Amino Acid Profile, Mineral Bioavailability, and Sensory Properties of Biscuits Produced from Composite Blends of Wheat, Coconut and Defatted Fluted Pumpkin Seed Flour

G. I. Oyet and B. S. Chibor

***Abstract* — The objective of this work was to produce biscuits from composite flour of wheat, coconut, and defatted fluted pumpkin seed and to evaluate the mineral bioavailability, amino acid profile and sensory properties of the composite biscuits. Wheat/coconut/defatted fluted pumpkin seed flours were blended in the ratio; 100/0/0, 80/10/10, 70/20/10, 60/30/10, 50/40/10, 40/50/10 and labelled as sample A, B, C, D, E and F respectively. Biscuits were baked with these composite flours using the creaming method. Lysine content ranged from 3.35-5.07g/100g, with sample F given significantly higher value followed by samples E (4.70g.100g) and D (4.33g/100g). All the essential amino acids increased significantly with increased substitution of coconut and fluted pumpkin seed flour, except phenyl-lanine and cysteine that decreased from 4.98 – 3.90 and 1.22 – 0.69g/100g respectively in sample A to E. Sample E gave higher total Ca, Fe, K and Zn of 26.89, 17.92, 86.02 and 2.82mg/100g respectively. Percentage bioavailability of Ca, Fe, Na, K and Zn were significantly higher in sample F with values of 59.67, 51.35, 58.22, 59.67 and 47.74% respectively. Sample E recorded the highest overall acceptability score of 6.38. This score was however not significantly different from scores obtained for samples A, B, C and D. Production of composite biscuits with 50/40/10% wheat/coconut/defatted fluted pumpkin seed flour is highly recommended.**

***Index Terms* — Biscuits, Amino Acids, Mineral, Sensory, coconut, Fluted Pumpkin.**

I. Introduction

Biscuits are inexpensive, ready-to-eat thin crisp food products which are majorly processed from wheat flour and other ingredients [1]. Biscuit consumption has been on the increase among all age groups in recent times because of its ready to eat nature [2]. In baking industry wheat is widely preferred cereal and most commonly used in biscuit production because of its unique rheological properties imparting positive effect on baking quality [3]. Most baked products made from wheat and other cereals are poor sources of protein and other essential nutrients [4]. Enrichment of cereal- based foods with other protein sources such as oil seeds and legumes has received considerable attention [5]. This is because oil seed and legumes are rich in proteins particularly essential amino acid which are limited in most cereals. The use of composite flour based on wheat and other cereals in bakery products are becoming popular because of the economic and nutritional advantages of composite flour [6]. The primary ingredient used for biscuit production (wheat) is low in essential minerals, which could be enhanced by supplementing the wheat flour. Coconut and fluted pumpkin seed flour (FPSF) can act as a supplement in baked products.

In recent times, there has been an increasing demand towards consumption of high fibre biscuits due to their health promoting properties. This has given rise to increased demand for health oriented and functional food products such as sugar-free, low calorie, low cholesterol and high fibre products as one of the advances to curb these health problems. Diets rich in fibre have been proven to have positive effect on health as their consumption has been related to decreased incidence of several diseases [7]. Several researchers have worked on the addition of dietary fibre to breakfast cereals and baked products particularly biscuits and bread in order to meet the consumer's health needs [8]. Thus, in order to meet the dietary fibre requirement, the enrichment of biscuits with higher dietary fibre content is an acceptable approach to increase daily intake of fibre.

Coconut flour is a significant source of dietary fibre, free of trans-fatty acids and is low in digestible carbohydrate [9]. Coconut flour is a unique product that is rich and healthy source of nutrient. It can be used as bulking agents, filling agents and as a substitute for wheat, rice and potato flour at certain levels. It was found that the glycemic index of coconut flour supplemented foods decreased with increasing levels of dietary fiber from coconut flour [10]. Coconut flour is loaded with numerous nutrients and it is free from gluten and phytic acid. The health benefits of coconut flour include protection against strokes, significant reduction in blood pressure, enhanced energy production and it also boosts thyroid function, balances blood sugar and insulin level, cleanses the body’s internal systems. Studies revealed that consumption of high fiber coconut flour increases fecal bulk [11].

Fluted pumpkin (*Tefairia occidentalis)* grows in many countries of West Africa, but cultivated mainly in Nigeria where it is used primarily in soups and herbal medicines. It is said to be indigenous to southern Nigeria [12]. The fruit pulp is not edible, but the seeds are rich in fat and protein, and can therefore be used in nutrient fortification, to enhance a well-balanced diet. [13] reported that fluted pumpkin seed contained 27% protein and 54% fat. Pumpkin seed flour have been used for nutritional enrichment and for maintaining the rheological and sensory properties of confectionery products

Published on December 12, 2020.
G. I. Oyet, Department of Food Science and Technology, Rivers State University, Nigeria.
(corresponding email: gogomaryo@yahoo.com)
B. S. Chibor, Department of Food Science and Technology, Rivers State University, Nigeria.

[14]. Pumpkin seed flour, unlike wheat, is rich in fibre (47.9%, dry mass) and thus, enhances intestinal functions and produce the feeling of satiety that is essential in body weight control [15]. It has potential for use as a functional agent in many formulated foods [16] [17]. Thus, use of Fluted pumpkin seed flour as wheat flour substitute in biscuit production has great potential to bridge the nutritional gap as might be presented in wheat. This could help to reduce protein-energy malnutrition in school children and adults [18], been that the major determinants of malnutrition in Africa are low availability of nutritious foods and inadequate consumption of protein rich diets. A lot of work has been done using many types of cereals and pulses to make composite flour for different types of bakery products but there is paucity of information on the use of FPSF even though it is cheaper than wheat. The objective of this study therefore was to determine the Amino acid content, total mineral and mineral bioavailability of biscuits produced from different inclusion levels of coconut and FPSF in wheat flour using conventional biscuit production recipe and to access its acceptability.

II. Materials and Methods

A. *Defatting of Fluted Pumpkin Seed Flour*

Fluted pumpkin seed was dehulled, cleaned and oven dried at 60 ºC for 24h [19] in a hot air oven (model QUB 305010G, Gallenkamp, UK), milled using a laboratory mill (model MXAC2105, Panasonic, Japan). The flour was defatted using the method described by [20], with slight modification. The milled seed flour was made into paste by adding warm and cold water intermittently. The paste was placed in boiling water and allowed to boil for 6 hours. Oil floating to the surface and kept to stand overnight in the refrigerator. To allow for oil crystallization making it easier to be skimmed off the mixture. Defatted pumpkin flour was dried in a hot air oven to 12–13% moisture content, sieved to fine particles.

B. *Preparation of Coconut Flour*

Coconut endosperm after the removal of shell and paring, was shredded, grated and oven dried at 60 ºC for 24h [19] in a hot air oven (model QUB 305010G, Gallenkamp, UK), milled using a laboratory mill (model MXAC2105, Panasonic, Japan) to obtain coconut flour.

C. *Composite Flour and Biscuit Production*

Six blends were prepared by mixing wheat, coconut and defatted fluted pumpkin seed flours in the following proportions: 100: 0: 0, 80: 10: 10, 70: 20: 10, 60: 30: 10, 50: 40: 10 and 40:50: 10% as shown in Table 1.

Composite biscuit was produced using the creaming method described by [21], The basic ingredient used were composite flour (100 g), fat (40 g), sucrose (50 g), milk (4 g), salt (2 g) and baking powder (1 g). The sugar and fat were initially creamed in a mixer to produce a creamy mixture before the flour and other dry ingredients were added. Thereafter, the mixture was thoroughly mixed with little water to form hard consistent dough. The dough was kneaded cut and baked at 160 °C for 20 min, cooled and packed as shown in Fig. 1.

TABLE 1: Production Blends for Wheat/Coconut/Fluted Pumpkin Seed Flour Biscuits

Samples	Ingredient								
	WF (g)	CNF (g)	DFPSF (g)	Sugar (g)	Fat (g)	Milk (g)	Salt (g)	H_2O (g)	Baking powder (g)
A	100	0	0	50	40	4	2	15	1
B	80	10	10	50	40	4	2	15	1
C	70	20	10	50	40	4	2	15	1
D	60	30	10	50	40	4	2	15	1
E	50	40	10	50	40	4	2	15	1
F	40	50	10	50	40	4	2	15	1

Key: A = 100% WF (control).
B = 80% WF + 10% CNF+10% DFPSF.
C = 70% WF + 20% CNF+10% DFPSF.
D = 60% WF + 30% CNF+10% DFPSF.
E = 50% WF + 40% CNF+10% DFPSF.
F = 40% WF + 50% CNF+10% DFPSF.
WF= wheat flour.

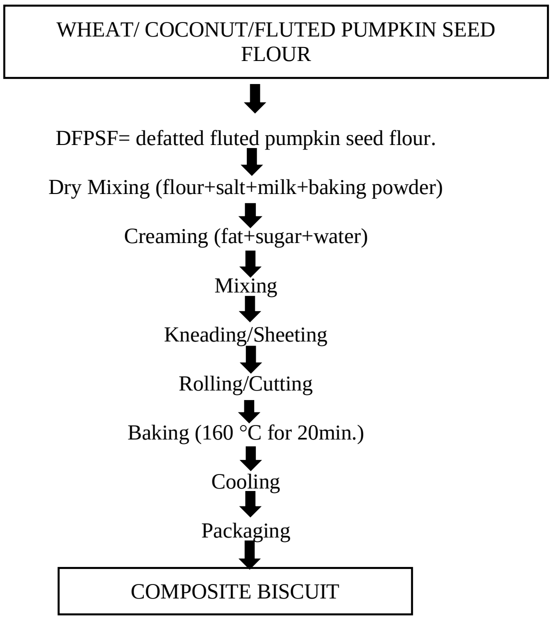

Fig. 1. Flow diagram for production of biscuit from composite flour.

D. *Amino Acid Profile*

Amino acid profile of the samples was determined using Ion Exchange Chromatography (IEC) as described by [22]. The sample was defatted, hydrolyzed and evaporated in a rotatory evaporator then injected into the Technicon sequential multisampling Amino Acid Analyzer.

E. *Mineral Content*

Mineral analysis was done by dry ashing according to procedure 14.013 of AOAC [23]. Muffle furnace (Model SKL, China) at temperature of 550 °C was used for ashing. After sample preparation, total mineral determination was done using Atomic Absorption spectrophotometer (AAS) (Hitachi Z-5300, polarized Zeaman, Hitachi Ltd; Japan). The light source was Hollow cathode lamp of each element, using acetylene and air combinations, with air pressure of 0.3 Mpa, and air flow rate of 6.5 L/min, acetylene pressure of 0.09 Mpa and a flow rate of 1.7 L/min was used. Other operating conditions such as wavelength and lamp current are given for each element as follows: Ca = 422.7 nm and 2 mA, Fe = 248.3 nm and 2 mA, K = 766.5 nm and 1 mA, mg = 285.2 nm and 1 mA, Na = 589.0 nm and 1mA. Phosphorous was determined by molybdenum blue method and the absorbance read at 700 nm using a spectrophotometer uv-visible (CELiL model CE2021 U.K).

F. Mineral Bioavailability

Mineral Bioavailability was determined using method described by [24]. About 1.0 g was extracted using 10 ml of 0.03N HCL with shaking at 37 ºC for 3 h. Thereafter, the extract was filtered and a clear filtrate obtained was dried at 100 ºC and then placed in a muffle furnace at 550ºC for 4 h, thereafter the sample was cooled and about 5 ml of 5N HCL was added and boiled gently for 10 min and then cooled and diluted with 100 ml of distilled water. The supernatant obtained was subjected to mineral analysis using atomic absorption spectrophotometer (AAS). The results obtained were used in calculating the percentage of minerals in the biscuit samples that are bioavailable in soluble fractions using the formula:

$$X\ (\mathrm{mg/100g}) = \frac{(\mathrm{Concentration}) \times 50 \times 1000}{104 \times \mathrm{Sample\ weight}}$$

Mineral Balance was calculated by difference [25].
Mineral balance (%) = 100 – Mineral Bioavailability (%).

G. Sensory Evaluation

Sensory evaluation of the biscuits was carried out after baking using the method of [26]. The sensory attributes; colour, taste, flavour, texture and general acceptability were evaluated using a 9 – point hedonic scale with 1 representing the least score (dislike extremely) and 9, the highest score (like extremely).

H. Statistical Analysis

All the analyses were carried out in duplicate. Data obtained were subjected to Analysis of Variance (ANOVA); differences between means were evaluated using Turkey's multiple comparison tests with 95% confidence level. The statistical package in Minitab software version 16 was used.

III. Result and Discussion

A. Amino Acid Profile

As shown in Table 2, Leucine content of sample F (8.29 g.100 g) was significantly (P<0.05) higher followed by sample E. Lysine content ranged from 3.35-5.07 g/100 g, with sample F given significantly higher value followed by samples E (4.70 g.100 g) and D (4.33 g/100 g). Lysine is essential for children as it is critical for bone formation, it is involved in hormone production, lowers serum triglyceride levels [27]. Increase in leucine and lysine content is due to increase in level of substitution of coconut and fluted pumpkin seed flour. Phenyl-lanine content of the composite biscuits decreased significantly from 4.98-3.90 g/100 g with increase substitution of coconut and fluted pumpkin seed flour, this is probably due to relatively higher phenyl-lanine fraction in wheat flour. Arginine content increased significantly with increased level of flour substation from 5.29-7.20 g/100 g, samples E and F were significantly (P<0.05) higher. Arginine is thought to be conditionally essential for children up to 5years old and the elderly 60 years and above while histidine is essential for children up to 5 years of age [28]. The result indicated that Arginine, lysine, histidine, glycine, isoleucine, leucine, glutamic acid and tyrosine were sufficient to meet nutritional needs of man based on FAO/UN/WHO reference pattern for amino acids [29]. Tyrosine content ranged from 3.11–5.53 g/100 g, with sample F given significantly higher value followed by samples E (4.44 g/100 g). The predominant non-essential Amino Acid found in the biscuits samples was glutamic acid, with values ranging from 11.81–15.20 g/100 g.

TABLE 2: Amino Acid Content Of Wheat/Coconut/Fluted Pumpkin Seed Flour Biscuit

Amino Acids (g/100 g)	Samples					
	A	B	C	D	E	F
*Leucine	$6.18^{f}\pm0.033$	$6.86^{e}\pm0.020$	$7.00^{d}\pm0.027$	$7.26^{c}\pm0.042$	$8.02^{b}\pm0.085$	$8.29^{a}\pm0.076$
*Lysine	$3.35^{f}\pm0.070$	$3.77^{e}\pm0.078$	$4.01^{d}\pm0.035$	$4.33^{c}\pm0.035$	$4.70^{b}\pm0.035$	$5.07^{a}\pm0.019$
*Isoleucine	$3.45^{c}\pm0.064$	$3.64^{b}\pm0.049$	$3.99^{a}\pm0.000$	$3.90^{ab}\pm0.034$	$4.03^{a}\pm0.034$	$3.93^{a}\pm0.000$
*Phenyl-lanine	$4.98^{a}\pm0.014$	$4.68^{b}\pm0.000$	$4.09^{c}\pm0.000$	$4.00^{d}\pm0.064$	$3.97^{d}\pm0.000$	$3.90^{e}\pm0.000$
*Tryptophan	$0.71^{d}\pm0.042$	$0.87^{c}\pm0.035$	$0.87^{c}\pm0.035$	$0.97^{a}\pm0.042$	$1.05^{a}\pm0.071$	$0.92^{ab}\pm0.035$
*Valine	$3.02^{d}\pm0.041$	$3.41^{c}\pm0.042$	$3.89^{ab}\pm0.042$	$3.95^{a}\pm0.035$	$3.95^{a}\pm0.035$	$3.98^{a}\pm0.027$
*Methionine	$1.12^{d}\pm0.030$	$1.31^{c}\pm0.035$	$1.30^{c}\pm0.000$	$1.31^{c}\pm0.035$	$1.53^{a}\pm0.035$	$1.43^{b}\pm0.000$
Proline	$3.51^{b}\pm0.085$	$3.45^{b}\pm0.000$	$3.91^{a}\pm0.071$	$4.06^{a}\pm0.000$	$3.91^{a}\pm0.071$	$3.65^{b}\pm0.000$
*Arginine	$5.26^{d}\pm0.099$	$6.45^{c}\pm0.020$	$7.00^{b}\pm0.020$	$7.02^{b}\pm0.020$	$7.19^{a}\pm0.020$	$7.20^{a}\pm0.020$
*Tyrosine	$3.11^{f}\pm0.062$	$3.27^{e}\pm0.000$	$3.78^{d}\pm0.000$	$3.96^{c}\pm0.000$	$4.44^{b}\pm0.000$	$5.53^{a}\pm0.020$
*Histidine	$2.12^{c}\pm0.078$	$2.21^{b}\pm0.050$	$2.14^{c}\pm0.042$	$2.24^{ab}\pm0.000$	$2.21^{b}\pm0.050$	$2.27^{a}\pm0.042$
*Cysteine	$1.22^{a}\pm0.007$	$0.95^{b}\pm0.000$	$0.84^{c}\pm0.042$	$0.83^{c}\pm0.085$	$0.71^{d}\pm0.085$	$0.69^{d}\pm0.028$
Alanine	$3.81^{f}\pm0.020$	$3.91^{e}\pm0.050$	$4.48^{c}\pm0.019$	$4.67^{b}\pm0.050$	$4.82^{a}\pm0.050$	$4.10^{d}\pm0.006$
Glutamic Acid	$11.81^{e}\pm0.219$	$13.95^{d}\pm0.006$	$14.00^{d}\pm0.012$	$14.54^{c}\pm0.031$	$15.00^{b}\pm0.012$	$15.20^{a}\pm0.025$
Glycine	$3.36^{e}\pm0.013$	$3.92^{e}\pm0.028$	$4.14^{d}\pm0.034$	$4.53^{b}\pm0.026$	$4.78^{a}\pm0.035$	$4.28^{c}\pm0.032$
*Threonine	$3.33^{e}\pm0.011$	$3.58^{d}\pm0.013$	$4.00^{b}\pm0.000$	$3.78^{c}\pm0.011$	$4.30^{a}\pm0.042$	$3.97^{b}\pm0.042$
Serine	$3.63^{d}\pm0.092$	$3.54^{e}\pm0.035$	$4.34^{a}\pm0.029$	$3.95^{b}\pm0.078$	$3.76^{c}\pm0.035$	$3.75^{c}\pm0.042$
Aspartic Acid	$6.63^{f}\pm0.006$	$7.90^{e}\pm0.042$	$8.04^{d}\pm0.042$	$8.17^{c}\pm0.0170$	$8.38^{b}\pm0.050$	$8.68^{a}\pm0.061$

Values are mean ± standard deviation of triplicate samples. Mean values bearing different superscript in the same row differ significantly (P<0.05).
Key: A = 100% WF (control), B = 80% WF + 10% CNF+10% DFPSF, C = 70% WF + 20% CNF+10% DFPSF, D = 60% WF + 30% CNF+10% DFPSF, E = 50% WF + 40% CNF+10% DFPSF, F = 40% WF + 50% CNF+10% DFPSF, WF= wheat flour, DFPSF= defatted fluted pumpkin seed flour, CNF= coconut flour.

B. Total Mineral Compositions (Mg/100g)

Potassium was the most abundant element in all the biscuits samples (Table 3). The highest potassium content (86.02 mg/100 g) was recorded in sample E. High potassium is of advantage, to protect against arterial hypertension [30]. The mineral content of the biscuit samples increased with coconut-flour substitution. Iron and Zinc content ranged from

9.59–17.92 and 1.67–2.82 mg/100 g respectively. Inadequate intakes of micronutrients (Zinc and Iron) have been associated with severe malnutrition, increased disease conditions and mental impairment [30] [31]. Calcium (Ca) content range from 11.64–28.89 mg/100 g, with sample E given significantly higher value followed by sample F (24.55 mg/100 g) and D (22.34 mg/100 g). Calcium content increased with increased substitution of coconut flour. Calcium is an important bone related macro element in human nutrition [32]. Calcium in addition with other micro minerals and protein can help in bone formation with calcium acting as principal contributor [33]. Calcium is important in blood clotting, muscles contraction and in certain enzymes in metabolic processes [34]. Magnesium content of the 100% wheat flour biscuit was significantly (P<0.06) higher at 19.87 mg/100 g, followed by sample B (19.00mg/100g). Sodium and magnesium content are shown to decrease with increased substitution of coconut flour, this is probably due to the higher content of Na and Mg in wheat flour. Magnesium is an essential micronutrient needed for nervous system health [35]. Inadequate intakes of micronutrients (Zinc and Iron) have been associated with severe malnutrition, increased disease conditions and mental impairment [31].

The results from this study showed that the biscuit samples would contribute substantially to the recommended dietary requirements for minerals [30].

TABLE 3: TOTAL MINERAL COMPOSITIONS (MG/100G) OF WHEAT/COCONUT/FLUTED PUMPKIN SEED FLOUR BISCUIT

Samples	Ca	Fe	Na	K	Mg	Zn
A	11.94^{f} ±0.014	10.55^{e} ±0.144	64.65^{a} ±0.116	51.19^{d} ±0.002	19.97^{a} ±0.003	1.67^{d} ±0.001
B	14.08^{e} ±0.013	9.59^{f} ±0.139	63.11^{b} ±0.001	56.66^{c} ±0.001	19.00^{b} ±0.003	1.68^{d} ±0.001
C	19.19^{d} ±0.013	10.85^{d} ±0.250	59.20^{c} ±0.217	56.68^{c} ±0.001	18.13^{c} ±0.001	1.90^{c} ±0.002
D	22.34^{c} ±7.183	14.91^{c} ±0.144	52.59^{d} ±0.000	78.45^{b} ±0.000	17.61^{d} ±0.002	2.12^{b} ±0.001
E	26.89^{a} ±0.007	17.92^{a} ±0.433	52.64^{d} ±0.001	86.02^{a} ±0.001	16.58^{e} ±0.000	2.82^{a} ±0.001
F	24.55^{b} ±0.006	16.46^{b} ±0.250	48.53^{e} ±0.002	78.45^{b} ±1.001	16.57^{e} ±0.001	2.80^{a} ±0.002

Values are means ± standard deviation of duplicate samples.
Mean values bearing different superscripts in the same column differ significantly (P<0.05)
Key: A = 100% WF (control), B = 80% WF + 10% CNF+10% DFPSF, C = 70% WF + 20% CNF+10% DFPSF, D = 60% WF + 30% CNF+10% DFPSF, E = 50% WF + 40% CNF+10% DFPSF, F = 40% WF + 50% CNF+10% DFPSF, WF= wheat flour, DFPSF= defatted fluted pumpkin seed flour, CNF= coconut flour.

C. Percentage (%) Bioavailable Mineral and Mineral Balance

The role of biscuits as source of minerals depend on the amount of the mineral available for absorption, which is also known as bioavailability. Bioavailability has been defined as the fraction of a compound that is released from its matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (enters the blood stream) [36]. Bioavailability includes the entire sequence of events that take place during the digestive transformation of food into material that can be assimilated by the body, the absorption/ assimilation into the cells of the intestinal epithelium, and lastly, the presystemic metabolism (both intestinal and hepatic) [37]. It refers to how well a nutrient can be absorbed by the body and used to reduce micronutrient malnutrition.

From the result in Table 4, percentage bioavailable calcium (Ca), iron (Fe) and sodium (Na) ranged from 54.15–59.62, 30.54-51.35 and 42.31–58.22% respectively. While the percentage bioavailable potassium (K) magnesium (Mg) and zinc (Zn) ranged from 42.93–59.03, 45.17–50.92 and 36.76–47.76% respectively. There was no significant difference (P>0.05) in the bioavailability of Ca in samples A and E. Sample F gave significantly (P<0,05) higher bioavailable Ca, Fe, Na, K and Zn, while percentage bioavailable Mg was higher in sample E this was probably due to the increase in soluble mineral content of biscuits enriched with coconut and defatted fluted pumpkin seed flour. According to [38] study on iron bioavailability of vegetables indicated that cooking increases iron bioavailability of certain vegetables 2 to 10 times. The cooking enhancing effect can be achieved with different heating processes including boiling, stir-frying and baking [39]. Bioavailability is the technical term used to convey the fact that not 100% of nutrients ingested will be absorbed, irrespective of whether consumed in the form of food or supplements. Low bioavailability diets (5% of the total iron absorbed) are based mainly on cereals and root vegetables with only very small quantities of meat, fish or vitamin C- containing foods [40]. Such diets often contain foods that inhibit iron absorption (maize, beans, whole-grain flour) and are dominant in many developing countries.

TABLE 4: PERCENTAGE (%) BIOAVAILABLE MINERAL IN WHEAT/COCONUT/FLUTED PUMPKIN SEED FLOUR BISCUIT

Samples	Ca	Fe	Na	K	Mg	Zn
A	58.76^{b} ±0.069	35.33^{d} ±0.004	47.70^{b} ±0.095	42.93^{d} ±0.003	47.26^{b} ±0.005	38.07^{d} ±0.073
B	54.38^{cd} ±0.004	42.00^{c} ±0.390	47.66^{b} ±0.071	44.06^{c} ±0.001	45.17^{d} ±0.000	36.76^{e} ±0.053
C	54.92^{c} ±0.001	30.54^{e} ±0.365	47.70^{b} ±0.114	53.10^{b} ±0.000	46.79^{c} ±0.005	42.89^{c} ±0.012
D	54.15^{d} ±0.718	46.80^{b} ±0.109	42.31^{c} ±0.000	44.56^{c} ±0.001	46.81^{c} ±0.003	44.21^{b} ±0.015
E	58.77^{b} ±0.007	46.57^{b} ±0.652	47.65^{b} ±0.001	53.13^{b} ±0.001	50.92^{a} ±0.003	44.42^{b} ±0.001
F	59.67^{a} ±0.019	51.35^{a} ±0.837	58.22^{a} ±0.000	59.67^{a} ±0.957	47.38^{b} ±0.000	47.74^{a} ±0.016

Values are means ± standard deviation of duplicate samples.
Mean values bearing different superscripts in the same column differ significantly (P<0.05).

Result for mineral balance of the biscuit samples as presented in Table 5, showed Ca ranging from 40.33–45.85%, with significantly (P<0.05) higher percentage noticed in sample D. Significantly High Mg and Zn balance of 54.83 and 63.24% were noticed in sample B while significantly (P<0.05) higher Fe balance of 69.46% and K balance of 57.07% were recorded in samples C and A respectively. Biscuits with high percentage balance in any particular mineral, is an indication of its low bioavailability. Increased percentage mineral balance is probably due to presence of anti-nutritional factors. As noted earlier by [25], anti-nutritional factors are shown to cause complexing, binding and inhibition of minerals, thereby decreasing their bioavailability and increasing the mineral balance. The mineral balance in a food substance is the insoluble mineral that is unavailable and cannot be absorbed [25].

TABLE 5: PERCENTAGE (%) MINERAL BALANCE OF WHEAT/COCONUT/FLUTED PUMPKIN SEED FLOUR BISCUIT

Samples	Ca	Fe	Na	K	Mg	Zn
A	41.24[c] ±0.069	64.67[b] ±0.004	52.30[b] ±0.095	57.07[a] ±0.003	52.74[c] ±0.005	61.93[b] ±0.073
B	45.62[ab] ±0.004	58.00[c] ±0.390	52.34[b] ±0.071	55.94[b] ±0.001	54.83[a] ±0.000	63.24[a] ±0.053
C	45.08[b] ±0.001	69.46[a] ±0.365	52.30[b] ±0.114	46.90[c] ±0.000	53.21[b] ±0.005	57.11[c] ±0.012
D	45.85[a] ±0.718	53.20[d] ±0.109	57.69[a] ±0.000	55.44[b] ±0.001	53.19[b] ±0.003	55.79[d] ±0.015
E	41.23[c] ±0.007	53.43[d] ±0.652	52.35[b] ±0.001	46.87[c] ±0.001	49.08[d] ±0.003	55.58[d] ±0.001
F	40.33[d] ±0.019	48.65[e] ±0.837	41.78[c] ±0.000	40.33[d] ±0.957	52.62[c] ±0.000	52.26[e] ±0.016

Values are means ± standard deviation of duplicate samples.
Mean values bearing different superscripts in the same column differ significantly (P<0.05).

D. Sensory Properties of Wheat/Coconut/Fluted pumpkin seed Flour Biscuit

Scores for colour ranged from 4.80 – 6.80, with samples E and D recording higher rating of 6.80 and 6.76 respectively. Colour is a significant parameter in judging well baked biscuits. It reflects the suitable raw material used for the preparation and also provides information about the formulation and quality of the product [41]. Kaur [42] also reported earlier that biscuit prepared from blends of refined wheat (25%), oat (50%) and chickpea (25%) had significantly higher colour values as compared with 100% wheat flour. Scores for taste, flavour and texture ranged from 4.20–6.60, 4.00–6.32 and 4.48–6.36 respectively. Taste is the primary factor that determines the acceptability of any product which has the highest impact as far as market success of product is concerned [43]. The colour, taste, flavour and texture rating of sample F were low, though not significantly (P>0.05) different from samples A, B and C. The variation in constituents or formulation causes the changes in textural properties [44]. Sample E recorded the highest overall acceptability score of 6.38. This score was however not significantly different (P>0.05) from scores obtained for samples A, B, C and D. This study showed that production of biscuits with composite flour of coconut, fluted pumpkin seed and wheat give products with acceptable quality comparable to wheat flour biscuits.

TABLE 6: SENSORY PROPERTIES OF WHEAT/COCONUT/FLUTED PUMPKIN SEED FLOUR BISCUIT

Samples	Colour	Taste	Flavor	Texture	Overall acceptability
A	6.08[ab]±1.120	5.72[ab]±1.407	5.52[ab]±1.293	5.56[ab]±1.678	5.56[ab]±2.001
B	6.20[ab]±1.160	5.44[ab]±1.219	5.56[ab]±1.238	6.44[a]±1.685	6.00[ab]±1.520
C	5.48[ab]±2.001	4.92[ab]±1.159	5.08[ab]±2.178	5.88[ab]±1.691	5.40[ab]±1.121
D	6.76[a]±1.615	5.40[ab]±1.384	6.320[a]±1.520	6.36[a]±1.753	5.36[ab]±1.705
E	6.80[a]±1.803	6.60[a]±1.483	5.00[ab]±2.550	6.20[a]±2.021	6.38[a]±1.272
F	4.80[b]±1.273	4.20[b]±1.614	4.00[b]±2.062	4.48[b]±2.257	4.48[b]±1.064

Values are means ± standard deviation of 25 responses.
Mean values bearing different superscripts in the same column differ significantly (P<0.05).
Key: A = 100% WF (control), B = 80% WF + 10% CNF+10% DFPSF, C = 70% WF + 20% CNF+10% DFPSF, D = 60% WF + 30% CNF+10% DFPSF, E = 50% WF + 40% CNF+10% DFPSF, F = 40% WF + 50% CNF+10% DFPSF, WF= wheat flour, DFPSF= defatted fluted pumpkin seed flour, CNF= coconut flour.

IV. CONCLUSION

From the results, leucine content of biscuit produced from 40% wheat in composite with 50% coconut and 10% defatted fluted pumpkin seed flour (sample F) was significantly (P<0.05) higher (8.29g.100g) followed by sample E, Sample F was also higher in other essential amino acids such as; lysine, arginine, isoleucine, valine and tyrosine while sample E was rich in arginine, valine, leucine, isoleucine and threonine. Increase in essential amino acid content was due to increase in level of substitution with coconut and fluted pumpkin seed flour. Sample E was significantly high in Ca, Fe, K and Zn while 100% wheat biscuit was significantly high in Mg. Sample F was also remarkably high in Zn (2.80mg/100g). Percentage bioavailability of all the minerals studied except Mg, were significantly higher in sample F, due to increased substitution of coconut flour. In terms of sensory quality, sample E was rated higher. This study showed that production of biscuits with composite flour of coconut, fluted pumpkin seed and wheat give products with acceptable quality comparable to wheat flour biscuits.

REFERENCES

[1] Noah A. 2017. Production, microbial and sensory qualities of biscuits produced from wheatcoconut-almond flour blend. *International Journal of Food Science and Nutrition*.2(6):135- 138.
[2] Adebowale AA, Adegoke MT, Sanni SA, Adegunwa MO, Fetuga GO (2012) Functional properties and biscuit making potentials of sorghum-wheat flour composite. American J. Food Technology 7: 372-379.
[3] Svec I, Hruskova M (2010) Evaluation of wheat bread features. Journal of Food Engineering 99: 505-510.
[4] Alobo, A. P. 2001. Effect of sesame seed on the millet biscuit characteristics. Plants Food Hum. Nutr. 56:195–202.
[5] Dhingra S, Jood S (2000) Organoleptic and Nutrition Evaluation of wheat breads supplemented with soybean and barley flour. Food Chemistry 77: 479-488.
[6] Neelam K, Rajni G (2000) Effect of composite flour fortification to wheat flour on the quality characteristics of unleavened bread. British Food J 11: 554-564.
[7] Akusu, O.M., Emelike, N.J.T. And Chibor, B.S. (2019). Physicochemical, Functional and Sensory Properties of 'Agidi' Produced from Maize, Millet and Sorghum Starch Blends. *Delta Agriculturist,* 11 (2/3): 50-59.
[8] Kabuo, N. O., Alagbaoso, O. S., Omeire, G. C., Peter-Ikechukwu, A. I., Akajiaku, L. O. and Obaise, A. C. (2018). Production and evaluation of biscuits from Cocoyam (Xanthosoma sagittifolium cv. Okoriko) -Wheat composite flour. Research Journal of Food and Nutrition, 2 (2): 53 - 61.

[9] Mishra, N. and Chandra, R. (2012). Development of functional biscuit from soy flour and rice bran. International Journal of Agricultural and Food Science, 2 (1): 14 – 20.

[10] Trinidad P. T., Aida C. Mallillin, D. H. Valdez, A. S. Loyola, F.C. Askali-Mercado, J. C. Castillo, R. R. Encabo, D. B. Masa, A. S. Maglaya, M. T. C. (2006). Dietary fiber from coconut flour: A functional food. Innovative Food Science and Emerging Technologies, 7, 309–317.

[11] Arancon, R.N., (1999) "Coconut flour", Cocoinfo International, 6(1), pg. 1–8.

[12] Akorode, M.O. and Adejore, M.A. (1990). Patterns of Vegetative and Sexual development of (*Telfaina occidentailis* Hook F). *Tropical Agriculture*, 67, 243-247.

[13] Giami, S. Y., Chibor, B. S., Edebiri, K. E. and Achinewhu, S. C. (1999). Changes in Nitrogenous and Other Chemical Constituents, Protein Fractions and In-Vitro Protein Digestibility of Germinated Fluted Pumpkin (*Telfairia occidentalis* Hook) Seed. *Plant Foods for Human Nutrition*, 53, 333-342

[14] Abdelghafor, R.F., Mustafa, A.I., Ibrahim, A.M.H. and Krishnan P.G. (2011). Quality of bread from composite Flour of Sorghum and Hard White Winter Wheat. *Advance Journal of Food Science and Technology* 3(1): 9-15.

[15] Dhiman, A.K., Sharma, K. and Atti, S. (2009). Functional Constituents and Processing of pumpkin: A review. Journal of Food Science and Technology-Mysore, 46(5):411-417.

[16] Giami, S.Y, and Barber, L. I. (2004). Utilization of Protein Concentrate from Ungerminated and Germinated Fluted Pumpkin (*Telfairia Occidentalis* Hook F) Seeds in Cookie Formulation. *Journal of the Science of Food and Agriculture*, 84(14), 1901-1907.

[17] Chibor, B.S., Kiin-Kabari, D.B. and Ejiofor, J. (2017). Physicochemical Properties and Fatty Acid Profile of Shea Butter and Fluted Pumpkin Seed Oil, a Suitable Blend in Bakery Fat Production. *International Journal of Nutrition and Food Sciences*. 6(3), 122-128.

[18] Bolarinwa IF, Abioye AO, Adeyanju JA, Kareem ZO (2016) Production and Quality Evaluation of Biscuits Produced from Malted Sorghum-Soy Flour Blends. Journal of Advances in Food Science and Technology 3: 107-113.

[19] Chibor B. S., Kiin-Kabari D. B and Eke-Ejiofor J. (2018). Comparative Assessment of the Physicochemical Properties and Fatty Acid Profile oF Fluted Pumpkin Seed Oil with Some Commercial Vegetable Oils in Rivers State, Nigeria. *Research Journal of Food and Nutrition*, 2(2): 32-40.

[20] Rosenthal, A., Pyle, D.I. and Niranjan, K. (1996). Aqueous and Enzymatic Processes for edible oil extraction. *Enzyme Microbiology and Microbial Technology*, 19, 402-420.

[21] Okaka, J. C. (1997). Cereals and legumes: storage and processing technology. Data and microsystem publishers, Enugu, Nigeria. Pp. 11 - 124.

[22] Onwuka, G. I. (2005). Food analysis and instrumentations theory and practice.1st edition. Lagos; Naphtali Prints. 29-73. 34.Schoen.

[23] AOAC (2012). Association for Official Analytical Chemist. Official Methods for Analysis, 19th Ed. Washington DC.

[24] Chauhan, B.M. and Mahjan, H. (1988). Effect of Natural Fermentation on the Extraction of Mineral from Pearl Millet Flour. *Journal of Food Science.* 53 (5):1576-1577.

[25] Akusu, O.M., Kiin-Kabari, D.B. and Isah, E.M. (2020). Anti-nutrients, Bioaccessibility and Mineral Balance of Cookies Produced from Processed Sesame Seed Flour Blends. *International Journal of Food Science and Nutrition Engineering*, 10(1): 1-11.

[26] Iwe, M. O. (2010). Hand Book of Sensory Methods and Analysis. Rojoint Communication Services Ltd, Enugu, Nigeria. pp. 35-40

[27] Gersten D. (2013). The 20 amino acids: What they are and how they keep you alive and vibrant. The Gersten Institute for Higher Medicine, San Diego, CA, USA.

[28] Sowers S. A (2009). primer on branched chain amino acids. Smart supplementation, literature education series on dietary supplements. Huntington College of Health Sciences.

[29] [29] FAO/UN/WHO. (1991). Food and Agricultural Organization of the United Nations World Health Organization. Protein quality evaluation. Report of joint FAO/WHO expert consultation. FAO Food and Nutrition Paper, 51 (FAO) Rome.

[30] Wardlaw GM (2004) Perspectives in nutrition (6th ed.). McGram Hill Companies, New York, U.S.A.

[31] Mannay S, Shadaksharaswany C.M. (2005). Foods: Facts and Principles. (2nd ed.). New Age International Ltd. Publishers. New Delhi, India.

[32] Soetan K.O., Olaiya C. O. and Oyewole O. E. (2010). The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science. 4(5): 200 – 222.

[33] Kiin-Kabari D.B., Hart A.D., Nyeche P.T. (2017). Nutritional Composition of Selected Shellfish Consumed in Rivers State, Nigeria. *American Journal of Food and Nutrition*, 5, 4, 142-146.

[34] Davies I.C and Jamabo N.A (2016). Determination of Mineral Contents of Edible Parts of Shellfishes from Okpoka Creeks in Rivers State, Nigeria.*International Journal of Fisheries and Aquaculture Research*, 2(2), 10-18.

[35] Abulude F.O, Lqual L.O, Ehikharmery G, Adesanya W.O and Ashafa S.L (2006). Chemical composition and functional properties of some pours from the Coastal Area of Ondo State, *Nigeria. Journal of Environmental, Agriculture and Food Chemistry*, 5, 1235-1240.

[36] Benito, P., and Miller, D. (1998). Iron absorption and bioavailability: an updated review. *Nutrition Research* 18(3):581–603.

[37] Ting R.S. and Loh S.P. (2016). In vitro bioaccessibility of calcium, iron and zinc from breads and bread spreads. International Food Research Journal, 23(5), 2175-2180.

[38] Yang R.Y. and Tsou S. C. (2006). Enhancing Iron Bioavailability of Vegetables through Proper Preparation Principles and Applications. Journal of International Cooperation 1(1):107- 119.

[39] Gashaw M T., Abebe, H. and Esayas. (2015). Bioavailability of Minerals in Cookies Developed from Blend of Moringa Leaf Powder and Wheat Flour for Iron Deficient Lactating Mothers. International Journal of Food Science and Nutrition Engineering, 5(6): 226-232.

[40] Baech, S.B, Hansen, M., Bhukhave, K., Jensen, M., Sorensen, S.S., Kristensen, L., Purslow, P.P., Skibsted, H.L., Sandstorm, B. (2003). Non heme iron absorption from a phtate-rich meal is increased by additional of small amount meat. *Am. J. Clin-Nut*, 2, 77:173-179.

[41] Mepba HD, Eboh L, Nwaojigwa SU (2007) Chemical composition, functional and baking properties of wheat-plantain composite flours. African Journal of Food Agriculture Nutrition and Development 7: 1-22.

[42] Kaur K, Kaur H, Bains K (2017) Development and Nutritional Evaluation of Cereal and Pulse Based Biscuits for Diabetic Patients. Br J Appl Sci Technol 21: 1-8.

[43] Feyera, M. (2019). Review on some cereal and legume based composite biscuits. *International Journal of Agricultural Science and Food Technology*, retrieved from www.peertechz.com, Nov. 2020.

[44] Pareyt B and Delcour JA (2008) The Role of Wheat Flour Constituents, Sugar, and Fat in Low Moisture Cereal Based Products: A Review on Sugar-Snap Cookies. *Critical Review in Food Science and Nutrition*, 48: 824-839.

Chibor Bariwere Samuel Place of birth: Buan, Rivers State, Nigeria. Date of birth: December 21st 1970. Ph.D. in Food Processing and Engineering, Micheal Okpara University of Agriculture, Umudike, Nigeria (2020; in view).
B.Sc. in Food Science and Technology, Rivers State University, Port Harcourt, Nigeria, 1994, PGD in Chemical Engineering, Rivers State University, Port Harcourt, Nigeria, 1997, M.Sc. in Food Processing and Storage Technology, Rivers State University, Port Harcourt, Nigeria, 2017.

Career: Process Engineer, Food product development and optimization consultant for private companies, a Researcher and Teacher, with long industrial experience in the Food Process sector as Senior Manager. published articles: He have over 17 publications appearing in all search gates (Google Scholar, Reasearchgate, Google Search, etc). Among them are:

1. Chibor, B.S., Eke-Ejiofor, J. and Kiin-Kabari, D.B. (2018). Effect of Chemical Interesterification on the Physicochemical Characteristics and Fatty Acid Profile of Bakery Shortening Produced from Shea Butter and Fluted Pumpkin Seed Oil Blend. American Journal of Food Science and Technology, Vol. 6, No. 4, 187-194.
2. Chibor, B.S., Kiin-Kabari, D.B. and Eke-Ejiofor, J. (2018). Comparative Assessment of the Physicochemical Properties and Fatty Acid Profile OF Fluted Pumpkin Seed Oil with Some Commercial Vegetable Oils in Rivers State, Nigeria. Research Journal of Food and Nutrition Vol. 2, Issue 2, 32-40.
3. Oyet, G.I and Chibor, B.S. (2020). Nutrient Composition and Microbiological Evaluation of Vended Street Foods in Parts of Lagos State, Nigeria. Asian Food Science Journal, 17(1): 1-14,

Mr. CHIBOR, Department of Food Science and Technology, Rivers State University, Port Harcourt, Nigeria, Secretary and Member Nigerian Institute of Food Science and Technology (NIFST).

Gogomary Oyet is the Group Head External Relations and Communications /EHSSQ at OVH Energy. He is an Adjunct Senior Lecturer at the University of Port Harcourt, Centre for Occupational Health Safety and Environment. A Researcher and Teacher with special interest in Food Safety and Environmental sustainability.

Dr. Oyet is from Nigeria and holds a B.Sc and M.Sc. degrees in Food Science and Technology, with option in Product Development. He holds a Doctorate degree in Food Science and Technology, with Specialization in Food Quality Control and Assurance from Rivers State University, Port Harcourt. He holds another Ph.D. Degree in Environmental Management from the Institute of Geoscience and Space Technology; Rivers State University of Science and Technology, Port Harcourt.

Dr. Oyet is a Specialist Fellow of the International Institute of Risk and Safety Management, SFIIRSM (UK), Fellow of the Nigerian Environmental Society (FNES), Fellow of the Occupational Safety and Health Association, FOSHA, (UK), Fellow of African Institute of Public Health Professionals (FALPHP), and a Fellow of the Institute of Administrator and Researchers of Nigeria (CFIAR). The Author has published several research articles in local and International Journals. He is a member of Nigerian Institute of Food Science and Technology (NIFST). Dr. Oyet was trained at the Massachusetts Institute of Technology (MIT) Cambridge, in Crisis Management, Business Continuity and Advance Business Resilience. Dr. Oyet is an alumnus of Henley's Business School, University of Reading, United Kingdom. He is an alumnus of the Harvard Kennedy School of Government, USA and London Business School, United Kingdom.