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Nutritional and Antioxidant Potential of Rice Flour Enriched with Kersting's Groundnut (*Kerstingiella geocarpa*) and Lemon Pomace

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Abstract

This study was designed to enhance the nutritional quality, antioxidant properties and product utilization potentials of locally produced 'Igbemo' rice flour by adding Kersting's groundnut and lemon pomace. Kersting's groundnut is an underutilized legume while lemon pomace is a byproduct of lemon utilization; both meant to enhance the protein quality, antioxidant potential and fibre contents of the composite flour. The dependent variables were minerals composition, amino acid profile, antioxidants and antinutrients properties, in-vitro protein digestibility and in-vitro carbohydrate digestibility. The result showed that blends with higher lemon pomace of 10.00 g had the best calcium, iron, potassium and magnesium contents and antioxidant contents, while blends with highest Kersting's groundnut (20.00 g) had the best zinc content. The anti-nutrients in the blends were generally low and safe for consumption.

Keywords: Antioxidants; Composite flour; Kersting's groundnut; Response surface methodology; Rice flour

1 Introduction

The increase in production and consumers' acceptability of rice flour in the production of non-gluten baked products is promoting further research into rice flour utilization. In addition to its non-gluten characteristics, rice flour has been found to possess good nutritional qualities. Rice, as a cereal, serves as a basic food source for over half the world population whilst it provides about 80 % of the food intake as ready-to-eat convenience and inexpensive gluten-free snacks (Awolu, Oluwaferanmi, Fafowora, & Oseyemi, 2015). Nutritionally, cereals are important sources of carbohydrates, dietary fibre and vitamins (Katina et al., 2005) but they are deficient in lysine. Cereal-based flours are therefore supplemented with legumes as credible source of lysine (Awolu et al., 2015; Awolu, Omoba, Olawoye, & Dairo, 2017). In addition, legumes serve as sources of protein and minerals needed for health growth and development.

Kersting's groundnut (*Kerstigiella geocarpa* Harms) is an underutilized legume; it is rich in essential minerals, protein and amino acids. The crop is indigenous to Africa and a viable alternative to high protein content foods (Bayorbor, Dzomeku, Avornyo, & Opoku-Agyeman, 2010). Kersting's groundnut has not been fully exploited and its nutritional importance has not been fully evaluated.

Lemon (*Citrus limon*) comes after orange and

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mandarin in the order of citrus importance. It is rich in vitamin C, minerals, dietary fibre, essential oils, organic acids, carotenoids and flavonoids (Gonzalez-Molina, Dominguez-Perles, Moreno, & Garcia-Viguera, 2010).
This study produced composite flour comprising rice, Kersting's groundnut and lemon pomace. The addition of Kersting's groundnut was meant to enhance its protein content and mineral compositions, whilst lemon pomace enhanced its fibre contents. Kersting's groundnut and lemon pomace has been found to be rich in antioxidants, hence, it was expected that their addition would enhance the antioxidant capacities of the composite flour.

2 Materials and Methods

2.1 Materials

Igbemo rice was sourced from Igbemo-Ekiti, Ekiti State. Kersting's groundnut (*Kerstingiella geocarpa Harms.*) was sourced from Oyingbo market, Lagos. Lemon fruits were sourced at Oja-Oba, Akure, Ondo State. All reagents were of analytical grade.

2.2 Preparation of Flours

Preparation of rice flour

About 5 g of Igbemo' rice grains were manually cleaned, dry-milled using hammer mill, sieved through 210 µm particle size sieves and then stored in a sealed plastic container at room temperature for further processing (Awolu et al., 2015).

Preparation of Kersting's groundnut flour

Kersting's groundnut seeds (2.0 kg) were parboiled for 35 min, manually dehulled, oven dried at 65 ^{o}C until constant weight was obtained and hammer-milled into fine particles. The flour was subsequently kept inside a sealed plastic container prior to usage (Awolu et al., 2015).

Preparation of lemon pomace flour

Lemon pomace was produced according to the method described by Kolodziejczyk, Markowski, Kosmala, Król, and Plocharski (2007). Fresh lemon fruits were washed with warm water to remove tough dirt and dust from the fruits. The washed fruit was pulverized with a sharp knife and blended into slurry using a Kenwood blender (BL-237). The juice was extracted from the slurry using a muslin cloth whilst the wet lemon pomace was oven dried (Gen-lab hot air oven, model DHG-9101.1SA) at 60 ^{o}C for 18 h. The dried pomace was blended into fine flour using the blender.

2.3 Experimental design for the development of flour blends

Optmization of the proximate properties of the composite flour was carried out using the optimal mixture model design of response surface methodology (Design expert 8.0.3.1, trial version). The independent variables were rice flour (70.30 – 85.00%), Kersting's groundnut flour (10.00 – 20.00%) and lemon pomace (5.00 – 10.00%) while the dependent variables were the proximate composition.

2.4 Proximate composition determination of the composite flour

The moisture content, crude protein, fat, ash content and crude fibre of the composite flour blends were determined according to the standard methods of AOAC (2005).

2.5 Minerals analysis

Mineral analysis was determined according to the AOAC (2005) method. The sample was ashed, and about 15 mL of 6 N HCl was added to it and transferred to a 100 mL volumetric flask. Distilled water was used to make up to the 100 mL mark. Atomic Absorption Spectroscopy (AAS) was used for analysis of all the minerals except potassium and sodium which were analyzed using a flame emission spectrophotometer

(Model A-6200, Shimadzu, Corporation and Kyoto, Japan). Standards for sodium and potassium were prepared from their chloride salts.

2.6 Amino acid profile determination

The sample was hydrolyzed using hydrochloric acid (6 N) for 24 h at 110 ^{o}C in a vial under vacuum and N_2 atmosphere, evaporated and dissolved in sodium citrate buffer (pH 2.2) and the hydrolysates were analyzed by using a HPLC combined with a Pickering PCX5200 derivatizer (Pickering Laboratories, Inc., USA) and ion exchange column (3.0 × 250 mm, 8 μm). The amino acids were identified spectrophotometrically by measuring at 570 nm (Benitez, 1989).

2.7 Evaluation of Antioxidants Properties

Determination of ABTS scavenging ability

Aqueous 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging ability solution (7.8 M) with $K_2S_2O_8$ (2.45 mM, final conc.) was left in the dark for 16 h and the absorbance adjusted at 734 nm to 0.700 with ethanol. About 0.2 mL of the appropriate dilution of the extract was added to 2.0 mL of ABTS solution and the absorbance was read at 732 nm after 15 min. The Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) equivalent antioxidant capacity was subsequently calculated (Re et al., 1999).

Determination of DPPH free radical scavenging ability

The stock reagent solution (1×10^{-3} M was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at 20 ^{o}C. The working solution (6×10^{-5} M) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm. Exactly 0.1 mL each of extract solutions of different concentrations were vortexed for 30 s with 3.9 mL of DPPH solution and left to react for 30 min; the absorbance at 515 nm was then recorded. A control with no added extract was also analyzed. DPPH Scavenging activity was calculated using Eq. (1) (Lee, Mulugu, York, & O'Shea, 2007).

$$DPPH = \frac{Ab_{control} - Ab_{sample}}{Ab_{control}} \times 100 \qquad (1)$$

Where Ab = Absorbance

Total flavonoids

About 0.5 mL aliquot of 20 g L^{-1} $AlCl_3$ ethanolic solution was added to 0.5 mL of extract solution. The absorbance at 420 nm was measured after 1 h at room temperature. The presence of flavonoids was indicated by a yellow colouration. Extract samples were evaluated at a final concentration of 0.1 mg mL^{-1} (Eq. 2) and expressed as quercetin equivalent (QE) based on the calibration curve (Ordonez, Gomez, Vattuone, & Lsla, 2006)

$$C = 0.00255 \times Ab \qquad (R^2 = 0.9812) \qquad (2)$$

Where Ab is the absorbance and C is the concentration (mg QE g^{-1} DW)

2.8 Determination of Antinutrients

Determination of oxalate content

About 1 g of sample was weighed into 100 mL conical flask, 75 mL 3 M $H_2SO_4$was added and stirred for 1 h with a magnetic stirrer. Exactly 25 mL of the filtrate (Whatman filter paper No.1) was taken and titrated hot against 0.05 M $KMnO_4$ solution until a faint pink colour which persisted for at least 30 sec was formed. The oxalate content was calculated by taking 1 mL of 0.05 M $KMnO_4$ as equivalent to 2.2 mg oxalate using Eq. (3) (Day & Underwood, 1986)

$$Oxalate(mg/100g) = \frac{Titrevalue \times 2.2 \times DF}{W} \qquad (3)$$

Where 2.2 mg = mass equivalent value of 1mL of 0.05 M $KMnO_4$ solution.
DF = Dilution factor (total volume of sample divided by volume of portion used for titration)
*W = Sample weight in g.

Determination of phytic acid content

About 2 g of sample was weighed into 250 mL conical flask; 100 mL of 2 % concentrated HCl was thereafter added, allowed to soak for 3 h and filtered. The filtrate (50 mL) was pipetted into 250 mL beaker, with 107 mL ammonium thiocyanate solution added as an indicator and titrated with standard iron III chloride $FeCl_3$ solution (containing 0.00195 g iron/mL) until a brownish yellow colour appeared and persisted for five minutes. The phytic acid content was calculated using Eq. (4) (Russell, 1980):

$$PHY = \frac{0.00195 \times V_{FeCl_3} \times DF}{W_{Sample}} \qquad (4)$$

PHY = Phyticacid (g/kg)
V_{FeCl_3} = volume of $FeCl_3$ consumed
W_{Sample} = sample weight
DF = Total volume of extraction solvent added/volume of aliquot taken for the titration.

Determination of tannin content

About 0.2 g of sample was placed in a test tube, 10 mL of 1 % HCl/methanol was added, the test tube was capped, continuously shaken for 20 min and then centrifuged at 2500 rpm for 5 min. Exactly 1 mL of the supernatant was pipetted into fresh tubes, the absorbance was set at zero and 1 mL blank solution was mixed with 5 mL 4 % HCl/methanol and 5 mL vanillin reagent in a test tube. The sample and blank test tubes were incubated for 20 min at 30 ^{o}C. Absorbance was read at 500 nm and concentration of condensed tannins was determined from standard curve. Tannin concentration was expressed in % as follows (Trease & Evans, 1978):

$$Tannic\ content = \frac{(C \times 10)}{200} \times 100 \qquad (5)$$

Where:
C= Concentration corresponding to the optical density
10 = Volume of extract (mL)
200 = sample weight (mg)

Determination of saponin content

About 20 mL of 20 % aqueous ethanol was added to 10 g of the ground sample and agitated with a magnetic stirrer for 12 h at 55 ^{o}C. The solution was filtered through Whatman No.1 filter paper and the residue re-extracted with 200 mL 20 % aqueous ethanol. The extract was reduced to 40 mL under vacuum and 20 mL diethyl ether added in a separating funnel and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The pH of the aqueous solution was adjusted to 4.5 by adding NaOH, and the solution shaken with 60 ml n-butanol. The combined butanol extracts were washed twice with 10 mL of 5 % aqueous NaCl and evaporated to dryness in a fume cupboard to give a crude saponin (Hudson & El-Difrawi, 1979).

Determination of trypsin inhibitor

Tris-buffer (0.05 M, pH 8.2) containing 0.02 M $CaCl_2$: 6.05 g tris- (hydroxymethyl) aminomethane and 2.94 g $CaCl_2{\cdot}2H_2O$ were dissolved in 500 mL of distilled water, the pH was adjusted to 8.2 and the volume made up to 1 L with distilled water. About 2.0 mL of trypsin solution was added to 1.0 g of the extracted sample in a test tube and then placed in a water bath at 37 ^{o}C. Exactly 5 mL hydrated Benzoyl-DL-arginene-p-nutoanilide (BAPA) solution was dissolved in dimethyl sulfoxide previously warmed to 37 ^{o}C. The reaction was terminated 10 min later by adding 1 mL of 30 % acetic acid. After thorough mixing, the contents of each tube were filtered through Whatman No.1 paper and the absorbance was measured against the blank (Kakade, 1974).

2.9 In-vitro protein (IVPD) determination

Composite flour samples (200 mg) were weighed into an Erlenmeyer flask and mixed with 35 mL of porcine pepsin (1.5 g of pepsin in 0.1 M KH_2PO_4, pH 2.0). Samples were digested for 2 h at 37 ^{o}C; digestion was stopped by addition of 2 mL of 2 M NaOH and the samples were centrifuged (4900 xg at 40 ^{o}C) for 20 min after

which the supernatant was discarded. The residues were washed and centrifuged twice with 20 mL of buffer (0.1 M KH_2PO_4, pH 7.0). Undigested nitrogen was determined using Kjeldahl method. Digestibility was calculated using Eq. (6) (Aboubacar, Axtell, Huang, & Hamaker, 2001).

$$IVPD = \frac{(N_{sample} - N_{Undigested})}{N_{sample}} \times 100 \quad (6)$$

2.10 In-vitro carbohydrate digestibility determination (IVCD)

Exactly 4 mL of phosphate buffer (pH 6.6), 1 mL of sodium chloride and 1 mL of α amylase enzyme was added to 5 mL of the sample at room temperature and mixed thoroughly. Aliquots (0.2 mL) of the mixture were taken at zero and 1.0 h (complete hydrolysis was predetermined) after addition of the enzyme and dispensed into 10 mL Lugol's iodine solution (1:100 dilution). The absorbance was measured at 620 nm and the in- vitro carbohydrate digestibility was calculated using Eq. (7) (Shekib, Eliraqui, & Abobakr, 1988).

$$IVCD = \frac{Abs_{time=0} - Abs_{time=1h}}{Abs_{time=0}} \times 100 \quad (7)$$

where Abs is the Absorbance

3 Results and Discussions

3.1 Proximate composition and functional properties of the composite flour

The result of the proximate composition is presented in Table 1. The moisture content of the composite flour (6.20 to 6.81 g/100g) was within the acceptable range ($\leq$ 10%) for flours to ensure shelf stability. In addition, the variation in moisture content of the composite flour had a low R-squared and the adjusted R-squared values of 0.3496 and 0.0244 respectively, which was an indication that it would not support moisture; hence, the composite flour would have a good shelf life.

The ash content of the composite flour ranged between 0.98 and 1.81 g/100g. The ash content increased significantly ($p \leq 0.05$) as the levels of lemon pomace incorporation increased. The R-squared and adjusted R-squared values were 0.7546 and 0.7168 respectively. The crude protein of the flour samples ranged between 7.51 and 12.99 g/100g. The protein content increased significantly ($p \leq 0.05$) as the level of Kersting's groundnut incorporation increased. A similar results have been obtained for composite flour consisting of rice, cassava and Kersting's groundnut flours (Awolu et al., 2015). The ANOVA indicated that the model and model term (linear mixture, AB, AB (A-B)) were significant ($p \leq 0.05$) while the R-squared and the adjusted R-squared values were 0.9975 and 0.9937 respectively. The high adjusted R-squared value for protein content showed that the protein had high positive effect on the composite flour.

The fat content of the flours ranged from 4.00 - 4.44 g/100 g. In comparison, wheat flour had fat content of 1.33 g/100g. The fat content decreased with increased incorporation of rice and lemon pomace flours. The composite flour may be better enhancer of flavour and fat-soluble vitamins than wheat flour. In addition, the fat values of the composite flour should not possess any negative effect in terms of rancidity. The crude fibre values ranged from 2.52 to 3.78 g/100g.The result indicated that the addition of Kersting's groundnut flour and lemon pomace increased the crude fibre content of the composite flour. The ANOVA indicated that the model and model terms (linear mixture, AB, AB(A-B) for crude fibre were significant ($p \leq 0.05$) while the R-squared and adjusted R-squared were 0.9975 and 0.9937 respectively.

The carbohydrates content of the flours ranged between 62.03 - 72.98 g/100g. The model and model terms (linear mixture component, AB, AC, A^2BC, ABC^2) were significant ($p \leq 0.05$) while the R-squared and adjusted R-squared were 0.9945 and 0.9882 respectively.

Table 1: Proximate Composition of Composite Flour

Run	Variables (g)			Proximate composition (g/100g)					
	A (g)	B (g)	C (g)	Moisture	Ash	Protein	Fibre	Fat	CHO
1	79.84	10.16	10.00	6.40	1.81	7.51	3.71	4.00	66.57
2	75.00	20.00	5.00	6.20	1.25	12.99	2.89	4.28	67.39
3	83.50	11.50	5.00	6.50	1.05	8.56	2.80	4.00	72.09
4	70.30	20.00	9.70	6.30	1.76	12.57	3.34	4.30	62.03
5	76.98	15.75	7.25	6.46	1.70	9.12	3.21	4.27	67.98
6	79.84	10.16	10.00	6.47	1.30	8.43	3.62	4.00	66.18
7	82.28	10.00	7.71	6.30	1.48	8.41	3.70	4.00	68.40
8	75.00	20.00	5.00	6.81	1.30	12.8	2.90	4.44	66.75
9	85.00	10.00	5.00	6.40	0.98	8.41	2.68	4.01	72.52
10	73.16	19.07	7.76	6.62	1.68	12.72	3.18	4.36	63.67
11	82.28	10.00	7.71	6.80	1.60	8.46	3.72	4.11	67.60
12	85.00	10.00	5.00	6.41	1.06	7.99	2.66	4.00	72.88
13	80.31	14.68	5.00	6.43	1.32	9.55	2.52	4.20	70.98
14	77.44	12.55	10.00	6.41	1.80	9.00	3.78	4.10	64.91
15	73.56	16.43	9.99	6.30	1.74	11.62	3.09	4.19	63.10
16	70.30	20.00	9.70	6.30	1.76	12.63	3.30	4.24	62.07

*A - Rice Flour; B - Kersting's groundnut; C- Lemon pomace; CHO - Carbohydrate

3.2 Minerals composition of optimized composite flour

The result of the mineral composition of the composite flour is presented in Table 2. Iron contents in the composite flour ranged between 2.82 and 3.47 mg/1 00g. Iron deficiency is the most common nutrient disorder worldwide as it accounts for 50 % of the cases of anaemia (World Health Organization, 2001). Iron contents reported in rice, Kersting's groundnut and lemon pomace flours were 0.82 mg/100g, 10.00 mg/100g and 147.65 mg/100g respectively (Adeyeye & Faleye, 2007; Atukorale, 2002; Janati, Beheshti, Feizy, & Fahim, 2012). The iron content in composite flour increased significantly ($p \leq 0.05$) as the level of lemon pomace increased.

Zinc values ranged from 2.37 to 2.67 mg/100g. Zinc content in the composite flour increased significantly ($p \leq 0.05$) as the levels of Kersting's groundnut increased. The values of zinc obtained in this study was higher than 0.58 – 0.66 mg/100g reported for wheat based composite flour enriched with 'orarudi' (*Vahigna sp*) (Onoja et al., 2014).

Magnesium values ranged between 4.54 and 4.64 mg/100g. The magnesium content increased significantly ($p \leq 0.05$) as the lemon pomace increased. Magnesium is a cofactor in about 300 enzyme systems which play regulatory roles in several biochemical reactions in the body such as protein synthesis, muscle and nerve function, blood glucose control and blood pressure regulation (Laurant & Touyz, 2000). It also promotes strong bones strong and keeps heart rhythm steady (Twum et al., 2015).

Potassium ranged between 121.77 -166.33 mg/100g. Potassium content increased significantly ($p \leq 0.05$) with increasing lemon pomace flour content. Potassium plays a vital role in maintaining osmotic balance and pH of the body fluids, regulating muscle and nerve irritability, controlling glucose absorption and enhancing normal retention of protein during growth (National Research Council, 1980).

Calcium values ranged from 55.65 to 65.10 mg/100g. Lemon pomace had been reported to contain about 8452.50 mg/100g calcium content (Janati et al., 2012). The calcium content of

the composite flour increased significantly ($p \leq 0.05$) as lemon pomace increased. Calcium functions primarily in the development of strong bones.

3.3 Antioxidant properties of the composite flour

The results of the antioxidant property of composite flour are presented in Table 3. The DPPH free radical scavenging ability ranged from 39.57 to 45.10 %. DPPH of the composite flour increased significantly ($p \leq 0.05$) as lemon pomace increased.
The ABTS scavenging ability of the composite flour ranged between 23.81 and 25.40 mMol/g. The ABTS also increased significantly ($p \leq 0.05$) as lemon pomace increased. The increase in both DPPH scavenging activities and ABTS as a result of the addition of lemon pomace is a justification for its addition, which is to improve antioxidant capacities. In addition, lemon pomace flour had positive effect on the flavonoids content of the composite flour; an increase in level of lemon pomace in the composite flour brought about a corresponding significant ($p \leq 0.05$) increase in the flavonoid contents. Flavonoids are major polyphenolic components of foods and display anti-inflammatory, anti-allergic and anti-cancer activities (Crozier, Clifford, & Ashihara, 2008).

Antinutritional properties of composite flour

The results of antinutritional properties of the composite flour are presented in Table 4. Phytate content of the composite flour ranged between 2.29 and 2.50 mg/100g. Run 2 with the highest Kersting's groundnut content (20 g/100g) had the overall highest antinutrients contents. However, the antinutrients contents were within recommended level that is safe for human consumption; the recommended toxicity level of phytates for humans is 2 – 5 g/day (Hassan, Umar, & Umar, 2004), while the phytate content in the composite flour was far less than this value.
There was no significant ($p > 0.05$) difference between the tannin contents of the composite flour of the samples. The tannin content obtained in this study was also very safe for consumption (Ikpeme, Ekpeyoung, & Igile, 2012). This low result indicated that the composite flour would have good protein digestibility as high protein contents interferes with protein digestibility.
The trypsin inhibitor activity of the composite flour ranged between 0.26 and 0.34 mg/100g. The higher the Kersting's groundnut content, the higher the trypsin inhibition activity. As with already mentioned antinutrients, the trypsin inhibition levels were very minimal and safe for human consumption. A trypsin inhibitor activity content ranging from 4.01 to 46.01 mg/100g has been reported for Acha-Soybean composite flour (Ikpeme et al., 2012).
Oxalates ranged from 0.73 to 0.87 mg/g. The level of oxalate obtained is also low and safe for human consumption. The toxicity of oxalates is 2 – 5 g/day (Hassan et al., 2004). The saponin content obtained in this study was also low and safe for human consumption.

In-vitro carbohydrate digestibility and in-vitro protein digestibility of the composite flour

Research has shown that nutrient composition of foods is not enough to determine nutrient bio-availability (Julian et al., 2007), hence the need for in-vitro (starch and protein) digestibility analyses. The result of in-vitro carbohydrate digestibility is presented in Table 5. The results showed that the sample with highest rice content (run 7) had the overall best carbohydrate digestibility. Rice content dictates the extent of the carbohydrate digestibility; the higher the rice content the higher the carbohydrate digestibility and vice versa. In addition, digestion time rather than digestion temperature enhanced carbohydrate digestibility. Carbohydrate digestibility was higher in samples with the same digestion temperature but higher digestion time; hence digestion at 60 min was higher than digestion at 30 min.
The results of in-vitro protein digestibility are presented in Table 6. Unlike carbohydrate digestibility where the sample with the highest rice (with highest carbohydrate content) had the highest digestibility, protein digestibility had the

Table 2: Minerals Composition of the Composite Flour

Sample	Calcium (mg/100g)	Potassium (mg/100g)	Zinc (mg/100g)	Iron (mg/100g)	Magnesium (mg/100g)
Runs 2	55.65 ± 1.21^{c}	121.77 ± 0.59^{c}	2.67 ± 0.05^{a}	2.82 ± 0.11^{b}	4.54 ± 0.12^{c}
Runs 7	61.11 ± 1.18^{b}	139.00 ± 0.58^{b}	2.55 ± 0.10^{a}	3.33 ± 0.14^{a}	4.58 ± 0.18^{b}
Runs 14	65.10 ± 0.96^{a}	166.33 ± 0.58^{a}	2.37 ± 0.05^{b}	3.47 ± 0.08^{a}	4.64 ± 0.12^{a}

*values are mean ± standard deviation of triplicate samples

*values on the same column with the same superscript are not significantly different at $p \leq 0.05$

*Run 2 = 75 g/100g rice; 20 g/100g Kersting's groundnut, 5 g/100g lemon pomace flours

*Run 7 = 82.28g/100g rice; 10 g/100g Kersting's groundnut, 7.71 g/100g lemon pomace flours

*Run 14 = 77.44 g/100g rice; 12.55 g/100g Kersting's groundnut, 10 g/100g lemon pomace flours

Table 3: Antioxidant Properties of the Composite Flour

Sample	DPPH (%)	ABTS (mMol/g)	Flavonoids (mg QE g^{-1})
Runs 2	39.57 ± 1.35^{c}	23.81 ± 0.08^{c}	1.21 ± 0.01^{c}
Runs 7	42.73 ± 3.05^{ab}	24.40 ± 0.40^{b}	1.28 ± 0.01^{a}
Runs 14	45.10 ± 1.89^{a}	25.40 ± 0.24^{a}	1.26 ± 0.01^{b}

*values are mean ± standard deviation of triplicate samples
*values on the same column with the same superscript are not significantly different at $p \leq 0.05$
*Run 2 = 75 g/100g rice; 20 g/100g Kersting's groundnut, 5 g/100g lemon pomace flours
*Run 7 = 82.28g/100g rice; 10 g/100g Kersting's groundnut, 7.71 g/100g lemon pomace flours
*Run 14 = 77.44 g/100g rice; 12.55 g/100g Kersting's groundnut, 10 g/100g lemon pomace flours

Table 4: Antinutritional Properties of the Composite Flour

Sample	Oxalate (mg/100g)	Saponin (%)	Tannin (mg/100g)	Phytates (mg/100g)	Trypsin (%)
Runs 2	0.87 ± 0.01^{a}	3.31 ± 0.20^{a}	0.03 ± 0.00^{a}	25.05 ± 0.02^{a}	0.34 ± 0.12^{a}
Runs 7	0.73 ± 0.02^{c}	2.82 ± 0.04^{b}	0.03 ± 0.00^{a}	24.03 ± 0.02^{b}	0.26 ± 0.02^{c}
Runs 14	0.81 ± 0.04^{b}	2.44 ± 0.01^{c}	0.03 ± 0.00^{a}	22.89 ± 0.01^{c}	0.27 ± 0.04^{b}

*values are mean ± standard deviation of triplicate samples *values on the same column with the same superscript are not significantly different at $p \leq 0.05$
*Run 2 = 75 g/100g rice; 20 g/100g Kersting's groundnut, 5 g/100g lemon pomace flours
*Run 7 = 82.28g/100g rice; 10 g/100g Kersting's groundnut, 7.71 g/100g lemon pomace flours
*Run 14 = 77.44 g/100g rice; 12.55 g/100g Kersting's groundnut, 10 g/100g lemon pomace flours

Table 5: In-vitro Carbohydrate Digestibility of Composite Flour

Sample	Digested Starch			
	50 ^{o}C for 60 min	50^{o}C for 30 min	40^{o}C for 60 min	40^{o}C for 30 min
Run 2	28.67 ± 0.80^{b}	10.48 ± 0.05^{c}	25.47 ± 0.12^{c}	10.05 ± 0.09^{c}
Run 7	30.00 ± 0.45^{a}	25.62 ± 0.03^{a}	28.44 ± 0.42^{a}	23.36 ± 0.42^{a}
Run 14	28.54 ± 0.02^{b}	15.78 ± 0.07^{b}	27.88 ± 0.11^{b}	12.45 ± 0.08^{b}

*values are mean ± standard deviation of triplicate samples *values on the same column with the same superscript are not significantly different at $p \leq 0.05$
*Run 2 = 75 g/100g rice; 20 g/100g Kersting's groundnut, 5 g/100g lemon pomace flours
*Run 7 = 82.28g/100g rice; 10 g/100g Kersting's groundnut, 7.71 g/100g lemon pomace flours
*Run 14 = 77.44 g/100g rice; 12.55 g/100g Kersting's groundnut, 10 g/100g lemon pomace flours

Table 6: In-vitro Protein Digestibility Determination (IVPD) of the Composite Flour

Sample	% Digestibility 10min	% Digestibility 15min
Runs 2	69.26 ± 0.01^{c}	67.27 ± 0.19^{c}
Runs 7	75.05 ± 0.23^{b}	73.78 ± 0.48^{b}
Runs 14	77.59 ± 0.04^{a}	78.31 ± 0.02^{a}

*values are mean ± standard deviation of triplicate samples *values on the same column with the same superscript are not significantly different at $p \leq 0.05$
*Run 2 = 75 g/100g rice; 20 g/100g Kersting's groundnut, 5 g/100g lemon pomace flours
*Run 7 = 82.28g/100g rice; 10 g/100g Kersting's groundnut, 7.71 g/100g lemon pomace flours
*Run 14 = 77.44 g/100g rice; 12.55 g/100g Kersting's groundnut, 10 g/100g lemon pomace flours

Table 7: Amino Acid Profile of the Composite Flour

Essential amino acid	Concentration: g/100g protein	Non-essential amino acid	Concentration: g/100g protein
Leucine	6.80	Glycine	3.89
Lysine	4.53	Alanin	4.09
Isoleucine	3.92	Serine	3.13
Phenylalanine	4.08	Cystine	1.21
Valine	3.97	Aspartic acid	7.01
Methionine	2.40	Glutamic acid	9.54
Histidine	2.55	Proline	2.84
Threonine	3.19	Hydroxyproline	5.76
Tryptophan	1.86	Citrulline	3.78
		Arginine	2.55

highest digestibility when Kersting's groundnut (main protein component) was only 12.55 % of the composite flour. The effect of higher antinutrients (though at safe levels) in run 2 must have accounted for the lowest protein digestibility. In fact, run 2 with the highest protein content had the lowest protein digestibility. As with carbohydrate digestibility, the higher the digestion time the higher the digestibility.

Amino acid profile of the composite flour

The amino acid profile of the composite flour is presented in Table 7. Leucine was the highest (6.80 mg/100g) essential amino acid followed by lysine (4.53 g/100g). Lysine is a major limiting amino acid in cereals and the increase in lysine in the composite flour could be as a result of Kersting's groundnut incorporation. Lysine promotes protein synthesis and thus, it is important for growth and maintenance of the body (Awolu et al., 2017). Glutamic acid and aspartic acid were the most abundant amino acids in the composite flour with values of 9.54 g/100g and 7.01 g/100g respectively. Glycine, together with other essential amino acids such as alanine, arginine, and phenylalanine forms polypeptides that promote growth and tissue healing (Davies & Reid, 1979).

4 Conclusions

The utilization of rice flour in the production of nutritionally rich baked products would be enhanced by the addition of Kersting's groundnut and lemon pomace. While Kersting's groundnut enhanced the protein content and minerals composition; lemon pomace enhanced its fibre content and antioxidant potentials. The addition of Kersting's groundnut at level of 20 g/100g of the composite flour was considered nutritionally safe.

In essence, composite flour consisting rice, Kersting's groundnut and lemon pomace at the blend ratios carried out in this study would be beneficial for consumption in terms of its nutritional composition and antioxidant capacities without negative antinutritional factors.

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