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ORIGINAL ARTICLE

In Vivo Glycemic Index Evaluation and Characterization of Maize and Kidney Bean Composite Flour

[1]Onyebuchi, Ferdinand Martina / [2*]Ochelle, Paul Ohini / [2]Ekeh, Mike Ojotu /

Authors' Affiliation

[1]Department of Veterinary Biochemistry, Federal University of Jos, Plateau State, Nigeria

[2]Department of Food Science and Technology, Federal University of Agriculture, Makurdi, Nigeria

Corresponding author

Ochelle, Paul Ohini

Department of Food Science and Technology, Federal University of Agriculture, Makurdi, Nigeria

Email: ochelleogbu1989@gmail.com

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Abstract

In vivo glycemic index using maize and kidney bean composite flour was carried out by applying cut-off value of greater than seventy (>70) to denote a high glycemic foods, 55-69 indicating medium glycemic foods and (<55) and below indicating low glycemic foods. Maize and kidney bean flours were obtained using the ratios A= (100 maize flour as control), B= (90 MF and 10% KBF), C= (85 % MF and 15% KBF), D= (80% MF and 20% KBF) and E= (75 % MF and 25% KBF). Products were analyzed for glycemic index using albino rats, full Amino acid profile, mineral and vitamin analysis. The glycemic analysis showed a decreased in the fortified samples against the control as the level of kidney bean flour substitution increased over time. There was also a considerable increase in the chemical analysis as the level of kidney bean flour increased. The chemical analysis showed increment in their respective ratios as the level of substitution increased. Results reviewed the problem of poor malnutrition in developing countries and provide a balanced meal.

Practical Application

Glycemic index (GI) is a measure of the blood glucose-raising potential of a carbohydrate-containing food after it is eaten. Substitution of kidney beans with maize flour can be used to improve the nutrient composition, blood glucose level for diabetic patients and other quality attribute as the fortified samples showed a positive effect on the reduction of blood glucose level. The results of the study also showed maize and kidney bean flours will help to reduce incidences of protein energy malnutrition in the world especially in developing countries.

Keyword: Maize flour, Kidney bean flour, glycemic index. Amino Acids.

1. Introduction

Maize just like other cereals is low in essential amino acids such as lysine, tryptophan and threonine, lysine being the most limiting and in protein fraction such as albumins (Ezeogu *et al.*, 2008). Maize is the common food product that is eaten in the family of carbohydrate after rice and wheat; it could be eaten roasted or boiled (Hugo, 2010, Olanipekun *et al.*, 2015; Adeyeye *et al.*, 2017)

Maize Tuwo is one of the food products that can be obtained from Maize in Nigeria.

It is essentially a food gel or dumpling which is stiff, has a yield value and can be moulded into shapes (Muller, 1970). However, the utilization of Tuwo and maize generally is limited by its extremely low protein content and so the consumption of its products has been implicated in malnutrition. Kidney beans, is a member of the species phaseolus vulgaris, have a long history in medicinal use and consumption worldwide also known as "common bean". Kidney beans are popular additions to various cuisines due to their high protein content and delicacy, along with the presence of some antioxidants, minerals and polyphenols. Kidney bean grown in subtropical regions of the world and are a staple food source for animals and human (Rycroft *et al.*, 2001).

Kidney bean are rich in protein; one cup of boiled kidney bean (177 g) contains approximately 15 g of protein, accounting for 27% of the total caloric content. Generally, the nutritional quality of bean is lower than in animal proteins.

Starch is predominantly made up of a long chains of glucose, called amylose and amylopectin. Bean have a relatively high proportion of amylose (30-40%) compared to most other dietary sources of starch. Amylose is not easily digestible as amylopectin (Thoma, Thompson & Jenkins, 1983). For this reason, bean starch is a slow-release carbohydrate, its digestion takes longer and it causes lower and more gradual rise in blood sugar than other types of starch, making kidney bean particularly beneficial for people with diabetes. It also rank very low on the glycemic index.

Resistant starch offers an exciting new potential as a food ingredient, it has been shown to possess physiological benefits similar to soluble fibers, to be used as a mechanism for sustained glucose release. Resistant starch has been defined as the fraction of starch, which escapes digestion in the large intestine (Englyst *et al.*, 1992). Several observational studies indicate that eating beans, or other foods that are low on the glycemic index, may cut the risk of becoming diabetic. These concepts are used to provide patients with diabetes in choosing foods. Staple foods from cereals however are not commonly used by patients due to their high glycemic index/glycemic load. As a result, supplementation in order to make this food type beneficial to diabetic patients have been explored. The aim of this study was to evaluate the invivo glycemic index, amino acid profile, mineral and vitamin analysis of maize and kidney bean composite flour.

2. Materials and Methods

Kidney beans and maize samples were collected randomly from the market in Plateau state during the period under review and the flour were prepared for the various analyses

2.1. Preparation of Maize and Kidney bean flour

The method described by Bolade *et al.* (2002) was used to prepare both flours. Picking out stones and some debris, was followed by grinding to homogenization using pestle and mortar and was passed through 250 mm sieve clothe to obtain the flour. Flour samples for chemical analysis were kept in air tight plastic bottles at 260 °C in the laboratory for the various analysis.

2.2. Preparation of Maize flour

The flow chart for the preparation of maize flour is showed in Figure 1.

2.3. Blend formulation for maize and kidney beans composite flours

The procedure for the preparation of the composite flour is showed in Figure 2.

2.4. Analyses

2.4.1. Effect of resistant starch on glycemic index/glucose of maize flour by Rat Bioassay

30 Albino rats were divided into six (6) groups of 5. Four different flour blends and control were prepared and used for animal feeding

experiments. The experimental rats were housed in a wire cages at room temperature and kept

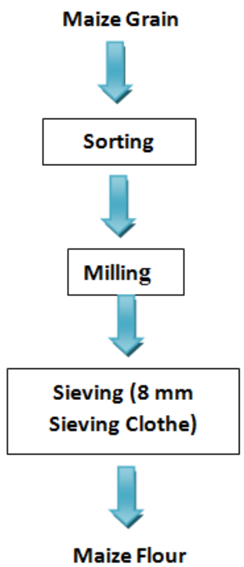

Figure 1: Flow chart for the production of maize flour. Source: (Bolade *et al.*, 2002)

under hygienic condition and fed according to the modified method of (Etuk *et al.*, 2010). After fasted overnight for 12 hours. The blood glucose of the rats were measured every day. Blood were collected from tails of rats, tails were embedded in 450 °C water bath and about one millimeter of its end was cut and a drop of blood was used for blood glucose test with the help of Acu Check Active Gluconometer (Roche Switzerland).

-Group (1): Control negative; rats fed on maize flour only

-Group (2): Rats fed on diet containing (90% maize flour and 10% kidney beans)

-Group (3): Rats fed on diet containing (85% maize flour and 15% kidney beans)

-Group (4): Rats fed on diet containing (80 % maize flour and 20% kidney beans)

-Group (5): Rats fed on diet containing (75% maize flour and 25% Kidney beans)

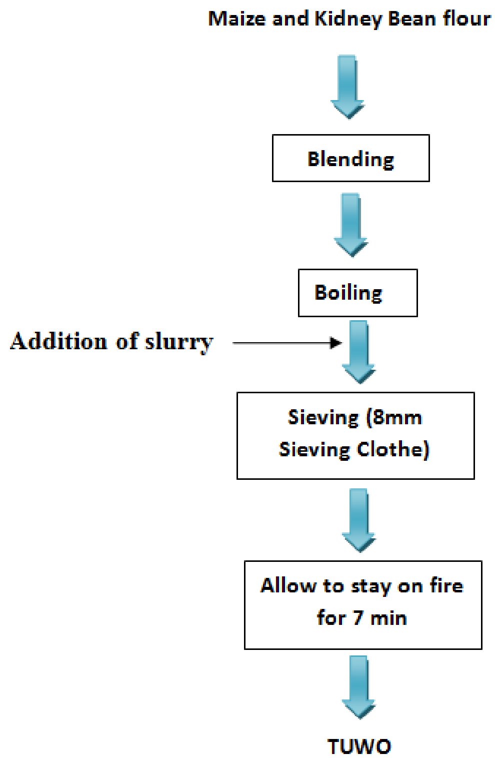

Figure 2: Flow chart for composite flour production. Source: Bolade *et al.* (2002) with slight modification.

2.4.2. Amino Acid Determination

Qualitative assessment of the amino acid (profile) composition of the food formulations was carried out using the method of Rosen (1957). The estimation of the amino acids was by the use of the guide strip technique where developed thin-layer chromatography plates were used in locating the positions of amino acids in unsprayed plates. The squares containing amino acids were cut-out and eluted with 5 ml distilled water at 700 °C for 2 hour; the cellulose powder was removed by centrifugation at 5,000 rpm for 5 min. The supernatant were decanted and kept for the colorimetric analysis of amino acid profiles against Food and Agricultural Organization (FAO) reference values of each amino acid.

Table 1: Flour Blends Formulation (%)

Samples	Maize Flour (MF)	(KBF)	Total
A	100	-	100
B	90	10	100
C	85	15	100
D	80	20	100
E	75	25	100

2.4.3. Mineral contents of the composite flours

The evaluation of potassium content was done using Flame Photometry method (AOAC, 2012). Determination of Iron, Zinc, Calcium and Sodium was done following the method described by AOAC (2012).

2.4.4. Vitamin Contents of the composite flours

Evaluation of Thiamine, Riboflavin, Niacin and Ascorbic Acid (Vitamin C) was done following the methods described by AOAC (2012).

2.5. Statistical analysis

Data obtained was subjected to Analysis of Variance (ANOVA) followed by Tukey's Least Significant Difference (LSD) test to compare treatment means; differences were considered significant at 95% level (P≤0.05) (SPSS V21 software).

3. Results and Discussion

3.1. Glycemic analysis

The result of the glycemic analysis is presented in Table 2, the glycemic index increased for the reference food while the reverse was the case for the fortified samples down the column. There was significant (p<0.05) increase among all the samples. The result of the glucose content is in agreement with the work (Dooshima *et al.*, 2015). Also, Akanbi & Ikujenlola (2016) who worked on the physicochemical composition and glycemic index of whole grain bread produced from composite flours of quality protein maize and wheat reported that increasing inclusion of quality protein maize resulted in decline in the blood glucose content (glycemic index) of the fortified products against the reference food. From this study, increasing addition of kidney beans flour in the fortified samples resulted in decreased in the glycemic index; this is because beans have a relatively high proportion of amylose compared to most other dietary sources of starch. Eating bean or other food that are low on the glycemic index may cut the risk of becoming diabetic (Jenkins *et al.*, 2002).

Low GI foods have been reported to have some physiological and metabolic advantages (Pasupuleti & Anderson, 2008) includes reduced rate of carbohydrate absorption in the small intestine, lower postprandial glucose rise; reduced daily insulin levels, fatter gastric inhibitory polypeptide response; decreased 24 hour urinary c-peptide output; prolong suppression of plasma free fatty acids among others. A person is regarded as diabetic if FBS > 7 mmol/l, 126 mg/dl or RBS >11mmol/200 mg/dl). Applying the cut-off value of greater than seventy (>70) to denote high GI foods (Brand-Miller *et al.*, 2003) it can be indicated that the control (100 % maize) flour can be classified as high GI foods.

Results of the processed maize flour showing high GI value are also similar to results of porridges made from refined maize called Agidi and Tuwo Masara, which had glycemic index values of 92.30 and 86.80 respectively. In general, these foods should not be eaten alone, there should be eaten in context with, legumes, vegetables or meat in a meal. However, studies have shown that glycemic index of a meal can be predicted by the glycemic indices of the different foods consumed within the meal (Brouns *et al.*, 2005). Therefore, data generated in this study can be useful in predicting the glycemic indices of mixed meals.

Proteins provide the structure for all living things as proteins participate in the vital chemical processes that sustain life. The incorporation of kidney bean flour to the maize flour improves the amino acid profile of the composite flours as depicted in Table 3. The result of essential amino acid composition and scores of the blends formulations reviewed lysine and tryptophan increased dramatically from 2.04 g/100 g to 4.21 g/100 g as compared to the FAO reference of 5.50 g/100 g. Tryptophan values increased from 1.00 g/100 g to 1.33 g/100 g in the composite flours as compared to FAO reference of 1.00g. This trend is in agreement with the work of Gernah *et al.* (2012) who worked on the nutritional and sensory evaluation of food formulations from malted maize fortified with defatted sesame flour.

Table 2: Glycemic analysis using albino rats by comparing the average blood glucose responses to glucose made from maize and kidney bean composite flour.

	Time (Minutes)					
Samples	**15**	**30**	**45**	**60**	**90**	**120**
A	$68.19^{e}\pm0.27$	$75.13^{e}\pm0.11$	$76.41^{e}\pm0.44$	$78.77^{e}\pm0.57$	$80.03^{d}\pm0.06$	$82.03^{e}\pm0.05$
B	$65.06^{d}\pm0.06$	$61.13^{d}\pm0.95$	$59.00^{d}\pm0.11$	$57.23^{d}\pm0.06$	$85.24^{e}\pm0.21$	$51.09^{d}\pm0.12$
C	$62.13^{c}\pm1.06$	$58.30^{c}\pm0.53$	$56.10^{b}\pm0.09$	$54.84^{c}\pm0.06$	$52.03^{c}\pm1.05$	$48.76^{c}\pm0.42$
D	$60.69^{b}\pm0.26$	$56.26^{b}\pm1.09$	$57.26^{c}\pm1.17$	$50.37^{b}\pm0.57$	$48.45^{b}\pm0.49$	$45.19^{b}\pm0.19$
E	$51.19^{a}\pm0.83$	$49.06^{a}\pm0.17$	$46.60^{a}\pm1.04$	$42.05^{a}\pm0.31$	$40.68^{a}\pm0.43$	$36.95^{a}\pm0.96$
LSD	0.99	1.05	0.08	1.22	1.50	1.99

Values are means standard of duplicate determinations
Means with same superscript in the same column are not significantly (p>0.05) different
LSD: Least Significant Difference
Keys: A = (100 % Maize flour control) B = (90 % Maize and 10% kidney beans flour) C = (85% Maize and 15% kidney beans flours) D (80% Maize and 20% Kidney beans flours), E (75% Maize and 25% Kidney beans flours).

3.2. Amino Acid analyses of Maize and Kidney beans composite flour

The result of the maize and kidney bean flours is shown in Table 3. Amino acids are the chemical building blocks that make up proteins.

Table 3: Amino Acid analyses of maize and kidney beans composite flour

Samples/parameters	A	B	C	D	E	LSD	FAO Reference
Alanine	$1.83^{a}\pm0.00$	$1.93^{c}\pm0.00$	$1.93^{c}\pm0.00$	$1.88^{b}\pm0.01$	$2.01^{d}\pm0.01$	0.01	-
Aspartic acid	$4.34^{a}\pm0.01$	$4.98^{b}\pm0.02$	$6.12^{e}\pm0.01$	$5.66^{c}\pm0.00$	$6.01^{d}\pm0.01$	0.06	-
Arginine	$2.01^{a}\pm0.02$	$3.09^{b}\pm0.00$	$3.09^{b}\pm0.00$	$3.25^{d}\pm0.03$	$3.17^{c}\pm0.01$	0.02	-
Glycine	$1.41^{a}\pm0.03$	$1.70^{b}\pm0.01$	$1.74^{b}\pm0.01$	$1.90^{d}\pm0.01$	$1.80^{c}\pm0.01$	0.05	-
Glutamic acid	$1.71^{a}\pm0.02$	$2.68^{b}\pm0.02$	$3.96^{c}\pm0.04$	$4.91^{d}\pm0.03$	$5.66^{e}\pm0.01$	0.09	-
Serine	$2.14^{a}\pm0.00$	$2.19^{a}\pm0.00$	$3.83^{d}\pm0.23$	$2.95^{b}\pm0.04$	$3.03^{c}\pm0.02$	0.06	-
Proline	$0.64^{a}\pm0.06$	$2.00^{b}\pm0.01$	$2.50^{d}\pm0.01$	$2.44^{c}\pm0.02$	$2.57^{e}\pm0.02$	0.05	-
Threonine	$1.70^{a}\pm0.01$	$2.83^{c}\pm0.05$	$2.74^{b}\pm0.01$	$2.77^{b}\pm0.01$	$3.88^{d}\pm0.02$	0.04	4.00
Tryptophan	$1.00^{a}\pm0.01$	$1.09^{b}\pm0.01$	$1.23^{c}\pm0.05$	$1.28^{cd}\pm0.01$	$1.33^{d}\pm0.04$	0.06	1.00
Isoleucine	$1.03^{a}\pm0.01$	$2.02^{b}\pm0.00$	$3.02^{c}\pm0.01$	$3.10^{d}\pm0.01$	$4.05^{e}\pm0.05$	0.07	4.70
Leucine	$3.25^{a}\pm0.05$	$3.25^{a}\pm0.05$	$4.37^{b}\pm0.01$	$5.41^{c}\pm0.03$	$6.07^{d}\pm0.04$	0.01	7.00
Lysine	$2.04^{a}\pm0.01$	$2.22^{b}\pm0.09$	$3.10^{c}\pm0.01$	$3.82^{d}\pm0.04$	$4.21^{e}\pm0.28$	0.08	5.50
Methionine	$0.98^{a}\pm0.07$	$1.52^{b}\pm0.06$	$1.89^{c}\pm0.04$	$2.06^{d}\pm0.04$	$3.06^{e}\pm0.05$	0.09	3.50
Phenalalanine	$2.11^{a}\pm0.01$	$2.13^{a}\pm0.01$	$3.43^{b}\pm0.01$	$4.85^{c}\pm0.21$	$3.41^{b}\pm0.03$	0.04	6.00
Tyrosine	$1.78^{a}\pm0.01$	$2.10^{b}\pm0.01$	$2.11^{b}\pm0.00$	$2.27^{c}\pm0.02$	$2.47^{d}\pm0.11$	0.01	-
Valine	$2.60^{a}\pm0.01$	$3.07^{b}\pm0.03$	$3.05^{b}\pm0.01$	$3.51^{c}\pm0.01$	$4.61^{d}\pm0.55$	0.03	5.00
Histidine	$0.88^{a}\pm0.00$	$0.93^{a}\pm0.01$	$0.99^{b}\pm0.00$	$1.00^{b}\pm0.00$	$1.13^{c}\pm0.00$	0.06	-

Values are means standard of duplicate determinations
Means with same superscript in the same column are not significantly (p>0.05) different
LSD: Least Significant Difference
Keys: A = (100 % Maize flour control) B = (90 % Maize and 10% kidney beans flour) C = (85% Maize and 15% kidney beans flours) D (80% Maize and 20% Kidney beans flours), E (75% Maize and 25% Kidney beans flours).

3.3. Mineral analyses of Maize and Kidney beans composite flours

The mineral composition of the composite flours is as shown in Table 4, down the column, the Calcium, Zinc and Iron content of the composite flours increased ($p<0.05$) significantly with increasing supplementation of kidney beans with maize. Their values ranged from 0.49-0.70, 0.48-0.71 and 1.85-2.40 mg/100 g while the potassium and sodium content ranged from 763.48-799.04 and 0.38-1.09 mg/100 g respectively. The trend is in arrangement with the work of (Ufot & Winifred, 2016). The variation observed in the Calcium, Zinc and Iron content of the composite flours could be due to the compositional difference in terms of mineral content between the crops used in the blends. Calcium aids in the formation and maintenance of bones and teeth, also for proper function of our blood vessels, muscles, nerves and hormones (FAO/WHO/UNU, 1994). Iron is a factor in red blood cell formation (FAO/WHO/UNU, 1994). The potassium trend showed that there was an increment in the amount of potassium as the amount of kidney beans with maize increased. The trend is not in arrangement with the work of (Ufot & Winefred, 2016). The increased in potassium content of the composite flours maybe due to the high potassium content in kidney beans. The physiological roles of mineral elements in human diets have been documented (Grosvernor & Smolin, 2002). The variation observed could be due to the compositional difference in terms of mineral content between the crops used in the blends; kidney beans is also rich in sodium. Sodium is important for fluid balance, required for normal cell function and regulation of blood volume (FAO/WHO/UNU, 1994).

1.35-3.14 mg/100 g respectively while Vitamin B2 didn't have a significant (p>0.05) difference, it ranged from 0.04-0.09 mg/100 g respectively. This trend supports the claim of (Agunbiade *et al.*, 2002; Chike & Anita, 2016). Who observed increase in vitamin B concentration of bambara fortified maize sorghum mix. Vitamin B1 releases energy from carbohydrate and also aids normal growth (UICEC/WHO, 2005). Niacin is a factor in energy metabolism and tissue formation which aids in normal growth and development (UICEC/WHO, 2005). Niacin is a part of the coenzyme, nicotinamide adenine dinucleotide (NAD) or its phosphate form, NADP plays a key role in energy production and in the synthesis of fatty acids and steroids.

Table 4: Mineral Contents (mg/100 g) of maize and kidney bean composite flour

Samples	Potassium	Iron	Zinc	Sodium	Calcium
A	$763.48^{a}\pm0.71$	$1.85^{a}\pm0.01$	$0.48^{a}\pm0.03$	$0.38^{a}\pm0.01$	$0.49^{a}\pm0.02$
B	$769.26^{b}\pm0.04$	$1.89^{ab}\pm0.02$	$0.50^{a}\pm0.01$	$0.57^{b}\pm0.04$	$0.55^{a}\pm0.01$
C	$773.91^{c}\pm0.11$	$1.91^{ab}\pm0.02$	$0.61^{b}\pm0.03$	$0.76^{c}\pm0.02$	$0.62^{b}\pm0.01$
D	$783.13^{d}\pm2.57$	$1.96^{b}\pm0.04$	$0.66^{c}\pm0.03$	$0.82^{d}\pm0.00$	$0.65^{bc}\pm0.01$
E	$799.04^{e}\pm0.07$	$2.40^{c}\pm0.01$	$0.71^{c}\pm0.04$	$1.09^{e}\pm0.04$	$0.70^{c}\pm0.03$
LSD	2.01	0.04	0.06	0.07	0.07

Values are means standard of duplicate determinations
Means with same superscript in the same column are not significantly (p>0.05) different
LSD: Least Significant Difference
Keys: A = (100 % Maize flour control) B = (90 % Maize and 10% kidney beans flour) C = (85% Maize and 15% kidney beans flours) D (80% Maize and 20% Kidney beans flours), E (75% Maize and 25% Kidney beans flours)

3.4. Vitamin Content of Maize and kidney bean flours

The Vitamin analysis of the composite bread is as shown in Table 5, down the column, the Vitamin increase significantly (p<0.05) for vitamin B1, B3 and Vit C content of the composite flours from 0.84-0.97, 0.45-0.67 and

The coenzyme is also involved in DNA replication, repair and cell differentiation. Deficiency of these very important vitamins lead to pellagra. Vitamin C is important factor in the development and maintenance of bones, cartilage, teeth and gums (FOA/WHO/UNU, 1994). The Riboflavin (B2) content of the

Table 5: Vitamin contents (mg/100 g) of maize and kidney bean composite flour

Samples	Thiamine	Riboflavin	Niacin	Vitamin C
A	$0.84^{a}\pm0.01$	$0.04^{a}\pm0.00$	$0.45^{a}\pm0.00$	$1.35^{a}\pm0.08$
B	$0.87^{a}\pm0.01$	$0.04^{a}\pm0.00$	$0.52^{b}\pm0.01$	$2.66^{b}\pm0.33$
C	$0.89^{a}\pm0.00$	$0.07^{a}\pm0.01$	$0.56^{b}\pm0.01$	$2.98^{c}\pm0.05$
D	$0.93^{b}\pm0.01$	$0.08^{a}\pm0.00$	$0.63^{c}\pm0.02$	$3.14^{d}\pm0.07$
E	$0.97^{b}\pm0.01$	$0.09^{a}\pm0.00$	$0.67^{c}\pm0.03$	$3.12^{d}\pm0.02$
LSD	0.10	0.06	0.06	0.03

Values are means standard of duplicate determinations
Means with same superscript in the same column are not significantly (P>0.05) different
LSD: Least Significant Difference
Keys: A = (100 % Maize flour control) B = (90 % Maize and 10% kidney beans flour) C = (85% Maize and 15% kidney beans flours) D (80% Maize and 20% Kidney beans flours), E (75% Maize and 25% Kidney beans flours).

composite flour support the claim (Uchechukwu *et al.*, 2017) who reported increase in the vitamin B2 properties of maize Ogi cofermented with pigeon pea. Riboflavin is a factor in energy metabolism and tissue formation (UICEC/WHO, 2005).

4. Conclusion

The fortified samples in this study showed a reduction of the invivo glycemic index when compared to the control and could be considered an interesting alternative to be used in weight and glycemic control diets in which a slower liberation of glucose is desired. The Amino acid profile, mineral and vitamin results revealed that maize and kidney flour blends will contribute significantly in preventing the problem of poor malnutrition in developing Countries.

Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

All animal care and experimental protocols were ethically reviewed and approved by the Department of Food Science and Technology, Federal University of Agriculture, Makurdi, Nigeria. This study does not involve any human testing.

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