 April, 2021

Accepted 20 May, 2021

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nutritional value. The traditional preparation processes of fermented cereal-based products are not only tedious and time-consuming, but they are generally unhygienic and labour intensive. Investigations into the effects of modification of the cereal grain biopolymer by fermentation on the nutritional composition and functional properties of the flour can assist in the characterization of the flour to facilitate product standardization, specification of preparation methods and overall increase in availability, safety and variety.

Maize is an important cereal grain, not only on account of its low amounts of anti-nutritional factors, but also its general acceptability and versatility that makes it a grain of choice for numerous food applications; hence it's ranking as the third most important cereal grain in the world after wheat and rice as staple food (Gwirtz and Garcia-Casal, 2014). Fermented maize finds application in several staple diets as well as snacks items in various parts of Nigeria and Africa. Products such as *Ndaleyi* and *Sinasin* in north-eastern Nigeria are staple diets with high ceremonial value in social occasions; while products like *koko*, *banga* and *akamu* are very common in all parts of the country as breakfast cereals and weaning foods (Nkama, 1993). In all these products, fermentation imparts certain attributes that give each product its specific character and quality. Availability of shelf-stable fermentation-modified maize flour that can give same attributes can reduce preparation time, increase level of hygiene and create an avenue for mass production of fermentation-modified maize flour for these and many other applications. Thus, the aim of this research was to study the impact of fermentative modification of maize grain biopolymer on functional properties, proximate composition, amino acids profile and protein quality of maize flour.

METHODOLOGY

Sample Preparations

Raw flour preparation

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. The sample flour thus obtained was sieved using a standard sieve with 300 μ m aperture and then kept in airtight polythene bags until needed.

Modified flour preparation

Natural fermentation was carried out at the prevailing ambient temperature for 48 hours for maize. Fermentation water was being discarded after every 12 hours to avoid undesirable microbial succession and the development of putrid odour. The fermented grains were decanted and dried in a solar dryer. 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 300 μ m aperture and then kept in airtight polythene bags until needed.

Determination of Functional Properties

Determination of Water Absorption Capacity (WAC)

Water Absorption Capacity was determined using the method of Sathe and Salunkhe (1981) with slight modifications. 10 ml of distilled water was 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/cm³. 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)

This was determined by the method of Sathe *et al.* (1981). Test tubes containing suspensions of 2, 4, 6, 8 up to 20% (w/v) flour in 5 ml distilled water were heated for 1 h in boiling water, followed by cooling in ice and further cooling for 2 h at 4°C. The least gelation 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)

This was determined according to the method described by Shinde (2001). 1g of flour sample was weighed accurately in triplicate and transferred to 20 ml screw capped tubes. 10 ml 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.* (1959) 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:

$$\text{Swelling power} = \frac{\text{weight of the paste}}{\text{weight of dry flour}}$$

Determination of Bulk Density

This was carried out using the procedure of Narayana and Narasinga (1984). 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:

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

Proximate Analyses and Amino Acids Profiling

Moisture Content

Moisture content of samples was determined by hot air oven drying method as recommended by AOAC (2000).

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 protein (AOAC, 2000).

Fat Content

This was carried out according to (AOAC, 2000). Diethyl ether at 50°C was used for the extraction under reflux for 5h using a Soxhlet apparatus.

Total Ash

The ash content in the sample was determined by incineration with the furnace at 550°C (AOAC, 2000) after a period of 31 - 32 h a white ash was removed and placed in a desiccator for 1 h and then weighed.

$$\text{Ash content (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100\%$$

Carbohydrate

Percentage Carbohydrate was obtained by difference (AOAC, 2000). The sum of Percentage Moisture, Fat, Protein and Ash was subtracted from 100 and the balance was recorded as percentage Carbohydrate.

Energy Value (kcal.)

Percentage Carbohydrate was obtained by difference (AOAC, 2000). The sum of Percentage Moisture, Fat, Protein and Ash was subtracted from 100 and the balance was recorded as percentage Carbohydrate.

Determination of Amino Acids

Amino Acids were profiled by the Isocratic HPLC - 2 methods (AOAC, 2000). Samples were solubilized, centrifuged and filtered through a 0.22µm membrane, and the filtrate was used for the experiment. Standard solutions of the amino acids were prepared and serially diluted to give 25pmol of each amino acid derivative. Chromatographic separation of samples was carried on a Buck scientific BLC10/11 - model HPLC equipped with UV 338nm detector.

Estimation of Protein Quality Indices

The Essential Amino Acid Index [EAAI]

was calculated using the method of Labuda *et al.* (1982) as reported by Steve (2012), according to the following equation:

$$EAAI = \sqrt[9]{\frac{[Lys \times Thr \times Val \times Met \times Ile \times Leu \times Phe \times His \times Trp]_a}{[Lys \times Thr \times Val \times Met \times Ile \times Leu \times Phe \times His \times Trp]_b}}$$

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 content of the same amino acids 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.* (1974) as used by Ogunmodimu *et al.* (2015).

$$PER = 0.06320 [X10] - 0.1539$$

Where: X10 = 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.* (2011) as a function of EAAI.

$$BV = 1.09 (EAA \text{ Index}) - 11.7.$$

Nutritional Index (NI)

The nutritional index of the food samples was calculated using the formula below as described by Crisan and Sands (1978).

$$\text{Nutritional Index}(\%) = \frac{EAAI \times \% \text{ Protein}}{100}$$

Amino Acid Score

The amino acid score was calculated using the ratio of a gram of the limiting amino acid in the food to the same amount of the corresponding amino acid in the reference diet multiplied by 100.

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

Essential-to-Total Amino Acids Ratio with Histidine and Arginine (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.

Essential-to-Total Amino Acids Ratio without Histidine and Arginine (TEAA/TAA)

This is similar to the above value only that histidine and arginine are not included in the sum of the available EAA.

Non-Essential-to-Total Amino Acids Ratio (TNEAA/TAA)

This is the percentage ratio of dispensable amino acids relative to all the amino acids found in the sample.

Total Sulphur-Containing Amino Acids Ratio (TSAA) (Meth + Cys g/100g)

This is the sum total of all the sulphur containing amino acids in the sample.

Total Aromatic Essential Amino Acids Ratio (TAEAA) (Phe + Tyr g/100g)

This expresses the total sum of the aromatic indispensable amino acids in the test material.

Essential-to-non-essential Amino Acids Ratio (TEAA/TNEAA)

This expresses the relative abundance of indispensable amino acids in relation to the non-essential ones in the sample material.

Statistical Analysis

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

RESULTS AND DISCUSSION

Table 1 shows the functional properties of the flour samples investigated which include *Water Absorption Capacity (WAC)*, *Least Gelling Concentration (LGC)*, *Gelatinization Temperature (GT)*, *Swelling Capacity (SC)* and *Bulk Density (BD)*. The influence of fermentation on these properties can be seen from the statistical analysis and from the percentage changes in the recorded values.

The results of the functional properties for the control samples compare favourably with the data recorded by Chandra and Samsheer (2013). However, fermentation did significantly reduce the functional properties of the maize flour

except for *LGC* which recorded some increase as a result of the process. It was observed that *Water Absorption Capacity (WAC)*, *Gelatinization Temperature (GT)*, *Swelling Capacity (SC)* and *Bulk Density (BD)* were all significantly reduced at $P < 0.05$ relative to the control samples. This reduction may be attributable to the transformation in the nature of the maize grain biopolymer due to the combined effects of microbial degradation and enzymatic hydrolysis during fermentation. When maize kernels are soaked in water for 1–3 days, *Corynebacterium* spp. will initiate the starch hydrolytic process to produce lactic acid and then *Aerobacter* spp. and *Lactobacillus* spp. in succession would continue the acid production (Opatpatanakit *et al.*, 1994). Such a process causes degradation of starch and soluble sugars by both intrinsic grain enzymes and enzymes of the fermenting media (Chavan and Kadam, 1989). Hydrolytic enzymes inherent in the maize kernel, like amylases contribute to the starch degradation process during the water imbibition stage just before the onset of microbial activities. α -Amylases breakdown the α -1,4 linkages of starch to give low molecular weight sugars and α -dextrins. Although the hydrolyzing effect of α -Amylases requires the starch to be gelatinized for more rapid action, they also degrade granular starch if given sufficient time as observed during cereal germination (Ojha *et al.*, 2018).

The observed -5.3% reduction in *WAC* of the fermentation-modified maize flour may be as a result of the disruption of the starch granules by the microbial and enzymatic actions which led to the solubilization of the amorphous region of the starch granules and weakened the crystalline region sufficient enough to distort the polymer network leading to a reduced ability of the modified polymer to associate with water molecules, the phenomenon that manifests itself as a reduction in *Water Absorption Capacity*. *WAC* of food materials is an indication of the relative availability of starch and fibre which is important in dietary formulation (Singh, 2001).

The -2.7% drop in the *Gelatinization Temperature (GT)* of the fermentation-modified maize flour observed in this work may be attributed to the breakdown of the shorter side chains of the amylopectin portion of the starch

granule which are shown to be important in gelatinization process (Tako *et al.*, 2014). In gelatinization process, the tetrahedral bonding capacity of water molecules is only partially involved in gelatinized starch solutions due to the higher kinetic energy of the short side chains (A and B1) of amylopectin molecules. As the shorter side chains are broken down, the higher kinetic energy of the side chains in the native starch are depleted; and the 4% starch molecules that adopt hexagonal conformation involving inter-molecular hydrogen bonding as proposed by Tako *et al.* (2014), might have increased due to the fermentative breakdown of the shorter side chains. Hence, more and more starch molecules would be able to engage larger number of water molecules into a network of tetrahedral hydrogen-bonding resulting in gelatinization at a lower temperature.

The reduction in the *Swelling Capacity (SC)* observed may be due to the fact that the grain's biopolymer is already hydrated during fermentation unlike the control samples (Narayana and Narsinga, 1982). Also, the loss of soluble compounds due the microbial degradation might have reduced the amount of water absorbing compounds that are thus unavailable for swelling thereby reducing the swelling capacity of the fermentation-modified maize flour by -10.8%. The extent to which a particular starch sample swells under given set of conditions is the *Swelling Capacity (SC)* of the starch which is important in process specification and material balance.

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 thus reduced which led to the -11.8% drop in the *Bulk Density (BD)* of the fermentation-modified maize flour observed in this work. Values of starch density recorded in literature ranges from 1.4g/cm^3 to 1.6g/cm^3 (Rooney and Pflugfelder, 1986).

A 17.9% increase in *Least Gelling Concentration (LGC)* was observed in this work which may be attributed to a decrease in “network-forming-units” in the modified starch

as a result of the enzymatic and microbial disruption of the starch amyloplast, solubilization and separation of amylose from amylopectin and the extraction of other minor granule constituents. Bemiller and Whistler (1996) explained that gel formation in cereal biopolymer involves three-dimensional network of connected molecules such as crystals or molecular aggregates/fibrils, entrapping a large volume of water in a sponge-like fashion, joined by hydrogen bonding, hydrophobic associations, ionic cross bridges, entanglements, or covalent bonds. For gelatinization to occur therefore, the starch molecules that adopt a hexagonal conformation involving intra- and inter-molecular hydrogen bonding (about 4% in raw starch) would have to engage large number of

the liquid water molecules into a “network” of tetrahedrally hydrogen-bonded water molecules (Tako *et al.*, 2014). The observed increase in *LGC* in this work may therefore be due to a decrease in the total number of “network-forming-units” that is necessary to produce the ‘cage effect’. Hence, higher concentration of the fermentation-modified maize flour was required to supply enough “network-forming-units” for gelation to take place. The lowest flour concentration at which starch gelatinization takes place is regarded as the least gelling concentration. This property is an indication of the nature of the starch and its density in a given sample which may vary from one grain to another and is important in dietary formulation.

Table 1: Functional properties of maize flours

Property	Raw Flour	Modified Flour	Percentage Change
WAC	190.33 ± 2.10 ^{adg}	180 ± 1.32 ^{afg}	-5.3
LGC	7.8 ± 1.0 ^{adg}	9.2 ± 0.76 ^{adg}	17.9
GT	62 ± 1.7 ^{adg}	60.3 ± 1.15 ^{afg}	-2.7
SC	15.8 ± 0.6 ^{adg}	14.1 ± 0.15 ^{afg}	-10.8
BD	0.51 ± 0.08 ^{adg}	0.45 ± 0.01 ^{afg}	-11.8

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

Table 2: Proximate composition of maize flours

Property	Raw Flour	Modified Flour	Percentage Change
Moisture (%)	10.5 ± 0.5 ^{adg}	10.5 ± 0.5 ^{adg}	0
Ash (%)	1.6 ± 0.4 ^{abdg}	0.8 ± 0.3 ^{abeg}	-50
Fat (%)	2.0 ± 0.2 ^{adg}	0.7 ± 0.2 ^{aeg}	-65
Protein (%)	7.0 ± 1.0 ^{adg}	10.0 ± 1.0 ^{afg}	42.9
Carbohydrate (%)	78.9 ± 1.7 ^{adg}	78.1 ± 1.4 ^{adg}	-1.0
Energy Value (Kcal)	315.6 ± 4.3 ^{abdg}	309.0 ± 1.6 ^{abdg}	-2.1

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

Table 2 below shows the result of the proximate composition analysis the energy value carried out in this work.

The result of *moisture contents* of the two flours did not show any significant difference since moisture of dried food materials is normally a function of drying conditions and the desired moisture level in the final product. The modification of the chemical structure of the flour due to fermentation did not manifest in significant difference in the drying characteristics of the flour. Hence the two flours dried under similar conditions exhibited similar final moisture contents. However, variation in variety and drying conditions can lead to significant variations in the moisture content.

Moisture values of 10.82%, 10.53% were reported in literature (Adeoti *et al.*, 2013; Adeneke *et al.*, 2014). *Percentage Ash* content observed from table 2 shows that fermentation process has significantly reduced the ash content of the maize flour by -50% compared to the control samples. This may be due to the loss of soluble matter during fermentation. Steve and Olufunmilayo (2011) reported ash contents of 0.87% for fermented corn which agrees closely with the values observed here for the fermented products.

Total Fat recorded in this work indicated a significantly reduction from that of control samples. The reduction may be due to microbial generation of several biological catalysts

including lipolytic enzymes like lipases and esterases which attack ester bonds of glycerides in the lipids of the cereal biopolymer leading to a reduction of total fat by -65% and also contributed in the development of flavour compounds (Ojha *et al.*, 2018). It was also observed that fermentation has significantly increased the *crude protein* content ($p < 0.05$) of the maize flour by more than 42% compared to the control sample. This increase may be as a result of the production of proteinaceous metabolites during fermentation including the single cell proteins themselves adding to the net value of protein in the samples (Chavan and Kadam, 1989).

The results of percentage carbohydrate and energy value showed no significant difference between the fermented and control flour samples. Although fermentation engenders carbohydrate hydrolysis and washing away of the soluble simple sugars with the fermentation liquor, this work revealed that such a reduction (-1%, for carbohydrate and -2.1% for energy value) is not statistically significant at $P < 0.05$. Since calorific values are 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 the statistically insignificant decrease in the carbohydrate content translates in similar insignificant decreases in the energy value.

The results of amino acids profiling were presented in Tables 3. The result showed that fermentative modification has improved the values of all the amino acids by different percentages. This is because fermentation process involves oxidation of carbohydrates and other minor components of cereal biopolymer to generate a range of products including organic acids, alcohol and carbon dioxide (Ray and Daeschel, 1992) which have preservative effect and a number of other desirable products including flavour compounds like diacetyl and acetaldehyde as well as vitamins, antioxidants and bioactive peptides which improve the nutritional quality of the food (Ross *et al.*, 2002).

It was observed that *tryptophan*, *methionine* and *histidine* recorded highest percentage increase of up to 100% and above as a result of fermentation. Tryptophan was increased by 142.5% followed by Histidine with 106% and

methionine was increased by 93.8%. *Leucine* and *isoleucine* contents of the modified flour were increased by 74.8% and 66.7% respectively, while *lysine* and *valine* contents were each improved by 52.9% and 48.0%. On their part, *phenylalanine* and *threonine* were the least improved amino acids with less than 10% increase. These changes can be attributed to the oxidation of carbohydrates and other minor components of the biopolymer which generates a range of metabolites with nutritional as well as preservative functions that are part of the advantages of fermentation process (Ross *et al.*, 2002). On the overall, fermentative modification of maize biopolymer has improved the total essential amino acid profile of the flour by 54.6% which is an important achievement. In a study reviewed by Boye *et al.* (2012), it was reported that fermentation increased the content of EAA in maize from 41 to 49g/100g. Specifically, *histidine*, *isoleucine*, *leucine*, *lysine* and *tryptophan* were increased by 0.8g/100g, 0.5g/100g, 1.5g/100g, 1.5g/100g and 0.12g/100g in that order. Total sulphur AA (Met & Cys) and total aromatic amino acids (Tyr & Phe) increased by 0.6 and 3.5 g/100g respectively, while Lys (0.72) and Trp (0.73) contents were improved to 0.98g/100g and 0.84g/100g respectively. However, in another study reviewed by Boye *et al.* (2012), it was reported that after maize fermentation the levels of some amino acids, notably *valine*, was reduced from 6.1 to 4.3g/100g.

The results of the calculated protein quality of the samples are presented in Table 4 and some interesting findings are observable from the table. The improvements recorded in both the essential and non-essential amino acids as a result of fermentative modification have translated into an overall improvement in the protein quality of the modified flour as calculated using recommended formulae.

Nutritional Index (NI) of maize flour was improved by about 130% which is quite remarkable considering the relative ease and the cost effective way in which the process was achieved. However the Biological Value (BV) is most remarkable as the increase of almost 3000% was recorded. Protein Efficiency Ratio (PER) was observed to have been improved by 72.4%, while the Essential Amino Acids Index (EAAI) was improved by 61.4%. Percentage Essential Amino Acid with histidine and arginine [(TEAA + His + Arg/TAA)%]

recorded 6.9% increase while the same value calculated without histidine and arginine [(TEAA/TAA)%] recorded 9%. Comparing essential to non-essential amino acids, the percentage non-essential amino acids [(TNEAA/TAA)%] recorded a drastic fall of -8.6% due the fact the fermentative modification process has increased the percentage total essential amino acids more than twice the increase in percentage total non-essential amino acids. For this reason, the Essential-to-Non-essential Amino Acids Ratio (TEAA/TNEAA) has increased by more than 18%. Total Sulphur-containing Amino Acids (TSAA) was increased by 60.6% while Total Aromatic Essential Amino Acids (TAEAA) recorded 12.4% increase. These observations of increase in protein quality

indices agree with available literature that fermentation has increased protein quality indicators as follows: true protein digestibility from 76.6% to 86.8%, protein efficiency ratio (PER) from 1.8 to 2.1 and PDCAAS from 55 to 83 % (Boye et al., 2012). Such observed increment due to fermentation is beneficial to the food industries as protein quality is related to true nitrogen and amino acid retention which is related to the capacity of the protein to provide nitrogen and indispensable amino acids (Lee et al., 2016); since it is the protein quality, not only the quantity, that exerts the true nutritional effects on body functions such as immunity, host defenses, linear growth, and the associated mental development and learning capacity (Protein Quality Workshop, 2013).

Table 3: Essential amino acids of maize flours (g/100g protein)

Amino Acid	Raw Flour	Modified Flour	Percentage Increase
Histidine	0.3 ± 0.000 ^{adg}	0.62 ± 0.014 ^{adg}	106.0
Isoleucine	0.825 ± 0.007 ^{adg}	1.375 ± 0.007 ^{adg}	66.7
Leucine	1.17 ± 0.014 ^{adg}	2.045 ± 0.007 ^{adg}	74.8
Lysine	0.87 ± 0.000 ^{adg}	1.33 ± 0.014 ^{adg}	52.9
Methionine	0.405 ± 0.007 ^{adg}	0.785 ± 0.007 ^{adg}	93.8
Phenylalanine	0.81 ± 0.014 ^{adg}	0.88 ± 0.014 ^{adg}	8.6
Threonine	0.675 ± 0.007 ^{adg}	0.705 ± 0.021 ^{adg}	4.4
Tryptophan	0.20 ± 0.014 ^{adg}	0.485 ± 0.007 ^{adg}	142.5
Valine	0.95 ± 0.014 ^{adg}	1.43 ± 0.014 ^{adg}	48.0
TEAA	6.21	9.61	54.6

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

Table 4: Protein quality and amino acids ratios of raw and modified maize flours

Property	Raw Flour	Modified Flour	Percentage Change
Nutritional Index	0.77 ^{adg}	1.77 ^{afg}	129.9
BV (%)	0.27 ^{adg}	7.61 ^{adg}	2718
PER (g/100g)	0.29 ^{adg}	0.50 ^{adg}	72.4
EAAI (%)	10.98 ^{adg}	17.72 ^{adg}	61.4
(TEAA + His + Arg/TAA)%	52.09 ^{adg}	55.68 ^{adg}	6.9
(TEAA/TAA)%	48.9 ^{adg}	53.32 ^{adg}	9.0
(TNEAA/TAA)%	51.1 ^{adg}	46.68 ^{adg}	-8.6
(TEAA/TNEAA)	0.96 ^{adg}	1.14 ^{adg}	18.8
TSAA: (Meth+Cys) (g/100g)	0.71 ^{adg}	1.14 ^{afg}	60.6
TAEAA:(Phe+Tyr) (g/100g)	1.45 ^{adg}	1.63 ^{afg}	12.4

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

Table 5: Amino Acid Score (AAS %) of raw and modified maize flours

Property	Raw Flour	Modified Flour	Percentage Change
Histidine	13.64 ^{adg}	28.18 ^{adg}	69.9
Isoleucine	15.28 ^{adg}	25.46 ^{adg}	66.6
Leucine	13.6 ^{adg}	23.78 ^{adg}	74.9
Lysine	12.43 ^{adg}	19.0 ^{adg}	52.9
Methionine	4.35 ^{adg}	8.44 ^{adg}	94.0
Phenylalanine	17.23 ^{adg}	18.72 ^{adg}	8.7
Threonine	14.36 ^{adg}	15 ^{adg}	4.5
Tryptophan	4.26 ^{adg}	10.32 ^{adg}	142.3
Valine	14.39 ^{adg}	21.67 ^{adg}	50.6

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

Table 5 below shows the results of the chemical scores of the indispensable amino acids as recorded in this work.

It was observed that of the nine essential amino acids, *tryptophan* recorded the highest percentage increase in chemical score (142.3%) as a result of fermentative modification similar to the value recorded in the increase of the amino acid itself. The increase in the chemical scores of *methionine*, *leucine*, *histidine* and *isoleucine*, in that order were 94%, 74.9%, 69.9%, and 66.6%. *Lysine* and *valine* recorded considerable increase of more than 50% in their chemical score while *phenylalanine* and *threonine* recorded the least with 8.7% and 4.5% respectively. The values of chemical score have serious implications in the food industry as regulatory bodies and health-care professionals have all acknowledged the fact that crude protein values only are no longer enough criteria for adequate human nutrition and have recognized the relevance of protein quality which is better demonstrated using the dietary indispensable amino acid score (IDF Fact Sheet, 2014).

Conclusion

Fermentative modification of maize grain biopolymer has led to appreciable changes in the functional properties, proximate composition and protein quality of the modified flour. Significant reduction was recorded in *water absorption capacity*, *swelling capacity*, *gelatinization temperature* and *bulk density* with beneficial consequences in flour storability and product formulation. However, *least gelling concentration* recorded a 17.9% increase which is attributable to a decrease in “network-

forming-units” in the modified starch as a result of enzymatic and microbial disruption of the starch amyloplast, solubilization and separation of amylose from amylopectin and the extraction of other minor granule constituents. The fermentation process also led to significantly reduction in the percentage ash and fat contents of the flours, but led to an increase in protein content by more than 42% compared to the control sample due to the production of proteinaceous metabolites during fermentation. The process however, did not significantly affect total carbohydrate content and energy value compared to control samples, although reduction of -1% and -2% respectively were observed in their values. Fermentative modification has improved the values of all the amino acids by different percentages due to oxidative degradation of the maize grain biopolymer and the generation of metabolites including flavour compounds, vitamins, and peptides with consequent improvement in the nutritional quality of the modified flour. The improvements recorded in both the essential and non-essential amino acids as a result of fermentative modification have translated into an overall improvement in the protein quality of the modified flour as well as its amino acids score. *Nutritional Index (NI)* of the modified maize flour was improved by about 130% which is quite remarkable considering the relative ease and the cost effective way in which the process was achieved. However the improvement in *Biological Value (BV)* was most remarkable as almost 3000% increase was recorded. *Protein Efficiency Ratio (PER)* was observed to have been improved by 72.4%, while the *Essential Amino Acids Index (EAAI)* was improved by 61.4%. Of the nine essential amino acids, *tryptophan* recorded the highest percentage

increase in chemical score (142.3%) as a result of fermentative modification similar to the value recorded in the increase of the amino acid itself. The chemical scores of *methionine*, *leucine*, *histidine* and *isoleucine*, in that order were 94%, 74.9%, 69.9%, and 66.6%. *Lysine* and *valine* recorded considerable increase of more than 50% in their chemical score while *phenylalanine* and *threonine* recorded the least with 8.7% and 4.5% respectively.

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