

Proximate Composition and Multi-enzyme *In vitro* Protein Digestibility of Maize-tilapia Flour Blends

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Abstract: The study aims at reducing the post harvest loss of Nile Tilapia (*Oreochromis niloticus*) in the riverine areas in Africa, by introducing the underutilized specie of fish into human food chain, to produce a high quality protein cereal gruel. *O. niloticus* were divided into 2 parts; one part fermented while the other was not. The fish samples, fermented Fish Flour (FF) and unfermented Fish Flour (UF) were introduced into fermented Maize flour (M) at various ratios to form Maize-Tilapia blends. The proximate composition of the blends were determined moisture content ranges from 59.0-95.5 g kg⁻¹, protein 140.9-160.0 g kg⁻¹, fat 62.0-64.0 g kg⁻¹ and carbohydrate 659.1-698.3 g kg⁻¹, Gross energy 15883.5-16774.8 kJ kg⁻¹. The blends were within the range prescribed by the FAO/WHO pattern for processed food. The K Na⁻¹ ratio was > 1 while the Ca/p ratio was <1 in all the blends. The hemicellulose content were between 8.05-10.20 g kg⁻¹; cellulose were 8.62-10.20 g kg⁻¹ and lignin content ranged from 0.10-0.60 g kg⁻¹. a multi enzyme digestion system consisting of trypsin, chymotrypsin and peptidase showed an improved protein digestibility in blends containing fermented Fish Flour (FF) at 15 min. with digestibility ranging from 80.1-83.7%.

Key words: Nile tilapia fish, maize, fermentation, proximate composition, protein digestibility

INTRODUCTION

Nile Tilapia (*O. niloticus*) is a widely cultured specie of fish in Africa having a world wide harvest of over 800,000 metric tons^[1]. They are often discarded in fish farms due to their tendency to over populate the pond, because of their high fecundity which results in stunted growth and hence fails to command a good market price, resulting in high post harvest loss of these specie of fish, which could have provide high quality protein if introduced into human food chain. Studies on the nutritional quality have shown Tilapia, to have a high crude protein content (57.69±2.05%), adequate amounts of all the essential amino acids and rich in calcium, phosphorus and iron^[2].

Soft porridge from cereals (either maize or sorghum) is a very popular weaning food for infants as well as breakfast cereal gruel for adults in Africa. It has a low nutritional value, the energy as well as nutrient density are further lowered with the introduction of large volume of water required during production. Efforts to improve the nutrient density in the past, has been based on fortification with legumes to boost the deficient amino acids^[3,5]. These resulted in products of variable

organoleptic properties and poor digestibility; which is attributed to the low solubility of plant protein^[6]. There is however a need to make use of the underutilized Tilapia fish for the development of highly digestible and proteinous cereal gruel thereby reducing the post harvest losses of the fish especially in the riverine areas in Africa, where the harvest is enormous.

This study reports on the proximate composition and multi-enzyme *in vitro* protein digestibility of Maize-Tilapia flour blends.

MATERIALS AND METHODS

Healthy yellow maize (*Zea mays*. L.) Seeds used were obtained from a local market in Akure, Ondo State Nigeria. The fresh Tilapia fish (*O. niloticus*) was purchased from the fish farm of the Ministry of Agriculture, Ibadan, Oyo State and transported in Frozen blocks to Food Science and Technology Laboratory of the Federal University of Technology, Akure, where it was processed.

Preparation of Flours: The healthy yellow maize seeds were processed into 'Ogi' a traditional Nigeria fermented weaning food using an improved method with greater

Table 1: Proximate composition (g kg⁻¹ DM) of Maize flour (M), Unfermented fish flour (UF) and Fermented Fish Flour (FF)

Component	MF	UF	FF
Moisture	116.9±0.30	47.5±0.20	47.6±0.3
Protein	076.3±0.28	63.7±0.18	78.7±0.1
Fat40.	400.1±0.14	02.2±0.24	06.7±0.2
Ash	016.2±0.12	07.8±0.12	04.0±0.1
Carbohydrate	867.4±0.30	18.8±0.03	03.3±0.2
Energy (kJ kg ⁻¹)	17369.900	36.00000	09.9000

Mean±standard deviation for at least 3 determinations

Table 2: Blend ratio obtained from Quattro pro 1997-statistical tools

Blends	Sample ratios		
	MF	UF	FF
M:UF ₁	6.25	1	-
M:UF ₂	8.87	1	-
M:FF ₃	6.25	-	1
M:FF ₄	9.30	-	1

protein recovery^[7]. The fresh Ogi was dried at 60°C in an oven (Gallenkamp, model OV-160) and milled in a laboratory mill into flour, sample was sieved in a 200-mm mesh sieve to obtain fermented Maize flour (M). The fresh fish samples were descaled, degutted and washed in clean water, they were steamed for 15 min. and minced. The minced fish was divided into two parts: one part, was dried in an oven (Gallenkamp, model OV-160) drier at 60°C milled in a laboratory mill and sieved with a 200-mm mesh sieve to obtain unfermented fish Flour (UF). The second minced portion was subjected to natural fermentation by spreading on a tray for 24 hours at room temperature (30±2°C) samples were then dried in an oven at 60°C and milled in a laboratory mill to obtain the fermented Fish Flour (FF). Resulting flours- M, UF and FF were packaged in airtight polythene satchets and stored in a cool (4°C) dry place till they were used in proximate analysis.

Formation of blends: Proximate composition of the resulting flours were carried out and results (Table 1) was subjected to Quattro pro 1997-statistical tool to obtain blend ratios (Table 2) with proximate composition comparable to a target reference diet "Cerelec"-a commercial cereal -based weaning product of Nestle foods (Nig) Ltd.

Proximate analyses: Moisture, Protein (N x6.25), Fat, Ash man were determined by standard procedures^[8]. Total carbohydrate was calculated by difference. All chemicals used were of analytical grade. Total energy was estimated using atwater factor (Smith and Ojofeitimi 1995). Total energy (kJ)=(Protein (g)x16.8)+(Carbohydrate (g)x16.8) + (Fat(g)x37.8). Sodium, Calcium, Magnesium and Potassium contents were determined by flame photometry (A.O.A.C1990). The concentration of Iron was determined after wet digestion with a mixture of perchloric and

nitric acid using Atomic Absorption Spectrophotometry (AAS, Model SP9, pye unicam, UK). Phosphorus was estimated colorimetrically by ammonium molybdate method. The cellulose, hemicellulose and lignin content were determined.

in vitro protein digestibility: The multi enzyme *in vitro* protein digestibility method of Hsu *et al.*,^[9] was used. The multienzyme system consist of trypsin, chymotrypsin and peptidase. The enzymatic activity was determined using sodium caseinate freshly made prior to digestibility determination.

About 1.75g of each of the blends (Table 2) were dissolved in 50ml distilled water to give 6.25mg protein per ml of sample suspension. The pH of the sample suspension was adjusted to 8.0 using 0.1m HCL or 01M NaOH. Each sample suspension was kept in water bath at 37°C with constant stirring. ml of the multienzyme solution of 1.6mg trypsin, 1.4mg peptidase and 3.1mg chymotrypsin (with adjusted pH of 8.00) was added. The pH of each of the sample suspension was recorded at 10min. and 15 min., respectively after adding the multienzyme solution.

The *in vitro* protein digestibility was calculated using equation proposed by Hsu *et al.*,^[9]

$$Y = 210.464 - 18.103 x$$

$$Y = \text{in vitro digestibility}\%$$

$$X = \text{pH of sample suspension}$$

Statistical Analysis: Data obtained were subjected to analysis of variance (ANOVA), Steel and Torrie^[10]. Where significant difference were discovered at P <0.05 mean separation was done by Duncan Multiple Range Test^[11].

RESULTS AND DISCUSSION

Blends containing unfermented fish (UF) had an average protein content of 158.6 g kg⁻¹ which was much higher than 76.3 g kg⁻¹ for Maize flour (M) Table.3. Protein ranged from 140.9 g kg⁻¹ (M:FF₄) to 160.0 g kg⁻¹. (M:UF₁). The result shows a substantial increase in protein of Maize flour (M) after blending with UF and FF, respectively, but protein content of blends are yet to conform with the minimum requirement of FAO/WHO pattern of > 167.0 g kg⁻¹, this suggest a further inclusion of other protein source. Fat content ranged from 62.0 g kg⁻¹ in M: FF₄ to 640.0 g kg⁻¹ in M: UF₂, the fat and energy value of blends were above the minimum recommendations of FAO/WHO pattern for weaning foods^[12]. The daily energy requirement

for adults is dependent on their physiological state, an average of 11,300 KJ is required daily. For an adult to

Table 3: Proximate composition (g kg⁻¹ DM) of Maize-Tilapia blends versus cerelac

Component	Maize-Tilapia blends				Cerelac	FAO/WHO Pattern
	M:UF ₁	M:UF ₂	M:FF ₁	M:FF ₄		
Moisture	095.5 ^a ±0.02	059.0 ^a ±0.01	059.90 ^a ±0.05	761.0 ^a ±0.03	50	<100.0
Protein	160.0 ^a ±0.03	157.2 ^b ±0.01	156.80 ^b ±0.02	140.9 ^a ±0.01	160	<167.0
Fat	062.4 ^b ±0.00	064.0 ^a ±0.03	063.80 ^a ±0.01	062.0 ^b ±0.02	90	>060.0
Ash	023.0 ^a ±0.05	022.5 ^a ±0.01	022.60 ^a ±0.04	022.7 ^a ±0.01	23	-
Carbohydrate	659.1 ^b ±0.01	697.3 ^a ±0.00	697.80 ^a ±0.01	698.3 ^a ±0.02	637	-
Gross energy (kJ kg ⁻¹)	16119.6	16774.8	16768.9	15883.5	167	>15750.0

Value in a row denoted by different superscripts differs significantly, Mean±S.E for 3 determinations

Table 4: Mineral composition of Maize-Tilapia blends (mg kg⁻¹DM) mineral composition

Blends	Na	K	K Na ⁻¹	ratioCa	P	Ca/P	ratioFe	Mg
M: UF ₁	50.8	262.3	5.2	40.2	160.0	0.3	3.8	86.1
M: UF ₂	50.0	278.3	5.6	41.0	179.2	0.2	3.6	89.1
M: FF ₁	48.0	255.6	5.3	40.0	179.8	0.2	4.0	90.0
M: FF ₄	49.0	260.2	5.4	40.6	180.3	0.2	4.1	90.0

Table 5: Hemicellulose, cellulose and lignin content of Maize-Tilapia blends (g kg⁻¹ DM)

Blends	Hemicellulose	Cellulose	Lignin
M: UF ₁	08.50	08.60	0.10
M: UF ₂	10.20	09.25	0.14
M: FF ₁	08.05	09.30	0.12
M: FF ₄	09.25	10.20	0.16

Table 6: Protein digestibility of Maize-Tilapia blends at different pH

Blends	pH		Protein digestibility%	
	10 min	15 min	10 min	15 min
M: UF ₁	7.8	7.8	69.36	74.7
M: UF ₂	7.9	7.8	67.50	69.3
M: FF ₁	7.5	7.2	74.70	80.1
M: FF ₄	7.3	7.0	78.30	83.7

meet the minimum daily requirement, about 701g of M:UF₁, 673g of M:UF₂, 673 g of M:FF₁, and 711 g of M:FF₄, would be required^[13,14].

The blends are rich in Na, K, Ca, P and Mg. The level of potassium present is higher. The K Na⁻¹ ratio of the blends ranged between 5.16-5.57, these values are greater than the recommended value (1)^[15], consumption could therefore be accompanied with NaCl to enhance balance of body fluid as consumption without salting may lead to mineral imbalance in those fed solely on it^[16]. The Ca and P in the blends are much lower than the recommended levels of infants and young children Passmore *et al.*^[17], this may be necessary if these blends are to be fed to these groups of children (Table 4).

Table 5 shows the partially nutritive (cellulose and hemicellulose) and non-nutritive (lignin) components of the blend formulations. Blends are less fibrous hence substantial amounts of the nutrients are contained in the form of cellular matter (i.e the nutritive components). The partially nutritive component, the cell wall carbohydrate,

was fractionated into cellulose and hemicellulose, these ranges from 10.20-8.62 g kg⁻¹ and 8.05-10.20 g kg⁻¹, respectively, these increases, the bioavailability of the nutrients by consumers.

in vitro Protein Digestibility (IVPD) (Table 6) shows are marked improvement in the digestibility by adding Fermented Fish (FF) to the Maize flour (M). This observation was more pronounced at 15 min. ranging from 80.1%-83.7%, depicting IVPD as a function of time in a multi enzyme system. Higher digestibility observed in blends containing Fermented Fish (FF) could be attributed to the effect of fermentation on digestibility. Fermentation improves digestibility of protein by destroying protease inhibitor and opening the protein structure through denaturation^[9]. Maize-Tilapia blends containing Fermented Fish (FF) would be preferred since it's digestibility is highest.

CONCLUSIONS

The results shown in this study demonstrate that there is a potential for using under-utilized Tilapia fish to make nutritionally adequate foods for both infants and adults in riverrine areas of developing countries where they are often discarded there by reducing post harvest losses.

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