



MINERAL AND ANTINUTRITIONAL PROPERTIES OF EXTRUDED SNACKS FROM FERMENTED MILLET-PUMPKIN LEAVES COMPOSITE FLOUR

***Oluwakemi Abosede OJO¹, Ekene Joachim OBINYERE¹, Adewale Olusegun OBADINA^{1,2}, Emmanuel Kehinde OKE³, Eniola Oluwayemisi ONI⁴**

¹Department of Food Science and Technology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, ojo.oluwakemia@funaab.edu.ng

²Department of Biotechnology and Food Technology, University of Johannesburg, Doornfontein Campus, South Africa

³Department of Food Science, University of Medical Sciences, Ondo City, Ondo State, Nigeria

⁴Department of Microbiology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

*Corresponding author

Received 29th December 2023, accepted 25th March 2024

Abstract: This study was carried out to determine the mineral and antinutrient content of fermented millet-pumpkin leaves composite flour extruded snacks. Pearl millet grains were carefully sorted to remove unwanted materials, washed and soaked and allowed to ferment for 24 h and 48 h, it was finally dried in an oven dryer and the dried grains were milled using a laboratory hammer mill. Calcium, potassium, phosphorus and iron ranged from 42.71 to 116.58 mg/100g, 138.72 to 218.76 mg/100g, 110.36 to 170.80 mg/100g, 62.51 to 78.67mg/100g for 24 h fermentation time and 46.36 to 125.97 mg/100g, 153.15 to 255.94 mg/100g, 98.64 to 146.73 mg/100g and 74.77 to 90.22 mg/100g for 48 h fermentation time, respectively. The interactive effect of fermented millet and pumpkin leave flour does not have a significant ($p>0.05$) effect on the calcium, potassium and iron content at both 24 h and 48 h fermentation time while it has a significant ($p>0.05$) effect on the trypsin inhibitor at 24 h fermentation time. The solution process to the optimization of extruded snacks from fermented millet and pumpkin leaves flour blends are 90% fermented millet flour and 10% pumpkin leaves flour and 87% fermented millet flour and 13% pumpkin leaves flour at 24 h and 48 h fermentation time, respectively. Result shows that fermentation and extrusion process could be used to enrich the nutritional potential of millet extruded snacks.

Keywords: Fermentation, Pumpkin, Leaves, Anti-nutrients, Response Surface Methodology

1. Introduction

Snacks are readily available small portion of food that is eaten in between meals [1]. Basically, they are produced from ingredients in accordance with predetermined plans to produce food products with specified functional properties. The consumption of snack is currently on the increase and this contributes to the calorie intake of individuals [2]. Proper intake of healthy snacks, therefore, can aid the supplementation nutrient requirements of humans.

The most commonly consumed snacks are cereal-based, which are mostly low in nutrient. Micronutrient deficiency is the main contributor to children mortality and morbidity [3]. Deficiency of iron is the most predominant micronutrient deficiencies and this problem is a concern to public health.

The immune system is weakened and it is prone to infection, retarded growth and impaired cognitive [4]. As a result of high poverty level in Nigeria, especially in rural areas, nutrient deficiencies are more common [5, 6]. In most developing countries, children are fed on foods that are

cereal based such as millet which is low in micronutrients [7, 8]. Studies have shown that cereals are high in anti-nutrients which hinder the availability and absorption of minerals [9]. In order to improve the value of cereal based food, there is need to fortify cereals with other food materials that are rich in micronutrients.

Millet grains are gluten-free [10], and a drought resistant crop, they are commonly processed into flour in Nigeria and can be utilized in the production of beverages, snacks and weaning foods. However, similar to other cereals, millet is deficient in essential amino acids and also has high level of anti-nutritional factors [11]. These forms compounds with protein vitamin and minerals thereby hindering the absorption of nutrients in the body [12]. In the process of fermentation, nutrients from the food are utilized by microorganisms for their growth and reproduction. The metabolic activities of the microorganisms and enzymes present cause changes in the food's chemical and physical properties. The nutritional and physical qualities of the food product in question are impacted by the alterations brought about by fermentation processes. These activities result into unique flavour, a longer shelf life and better nutrition. Additionally, fermentation improves colour and texture. Microbial fermentation results in increased amounts of B vitamins and mineral bioavailability, as well as better protein and carbohydrate digestibility [13]. Addition of foods that are rich in micronutrient such as pumpkin leaves is a way of to address nutrient deficiency in children [14].

Pumpkin is commonly grown in Nigeria and belongs to the cucurbitaceous family, [15]. Pumpkin is cultivated for its leaves consumption by humans and for feed while the seeds are regarded as waste [16]. Studies shows that pumpkin leaves have good nutritional quality and its incorporation into regularly consumed snacks could have a nutritional benefit in terms of minerals

availability [17]. Pumpkin leaves are a good source of iron, carotene and ascorbic acid [18]. Researchers have made efforts to utilize pumpkin leaves flour in addressing macronutrient deficiencies but not in the deficiency of micronutrients. Although pumpkin seed flour have been largely exploited in bakery foods [19] but not much attention has been given to its use in the development of extruded snacks. Depending on the material used and process, extruded products may go through a variety of physicochemical and nutritional changes. These changes include protein denaturation, lipid oxidation, starch gelatinization, cross-linking and dextrinization, denaturation of enzymes, browning, degradation of vitamin and flavour formation. The qualities of the finished product are result of all these alterations and are also affected by the processing conditions. Consequently, by altering feed formulation and conditions on the same equipment, extrusion technology enables quick and effective conversion of varied raw materials into numerous appetizing food items [20]. Extrusion processing has found use with a variety of food groups, including fruits, vegetables, fish and meat products, roots and tubers and oil seeds. However, there is scarcity of information on the mineral and antinutritional properties of extruded snacks from fermented pearl millet and pumpkin leaves flour blends. The main objective of the study is to determine the mineral and antinutritional properties of extruded snacks from fermented pearl millet and pumpkin leaves flour blends.

2. Materials and Methods

Materials

Millet grains, fresh pumpkin leaves, sugar, salt and milk powder were obtained from Elewera market, Abeokuta, Ogun state, Nigeria.

Production of fermented millet flour

The preparation of fermented millet flour sample was done according to the method described by [21]. The millet grains were carefully sorted to remove any unwanted materials. They were then rinsed with clean water, allowed to ferment for 24 or 48 hours, and finally dried in an oven dryer until all moisture had been removed. The grains (dried) were milled into flour using a laboratory hammer mill.

Preparation of pumpkin leaves flour

The preparation of pumpkin leaves flour sample was done according to the method described by [22]. Pumpkin leaves were sorted, oven-dried at 60 °C for 5 h, milled into powder using electric blender and stored at low temperature until needed.

Formulation of fermented millet-pumpkin leaves composite flour for extruded snacks

Using D-optimal mixture design, fermented millet and pumpkin leaves were combined at 100:0, 90:10, 97:3.0, 95.25:4.75, 93.50:6.50, 91.75:8.25, 97:3.0, 90:10, 97:3.0 and 93.50:6.50, respectively.

Production of extruded snacks from fermented millet-pumpkin leaves composite flour

The extruded snacks were produced according to the method of [23]. 100 g of flour samples and composite flour samples were added to other ingredients (7.5% sugar, 0.75% salt, 21% milk powder and 50 ml of hot water at 80–90°C). It was left for 2 min for uniform hydration. The extruder used had a screw length per diameter of 16.43:1, a screw diameter of 18.5 mm, and a length of 304 mm. Band heaters was used to heat the barrel section. It was allowed to operate at full speed with a constant condition of barrel temperature (80°C), screw speed (60 rpm) and feed moisture of 27% (wet basis). Extruded snacks were cut into smaller pieces (2 cm length) and were allowed to dry in a hot air oven (Genlab, OV/ DIG, UK) at 60°C for 3 h to get the

fermented millet-pumpkin extruded snacks. Immediately after extrusion, the snacks was allowed to cool at ambient temperature, it was packaged in high density polyethylene bags, and stored.

Mineral composition of extruded snacks from fermented millet-pumpkin leaves composite flour

The mineral contents of the finely ground extruded samples were determined as described by [24]. The atomic absorption spectrophotometer (712354) was used to measure calcium, phosphorus, potassium, and iron (Thermo Scientific S Series Model 712354). Under a fume hood, 0.5 grams of extruded snack were weighed and placed into a 125 mL Erlenmeyer flask, this was followed by the addition of perchloric acid (4 mL), concentrated HNO₃ (25 mL), and concentrated sulfuric acid (2 mL). In a digester (Buchi Digestion unit K-424) at low temperature, under a perchloric acid fume hood, the contents were thoroughly mixed and gently heated until fumes appeared. The heat was sustained for 30 seconds followed by a cooling period and the addition of 50 ml of distilled water. Afterwards, the mixture was cooled, filtered, and made up with distilled water in a Pyrex volumetric flask. The solution was then measured using an Atomic Absorption Spectrophotometer.

Anti-nutritional properties of extruded snacks from fermented millet-pumpkin composite flour

Determination of tannin

For the determination of tannin content in the extruded snacks, the method described in [25] was used. 0.2 g of extruded snacks sample was placed in a 50 mL beaker. For an hour, 20 mL of methanol (50%) was added, covered with paraffin and placed in a water bath (77–80°C) with constant stirring with a rod to avoid lumping. A double-layered Whatman No.1 filter paper was used to filter the extract quantitatively into a volumetric flask (100 mL) and 50%

methanol was used to rinse it. To make it up, distiller's water was added, and it was thoroughly stirred. In a volumetric flask (50 mL), extract (1 mL) was pipetted into Folin-Denis reagent, 2.5 mL of distilled water, and 17% Na₂C₂O₃ (10 mL) were then added and mixed thoroughly. After mixing thoroughly, the mixture was left for another 20 minutes for blue-green coloration to develop. The absorbance of the tannic acid standard solutions and that of sample were read after the development of colour on a 21D Spectrophotometer at $\lambda = 760$ nm.

Determination of trypsin inhibitor activity

1 g of extruded snacks was extracted with 50 ml of 0.01N NaOH for an hour, and the pH was determined. The suspension was mixed with distilled water to a point where 1 ml produces trypsin inhibition of 40 to 60%. In addition, 2 ml of trypsin solution was added to the test tube, the tubes were placed in a water bath (37°C) for 10 minutes, and 1 ml of 30% acetic acid was added and mixed thoroughly. The contents were filtered with Whatman No. 3 and absorbance was measured using spectrophotometer at $\lambda=410$ nm against a blank reagent.

Determination of total phenol

Using a mortar and pestle, 10 ml of ethanol (80%) was mixed with one gram of extruded snacks flour. For 20 minutes, it was centrifuged at 10000 rpm. The supernatant was stored and the residue was extracted again with 10 ml of ethanol (80%). After evaporating the supernatants to dryness, the residue was dissolved in 5 ml of distilled water. Different quantities of samples (0.2-2ml) were taken in the test tubes. Volume of each test tube was made to 3 ml with distilled water. To each test tube, 0.5 ml of Folin Ciocalteu reagent was added, followed by 2 ml of sodium carbonate (20%) after 3 minutes. After thoroughly mixing the contents of the test tube, the contents were placed in a boiling

water bath for 1 minute. It was allowed to cool, and the absorbance were measured at $\lambda= 650$ nm against a blank reagent.

Determination of phytate

A sample of finely ground extruded snacks (2g) was soaked in 20 ml of HCl (0.2 N) for 3 hours and then filtered. A solution of ferric ammonium sulphate (1 ml) was mixed with the filtrate after filtration (0.5 ml). After boiling for 30 minutes, cooling, and centrifuging at 3000 rpm for 15 min, a supernatant volume of 1 ml was added to a 2, 2-pyridine solution of 1.5 ml and the absorbance was read with the aid of a spectrophotometer at $\lambda= 650$ nm against a blank reagent.

Statistical analysis

Data obtained were analysed using SPSS version 21.0 and Duncan multiple range tests were used to evaluate the differences between mean values at $p<0.05$. A design expert version (8.0) was used to evaluate the effects of the optimization procedure and at 5% confidence levels, significant effects were detected.

3. Results and Discussion

Mineral composition of extruded snacks from fermented millet- pumpkin leaves composite flour

The mean value and the interactive effect of the mineral composition of the extruded snacks produced from fermented millet and pumpkin leaves composite flour blends is presented in Table 1 and 3. Calcium content ranged from 42.71 mg/100g to 116.58 mg/100g and 46.36mg/100g to 125.97 mg/100g for 24h and 48h fermentation time, respectively. Extruded snacks from fermented millet of 100% had the least calcium content while extruded snacks from fermented millet of 91.75% and pumpkin leaves of 8.25% had the highest value for all the minerals. The interactive effect of fermented millet- pumpkin leaves does not have a significant ($p>0.05$) effect on the

calcium content at both 24 h and 48 h fermentation time. It was observed that as the pumpkin leaves flour increases the calcium content increases which could be as a result of leafy vegetable that is rich in

minerals composition. Also, it could be due to high extrusion temperature resulting in increase in amount of minerals in extruded products [26].

Table 1
Mineral Composition of Extruded Snacks from Fermented Millet-Pumpkin Leaves Flour at 24 h
Fermentation Time

FM (%)	PF (%)	Calcium (mg/100g)	Potassium (mg/100g)	Phosphorus (mg/100g)	Iron (mg/100g)
100	0.00	42.71 ^a	138.72 ^a	110.36 ^a	62.51 ^a
90.00	10.00	94.70 ^b	196.50 ^b	126.24 ^b	71.34 ^b
97.00	3.00	109.74 ^c	200.90 ^c	131.39 ^c	73.27 ^c
95.25	4.75	114.70 ^d	204.47 ^e	137.54 ^d	75.36 ^d
93.50	6.50	114.73 ^d	216.75 ^f	137.75 ^d	76.11 ^e
91.75	8.25	116.58 ^e	218.76 ^f	130.80 ^e	78.67 ^f
97.00	3.00	109.73 ^c	201.90 ^c	131.39 ^c	73.27 ^c
90.00	10.00	94.95 ^b	196.72 ^b	126.74 ^b	71.31 ^b
97.00	3.00	109.54 ^c	200.95 ^c	131.19 ^c	72.87 ^c
93.50	6.50	114.98 ^d	215.95 ^f	137.55 ^d	76.15 ^e

Mean values with different superscripts within the same column are significantly different ($p < 0.05$); FM- Fermented millet, PF- Pumpkin flour

Table 2
Regression Coefficient of Mineral Composition of Extruded Snacks from Fermented Millet-Pumpkin Leaves Flour at 24 h Fermentation Time

Parameter	Calcium	Potassium	Phosphorus	Iron
A	115.99	218.90	132.63	78.62
B	100.54	187.97	122.57	71.89
AB	-54.66	-61.33	18.98*	-13.99
F-value	0.57	2.04	1.07	2.24
R ²	0.5604	0.4053	0.5636	0.4274

*Significant at ($p < 0.05$): A- Fermented millet flour, B- Pumpkin leaves flour, AB- Interaction effects of fermented millet and pumpkin leaves flour, R²- Coefficient of determination

Table 3
Mineral Composition of Extruded Snacks from Fermented Millet-Pumpkin Leaves Flour at 48 h
Fermentation Time

FM (%)	PF (%)	Calcium (mg/100g)	Potassium (mg/100g)	Phosphorus (mg/100g)	Iron (mg/100g)
100	0.00	46.36 ^a	153.15 ^a	98.64 ^a	74.77 ^a
90.00	10.00	94.81 ^b	212.71 ^b	108.62 ^b	75.96 ^a
97.00	3.00	109.12 ^c	229.59 ^c	118.23 ^c	80.67 ^b
95.25	4.75	114.30 ^d	231.04 ^d	118.65 ^d	85.48 ^c
93.50	6.50	116.31 ^d	242.07 ^e	126.70 ^e	88.48 ^{cd}
91.75	8.25	125.97 ^f	255.94 ^f	146.73 ^f	90.22 ^d
97.00	3.00	108.12 ^c	229.59 ^c	118.10 ^c	80.77 ^b
90.00	10.00	94.69 ^b	213.71 ^b	108.42 ^b	75.98 ^a
97.00	3.00	109.52 ^c	230.59 ^c	117.23 ^c	80.57 ^b
93.50	6.50	115.66 ^e	241.07 ^e	126.70 ^e	88.48 ^{cd}

Mean values with different superscripts within the same column are significantly different ($p < 0.05$); FM- Fermented millet, PF- Pumpkin flour

Table 4

Regression Coefficient of Mineral Composition of Extruded Snacks from Fermented Millet-Pumpkin Leaves Flour at 28 h Fermentation Time

Parameter	Calcium	Potassium	Phosphorus	Iron
A	115.42	257.64	110.32	80.67
B	100.54	202.65	122.69	74.97
AB	-52.06	-42.51	-10.68*	-1.22
F-value	0.60	3.13	1.26	2.28
R ²	0.1662	0.5110	0.2955	0.4321

*Significant at ($p < 0.05$): A- Fermented millet flour, B- Pumpkin leaves flour, AB- Interaction effects of fermented millet and pumpkin leaves flour, R²- Coefficient of determination

It was observed that the calcium content of extruded snacks from fermented millet of 91.75% and pumpkin leaves of 8.25% was higher than the value (52.95 mg/100g) reported by [27]. This could be as a result of location where the sample was cultivated, since genetic and environment have effect on nutrient composition of plant [27].

Potassium content ranged from 138.72mg/100g to 218.76 mg/100g and 153 mg/100g for 24 h and 48 h 255.94 mg/100g for 24 h and 48 h fermentation time respectively. The interactive effect of fermented millet and pumpkin leaf flour does not have a significant ($p > 0.05$) effect on the potassium content at both 24 h and 48 h fermentation time. Phosphorus content ranged from 110.36 mg/100g to 137.75 mg/100g and 98.64 mg/100g to 146.73 mg/100g for 24 h and 48 h fermentation time, respectively. The interactive effect of fermented millet- pumpkin leaves flour has a significant ($p < 0.05$) effect on the phosphorus content at both 24 h and 48 h fermentation time (Table 3 and 4). This could be as a result of minerals being heat stable and most likely not affected during extrusion cooking. Iron content ranged from 62.51 mg/100g to 78.67 mg/100g and 74.77 to 90.22 mg/100g for 24 h and 48 h, respectively. The interactive effect of fermented millet- pumpkin leaves flour does not have a significant ($p > 0.05$) effect on the iron content at both 24 h and 48 h fermentation time. The value of iron content is higher when compared with the value (4.68 mg/100g) reported by [23].

This indicates the presence of pumpkin leaves which is rich in iron content. Iron is needed in the body for haemoglobin and myoglobin synthesis which are the carriers of oxygen in the blood. It was also noticed that the entire mineral except phosphorus increased with increase in fermentation time which confirms that fermentation reduces chelating agents thereby promoting mineral availability [28].

Anti-nutritional properties of extruded snacks produced from fermented millet and pumpkin leaves composite flour

Anti-nutritional factors are regarded as compounds that unfavourably affect bioavailability, digestion, utilization nutritional potential of a food material [29]. The mean value and the interactive effect of the antinutritional composition of the extruded snacks produced from fermented millet and pumpkin leaves composite flour blends is presented in Table 5 and 6. Tannin content ranged from 1.102 to 3.1119% and 0.552% to 2.059 % for 24 h and 48 h, respectively. The interactive effect of FMF and PF had no significant ($p < 0.05$) effect of the tannin content at 24 h and 48 h fermentation time. The tannin content of fermented millet of 91.75% and pumpkin leaves of 8.25% (3.119% and 2.059%) were low compared to the value (9.52%) reported by [29]. Tannin has an astringent taste and is important in the healing of wound. As the fermentation time increases, the tannin content decreases and this could be as a result of increased in fermentation time, as

fermentation has the potential to reduce tannin content in cereals.

Trypsin inhibitor affects protein digestion adversely by inhibition of proteolytic enzymes. Trypsin inhibitor content of the extruded snacks ranged from 0.029 to 0.025% and 0.012 to 0.025% for 24 and 48 h fermentation time, respectively. The result shows as the pumpkin leaves proportion increases, the trypsin inhibitor content decreases. This result is relatively lower compared to that of yellow maize and soy bean flour blends (0.06%) reported by [30]. The interaction of fermented millet flour and the pumpkin leaf blend on trypsin inhibitor content of the extruded snacks produced from fermented millet grains and pumpkin leaves have a negative

significance ($p < 0.05$) effect for 24 hours of fermentation. Trypsin inhibitor is highly sensitive to heat and may be affected with application of heat. The phenolic content of the extrudates ranged from 0.055 to 0.098% and 0.043 to 0.088% for both 24 and 48 hours of fermentation, respectively. The regression coefficient for phenolic content has the coefficient of determination (R^2) of 0.7714 and 0.8574 for 24 and 48 hours, respectively. It was observed that as the pumpkin leaves flour increases, the phenolic content decreases for 24 hours of fermentation. However, as the proportion of pumpkin leaves flour increases, the phenolic content decreases for 48 hours fermentation time.

Table 5

Anti-nutritional properties of extruded snacks from fermented millet and pumpkin leaves composite flour blends at 24 h fermentation time

FM (%)	PF (%)	Tannin (%)	Trypsin inhibitor (%)	Phenolic (%)	Phytate (%)
100	0.00	1.100 ^a	0.031 ^e	0.098 ^f	0.044 ^b
90.00	10.00	1.102 ^a	0.030 ^e	0.078 ^b	0.041 ^{ab}
97.00	3.00	2.105 ^b	0.028 ^{cd}	0.090 ^{cd}	0.041 ^{ab}
95.25	4.75	2.206 ^b	0.028 ^{cd}	0.088 ^c	0.033 ^{ab}
93.50	6.50	2.414 ^c	0.027 ^c	0.090 ^d	0.032 ^b
91.75	8.25	3.119 ^d	0.027 ^c	0.055 ^a	0.032 ^{ab}
97.00	3.00	2.165 ^b	0.025 ^a	0.095 ^e	0.041 ^{ab}
90.00	10.00	1.102 ^a	0.026 ^b	0.076 ^b	0.040 ^{ab}
97.00	3.00	2.165 ^b	0.025 ^a	0.076 ^b	0.041 ^{ab}
93.50	6.50	2.424 ^c	0.029 ^{cd}	0.089 ^{cd}	0.032 ^{ab}

Mean values with different superscripts within the same column are significantly different ($p < 0.05$); FM- Fermented millet, PF- Pumpkin flour

Table 6

Regression Coefficient of Anti-nutritional Properties of Extruded Snacks from Fermented Millet and Pumpkin Leaves Composite Flour at 24 h Fermentation Time

Parameter	Tannin	Trypsin Inhibitor	Phenolic	Phytate
A	0.12	0.028	0.088	1.352×10^{-3}
B	0.094	0.025	0.073	1.064×10^{-3}
AB	-0.011	-0.027*	6.088×10^{-3}	2.269×10^{-3}
F-value	0.88	3.78	1.12	1.15
R^2	0.8264	0.6578	0.7714	0.7771

*Significant at ($p < 0.05$): A- Fermented millet flour, B- Pumpkin leaves flour, AB- Interaction effects of fermented millet and pumpkin leaves flour, R^2 - Coefficient of determination.

Table 7
Antinutritional Properties of Extruded Snacks from Fermented Millet and Pumpkin Leaves Composite Flour at 48 h Fermentation Time

FM (%)	PF (%)	Tannin (%)	Trypsin Inhibitor (%)	Phenolic (%)	Phytate (%)
100	0	0.552 ^a	0.023 ^e	0.088 ^f	0.004 ^a
90.00	10.00	1.054 ^b	0.020 ^d	0.067 ^c	0.002 ^c
97.00	3.00	1.054 ^b	0.017 ^{bc}	0.059 ^b	0.002 ^e
95.25	4.75	1.556 ^c	0.017 ^{bc}	0.070 ^d	0.001 ^a
93.50	6.50	1.606 ^c	0.016 ^b	0.072 ^e	0.002 ^c
91.75	8.25	2.059 ^d	0.012 ^a	0.043 ^a	0.001 ^a
97.00	3.00	1.064 ^b	0.014 ^b	0.058 ^b	0.001 ^d
90.00	10.00	1.055 ^b	0.021 ^d	0.067 ^c	0.002 ^{bc}
97.00	3.00	1.058 ^b	0.015 ^b	0.059 ^b	0.002 ^c
93.50	6.50	1.608 ^c	0.015 ^b	0.071 ^d	0.001 ^{ab}

Mean values with different superscripts within the same column are significantly different ($p < 0.05$); FM- Fermented millet, PF- Pumpkin flour

Table 8
Regression Coefficient of Antinutritional Properties of Extruded Snacks from Fermented Millet and Pumpkin Leaves Composite Flour at 48 h Fermentation Time

Parameter	Tannin	Trypsin Inhibitor	Phenolic	Phytate
A	0.11	0.024	0.060	5.470×10^{-3}
B	0.088	0.020	0.062	2.123×10^{-3}
AB	-0.091	-0.018	0.016	-0.011
F-value	0.64	3.60	0.18	4.06
R ²	0.5480	0.6454	0.8574	0.6748

*Significant at ($p < 0.05$): A- Fermented millet flour, B- Pumpkin leaves flour, AB- Interaction effects of fermented millet and pumpkin leaves flour, R²- Coefficient of determination

Recent reports have shown that phenolic compounds are antioxidants that can serve as protection to human body against free radicals, and this formation results from metabolism of the cells [31]. The phytate content of the extruded product ranged from 0.032% to 0.044% and 0.001 to 0.004% for both 24 and 48 hours of fermentation, respectively. From the regression table, the regression coefficient obtained showed the quadratic model developed for phytic content has the coefficient of determination (R²) of 0.7771 and 0.6748 for 24 and 48 hours of fermentation, respectively. There were no significant ($p > 0.05$) difference in the phytate content of the extrudates for both 24 and 48 hours of fermentation, respectively. From the nutrition standpoint, phytic acid is considered as an anti-nutritional compound because it prevents the bioavailability of minerals, but on the

other hand it provides resistance to the grain during storage against the bruchid beetle. It was observed that as the pumpkin leaves blend proportion increases, the phytate content decreases for both 24- and 48-hours fermentation periods, respectively. In this study, the decrease in content of the antinutritional composition of the extruded snacks may largely be caused by the action of leaching during fermentation and by extrusion process which has been previously reported. These results are similar to previous findings of [23] who observed significant reduction in the amount of anti-nutrients for lentil split extrusion.

Optimization process of extruded snacks from fermented millet and pumpkin leaves composite flour

The extruded snacks were optimized based on some quality parameters to extruded snacks. Calcium, potassium, phosphorus, iron, tannin, trypsin inhibitor, phenolic and phytate were quality parameter studied in this work and were also attributes based on desirability concept as well and this serves as the constraints to the optimization process. Calcium, phosphorus, potassium and iron were maximized while tannin, phenolic, trypsin inhibitor and phytate were set at none. The solution to the optimized extruded snacks from fermented millet and pumpkin leaves composite flour showed desirability of 0.74 and 0.79 for 24 and 48 h fermentation (Table 9).

4. Conclusions

Extruded snacks from fermented millet and pumpkin leaves flour had a desirable mineral composition and reduced antinutritional properties. However, the optimized solution of extruded snacks from fermented millet-pumpkin leaves flour for 24 and 48h were 0.74 and 0.79 respectively. Further studies should be carried out on the sensory and storage stability of the extruded snacks.

Table 9

Solution Process to the Optimization of Extruded Snacks from Fermented Millet-Pumpkin Leaves Flour

Time (h)	Ca	K	P	Fe	Tannin	TI	Phenolic	Phytate	Desirability
24	114.73	218.76	170.80	76.15	1.10	0.02	0.05	0.03	0.74
48	125.97	242.07	146.73	90.22	0.55	0.12	0.04	0.01	0.79

Ca-Calcium, K-Potassium, P-Phosphorus, Fe- Iron, TI- Trypsin inhibitor

5. References

[1]. ADEBIYI J. A, OBADINA, O. A., ADEBO O.A. & KAYITESI, E. Fermented and malted millet products in Africa: Expedition from traditional/ethnic foods to industrial value-added products. *Critical Reviews in Food Science and Nutrition*, 58(3), 463-474 (2018)

[2]. AIRES B. Anthropometric and biochemical assessment of nutritional status and dietary intake in school children aged 6-14 years, Province of Buenos Aires, Argentina. *Arch Argent Paediatrics* 116(1), 34-46 (2018)

[3]. FAO/WHO. Human vitamin and mineral requirements. *Geneva: World Health Organization* (2nd ed). pp. 341 (2005)

[4]. FABER, M., BERTI, C. & SMUTS, M. Prevention and control of micronutrient deficiencies in developing countries: current perspectives. *Nutrition and Dietary Supplements*, 41 (2014)

[5]. FERGUSON, E., CHEGE, P., KIMIYWE, J., WIESMANN, D. & HOTZ, C. Zinc, iron and calcium are major limiting nutrients in the complementary diets of rural Kenyan children. *Maternal and Child Nutrition*, 11,6-20 (2015)

[6]. EBERWEIN, J. D., KAKIETEK, J., BENI, D. DE, PEREIRA, A., AKUOKU, J. K. & VOLEGE, M. An investment framework for nutrition in Kenya: reducing stunting and other forms of child malnutrition. In Martin & Lutalo (Eds.), World Bank Group (2016)

[7]. MAINA, M. J. Iron and nutritional status of children 12-59 months in Migwani Division, Mwingi District -Kenya. University of Nairobi (2011)

[8]. PELTO, G. & THUITA, F. (2014), Kenya policy brief: feeding infants and young children in Kitui county nutrition in infancy and early childhood (2014)

[9]. EZE, C., OKAFOR, G., OMAH, E. & AZUKA, C. Micronutrients, antinutrients composition and sensory properties of extruded snacks made from sorghum and charamenya flour blends. *African Journal of Food Science*, 14(1), 25-31 (2020)

[10]. TUFA, M. A., URGU, K., WELEDESEMAYAT, G. T. & MITIKU, B. G. Development and nutritional assessment of complementary foods from fermented cereals and soybean. *Food Science and Nutrition*, 2(2),1-8 (2016)

[11]. TAMILSELVAN, T. & KUSHWAHA, A. Effect of traditional processing methods on the

Oluwakemi Abosede OJO, Ekene Joachim OBINYERE, Adewale Olusegun OBADINA, Emmanuel Kehinde OKE, Eniola Oluwayemisi ONI, Mineral and antinutritional properties of extruded snacks from fermented millet-pumpkin leaves composite flour, Food and Environment Safety, Volume XXIII, Issue 1 – 2024, page 33 – 42

- nutritional composition of sorghum (sorghum bicolor L. moench) flour. *European Journal of Nutrition & Food Safety*, 12(7), 69-77 (2020)
- [12]. MAKAWI, A. B., MUSTAFA, A. I., ADIAMO, O. Q. & MOHAMED AHMED, I. A. Physicochemical, nutritional, functional, rheological, and microbiological properties of sorghum flour fermented with baobab fruit pulp flour as starter. *Food Science and Nutrition*, 7(2), pp. 689-699 (2019)
- [13]. ALMAIMAN, S. A., RAHMAN, I. A., GASSEM, M., DALAL, ALKHUDAYRI, ALHUTHAYLI, H. F., MOHAMMED, M. A., HASSAN, A. B. & FICKAK, A., OSMAN, M. Biochemical changes during traditional fermentation of Saudi sorghum (*Sorghum bicolor* L.) cultivars flour into Khamir (local gluten free bread). *Journal of Oleo Science*, 70(3), 409-415 (2021)
- [14]. FAO/WHO, C. A. (2017). *Guidelines on Formulated Complementary Foods for Older Infants and Young Children*. Retrieved from <http://www.fao.org/fao-who-codexalimentarius>
- [15]. RASHWAN, A. K., YONES, H. A., KARIM, N., TAHA, E. M. & CHEN, W. Potential processing technologies for developing sorghum-based food products: An update and comprehensive review. *Trends in Food Science and Technology*, 110, 168-182 (2021)
- [16]. MUTIE, F.M., RONO, P.C., KATHAMBI, V., HU, G.W. & WANG, Q. F. Conservation of wild food plants and their potential for combating food insecurity in Kenya as exemplified by the dry lands of Kitui country. *Plants*, 9(8), 1-25 (2020)
- [17]. AKINOLA, S. Effect of Fermented Sorghum and Pumpkin seed Flour Addition on Quality Characteristics of Maize. *Nigerian Food Journal*, 33(2), 116-126 (2016)
- [18]. SETIAWAN, B., AULIA, S. S., SINAGA, T. & SULAEMAN, A. Nutritional content and characteristics of pumpkin cream soup with tempeh addition as supplementary food for elderly. *International Journal of Food Science* (2021)
- [19]. PONGJANTA, J., NAULBUNRAN, A., KAWNGDANG, S., MANON, T. & THEPJAİKAT, T. Utilization of pumpkin powder in bakery products. *Songklanakarin Journal of Science and Technology*, 28, 71-79 (2006)
- [20]. RIAZ, M.N. Selecting right extruder, in R. Guy (Ed.), *Extrusion Cooking Technologies and Applications (Wood head Publishing in Food Science and Technology Ltd. CRC Press LLC. New York, Washington, DC, 2001)*, 29-49 (2001)
- [21]. ADEBAYO-OYETORO A., ADEYEYE, S. A., OLATIDOYE, O. P., OGUNDIPE, O.O & ADENEKAN, E.O. Effect of co-fermentation on the quality attributes of weaning food produced from sorghum (*Sorghum bicolor*) and pigeon pea (*Cajanus cajan*). *Journal of Culinary Science & Technology* (2017)
- [22]. LAWAL, O.M., SANNI, O., OLUWAMUKOMI, M., FOGLIANO, V. & LINNEMANN, A.R. The addition of fluted pumpkin (*Telfairia occidentalis*) leaf powder improves the techno-functional properties of cassava pasta. *Food Structure*, 30, 1-11 (2021)
- [23]. KAREEM, S.T., ADEBOWALE, A.A., SOBOKOLA, O.P., ADEBISI, M.A., OBADINA, O.A., KAJIHAUSA, O.E., ADEGUNWA, M.O., SANNI, L.O. & KEITH, T. Some quality attributes of high quality cassava – Tigernut composite flour and its extruded snacks. *Journal of Culinary Science and Technology*, 13, 242–262 (2015)
- [24]. AOAC. International 17th Edition, *Journal of the Association of Official Analytical Chemists*, Gaithersburg, MD, USA (2000)
- [25]. SWAIN, T. Tannins and lignins. In: G Rosenthal and D H Jansen (Editors) *Herbivores, Their interaction with secondary plant metabolites*. Academic Press, New York, 257 (1979)
- [26]. ANUONYE, C. J., JIGAM, A. A. & NDACEKO, M. G. Effects of extrusion-cooking on the nutrient and anti-nutrient composition of pigeon pea and unripe plantain blends, *Journal of Applied Pharmaceutical Science*, 2(5), 158-162 (2012)
- [27]. SANNI L. O, IKUOMOLA D. P. & SANNI S. A. Effect of length of fermentation and varieties on the quality of sweet potato gari. *Proceedings of the 8th triennial symposium of the International Society for Tropical Root Crops Africa Branch*; 3, 208-211 (2001)
- [28]. ADEBIYI J. A, OBADINA, O. A., ADEBO O.A. & KAYITESI, E. Fermented and malted millet products in Africa: Expedition from traditional/ethnic foods to industrial value-added products. *Critical Reviews in Food Science and Nutrition*, 58(3), 463-474 (2018)
- [29]. DAHIYA P.K., LINNEMANN, A.R., VAN BOEKEL, MA, KHETARPAUL, N., GREWAL, R. B. & NOUT, M.J. Mung Bean: Technological and Nutritional Potential, *Critical Reviews in Food Science and Nutrition*, 55(5), 670-688 (2015)
- [30]. NKESIGA J. Production and evaluation of extruded snacks from yellow maize soybean carrot and amaranth leaf flour blends. A master thesis submitted to the Department of Food Science and Technology, Faculty of Agriculture, University of Nigeria, Nsukka. (2014).
- [31]. OBOH G. & ROCHA J. B. (2007). Polyphenols in red pepper [*Capsicum annum* var. Aviculare (Tepin) and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver]. *European Food Research and Technology*, 225, 239-247
- Oluwakemi Abosede OJO, Ekene Joachim OBINYERE, Adewale Olusegun OBADINA, Emmanuel Kehinde OKE, Eniola Oluwayemisi ONI, Mineral and antinutritional properties of extruded snacks from fermented millet-pumpkin leaves composite flour**, *Food and Environment Safety*, Volume XXIII, Issue 1 – 2024, page 33 – 42