ISSN: 2992-5762

ASUPFPE JOURNAL OF MULTIDISCIPLINARY RESEARCH AND INNOVATION

VOLUME 2, NUMBERS 1 - 4
APRIL 2024

AGRICULTURE, ENVIRONMENT AND APPLIED SCIENCES SERIES

A PUBLICATION OF

ACADEMIC STAFF UNION OF POLYTECHNICS
FEDERAL POLYTECHNIC, EKOWE CHAPTER
BAYELSA STATE, NIGERIA

Printed and Published in Nigeria by:

ICIDR Publishing House
International Centre for Integrated Development Research
3, Alderton/Ibo Hall Road P.
O. Box 456, Ikot Ekpene
Akwa Ibom State.
Tel: +234-8064087345, 08185053436
E-mail: icidresearch@gmail.com
Website: www.icidr.org

This Work is a Publication of
Academic Staff Union of Polytechnics,
Federal Polytechnic, Ekowe Chapter,
Bayelsa State,
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Curbing Food Insecurity through Composite Blend in the Production of Bread From wheat, *acha, uzaaku* and *Unere* flours

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ABSTRACT

The aim of this study was to evaluate the proximate and micronutrient composition, physical, biochemical and sensory properties of the bread produced from blends of wheat, acha, uzaaku and unere composite flours. Six samples (A-F) were produced in the following ratios A= 100:0:0 (100% Wheat), B= 80% wheat flour, 10% acha, 5% uzaaku flour, 5% unere; C = 70% wheat flour, 10% acha, 10% uzaaku flour, 10% unere; D = 60% wheat flour, 15% acha, 15% uzaaku flour, 10% unere; E = 50% wheat flour, 20% acha, 15% uzaaku flour, 15% unere; F = 40% wheat flour, 20% acha, 20% uzaaku flour, 20% unere. The parameters were analyzed using standard methods. The proximate composition of the bread showed significant increase (p<0.05) in protein, fat, crude fibre, ash, and caloric (energy) value which ranged between 7.69-18.32%, 2.41-3.70%, 2.473.86%, 3.09-4.03%, and 285.25-304.06 (Kcal/kg) and a significant decrease (p<0.05) in moisture content (20.72-26.14%) and carbohydrate (49.37-58.20%), respectively. The micronutrient content showed increase in calcium, magnesium, potassium, iron, zinc, phosphorus, vitamin B_1, B_2, B_3, A and C as the amount of substituted flour blends increases. The result of the physical properties showed decrease in the loaf weight, loaf height, loaf volume and specific volume ranged from 240.60-218.80 g, 3.33-6.66 cm, 155.00-205.00 cm^3, 0.71-0.85 cm^3/g, respectively. The result of the serum biochemical parameters of rats fed the composite bread samples showed significant decrease (p<0.05) in AST, ALT, ALP, Bilirubin, creatine, urea and albumin values, respectively. The sensory attributes of the bread showed significant differences (p<0.05) in colour, aroma, taste, crumb texture and general acceptability such that the values obtained ranged from 6.05-8.90, 6.008.45, 6.40-8.60, 5.85-8.30 and 6.10-8.35, respectively. The findings of the study showed that supplementation of wheat with 10% acha, 10% uzaaku and 10% unere flour blends could produce well accepted bread samples.

Keywords: *Food insecurity, Composite blend, Wheat, acha, uzaaku and Unere*

INTRODUCTION

Food insecurity is on the increase recently and this has been attributed to postharvest losses in developing countries like Nigeria. Access to safe, nutritious, and sufficient food is a basic human need. Poor handling and processing of agricultural crops are some of the major contributors to this global food insecurity challenge. Thus, raw foods must be conserved against further loss by applying the appropriate processing, packaging, distribution and storage techniques/methods. That is among the roles of Food Scientists and Technologists in ensuring food security. Another means by which food insecurity could be ameliorated is in the field of composite flour utilization in the making of foods confectionaries (Olaoye et al., 2006; Olaoye and Onilude, 2008). This is the act of blending two or more flours obtained from roots, tubers, cereals and legumes with or without the addition of wheat flour (Shittu *et al*., 2007). Composite flours have been reported to be more nutritious than whole-wheat flour or any other flour from single crop (Ndife *et al*., 2011). In developing countries including Nigeria, bread constitutes a significant portion of daily snacks. It is a snack containing high nutrients prepared through the application of heat in the oven where it is transformed into appealing products from rough or unappetizing dough. As a fermented confectionary product produced mainly from wheat flour, water, yeast and salt by series of processes involving mixing, kneading, proofing, shaping and baking, it is an instant, fast and economical food product with great dietary and digestive principles (Dewettinck et al., 2008).

Local raw materials supplementation with wheat flour in bread making is increasing due to the growing market for confectioneries and consumers growing consciousness for nutritious foods (Noor *et al*., 2012). By this process the use of local vegetable crops is encouraged and could lead to increased profit margins. Wheat (*Triticum aestivum*) is one of the most useful and valuable crops grown around the world (Ikhtiar and Alam, 2007). The usefulness of wheat depends partly on its gluten protein fraction which confers the visco-elastic properties that allow dough to be processed into bread, pasta, noodles and other food products (Browns *et al.,* 2011). Acha is a cereal, traditionally consumed whole as ―tuwo‖, couscous, ―gwate‖, acha jollof and kunun acha (Oburuoga and Anyika, 2012) in Northern Nigeria. The seeds are rich in methionine and cysteine, amino acids vital to human health which are deficient in most cereals (Omeire *et al*., 2014). The seeds also contain 7.9% protein, 1.8% fat, 71% carbohydrate and 6.8% fibre (Oburuoga and Anyika, 2014).

Legumes usually improve the quality of the cereal protein by supplementing them with limiting amino acids such as lysine and sometimes methionine (Chinma and Akpapunam 2007). *Uzaaku* is a legume known as African yam bean (*Sphenostylis stenocarpa*) has many nutritional benefits which could improve the level of malnutrition, boost food security and serve as a good functional food in formulating food products that possess some health benefits.

Unere commonly known as banana has been reported to be helpful in resolving constipation without necessarily resorting to laxatives due to its high fibre content (Ahwange *et al*., 2009) and in addition its pulp helps in preventing anaemia by stimulating the production of haemoglobin due to its iron content. The incorporation of acha, African yam bean and banana flours in wheat flour for the production of bread would increase both the micro and macronutrient contents of the bread, diversify the use of the crops, and inspire farmers to produce local crops which would increase their economic power. Since substitution of wheat with local raw materials is better to increase the availability of bread. Thus, this targeted at the production and evaluation of the nutrient composition, haematological, physical and sensory properties of bread from composite flours of wheat, acha, African yam bean and banana flours.

MATERIALS AND METHODS

Sources of Raw Materials

Wheat flour, acha, uzaaku, unere and all other baking ingredients were purchased from Ogbete main market, Enugu. All chemicals to be used were of analytical grade.

Preparation of Sample flours

Acha flour was prepared according to the method described by Adegoke (2004) with slight modification. *Uzaaku* flour was prepared according to the methods described by Enwere (1998) with slight modification. *Unere* flour was prepared according to the method described by Chinmah *et al*. (2004) with slight modifications. The flour produced from each sample was packaged in an airtight plastic container, labeled and kept in a refrigerator until needed for further use.

Formulation of flour blends

The wheat, acha, AYB and banana flours were blended to fit into the experimental design as shown in Table 1. The flours were thoroughly mixed at predetermined ratios; A= (100:0:0:0), B= (80:10:5:5), C= (70:10:10:10), D= (60:15:15:10), E= (50:20:15:15) and F= (40:20:20:20) to give samples A-F using a Panasonic MX-AC 2105 blending machine to obtain homogenous blends. The composite flour blends produced were separately packaged in lidded plastic containers labeled and stored in a refrigerator until needed for analysis. The whole wheat flour without any substitution (Sample A) served as control.

Table 1: Selected Composite flour blends of wheat, Acha, AYB and Banana flours

	Flour blends/ Ratios			
Samples	**WF**	**Acha**	**AYBF**	**Banana**
A	100	0	0	0
B	80	10	5	5
C	70	10	10	10
D	60	15	15	10
E	50	20	15	15
F	40	20	20	20

Key: WF = wheat flour; AF = Acha flour; AYBF = African yam bean flour, BF = Banana flour.

Bread preparation

The straight-dough method by (Chauhan et al., 1992) was used to prepare five different samples of bread, of which four were having varying amounts of wheat and brown hamburger bean flours with constant amount of water yam flour. The sample with 100% wheat flour served as the control. All the ingredients (flour, salt, sugar, yeast and water etc.) were mixed thoroughly to form the dough.

The dough was adequately kneaded to smooth consistently, divided into equal sized pieces, moulded and transferred into clean baking pans that have its inside walls smeared with vegetable oil. The dough was allowed to proof at ambient temperature and then baked at 220ºC for 45 min.

Proximate Composition of the Bread Samples

The moisture content, ash, crude fibre, crude protein and fat content of the bread produced from wheat, water yam and brown hamburger bean were determined using the method of AOAC (2012). Total carbohydrate was calculated by difference. The caloric value was determined using the Atwater factors of protein (4), fat (9), and carbohydrate (4). These factors were used to multiply the values determined for the stated nutrients and the sum total of the multiplied values recorded.

Determination of Micronutrient Composition

The vitamins (B-complexes, A, C) and some mineral elements (Ca, K, Na, Mg, Fe, Zn and P) were determined by the method of AOAC (2005) using the Atomic Absorption Spectrophotometer (AA 800 Perkin-Elmer Germany).

Physical Properties of Bread Loaves

The physical properties of bread loaves were determined by the method of AACC (2000). Bread loaves were weighed after baking using the electronic weighing balance and the loaf weights were recorded in grams. The bread loaf height was measured by using a measuring ruler. The loaf volume was determined using the Rape seed displacement method. The specific volume was calculated as loaf volume divided by loaf weight (cm^3/g).

Animal Model

Twenty-five male Wistar rats (80-90 g) were procured from animal house of the Department of Veterinary Medicine, University of Nigeria Nsukka, Nigeria. They were housed in cages made of plastics with a prepared gauze cover to enable ventilation. The animals were fed rat chow and water for 7 days of acclimatization. The animals were weighed each day throughout the period of the experiment. After acclimatization, the mean weights of the male Wistar rats (n = 25) at the beginning of the experiment were taken. The rats were divided into five diet groups with each group having (5) rats namely; group A (control) and groups B, C, D and E (test). The rats were housed in metabolic cages. The rats of the group A (control) during the 14 days of the experiment received the 100% wheat bread-diet while the rats of the test groups (B,C,D,E) received diets made with bread samples B,C,D,E from table1, respectively. All rats were fed once a day *ad libitum*, having unrestricted access to drinking water.

Blood collection and serum Analysis

At the end of the experiment period (day 21), the rats were made to starve for 24 hours and thereafter sacrificed using cervical dislocation. Blood was collected into heparinized tubes via ocular puncture using capillary tube then followed by centrifugation at 3000 rpm for 10 mins. Clear serum was collected separately for each sample and subsequently used for further biochemical analysis. The serum chemistry evaluation included total protein, albumin, globulin, urea, creatinine, Aspartate amino-transferase (AST), Alanine amino-transferase (ALT), Alkaline phosphatase (ALP) and glucose level as described by Brij *et al*. (1990).

Sensory Evaluation

The bread samples were presented to a 20-member panel of Judges that comprised of the students and staff of the Department of Food Science and Technology, Enugu State University of Science and Technology (ESUT) Enugu, Enugu State, Nigeria. The samples were assessed for crust appearance, crumb colour, crumb texture, aroma, and overall acceptability using a nine-point hedonic scale, where 9 indicated ―liked extremely‖ and 1 indicated ―dislike extremely‖ according to Ihekoronye and Ngody (1985).

Statistical analysis

The data generated will be subjected to one-way analysis of variance (ANOVA) using Special Package for Social Science (SPSS Version 20) software. Duncan‘s New multiple range test will be used to separate significant difference at $p<0.05$.

RESULTS AND DISCUSSION

Proximate Composition

The result for the proximate composition of bread produced from the flour blends is shown in Table 2.

Table 2: Proximate composition of (%) of bread samples from the composite blends

Sample	Moisture	Crude Protein	Crude Fat	Crude Fibre	Ash	Carbohydrate	Energy (Kcal/100g)
100:0:0:0	26.14±0.16^{a}	7.69±0.03^{f}	2.41±0.04^{f}	2.47±0.00^{f}	3.09±0.01^{f}	58.20±0.25^{a}	285.25±0.01^{f}
80:10:5:5	26.05±0.01^{b}	10.48±0.01^{e}	2.83±0.11^{e}	2.81±0.02^{e}	3.43±0.01^{e}	54.40±0.08^{b}	284.99±0.01^{e}
70:10:10:10	24.88±0.13^{c}	12.76±0.06^{d}	3.06±0.02^{d}	3.15±0.03^{d}	3.86±0.02^{d}	52.29±0.15^{c}	287.74±0.13^{d}
60:15:15:10	23.71±0.12^{d}	14.01±0.06^{c}	3.28±0.03^{c}	3.49±0.02^{c}	3.92±0.02^{c}	51.59±0.04^{d}	291.92±0.02^{c}
50:20:15:15	21.36±0.12^{e}	16.23±0.06^{b}	3.39±0.03^{b}	3.55±0.02^{b}	3.95±0.02^{b}	51.52±0.04^{e}	301.51±0.02^{b}
40:20:20:20	20.72±0.12^{f}	18.32±0.06^{a}	3.70±0.03^{a}	3.86±0.02^{a}	4.03±0.02^{a}	49.37±0.04^{f}	304.06±0.02^{a}

Values are expressed as mean ± standard deviation. Values with different superscript within the column are significantly different ($p<0.05$). 100:0:0 = 100% wheat flour; 80:10:5:5 = 80% wheat flour, 10% acha, 5% uzaaku flour, 5% unere; 70:10:10:10 = 70% wheat flour, 10% acha, 10% uzaaku flour, 10% unere; 60:15:15:10 = 60% wheat flour, 15% acha, 15% uzaaku flour, 10% unere; 50:20:15:15 = 50% wheat flour, 20% acha, 15% uzaaku flour, 15% unere; 40:20:20:20 = 40% wheat flour, 20% acha, 20% uzaaku flour, 20% unere.

Proximate composition of (%) of bread samples from the composite blends

The moisture content of the bread samples differed significantly at ($p<0.05$) and it ranged from 20.72-26.14%. The control (sample A) had the highest moisture content of (26.14%) while sample E had the least moisture content of (20.72%). The moisture content of the bread samples decreases as substituted with acha, uzaaku and unere flours increases. It was reported by Palmer (2001) that moisture content of bread decreases with increase in composite flours in wheat, acha and mung beans composite bread. The decrease in moisture could be explained on the lower content of gluten in non-wheat flours which result in formation of gluten network with weak cell structure that embed lower moisture. The decreased moisture content would also enhance longer shelf life. The protein content of the samples which ranged from 7.69 to 18.32% was observed to increase with increased substitution of acha, uzaaku and unere flours in the products. The protein contents of all the substituted bread samples were higher than that of the control (Bread samples made with 100% wheat bread). This agrees with the work of Alozie *et al*. (2009) where protein increased as a result of increase in Bambara nut in wheat- Bambara nut composite bread.

The fat content of the bread samples differed significantly at ($p<0.05$) as it ranged from 2.41 to 3.70%. The fat content of the control sample (Bread samples made with 100% wheat flour) was significantly ($p<0.05$) lower than the fat content of all the substituted samples. This implies that foods prepared using this composite flour would be energy dense foods suitable for people with high energy needs (Igbabul *et al*., 2014). The ash content of the samples ranged from 3.09 to 4.03% with the control (Bread samples made with 100% wheat flour) and the sample substituted with 20% acha, 20% uzaaku and 20% unere flours having the least (3.09%) and highest (4.03%) values, respectively. The increase in the ash content observed in substituted samples could suggest high mineral contents of the unit crops (acha, uzaaku and unere) used in the preparation of the bread samples (Christiana, 2019).

The crude fibre content of the samples ranged from 2.47% in the control sample to 3.86% for the sample substituted with 20% acha, 20% uzaaku and 20% unere flours, respectively. The crude fibre content increased as the levels of substitution with acha, uzaaku and unere flours increased and this is in agreement with the report that African yam bean and banana fruits are rich sources of dietary fibre (Iwe, 2003; Al-Farsi and Lee, 2008). The carbohydrate content of the samples differed significantly at ($p<0.05$) as it ranged from 49.37 to 58.20%. The control sample had the highest (58.20%) while sample F (substituted with 20% acha, 20% uzaaku and 20% unere flours) had the least carbohydrate vale (49.37%), respectively. Similar decrease in carbohydrate content was reported by Onweluzo and Nnamuchi (2009) for bread samples produced from *Treculia africana* and *Sorghum bicolor*.

The energy content of the bread samples which ranged from 285.25 to 304.06 KJ/100g. The energy content of the bread samples increased significantly ($p<0.05$) with increase in substitution of acha, uzaaku and unere flours in the products. The control sample (Bread samples made with 100% wheat flour) had the least energy value (285.25 KJ/100g), while the sample substituted with 20% acha, 20% uzaaku and 20% unere flours had the highest energy value (304.06 KJ/100g). The significant difference ($p<0.05$) in the energy content could be due to differences in protein, fat and carbohydrate contents of the samples. The substitution of wheat flour with acha, uzaaku and unere flours in the preparation of bread samples greatly increase the protein, fat, ash and crude fibre contents of the products.

Table 3: Mineral composition (mg/100g) of bread samples from the composite blends

Sample	Calcium	Magnesium	Potassium	Iron	Zinc	Phosphorus
100:0:0:0	$18.60^{f}\pm0.07$	$15.54^{f}\pm0.02$	$18.47^{f}\pm0.06$	$1.60^{f}\pm0.01$	$1.32^{f}\pm0.01$	$17.01^{f}\pm0.06$
80:10:5:5	$22.50^{e}\pm0.01$	$22.47^{e}\pm0.01$	$19.52^{e}\pm0.04$	$1.63^{e}\pm0.01$	$1.41^{e}\pm0.04$	$23.71^{e}\pm0.07$
70:10:10:10	$31.10^{d}\pm1.31$	$37.45^{d}\pm0.01$	$23.36^{d}\pm0.02$	$1.77^{d}\pm0.04$	$1.48^{d}\pm0.00$	$29.24^{d}\pm0.09$
60:15:15:10	$45.70^{c}\pm0.06$	$41.73^{c}\pm1.26$	$30.83^{c}\pm1.26$	$1.85^{c}\pm0.02$	$1.56^{c}\pm0.06$	$35.88^{c}\pm1.35$
50:20:15:15	$60.50^{b}\pm0.06$	$43.67^{b}\pm1.40$	$45.79^{b}\pm0.07$	$2.48^{b}\pm0.03$	$1.67^{b}\pm0.01$	$47.87^{b}\pm1.28$
40:20:20:20	$62.50^{a}\pm0.09$	$50.30^{a}\pm1.40$	$52.30^{a}\pm0.06$	$3.24^{a}\pm0.05$	$1.80^{a}\pm0.08$	$48.60^{a}\pm1.27$

Values are expressed as mean ± standard deviation. Values with different superscript within the column are significantly different (p<0.05). 100:0:0 = 100% wheat flour; 80:10:5:5 = 80% wheat flour, 10% acha, 5% uzaaku flour, 5% unere; 70:10:10:10 = 70% wheat flour, 10% acha, 10% uzaaku flour, 10% unere; 60:15:15:10 = 60% wheat flour, 15% acha, 15% uzaaku flour, 10% unere; 50:20:15:15 = 50% wheat flour, 20% acha, 15% uzaaku flour, 15% unere; 40:20:20:20 = 40% wheat flour, 20% acha, 20% uzaaku flour, 20% unere.

Mineral Composition of Bread samples

The mineral compositions of the bread samples are presented in Table 3. The calcium content of the bread samples ranged from 18.60 to 62.50mg/100g. The control sample (Bread samples made with 100% wheat flour) had the least value (18.60 mg/100g), while the sample substituted with 20% acha, 20% uzaaku and 20% unere flours had the highest calcium content (62.50mg/100g). The increase in calcium content observed in all the substituted samples could be attributed to the substitution effect caused by high levels of calcium in acha, uzaaku and unere flours as reported by Baliga *et al.* (2011). Calcium is a constituent of bones and it helps the body in muscular contraction and blood clothing.

The magnesium content of the bread samples differed significantly at ($p<0.05$) as it ranged from 15.54 to 50.54mg/100g with the control sample having the least (15.54mg/100g) and the sample substituted with 20% acha, 20% uzaaku and 20% unere flours and highest (50.54mg/100g) values, respectively. The increase in magnesium content observed in all the substituted samples could be attributed to the substitution effect caused by high levels of magnesium in acha, uzaaku and unere flours. The increase in the magnesium content of the samples is an indication that acha, uzaaku and unere are good sources of magnesium (Al-Farsi and Lee, 2008; Agunbiade and Ojezele, 2010). Magnesium is beneficial in the control of high blood pressure it is also an important component of bone which contributes to its structural development (Jacob *et al.*, 2015).

The potassium content of the bread samples which ranged from 18.47 to 52.30mg/100g increased significantly ($p<0.05$) as the levels of acha, uzaaku and unere flours increased in the products. The control sample (100% wheat flour) had the least value (18.47mg/100g) and the sample substituted with 20% acha, 20% uzaaku and 20% unere flours had highest (52.30mg/100g) values, respectively. The observed increase in the potassium content is an indication that acha, uzaaku and unere are good sources of potassium (Khan *et al.,* 2003; Polak *et al.,* 2015). Potassium is essential in blood clotting and muscle contraction in humans.

The iron content of the bread samples ranged from 1.60 to 3.24mg/100g. The iron content of the samples varied significantly ($p<0.05$) from each other. The sample substituted with 20% acha, 20% uzaaku and 20% unere flours had the highest iron values (3.24mg/100g), while the control sample had the least iron content (1.60mg/100g). The values (1.60-3.24mg/100g) obtained in this study were lower than the iron content (3.22-5.64mg/100g) of bread samples produced from blends of acha and fermented soybean paste reported by Mbaeyi-Nwaoha and Uchendu (2015). Regular consumption of food that is rich in iron has the potential to prevent anaemia in infants and young children. Iron is also an essential element that is needed in humans and plants. The zinc content of the bread samples differed significantly at ($p<0.05$) as it ranged from 1.32 to 1.80 mg/100g. There was an increase in the zinc content as the amount of acha, uzaaku and unere substituted increased. The increase in zinc content could be attributed to the addition of high proportions of acha, uzaaku and unere flours in the products (Oloye, 2014; Boudries *et al.,* 2007). Zinc plays an important role in the growth and development during pregnancy, childhood and adolescence (Park *et al.,* 2010). The substitution of wheat-bread with acha, uzaaku and unere flours generally increased the mineral contents of the products.

The phosphorus content of the bread samples differed significantly at ($p<0.05$) as it ranged from 17.01 to 48.60mg/100g with the control sample (100% wheat flour) had the least value (17.01mg/100g) and the sample substituted with 20% acha, 20% uzaaku and 20% unere flours had highest (50.76mg/100g) values, respectively. The increase in the phosphorus content of the samples is an indication that acha, uzaaku and unere are good sources of phosphorus (Agunbiade and Ojezele, 2010; Al-Farsi and Lee, 2008). Phosphorus is an important mineral that plays a significant role in the formation of Adenosine Triphophate (ATP) in the body (Okaka *et al*., 2006).

Table 4: Vitamin Composition (mg/100g) of bread samples from the composite blends

Sample	Thiamine (B_1)	Riboflavin (B_2)	Niacin (B_3)	Vitamin A	Vitamin C
100:0:0:0	$3.03^e \pm 0.01$	$6.35^f \pm 0.01$	$4.34^e \pm 0.03$	$7.10^f \pm 0.01$	$9.74^f \pm 0.03$
80:10:5:5	$6.08^e \pm 0.01$	$7.93^e \pm 0.16$	$10.36^d \pm 0.03$	$8.44^e \pm 0.03$	$13.83^e \pm 0.01$
70:10:10:10	$8.12^d \pm 0.01$	$8.55^d \pm 0.04$	$14.43^f \pm 0.01$	$12.71^d \pm 0.07$	$24.02^d \pm 0.01$
60:15:15:10	$11.17^c \pm 0.01$	$8.90^c \pm 0.02$	$16.49^c \pm 0.01$	$14.94^c \pm 0.07$	$33.25^c \pm 0.01$
50:20:15:15	$12.24^b \pm 0.01$	$9.15^b \pm 0.04$	$18.54^b \pm 0.01$	$16.04^b \pm 0.03$	$39.45^b \pm 0.04$
40:20:20:20	$14.33^a \pm 0.01$	$12.27^a \pm 0.01$	$22.63^a \pm 0.01$	$20.17^a \pm 0.02$	$42.77^a \pm 0.01$

Values are expressed as mean ± standard deviation. Values with different superscript within the column are significantly different (p<0.05). 100:0:0 = 100% wheat flour; 80:10:5:5 = 80% wheat flour, 10% acha, 5% uzaaku flour, 5% unere; 70:10:10:10 = 70% wheat flour, 10% acha, 10% uzaaku flour, 10% unere; 60:15:15:10 = 60% wheat flour, 15% acha, 15% uzaaku flour, 10% unere; 50:20:15:15 = 50% wheat flour, 20% acha, 15% uzaaku flour, 15% unere; 40:20:20:20 = 40% wheat flour, 20% acha, 20% uzaaku flour, 20% unere.

Vitamin Composition of bread samples from the composite blends

The vitamin composition of bread samples from the composite blends were presented in table 4. The thiamine content of the bread samples ranged from 3.03 to 14.33mg/100g. There is a significant increase ($p<0.05$) with increase addition of acha, uzaaku and unere flours in the formulations. The result showed that the sample substituted with 20% acha, 20% uzaaku and 20% unere flours had the highest thiamine content (14.33mg/100g) while the control sample had the lowest thiamine content (3.03mg/100g). Thiamine functions as a co-enzyme in energy metabolism in the body. It also helps in the treatment of beriberi and in the maintenance of healthy mental attitude in young children and adolescents (Okaka *et al*., 2006).

The riboflavin content of the bread samples ranged from 6.35 to 12.27mg/100g. The sample substituted with 20% acha, 20% uzaaku and 20% unere flours had the highest riboflavin content (12.27mg/100g) while the control sample (bread made with 100mg/100g wheat flour) had the lowest niacin content (6.35mg/100g), The riboflavin content of the custard samples increased significantly ($p < 0.05$) with increase in substitution with acha, uzaaku and unere flours in the formulations. The increase in the riboflavin content could be due to substitution effect. Riboflavin functions as part of a group of enzymes called flavoproteins (Potter and Hotchkiss, 2006).

The niacin content of the bread samples ranged from 4.23 to 22.63mg/100g. The result showed that the sample substituted with 20% acha, 20% uzaaku and 20% unere flours had the highest niacin content (22.63mg/100g) while the control sample had the lowest niacin content (4.23mg/100g). The increase in niacin content could be attributed to the addition of high proportion of acha, uzaaku and unere flours in the products. Niacin which is equally a member of the Bcomplex vitamin functions as a co-enzyme (NAD and NADP) in the body (Potter and Hotchkiss, 2006).

The vitamin A content of the bread samples ranged from 7.10 to 20.17 mg/100g. The control sample (bread made with 100% wheat flour) had the least (7.10mg/100g) value while the sample substituted with 20% acha, 20% uzaaku and 20% unere flours had the highest (20.17mg/100g) values, respectively. Vitamin A helps in the maintenance of normal vision of the eyes (Potter and Hotchkiss, 2006).

The ascorbic acid content of the bread samples ranged from 9.74 to 42.77mg/100g. The sample substituted with 20% acha, 20% uzaaku and 20% unere flours had the highest ascorbic acid content (42.77 mg/100g) while the control sample (bread made with 100 % wheat flour) had the lowest value (9.74mg/100g). The result showed that the ascorbic acid content of the samples increased with increased substitution of acha, uzaaku and unere flours in the products. Ascorbic acid is easily destroyed by oxidation, especially at high temperatures and it is the most easily destroyed vitamin during food processing (Potter and Hotchkiss, 2006).

Table 5: Physical Properties of bread samples from the composite blends

Sample	Loaf weight (g)	Loaf height (cm)	Loaf volume (cm^3)	Specific volume (cm^3/g)
100:0:0:0	$240.60^a \pm 0.04$	$6.66^a \pm 0.04$	$205.00^a \pm 0.02$	$0.85^a \pm 0.00$
80:10:5:5	$238.10^b \pm 0.04$	$4.55^b \pm 0.07$	$200.00^b \pm 0.07$	$0.84^b \pm 0.02$
70:10:10:10	$234.30^c \pm 0.07$	$4.30^c \pm 0.02$	$185.00^c \pm 0.04$	$0.79^c \pm 0.01$
60:15:15:10	$232.40^d \pm 0.05$	$3.55^d \pm 0.05$	$175.00^d \pm 0.00$	$0.75^d \pm 0.02$
50:20:15:15	$227.40^e \pm 0.07$	$3.42^e \pm 0.02$	$168.00^e \pm 0.02$	$0.73^e \pm 0.01$
40:20:20:20	$218.80^f \pm 0.07$	$3.33^f \pm 0.02$	$155.00^f \pm 0.02$	$0.71^f \pm 0.03$

Values are expressed as mean ± standard deviation. Values with different superscript within the column are significantly different (p<0.05). 100:0:0 = 100% wheat flour; 80:10:5:5 = 80% wheat flour, 10% acha, 5% uzaaku flour, 5% unere; 70:10:10:10 = 70% wheat flour, 10% acha, 10% uzaaku flour, 10% unere; 60:15:15:10 = 60% wheat flour, 15% acha, *15% uzaaku flour, 10% unere; 50:20:15:15 = 50% wheat flour, 20% acha, 15% uzaaku flour, 15% unere; 40:20:20:20 = 40% wheat flour, 20% acha, 20% uzaaku flour, 20% unere.*

Physical properties of bread samples from the composite blends

The physical properties were presented in table 5. The result showed that the loaf weight ranged from 218.80 – 240.60g. There was a significant difference ($p<0.05$) in the weight of the samples. The weight of the bread samples decreased with increase in the level of acha, uzaaku and unere flour in the formulation. This may be the directional increase in the protein content from the incorporation of acha, uzaaku and unere flours. The loaf height of the bread ranged from 3.33-6.66 cm with decrease in the loaf volume from sample A to F. The result of the study showed that values for loaf height increased with increase in the level of composite flour. This may be the directional increase in the protein content from the incorporation of acha, uzaaku and unere flours. The loaf volume varied from 155.00-205.00 cm^3. Sample A has the highest volume (205.00 cm^3) and Sample E has the least value (155.00 cm^3), while the other samples decreased significantly. The decrease may be as a result of the increase in the amount of acha, uzaaku and unere flours which do not contain gluten and thus dilutes the gluten content of wheat. The result showed that the specific volume ranged from 0.71 – 0.85cm^3/g. There was a significant difference ($p<0.05$) in the specific volume of the samples such that the values decrease as the level of substitution of wheat with acha, uzaaku and unere flours increases. The finding is in agreement with the report of Ndife et al. (2011) which stated that progressive inclusion of soy bean (legumes) flour to wheat flour decreased the bread volume. This might be attributed to the different flour types used for bread production. As the most important parameter in bread making, specific volume indicates final gas retention in the bread and this affects consumer‘s preference (Ranawana *et al*., 2020).

Table 6: Effect of composite bread samples on some Biochemical Parameters of Wistar rats

Sample	**AST(IU/L)**	**ALT(IU/L)**	**ALP(IU/L)**	**BIL(mg/dl)**	**Creatine (mg/dl)**	**UREA (mg/dl)**	**Albumin (mg/dl)**
100:0:0:0	18.60^a±0.41	19.80^a±0.41	38.20a±0.41	0.40^a±0.41	0.28^a±0.41	26.15±0.20	2.42±0.36
80:10:5:5	19.40^a±0.41	16.80^a±0.41	26.20a±0.41	0.31^a±0.41	0.33^a±0.41	26.60±0.65	3.20±0.65
70:10:10:10	21.80^a±0.41	16.60^a±0.41	34.40a±0.41	0.37^a±0.41	0.36^a±0.41	27.28±0.36	3.60±0.24
60:15:15:10	18.60^a±0.41	16.00^a±0.41	30.20a±0.41	0.33^a±0.41	0.38^a±0.41	28.63±0.24	3.85±0.20
50:20:15:15	13.40^a±0.41	15.80^a±0.41	27.60a±0.41	0.36^a±0.41	0.40^a±0.41	29.15±0.44	4.60±0.44
40:20:20:20	13.80^a±0.41	14.80^a±0.41	26.40^a±0.41	0.30^a±0.41	0.43^a±0.41	30.75±0.33	4.80±0.33

Values are expressed as mean ± standard deviation. Values with different superscript within the column are significantly different ($p<0.05$). 100:0:0 = 100% wheat flour; 80:10:5:5 = 80% wheat flour, 10% acha, 5% uzaaku flour, 5% unere; 70:10:10:10 = 70% wheat flour, 10% acha, 10% uzaaku flour, 10% unere; 60:15:15:10 = 60% wheat flour, 15% acha, 15% uzaaku flour, 10% unere; 50:20:15:15 = 50% wheat flour, 20% acha, 15% uzaaku flour, 15% unere; 40:20:20:20 = 40% wheat flour, 20% acha, 20% uzaaku flour, 20% unere.

Effect of composite bread samples on some Biochemical Parameters of Wistar rats

The result of serum biochemistry of the experimental rats is presented in Table 6. Alanine Aminotransferase (ALT), Alanine Phosphatase (ALP) and Aspartate Aminotransferase (AST) which is also known as Serum Glutamic oxaloacetic Transferase (SGOT) are pointers of the condition of the liver. They are low in the blood when the liver is normal and high when the liver is damaged. The results of Aspartate Aminotransferase (AST) concentration ranges from 13.80 to

19.80 IU/L. AST was significantly higher in the control than the fortified groups. The results of Alanine Aminotransferase (ALT) concentration range from 14.80 to 19.80 IU/L. ALT value was significantly higher in the control than the fortified groups. The results of Alanine Aminotransferase (ALP) concentration ranges from 26.40 to 38.20 IU/L. Low values of AST, ALT and ALP are pointers to improved liver function. Serum ALT and AST activities were bioindicators of liver function, though ALT is specific while AST which is not could in addition indicate dysfunction of other high metabolic organs, including the heart, kidney, skeletal muscle and brain (Shivaraj, 2009). Rats in the control treatment group had significantly higher values of ALT, ALP, and AST compared with the fortified fed groups. This indicated that the fortified fed samples have no adverse effects on the livers of the animals. Tissue damage is usually associated with the release of enzymes specific to the affected tissue or organ in circulation. The consequence is an increase in the activity of such enzymes in body fluids (Aliyu *et al*., 2006). The significant reductions observed in the activity of ALT and ALP indicate that the extracts of fortified bread samples were not harmful to the liver. The result is in agreement with the study of Atawodi and Iliemene, (2014) who reported that the AST and ALT were significantly lowered by administration of *A. africana* aqueous extract to CCl_4-induced rats.

Diseases associated with the kidney often manifest as water imbalance with alteration in fluid intake following polyuria or oliguria (Alcázar, 2008). The results of bilirubin concentration range from 0.30 to 0.40 mg/dl. Bilirubin was significantly lower in the control than the fortified groups. Low value of bilirubin is a pointer to improved kidney function. Bilirubin is excreted by the liver; hence its level in the blood is an index of liver function (Colleen, Allan and Micheal, 2003). Thus, strongly fortified feed samples protected and ameliorated rat liver damage as much as vitamin E did. The results of creatinine concentration range from 0.28 to 0.43 mg/dl. Creatinine was significantly lower in the control than the fortified groups 0.43 mg/dl respectively. Low value of creatinine is a pointer to improved kidney function.

The result of serum urea shows an increase the values recorded significant increase (p<0.05) in serum urea concentration when compared to the normal control. The results indicate no observable muscular wastage. The control group had 26.15 mg/dl while the group F fed (40% wheat, 20% acha, 20% uzaaku and 25% unere flours) had 30.75 mg/dl urea concentration. The result of the study indicates that all fortified groups showed significant increase (p<0.05) in serum albumin concentration when compared to the normal control. The control group had 2.42 mg/dl while the group F fed (40% wheat, 20% acha, 20% uzaaku and 25% unere flours) had 4.80 mg/dl albumin concentration. This is an indication of the good quality of the protein it supplies.

Christopher *et al*., (2020) stated that protein reserve in an animal gives an indication of the total protein value in the diet. The higher values of the albumin of rats in the fortified diet are a reflection of the adequacy of the dietary protein. This increase can be attributed to the absence of an anti-nutrient (phytate) which binds protein and makes it unavailable to the animals.

Table 7: Sensory properties of composite bread samples

Samples	Crumb Colour	Aroma	Taste	Texture	Overall acceptability
A	$8.90^{a}\pm0.10$	$8.45^{a}\pm0.3$	$8.60^{a}\pm0.3$	$8.30^{a}\pm0.1$	$8.35^{a}\pm0.3$
B	$7.75^{b}\pm0.2$	$7.85^{b}\pm0.1$	$7.80^{b}\pm0.4$	$7.45^{b}\pm0.1$	$7.05^{b}\pm0.3$
C	$6.80^{c}\pm0.2$	$6.75^{c}\pm0.3$	$6.80^{c}\pm0.2$	$6.75^{c}\pm0.4$	$6.75^{c}\pm0.07$
D	$6.40^{d}\pm0.2$	$6.65^{d}\pm0.2$	$6.60^{d}\pm0.3$	$6.30^{d}\pm0.4$	$6.50^{d}\pm0.1$
E	$6.20^{e}\pm0.07$	$6.10^{e}\pm0.3$	$6.55^{e}\pm0.2$	$5.90^{e}\pm0.4$	$6.40^{e}\pm0.4$
F	$6.05^{f}\pm0.05$	$6.00^{f}\pm0.4$	$6.40^{f}\pm0.2$	$5.85^{f}\pm0.4$	$6.10^{f}\pm0.2$

Values are expressed as mean ± standard deviation. Values with different superscript within the column are significantly different (p<0.05). 100:0:0 = 100% wheat flour; 80:10:5:5 = 80% wheat flour, 10% acha, 5% uzaaku flour, 5% unere; 70:10:10:10 = 70% wheat flour, 10% acha, 10% uzaaku flour, 10% unere; 60:15:15:10 = 60% wheat flour, 15% acha, 15% uzaaku flour, 10% unere; 50:20:15:15 = 50% wheat flour, 20% acha, 15% uzaaku flour, 15% unere; 40:20:20:20 = 40% wheat flour, 20% acha, 20% uzaaku flour, 20% unere.

Sensory properties of bread samples from the composite blends

The sensory evaluation of the composite bread samples was presented in Table 7. The crumb colour ranged from 6.05 to 8.90. There was significant difference ($p<0.05$) among the samples in terms of colour. Addition of acha, uzaaku and banana flours reduced the colour of the bread samples. Despite the high nutritionally quality of acha, uzaaku and unere, there flours colour could not be compete favourably with whole-wheat in bread products. Similar results were reported by Olanipekun *et al*. (2018) who pointed out that the incorporation of other types of flours in the manufacture of wheat bread affects the organoleptic properties of the breads produced. Colour is a key parameter in evaluating well prepared and baked food products.

The aroma scores of the bread samples ranged from 6.00 to 8.45. There was a significant difference ($p<0.05$) among the samples in terms of aroma such that the control sample (100% wheat bread) had the highest score (8.45) while sample F (50% wheat flour, 20% acha, 20% uzaaku and 20% unere flours) had the least score (6.00). Aroma is the main criterion that makes the product to be liked or disliked. Adequate consumption of any bread product is hugely dependent on the quality of the flavour. Aroma is a fundamental sensory quality that relates to the sensations in the nostrils caused by volatile substances rising from food or drink (Okache *et al*., 2020). The taste scores of the bread samples ranged from 6.40 to 8.60. There was a significant difference ($p<0.05$) among the samples such that the highest taste score was observed in bread made with 100% wheat. This is quite explainable because wheat has been the major flour for bread making therefore influencing the sensory judgment of the panelists.

Taste is the desirable quality of the foods. This is also where the judges sample the food orally. Bread texture value was observed to increase from 5.85 to 8.30. There was significant difference ($p<0.05$) among the samples in terms of texture such that the control sample (100% wheat bread) had the highest score (8.30). The texture of the bread samples talks about the physical feel of the food. This defines the smoothness or roughness of the food when eaten. The texture scores have shown that high amount of acha, uzaaku and unere flour blends (up to 15% each) in bread products will affect the texture of the bread. However, very pleasant texture was observed in the breads obtained with an incorporation rate of acha, uzaaku and unere flours up to 10% each.

The same remarks were reported by Ouazib (2017) during the evaluation of the effect of the partial substitution of wheat flour by chickpea flour on bread quality. Overall acceptability (OA) scores ranged from 6.10 to 8.50. There was significant difference (P<0.05) among the samples such the control sample (100% wheat bread) had the highest score (8.50). The 100% whole wheat bread was score higher and most preferred than all the other substituted samples. This is based on the fact that wheat has been the major flour for bread making therefore influencing the sensory judgment of the panelists. Generally, the result of the sensory evaluation has shown that delicious and appealing bread samples can be obtained with an incorporation rate of acha, uzaaku and unere flours up to 10% each.

CONCLUSION

The study has demonstrated that substitution of wheat flour with acha, uzaaku and unere flours in the preparation of composite flour blends and bread greatly improved the nutrient profile of the final products with no traceable health side-effect. Also, the findings from the sensory properties of the bread samples revealed that supplementation of wheat with 10% acha, 10% uzaaku and 10% unere flour blends could produce well accepted bread samples. Thus, it is recommended that inclusion of acha, uzaaku and unere in bread will go a long way in enhancing nutrition, health and wellbeing of the consumers and reduce the dependence on wheat flour, thereby saving the huge foreign exchange used in importing wheat, for other projects. It will also reduce food insecurity and diversify the use of acha, uzaaku and unere.

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