

EFFECTS OF GERMINATION AND FERMENTATION ON THE QUALITY CHARACTERISTICS OF MAIZE/MUSHROOM BASED FORMULATION

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ABSTRACT

Maize grains were divided into four treatment groups, namely: germinated-fermented maize (GFM), germinated non-fermented maize (GNFNM), non-germinated fermented maize (NGFM) and non-germinated non-fermented maize (NGNM). Maize and mushroom (MR) based products (GFMR, GFNMR, NGFMR and NGNMR) from all four maize grains were formulated. Germination and fermentation were investigated as methods of improving the nutritional and organoleptic properties of the formulations. Inocula recycling (use of 50% fermenting mixture as starter) resulted in a pH reduction from 5.99 to 3.30 in non-germinated products and from 5.88 to 3.29 in germinated samples during fermentation. The increase in titratable acidity (expressed as g lactic acid/100g sample) from 0.14 to 0.17 in non-germinated products and from 0.14 to 0.18 in germinated samples was not significant ($p < 0.05$). The crude protein values ranged from 16.0g/100g for the NGFMR to 16.3g/100g for GNFMR product. The GNFMR had the lowest carbohydrate content (60.8g/100g) while the GFMR had the highest value of 66.0g/100g. No significant difference ($p < 0.05$) was obtained in the variation of the ash content between 5.2g/100g for GFMR to 5.4g/100g for GNFMR and NGFMR had the highest energy value of 340KJ/100g while the GNFMR had the lowest (336.8 KJ/100g). Germination resulted in a significant ($p < 0.5$) increase in digestibility. A combination of germination and fermentation further improved protein digestibility (%). The PER of the germinated and fermented product (1.87) was significantly ($p < 0.5$) higher than that of the non-germinated non-fermented product (PER=0.82). Sensory scores for flavor ranged from 3.30 out of 7 (fair) for GFMR to 4.7 (fairly good) for GNFMR. The GNFMR was characterized as having a sweet taste while the GFMR and NGFMR were characterized as having fairly sour flavors. The appearance score of 5.20 (pleasant) for GNFMR was significantly ($p < 0.05$) higher compared with the other products. The product made from GNFMR had the highest score of 5.50 (like very much) while NGFMR had a value of 3.70 (neither like nor dislike) for overall acceptability. Germination and/or fermentation of maize with added mushroom have been used to formulate acceptable products which could be used as a weaning food. The addition of mushroom implies that the final products have potential for improved protein quality beneficial to its consumers.

Key words: Fermentation, germination, maize/mushroom, porridge

INTRODUCTION

Maize is widely grown and consumed in Nigeria as one of the staple food commodities. Various kinds of food products are obtained through processing maize. On average, maize grains contain about 8.06g crude protein / 100g, 3.94g ether extract /100g, 1.40g ash /100g and 73.4g carbohydrates /100g while mushroom contains about 26.0g crude protein /100g: ether extract /100g: 6.0g ash /100g and 19.3g carbohydrates /100g [1, 2]. Studies by Oei [2] indicated that edible mushrooms in general, can furnish most of the essential amino acids needed in human diets. They are particularly high in lysine (5.0mg /100g dry weight) along with the sulphur containing amino acids [3]. Maize is low in the sulphur amino acids. The quality of a protein is primarily a function of its essential amino acid composition. Therefore, on the basis of complementarity, the combination of maize and mushroom in food formulations could adequately provide most of the essential amino acids needed in human diets.

In Nigeria edible mushrooms are mainly eaten as a delicacy and as a substitute for meat and fish in rural areas. Some are used for medicinal purposes; for example, *Pleurotus tubberegium* is used in ethno-medicine among the Igbos [4]. There is a growing awareness of this edible fungus because of its ease of cultivation on simple substrata, both on the industrial and home scale. Maize utilization in developing countries cannot be over emphasized. The presence of anti-nutritional factors (ANFS) such as tannins and phytate reduces the nutritional quality of maize in addition to its low quality of amino acids. This has led to adaptation of local fermentation methods for ANFS reduction [5, 6,]. Nout *et al.* [7, 8] achieved a natural selection of lactic acid bacteria in fermenting mixtures of cereals tubers, legumes and water by repetitive fermentation.

While there is abundant information on the effects of germination and/or fermentation on the nutritional and sensory characteristics of maize products, such information on maize and mushroom based food formulation is lacking. The objectives of this study were to formulate various maize and mushroom- based food products and evaluate their nutritional and sensory properties using germination and/or a fermentation process.

METHODOLOGY

Materials

Maize (*TZRS-W*) grains at about 10% moisture content were purchased from Modern Market, Makurdi, Nigeria. The mushroom (*Pleurotus ostreatus*) was cultivated (grown) indoors using rice straw (Figure 1). Corn starch, and multivite (a multivitamin mix containing: vitamin A, 100 IU; vitamin B1, 2.5mg; vitamin B2, 0.5mg; vitamin B6, 0.25mg; vitamin B12, 2.5mg; nicotinamide, 10mg; vitamin C, 30mg and vitamin D, 250 IU; Evans Medical Plc, Lagos, Nigeria) were purchased from a local supermarket in Makurdi. Rice husks were obtained from a local mill in

Makurdi. Twenty-four 3-week-old weanling Wistar albino male rats of 65-66g body weights were purchased from Veterinary Research Institute, Vom, Nigeria.

Sample preparation

Maize grains were manually sorted and winnowed to remove stones, debris and defective grains. The cleaned grains were packaged in 10L plastic buckets and covered with lids. Harvested mushrooms were dried in an air draft oven at 100⁰C and milled into flour (R) using a hammer mill (Brook Crompton 2000, Christy Hunt Agricultural ltd, England) Rice husks were dried in an air draft electric oven (Model T12H, Genlab Widnes, UK) at 100⁰C to a constant weight followed by sieving to pass through 0.2 mm mesh. The husks were then packaged in polyethylene bags of 0.28 mm thickness and sealed with an electric impulse sealer (Model 210-8, TEW Heating Equipment Co., Clamco Corp., Cleveland, OH). Cleaned maize grains were divided into four treatment groups, namely: germinated, fermented maize (GFM); germinated, non-fermented maize (GNFNM); non-germinated, fermented maize (NGFM) and non-germinated, non-fermented maize (NGNM) (Figure 3). All the prepared samples were stored in a refrigerator at 8±2⁰C and used for product formulation within 2 weeks.

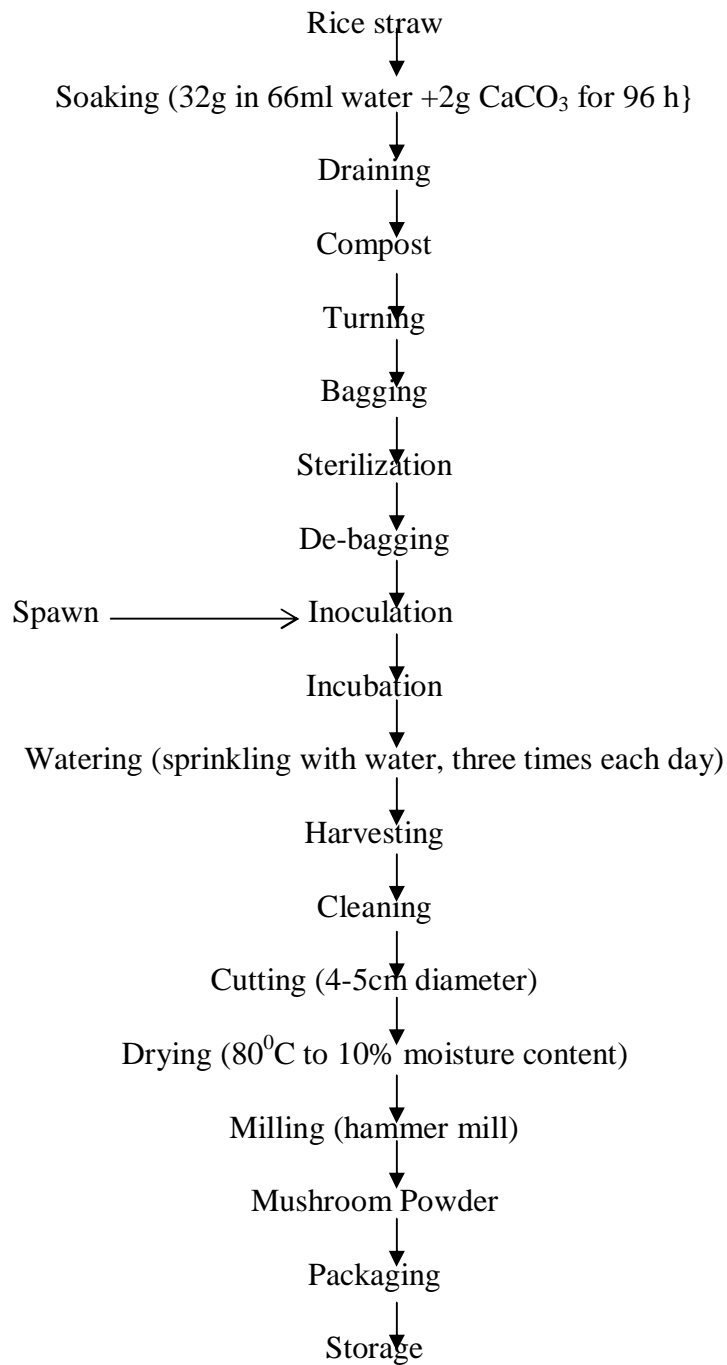


Figure 1: Flow diagram for mushroom (*Pleurotus ostreatus*) horticulture and production of its powder

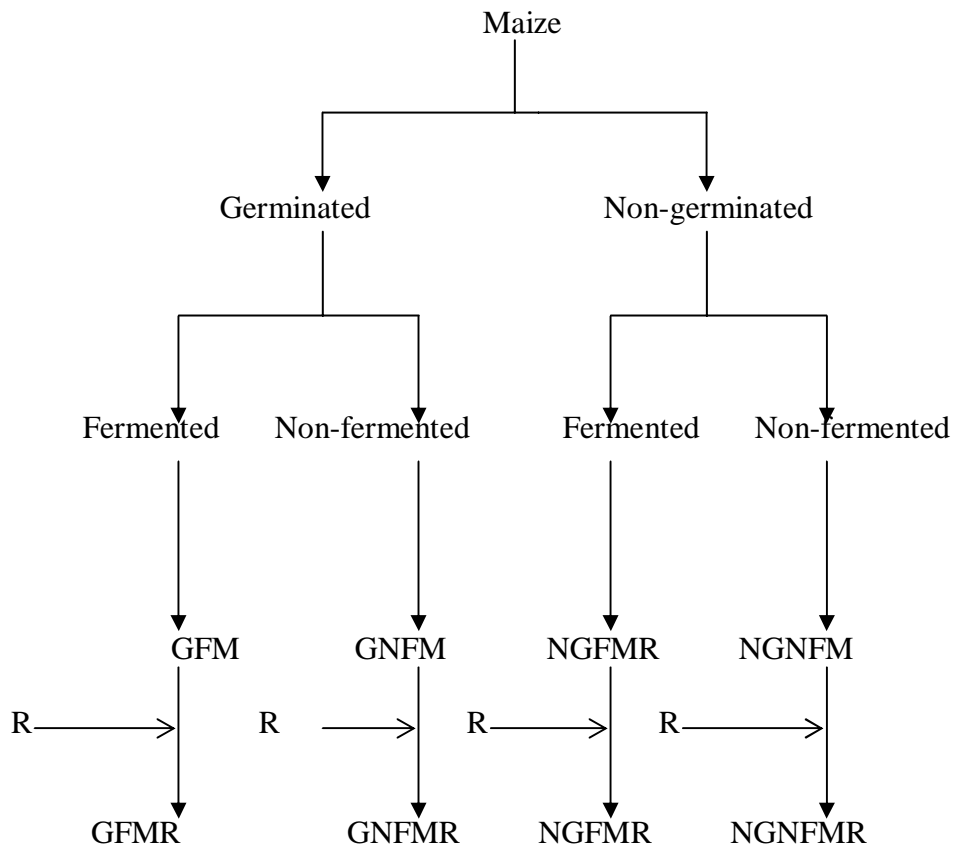


Figure 2: Flow diagram for the production of various maize + mushroom Products

Figure legend:

GFMR= Germinated, fermented maize + Mushroom; GNFM= Germinated, non-fermented maize + Mushroom; NGFMR= Non-germinated, fermented maize + mushroom; NGNFM= Non-germinated, non-fermented maize + Mushroom and R= Mushroom (*Pleurotus ostreatus*)

Germination

The optimal conditions for steeping and germination of the maize were determined by adopting the method of Malleshi *et al.* [9]. The maize grains were washed with tap water followed by steeping at room temperature ($30 \pm 2^{\circ}\text{C}$) in plastic buckets. The steep water was changed after every 4 hours. Germination was achieved by layering the seeds on moistened jute bags placed in a wooden dark cupboard for 72 hours. The germinated grains were dried to 10% moisture in an electric oven at 100°C and milled.

Fermentation

About 120g flour from the germinated or non-germinated grain was mixed with 80ml of distilled water in a 500ml beaker, covered and allowed to undergo natural fermentation at room temperature ($30 \pm 2^{\circ}\text{C}$). Fermentation was accelerated by adding 50% fermenting mixture as starter to fresh concentrate at 2 hours intervals over a period of 4 days when the pH stabilized. The fermented concentrates were dehydrated in an air draft oven at 80°C to 10 % moisture content to obtain GFM, and NGFM, respectively. The non-fermented controls designated as GNFM and NGNFM were also produced.

Product formulation

Material balancing was used to compute the amount of each maize and mushroom powder required to obtain 16g protein/100g from their respective proximate composition to obtain four blends of maize and mushroom powder consisting non-germinated, non-fermented maize + mushroom [NGNFM], germinated, non-fermented maize + mushroom (GNFM), non-germinated, fermented maize + mushroom (NGFM) and germinated fermented maize mushroom (GFMR) in accordance with material balancing described by Toledo [10]. In this method, the amount of ingredients required in the final product is obtained by calculation based on pre-determined percentage composition.

Chemical determinations

Proximate composition

Standard procedures for moisture content, ash, crude fiber, crude fat, and protein were used for determination of proximate compositions [11]. Carbohydrates were calculated by difference. Energy values were estimated by the Atwater factor method (4 x g protein, 9 x g fat, and 4 x g carbohydrates). Food grade analytical chemicals obtained from ZAYO international Ltd were used for the analyses.

Feeding trials

The animal feeding experiments were performed using weanling wistar male rats following a modification of the method described by Pellet & Young [12]. Instead of one animal per cage, 20 animals were randomly distributed to five metallic wire mesh cages with 4 animals per cage. Approximately 62.5g of each of the formulated maize/mushroom blends and Nutrend (Manufactured by Nestle foods, Nigeria) were blended with 37.5g of a formulated basal diet to give 10g protein/100g of each test diet. The basal diet (protein free diet) consisted of cornstarch, 80g/100g; corn oil,

10g/100g: rice husks, 5g/100g, common table salt, 4g/100g and vitamin mix, 1g/100g (Supplied by ZAYO international Ltd).

Each animal group was fed one of the test diets while the fifth group was fed the basal diet. Food and water were given *ad libitum*. The feed intake and weight of the rats in the various groups were recorded at 3 day intervals over a period of 28 days. Plastic containers were placed underneath the feeding troughs to collect and account for spillages. The weight gain (g) and food intake (g) for each group were used for estimating net protein ratio (NPR) and protein efficiency ratio (PER) after 14 and 28 days of feeding, respectively.

$$\text{NPR} = \frac{\text{Weight gain of test animals on a given diet} + \text{Weight loss of animals on basal diet}}{\text{Protein consumed by test animals on the given diet}}$$

$$\text{PER} = \frac{\text{Weight gain of test animals on a given diet}}{\text{Protein consumed by the test animals}}$$

The relative PER (R-PER) and relative NPR (R-NPR) values were obtained by relating the PER and NPR values, respectively to those of the Animal Nutrition Research Council (ANRC) casein which are 2.5 for PER [13] and 4.02 for NPR [14].

R-PER and R-NPR were calculated using:

$$\text{R-PER} = \frac{\text{PER of diet}}{\text{PER of ANCR- casein}}$$

and

$$\text{R-NPR} = \frac{\text{NPR of diet}}{\text{NPR of ANCR- casein.}}$$

Sensory evaluation

Sensory evaluation was performed using descriptive analysis and effective testing [15]. The panelists consisted of 20 mothers familiar with commercial weaning foods. They were staff members and graduate students of the University of Agriculture, Makurdi, Nigeria. A 7-point unstructured descriptive scale (1=very poor, 7 = excellent) was used to rate the sensory attributes of appearance and flavor. The panelists (semi-trained) were asked to identify flavor as rancid, putrid, beany, sour, sweet or other. A 7-point structured hedonic scale (1 – dislike extremely, 7 = like extremely) was used to score overall acceptability of the products. Each attribute was evaluated separately (at a time) at daily intervals between 10.00 a.m. - 11.00 a.m from

Monday to Friday. The assessments were conducted under fluorescent light in a special room with cubicles (separate partitions for each judge) for sensory evaluations. At each session, each panelist judged five samples. The order of presentation of the samples to the panel was randomized [15].

The formulated products were each cooked with distilled water (1:20w/v) by boiling and stirring with a glass rod to prevent sticking and lump formation for 10 min into smooth gruels. The porridges were stored in insulated 2L food flasks (Eleganza, Nigeria Plc, Lagos) from which they were served to the panelists. The gruels were served in 100ml colourless transparent plastic cups coded with 4-digit random numbers. About 50ml of each sample was served hot (70-80⁰C). Colourless transparent plastic spoons were provided for testing the samples. Fresh tap water was provided to rinse the mouth between evaluations. The familiarity of panel members with commercial weaning foods and the added training (semi-trained panelists) served as control to calibrate the panel.

Statistical analysis

Significant ($P < 0.05$) differences in chemical composition, *in vivo* protein digestibility, PER, NPR and sensory attributes were determined by analysis of variance [16]. Duncan's multiple range and Tukey's tests [15, 16] were used for separating the means.

RESULTS

Changes in pH and titratable acidity

Variations in pH and titratable acidity of the maize mushroom products during accelerated natural fermentation are shown in Table 1. Inocula recycling resulted in a pH reduction from 5.99 to 3.30 in non-germinated products and from 5.88 to 3.29 in germinated samples during fermentation. The increase in the titratable acidity (expressed as g lactic acid/100 g sample) from 0.14 to 0.17 in non-germinated products and from 0.14 to 0.18 in germinated samples was not significant ($p < 0.05$).

Proximate composition

The data on the energy values along with proximate composition of the various maize mushroom based formulated food products are shown in Table 2. No significant difference ($p < 0.05$) was observed in the crude protein values, which ranged from 16.0g/100g for the NGFMR to 16.3g/100 for NGNFMR product. The GNFMR had the lowest carbohydrate content (60.8g/100g) while the GFMR had the highest value of 66.0g/100g. The ash content varied between 5.2g/100 for GFMR to 5.4g/100g for GNFMR and NGFMR has the highest energy value of 340KJ/100 g while the NGNFMR had the lowest (336.80KJ/100 g).

Apparent digestibility

The effects of germination and fermentation on *in vivo* protein digestibility are shown in Table 3. Germination resulted in a significant ($p < 0.05$) increase in digestibility. A combination of germination and fermentation further improved protein digestibility.

For example, the GFMR had apparent digestibility of 88% which was found to be significantly ($p < 0.05$) higher than the 64% for NGNFMR.

***In vivo* protein quality evaluation**

The PER and NPR values are shown in Table 3. The PER of the germinated and fermented product (1.87) was significantly ($p < 0.5$) higher than that of the non-germinated non-fermented product (PER=0.82). Germination and fermentation resulted in a higher NPR of 2.83 compared to 2.35 for NGNFMR. The PER indicates the relationship between weight gain in the test animals and the corresponding protein intake, while NPR relates the weight changes in the animals fed the test diet to those fed the basal diet.

Sensory properties

Table 4 shows the sensory scores for porridges made of maize/mushroom and the control formulation. Flavor scores ranged from 3.30 out of 7 (fair) for GFMR 4.7 (fairly good) for GNFMR. The GNFMR was characterized as having a sweet taste while the GFMR and NGFMR were characterized as having fairly sour flavor. However there is a significantly lower score for appearance of the fermented products (4.0 and 4.30) than the non-fermented products (5.0 and 5.20).

The porridge made from GNFMR had the highest score of 5.50 (like very much) while NGFMR had a value of 3.70 (neither like nor dislike) for overall acceptability.

DISCUSSION

Lactic acid is used as an index of activity for certain bacteria such as: *Streptococcus*, *Leuconostoc* and *Lactobacillus* species, which were isolated from the maize/mushroom products [17, 18]. These microorganisms produce lactic acid in considerable amounts and are used in the manufacture of acid foods [16]. Pure cultures of these bacteria are added to the raw material or the naturally occurring bacteria are encouraged to multiply as is done with accelerated fermentations [18]. The observed increase in titratable acidity (Table 1) is likely the result of this fermentation. These observations are in conformity with earlier studies by Nout *et al.* [7]. The lower pH and higher acidity observed in the germinated meal could be due to the availability of more readily naturally fermentable sugars as germination proceeds.

The lower protein, carbohydrates, ether extracts and energy values of the fermented products (Table 2) could be due to use of the nutrients by microorganisms for growth. Earlier workers [7, 8, 19, 20] reported an increase in vitamin content as one of the nutritional benefits of germination and fermentation processes. The daily protein requirement of a 10kg infant is about 15g [21]. The PERs of GFMR, GNFMR, NGFMR (Table 3) were lower than the value of 2.1 recommended by the Protein Advisory Group (PAG) for weaning foods PAG [22].

The increase in protein digestibility as shown in Table 3 could be due to processing and other factors playing significant roles in improving protein digestion in the

products. Cooking with water or moist heat, for example, tends to soften and break down indigestible fibres, generally increases digestibility of nutrients and phytochemicals. Fermentation has also been identified to significantly improve the nutritional value of maize-based foods and as well reduce their anti-nutritional factors (23, 24).

Comparison of the PER and NPR values of the test diets with the corresponding literature values for ANRC-casein resulted in the R-PER and R-NPR data, as also shown in Table 3. The R-PER and R-NPR values, respectively, ranged between 0.75-0.33 and 0.70-0.58 with the NGNFSB being significantly ($p < 0.05$) lower than the GFSB at upper levels of each of the parameters. The higher protein digestibility, PER and NPR values observed in the germinated and fermented products could be due to enzymatic degradation of protein and carbohydrate macromolecules into smaller units, thereby, increasing the surface area of the substance for a facilitated digestion and subsequent absorption by the weaning animals. These observations were consistent with earlier reports of significant increases in PER in rats as a result of germination and fermentation of cereals and legumes [9, 19, 20, 23].

It is noteworthy that although the fermented products had lower scores for flavor, they were still acceptable to the panelists probably because sour gruels are common in local food preferences. Spontaneous lactic acid fermentation is widely applied in the processing of cereals for the preparation of a wide variety of dishes in Africa and it contributes to the development of acceptable texture, flavour and improves the safety of foods [24].

The results suggest that the germinated products have certain advantages over the non-germinated ones; weight gain in rats was higher, and the sensory evaluation was better. There is some trade off in that weight gain in rats was highest in the germinated, fermented

variety; but the sensory properties of the germinated, non-fermented was better.

CONCLUSION

Acceptable protein quality products, which could be used as a weaning food, can be formulated from maize and mushrooms using germination and/or fermentation technologies. Fermentation and germination increased the protein quality of the product. The sensory properties of porridges from flours of the germinated seeds are superior to those from the fermented flours.

The use of an oven dryer for dehydration of the fermented concentrates due to limitation of equipment available exposed the products to unduly long periods of drying which could have affected their nutritional and organoleptic properties. The use of a drum dryer with its attendant advantage of a high temperature and short time (HTST) processing could potentially further improve the nutritional and sensory properties of the fermented maize mushroom food formulations.

Table 1: Changes* in pH and titratable acidity with fermentation times of concentrates of germinated and non-germinated maize

Parameter	Maize concentrate	Fermentation time (days)					
		0	1	2	3	4	5
pH	Germinated	5.88±0.01	4.30±0.8	3.92±0.02	3.28±0.09	3.28±0.03	3.27±0.01
	Non-germinated	5.99±0.10	4.71±0.06	4.01±0.04	3.30±0.01	3.30±0.01	3.30±0.01
lactic acid (g/100g)	Germinated	0.14±0.12	0.16±0.09	0.17±0.03	0.18±0.01	0.18±0.01	0.18±0.01
	Non-germinated	0.14±0.03	0.16±0.10	0.17±0.02	0.17±0.01	0.17±0.01	0.17±0.01

*Values are means and standard deviations from duplicate determinations

Table 2: Proximate compositions, and energy values* of various maize/mushroom*¹ formulations

Parameter (g/100g)	Product			
	GFMR	GNFMR	NGFMR	NGNFMR
Moisture	9.8±0.2 ^a	9.8±0.2 ^a	9.7±0.6 ^a	10.4±0.8 ^a
Dry Matter	89.0±1.0 ^a	89.5±1.2 ^a	89.5±1.4 ^a	90.0±1.6 ^a
Crude protein	16.2±0.8 ^a	16.1±0.4 ^a	16.0±1.4 ^a	16.3±1.2 ^a
Ether extract	8.6±2.8 ^a	9.6±3.0 ^b	8.4±3.2 ^a	9.4±3.4 ^b
Ash	5.2±0.5 ^a	5.3±0.7 ^a	5.4±0.3 ^a	5.4±1.0 ^a
Fiber	4.2±0.1 ^a	4.1±0.2 ^a	4.5±0.1 ^a	3.9±0.2 ^b
Carbohydrate	66.0± ^a	60.8 ^b	65.3 ^a	64.5 ^a
Energy (KJ/100g)	363.2± ^a	366.0 ^a	358.8 ^a	360.8 ^a

*¹ (*Pleurotus ostreatus*) = variety of mushroom

*Values are mean ± standard deviation of duplicate determinations

Values with common superscript letter in each row are not significantly
 (P>0.05) different

Key:

GFMR= germinate-fermented maize mushroom; GNFMR= germinated-non
 fermented maize mushroom; NGFMR= non-germinated, fermented maize mushroom;
 NGNFMR non- germinated, non fermented maize mushroom food formulations.

Table 3: Nutritional indices of maize and mushroom*¹ based formulated food products

Nutritional Index	Product			
	NGNFMR	GNFMR	NGFMR	GFMR
PER	0.83±0.01 ^a	1.06±0.01 ^b	1.12±0.04 ^b	1.87±0.04 ^b
R-PER	0.33±0.02 ^a	0.42±0.03 ^a	0.45±0.02 ^a	0.75±0.02 ^b
NPR	2.35±0.02 ^a	2.60±0.01 ^b	2.50±0.01 ^b	2.83±0.02 ^c
R-NPR	0.58±0.01 ^a	0.65±0.04 ^b	0.64±0.03 ^b	0.70±0.04 ^b
Apparent Digestibility (%)	64±0.34 ^a	70±1.2 ^b	75±0.09 ^c	88±1.11 ^d

*¹ (*Pleurotus ostreatus*) = variety of mushroom

Values are mean ± standard deviation of duplicate determinations

Values with common superscript letter in each row are not significantly (P>0.05) different

Key:

GFMR= germinated, fermented maize mushroom GNFMR= germinated, non-fermented maize mushroom; NGFMR= non-germinated, fermented maize mushroom; NGNFMR non- germinated, non-fermented maize mushroom food formulations
 PER= Protein Efficiency Ratio, NPR= Net Protein Ratio, R= Relative

Table 4: Sensory scores* for various maize mushroom based food formulations

Attribute	Product			
	GFMR	GNFMR	NGFMR	NGNFMR
Flavor	3.30±0.73 ^a	4.70±0.74 ^b	3.50±0.53 ^a	3.30±0.52 ^a
Appearance	4.00±0.74 ^a	5.00±0.63 ^b	4.30±0.67 ^a	5.20±0.72 ^b
Overall Acceptability	4.80±0.70 ^a	5.50±0.72 ^b	3.70±0.72 ^c	3.80±0.71 ^c

*Each result is the mean score of 20 panelists based on a hedonic scale where, 1= dislike extremely and 7= like extremely. Highest possible score =7
 Values with common superscript letters in each row are not significantly (p>0.05) different.

Key:

GFMR=germinated, fermented maize mushroom GNFMR= germinated, non-fermented maize mushroom NGFMR= non-germinated, non-fermented maize mushroom, NGNFMR= non- germinated, non-fermented maize mushroom food formulations

- The GFMR and NGFMR were characterized as having fairly sour flavour

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