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Effects of Soaking Temperature and pH on Proximate and Mineral Composition of Bambara Nut (*Vigna subterranea*) Flour

Owoseni J.A.[1*]; Olanipekun B.F. [1]; Olasoju S.A. [2]

[1]Department of Food Science, Faculty of Food and Consumer Sciences Ladoke Akintola University of Technology, Ogbomoso
[2]Department of Agricultural Science Education, Lagos State University of Education, Lagos

Corresponding Author: Owoseni JA

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Abstract: Bambara nut (*Vigna subterranea*) is a drought tolerant legume containing a wide range of nutrients important in human nutrition. It is however relatively underutilized due to its hard-to-cook nature and anti-nutritional properties. Hence, this study investigated the influence of soaking temperature and pH on proximate and anti-nutritional properties of Bambara nut flour. A cream colored Bambara nut seeds were procured from Bodija Market, Ibadan, Oyo State. The seeds were cleaned, washed and soaked at different soaking temperatures (30 – 90 °C) and pH (3.0-10.0) for 12 h, dehulled, dried (60 °C for 48 h) and milled into flour. The soaking temperatures and pH were imputed into Response Surface Methodology (RSM) and thirteen runs were generated. The flour samples were analyzed for proximate and mineral composition. Data obtained were subjected to Analysis of Variance and means separated using New Duncan Multiple Range Test. Protein, crude fat, moisture content, carbohydrate, crude fiber and ash contents were in the ranges of 18.04-21.95, 7.92-8.48, 9.6-11.6, 55.09-59.02, 1.0-1.8 and 1.91-3.01%, respectively. Sodium, calcium, magnesium, zinc, iron and potasium were 1.04-2.43, 0.15-0.73, mg/100g, 4.62-5.83 mg/100g and 0.35-0.76, and 6.03-9.32 mg/100g respectively. This study has established the optimum soaking temperature (67.67 °C) and pH (3.1) and improve the bioavailability of its nutrients.

Keywords: *Bambara Nut, Proximate, Anti-Nutritional, Soaking Temperature, pH.*

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I. INTRODUCTION

Bambara nut (*Vigna subterranea*) is a leguminous plant which is indigenous to African and it is cultivated across the semi and sub-Sahara Africa (Tan *et al.*, 2020). It is commonly consumed in different parts of Africa and has different names based on the locality. In Nigeria, the common names are *Okpa* (Igbo), *Eparoro* (Yoruba) and *Gurjiya* (Hausa). In Malawi, it is known as *Nzama*, in Ghana as *Aboboi*, while in South African as *Jugo* beans (Nwadi et *al.*, 2023). Bambara nut is a drought tolerant plant with remarkable resistance to pest and diseases, exhibiting good yield performance in unfertilized soil (Ngui *et al.*, 2023). Beside this, it is a potential source of protein in local households, often formulated in sauces and consumed with cereals, roots and tuber (Tan *et al.*, 2020).

Bambara nut is a nutrient-dense legume and it's sometimes termed a "complete food" due to its balanced macronutrient composition. It contains 23.6% protein, 64.4% carbohydrate, 5.5% fiber and 6.5% fat, and it is rich in minerals (Azman *et al.*, 2019). It is relatively underutilized compared with other legumes such as cowpea and has often been associated with small-scale, subsistence farming, with women being the major producers and processors (Mbosso *et al.*, 2020). Bambara nut is normally referred to as a "poor man's crop" or "women's crop" grown primarily to supply the family with sufficient food, however, the crop was lately recognized as one of the crops for the new millennium due to its nutritional value, drought tolerance traits, and its capacity to grow and yield in poor soils which reduced the use of inorganic nitrogenous fertilizers (Chelangat *et al.*, 2023).

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In Nigeria Bambara nut has found various food uses; such as snacks, flour, porridge and various fried or steamed products such as "Akara". It has also been used in the preparation of local food drink such as "Kunu" and "Tuwo". Nwadi *et al.* (2022) reported that bambara nut flour has been used in making bread in Zambia and it has also been observed that milk prepared from Bambara nut gave a preferred flavour when compared with milk from cowpea, soyabean and pigeon pea. The animal feed potential of bambara nut has long been discovered and its suitability for animal grazing which is basically due to abundance of nitrogen and phosphorus in its kernels (Tan *et al.,* 2020).

Bambara nut, have desirable functional properties such as emulsion capacity, foaming capacity, water and oil absorption which are beneficial to the food industry. Ngui *et al*. (2023) reported that the high content and quality of protein and carbohydrate in bambara nut makes it suitable for use as an emulsifier, therefore its incorporation into foods has high prospect. The bambara protein is rich in essential amino acids with substantial level of lysine and methionine that greatly influence the functions and structure primordial in food formulation (Tan *et al.,* 2020).

Flours from bambara nut has been used to produce *moin-moin* (steamed bambara nut pudding) which is known as *Okpa* among the Ibo's and *akpekpa* among the Tiv's in Nigeria. *Okpa* is a typical delicacy produced from bambara nut flour which is very popular in the south eatern part of Nigerian (Ugwu *et al.,* 2023). It would appear that the high water and oil absorption capacity of the bambara nut flour contributes to the desirable texture and overall quality of "okpa" which has plays an important role in contributing to dietary protein and vitamin A intake among the school children (Nwadi *et al*., 2023).

Despite its ability to adapt to harsh climate and contribute to household protein requirement, bamabara nut is under little exploitation. In addition to climate change adaptation, bamabara nut flour incorporates perfectly as a protein ingredient in cookies and vegetable diary formulation (Tan *et al.,* 2020). The successful incorporation of bamabara flour in industrial food formulation depends on its physical, chemical and functional properties (Mune-Mune *et al.,* 2018). However, limited scientific evidence elucidates the major factors affecting the successful exploitation of this flour in food. Bambara nut has been reported by many researchers as a crop that has desirable potentials including functional and nutritional quality. However, more research is still needed to exploit and open up more profitable usage of this nutrient dense legume. Hence, the essence of this research is to study the effect of soaking temperature and pH on some quality attributes of bambara nut flour in order to exploits it usefulness and potentials in food formulation.

II. MATERIALS AND METHODS

A cream colored Bambara nut seeds used in this research were purchased from Bodija market, Ibadan, Oyo State, Nigeria.

- *Experimental Design*

A Response Surface Methodology (RSM) in Design Expert was adopted in this study. The two factors considered were the soaking temperature and pH. The proximate and mineral composityon of the flours were the response variables measured at levels expected to yield satisfactory experimental results.

- *Preparation of Bambara nut flour*

The Bambara nut seeds were sorted, cleaned, washed and then divided into thirteen portions. Each portion was soaked in cleaned water at temperature of 30, 40, 50, 60, 70, 80 and 90 ^{0}C at pH of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and duration of soaking time were between 10 -.12 h. It was manually dehulled and dried at 60 ^{0}C for 48 h in air draught dryer to moisture content of 10 %. It was milled and sieved through 0.8 mm sieve size. The flour samples were packed in high density polyethylene and stored at room temperature for further analysis.

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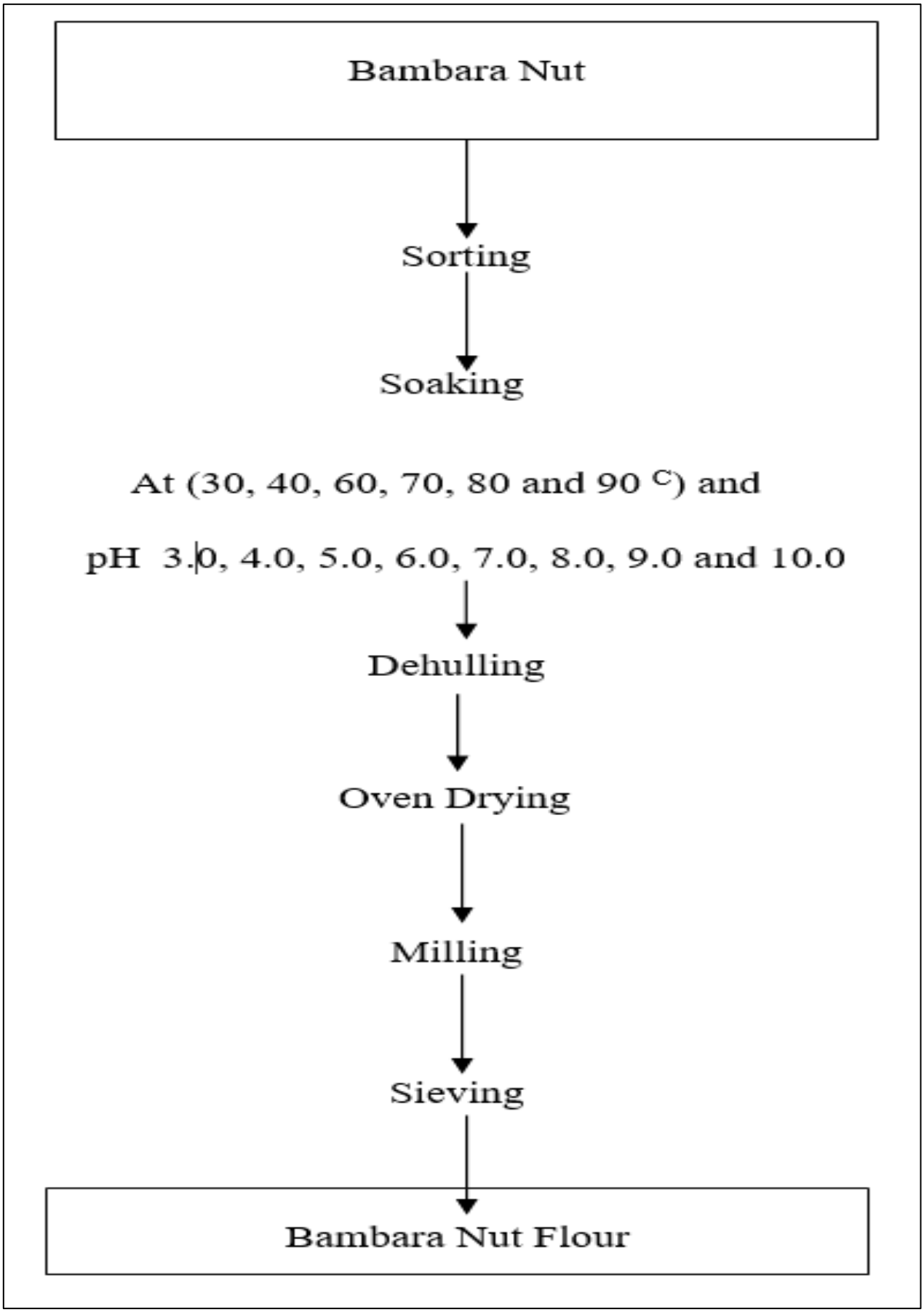

Fig 1 Flow Diagram for the Production of Bambara Nut Flour
Source; (Alagbaoso *et al.*, 2015)

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➢ *Preparation of pH Concentration Using Sodium Hydroxide (NaOH) and Hydrogen Chloride (HCl)*

The method described by Ngui *et al.* (2021), was used. Exactly 0.5 g of NaOH was dissolved in 100 ml distilled water and the pH was adjusted according to the concentration and determined by a pH meter.

➢ *Proximate Composition of Bambara Nut Four*

The procedure of AOAC (2020) was used to determine the moisture, crude protein, ash, crude fiber, carbohydrate and crude fat contents of the flour samples.

➢ *Determination of Mineral Composition*

The mineral composition determined were sodium, calcium, magnesium, iron, zinc and potassium according to the procedure of AOAC (2020).

➢ *Minerals Content Analysis*

The mineral contents of the flour sample were determined using the method described in the AOAC (2020). Exactly 0.5 g of the flour sample was weighed into a digestion tube and 10 ml of nitric/per chloric acid was added prior to mineral determination. The samples were digested at 150 °C until a clear fume is obtained and washed into a standard 50 ml volumetric flask and distilled water was added up to the mark. Atomic absorption spectrophotometer (210VGP, Buck Scientific incorporation, Norwalk, Connecticut, USA) was used in the determination of calcium (Ca), magnesium (Mg), zinc (Zn), and iron (Fe). Sodium (Na) and potassium (K) were determined using a flame photometer (2655-00, Coring Inc., USA.).

➢ *Statistical Analysis and Optimization Technique*

All data collected were subjected to analysis of variance (ANOVA) and means were separated using New Duncan Multiple Test. Design Expert version 6.0 was used to design the experiments and replication covering the full design of two factors was used for building quadratic model, which produced reasonable amout of information for testing the conditions well suited for model accuracy.

➢ *Production of Moin-Moin from Optimized Bambara Nut Flour*

The optimized Bambara nut flour was use in the production of *main- main* while the unoptimized flour sample was used as control. The *Moin-Moin* was prepared according to the method described by (Alagbaoso *et al.*, 2015) as shown in Figure 3.2. Flour samples of 50g was used in preparing *Moin-Moin* by adding two spoonful of power horse vegetable oil and 5g of onions. Two gram (2g) each of salt and pepper, seasoning cube of 8.03g was also added and mixed till a homogenous mixture (slurry) is obtained. The paste was then put in aluminum cups, covered and steamed for 2 hours using a gas cooker. After steaming it was allowed to cool for 30 minutes before the sensory evaluation was carried out.

➢ *Sensory Evaluation of Moin -Moin*

Sensory evaluation of *moin-moin* samples was conducted using 30 trained member panel. The panel comprise of a broad cross section of adult population (students and staff) of Ladoke Akintola Univrsity, Ogbomoso, with panelists that spread across a wide range of age and education groups. Samples was served in a randomized order in plates, with portable water for rinsing mouth in between tasting of samples to minimize rating errors, due to carry over of perceived attributes of previous sample. The panelists were asked to evaluate each sample based on the following parameters of aroma, colour, texture, taste and overall quality using a 9-point Hedonic scale as described by Onwuka (2018).

III. RESULTS AND DISCUSSION

The results of the proximate composition obtained are shown in Table 1. The minimum moisture content of 9.60 % was obtained at soaking temperature of 60 ^{0}C and pH 6.5 at experimental Run 1, while the maximum moisture content of 11.6 % was obtained at experimental Runs 7, soaking temperatures of 30 ^{0}C and pH 6.5. The result showed that maximum and minimum moisture content were obtained at constant pH of 6.5. This showed that pH did not have much influence on the moisture content of bambara nut flour. The lower the moisture content of the flour, the better its shelf life stability (Demba *et al.*,2022).

The effects of soaking temperature and pH on crude fat showed that the minimum value of 7.92 % was obtained at experimental Run 2 (soaking temperature of 60 ^{0}C and pH 3.0) while the maximum value of 8.48 % was obtained at experimental Run 7 (Soaking temperature of 30 $^\circ$C and pH 6.5). The crude fat content decreased from 8.48 to 7.92 % as soaking temperature and pH increases. The result showed that the crude fat content of bambara nut flour decreased with increased in soaking temperature and pH. This was similar to the work done by Oyeyinka and Oyeyinka (2018).

Table 1 Effect of Soaking Temperature and pH on the Proximate Composition of Bambara Nut Flour

Run	pH	Temp	Moisture	Ash	CrudeFibre	Crude Fat	Crude.Protein	CHO
		ºC	%	%	%	%	%	%
1	6.5	60	9.60±0.01^{a}	1.95±0.01^{a}	1.61±0.01^{d}	8.44±0.01^{g}	21.95±0.02^{i}	56.46±0.01^{b}
2	3.0	60	11.13±0.01^{e}	3.09±0.00^{d}	1.17±0.02^{b}	7.92±0.01^{a}	18.54±0.02^{c}	58.15±0.06^{c}
3	3.0	90	9.64±0.00^{a}	2.31±0.01^{b}	1.02±0.01^{a}	8.28±0.02^{de}	19.75±0.01^{e}	59.00±0.06^{d}
4	6.5	90	11.59±0.02^{a}	2.86±0.01^{cd}	1.34±0.01^{c}	8.21±0.01^{c}	20.92±0.01^{g}	55.09±0.01^{a}
5	10.0	30	10.96±0.01^{d}	2.86±0.01^{cd}	1.84±0.02^{g}	8.35±0.06^{f}	18.04±0.01^{a}	57.95±0.01^{c}
6	10.0	60	10.55±0.04^{b}	2.66±0.02^{c}	1.79±0.24^{f}	8.07±0.07^{b}	21.45±0.11^{h}	55.44±0.18^{a}
7	6.5	30	11.60±0.06^{g}	2.91±0.11^{cd}	1.73±0.01^{e}	8.48±0.04^{g}	20.07±0.07^{f}	55.21±0.08^{a}
8	10.0	90	10.55±0.01^{c}	1.91±0.07^{a}	1.00±0.01^{a}	8.32±0.03ef	18.97±0.01^{d}	59.03±0.07^{d}

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9	3.0	30	11.51±0.01^{f}	2.91±0.57^{cd}	1.02±0.01^{b}	8.24±0.63^{cd}	18.12±0.01^{b}	58.022±0.62^{c}

Variables with the same superscript along the same column are not significantly different (p>0.05).

The ranges of value obtained for protein were 18.04 - 21.94 % which is within the range of 9.60 – 40.0 % reported by Nwadi *et al* (2020) and Tan *et al.,* (2020). The variation in the value obtained by different researchers is attributed to differences in genetic background, growing conditions, and analytical techniques (Tan *et al.,*2020).

The crude fiber of the flour decreased with increase in soaking temperature. The pH remains constant at the lower and upper limits of the soaking temperature ranges indicating that pH did not have much effect on the crude fibre content. The result obtained in this study was within the range of 1.4 to 10.3% reported by Nwadi *et al.,* (2020) and Tan *et al., (*2020). The concentration and composition of dietary fibre are influenced by maturity stage and processing methods. The low level of dietary fibre in bambara nut flour is desirable, because non digestible dietary fibre can reduce the bioavailability of essential minerals (Damba *et al.,* 2022).

The highest value of carbohydrate 59.03 % was obtained at soaking temperature of 60 ºC and pH 10.0 (exprimental Run 8) while the lowest value of 55.09 % was obtained at soaking temperature of 90 ˚C and pH 6.5 (exprimental Run 4). The result obtained is within the range of those reported by Tan *et al.,* (2020). The results indicate that the carbohydrate content dereased with increase in soaking temperature and pH. Damba *et al.* (2022) reported that increase in fat content and techniques of soaking and dehulling had greater effect and causes reduction in the carbohydrate composition. This account for variation in carbohydrate composition of bambara nut flour. (Vhulenda *et al.,* 2023).

The ash content decreased with increase in soaking temperature and pH. The reduction in ash content could be as a result of leaching, where the ash content was lost in soaked water (Damba *et al.,* 2022).

- *Effect of Soaking Temperature and pH on Mineral Content of Bambara Nut Flour*

The responses obtained for the effect of soaking temperature and pH on the mineral content of Bambara nut flour are presented in Table 2.0

The responses obtained for sodium (Na) content of Bambara nut flour at different soaking temperature and pH indicated that the least value of sodium (1.04 mg/100g) was obtained at experimental Run 1, (Soaking temperature of 60 °C and pH 6.5) while the highest value for sodium (2.43 mg/100g) was obtained at experimental Run 9. The result obtained was within the range of 2.9 and 12.0 mg/100g reported by Mashau *et al.* (2023). The increase observed may be attributed to the absorption of sodium ions from the soaking medium into the seeds to form complexes with the sodium available in the aleurone layer of the seed (Damba *et al.,* 2022). However, this changes do not have significant effect on the sodium content of bambara nut flour.

Table 2 Effect of Soaking Temperature and pH on Mineral Composition of Bambara Nut Flour

Run	pH	Temp	Na	Mg	Ca	Fe	Zn	K
		^{0}C	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g
1	6.5	60	1.05±0.00^{a}	5.64±0.00^{d}	0.15±0.00^{a}	0.75±0.00^{h}	1.51±0.00^{i}	7.84±0.0C
2	3.0	60	2.02±0.53^{f}	4.62±0.02^{a}	0.73±0.00^{h}	0.10±0.00^{a}	0.36±0.00^{b}	6.03±0.00^{a}
3	3.0	90	1.19±0.00^{b}	5.65±0.00^{d}	0.32±0.00^{d}	0.41±0.00^{d}	0.61±0.00^{g}	8.43±0.00^{f}
4	6.5	90	1.83±0.00^{e}	5.83±0.00^{f}	0.39±0.00^{f}	0.49±0.00^{e}	0.49±0.00^{e}	8.56±0.00^{g}
5	10.0	30	1.99±0.01^{f}	5.62±0.00^{d}	0.30±0.0C	0.63±0.00^{f}	0.43±0.00^{d}	9.32±0.00^{e}
6	10.0	60	2.43±0.00^{h}	5.79±0.01^{e}	0.32±0.00^{e}	0.76±0.00^{i}	0.56±0.00^{f}	9.17±0.00^{h}
7	6.5	30	1.44±0.0C	5.77±0.00^{e}	0.21±0.00^{b}	0.66±0.00^{g}	0.76±0.00^{h}	8.13±0.00^{d}
8	10	90	2.26±0.00^{g}	5.54±0.0C	0.39±0.00^{f}	0.30±0.0C	0.35±0.00^{a}	8.15±0.00^{e}
9	3.0	30	1.65±0.11^{d}	4.79±0.16^{b}	0.41±0.00^{g}	0.22±0.00^{b}	0.41±0.0C	7.22±0.00^{b}

Variables with the same superscript along the same column are not significantly different (p-value>0.05) Na-Sodium, Mg-Magnesium, Ca-Calcium, Fe-Iron, Zn-Zinc, K-Potasium.

The responses obtained for magnesium (Mg) content indicates that the least value of magnesium (4.62 mg100/g) was obtained at experimental Run 3 (Soaking temperature of 60 °C and pH 3.0) while the highest value for magnesium (5.83 mg/100g) was obtained at experimental Run 5 (Soaking temperature of 90 ^{C} and pH 6.5). The result obtained in this study was higher than 3.90 mg/100g reported by Musah *et al.,* (2021). The result indicates that increase in soaking temperature and pH causes significant increase in the magnesium content of bambara nut flour.

The calcium contents of bambara nuts was not significantly affected by the soaking temperatue and pH. The maximum value of calcium obtained was 0.73 mg100/g at soaking temperature of 60 ^{C} and pH of 3.0 (exprimental Run 3) while the minimum value of 0.15 mg/g was obtained at temperature 60 ºC and pH of 6.5 (exprimental Run 1, respectively). The value of calcium obtained in this report was lower than the range of 4.72-6.88 g/100mg reported by Oyeyinka *et al.* (2019) and 3.5 mg/100g reported by Musah *et al* (2021). Damba *et al.,* (2022) reported that the bioavailability of minerals has intricate factors surrounding

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them, soaking has a great effect on mineral availability, either positive or negative since it facilitates the hydrolysis of anti-nutrients and in the same vein could lead to leaching of minerals. This accounts for variation observed in the value obtained by different researchers.

The result of the Iron (Fe) composition of Bambara nut flour shows that as the soaking temperature increases the iron content also increases. This was observed at experimental Run 3 (soaking temperature of 60 ºC and pH of 3.0) and experimental Run 9 (soaking temperature of 60 ^{C} and pH of 10.0) which represent the minimum value of 0.10 and maximum value of 0.76 respectively. The result indicates that soaking temperature does not affect the iron content as both the minimum and maximum iron content occur at the same soaking temperature. However, as the pH increases from 3.0 to 10.0, the iron content also increases. Thus, it can be stated that increase in alkalinity of the soaking medium causes increase in the iron content of the flour sample. Also the value of iron obtained in this study is lower than 2.11-3.93 mg/100g reported by Chelangat *et al*. (2023). The variation observed may be due to leaching of the nutrient in the soaking medium (Damba *et al.,* 2022).

The responses obtained for Zinc (Zn) content of bambara nut flour at different soaking temperature and pH indicated that the least value of Zinc 0.36 mg/100g was obtained at exprimental Run 3 (Soaking temperature of 60 ^{C} and pH 3.0) while the highest value for Zinc 1.51mg/100g was obtained at exprimental Run 10 (Soaking temperature of 30 ºC and pH 6.5). The result shows that Zinc content tends to decrease with increase in soaking temperature while increase in pH causes the zinc content to increase. The result obtained in this study is higher than the range 0.006-0.42 mg/100g reported by chelangat *et al* (2023). The variation may be influenced by factors such as storage period, processing method, location of the mineral in the seeds and the degree of mineral chelation (Gwala *et al* 2020).

The responses obtained for Potassium (K) content of bambara nut flour at different soaking temperature and pH shows that the least value of Potasium 8.56 mg/100g was obtained at the Experimental Run 1, (Soaking temperature of 60 ºC and pH 6.5) while the highest value for Potasium 9.32 mg/100g was obtained at Experimental Run 8 (Soaking temperature of 30 °C and pH 10.0). The result obtained in this research shows that maximum value for potassium was obtained at lower temperature and higher pH, hence soaking temperature and pH have significant effect on the Potasium content of bambara nut flour.

IV. CONCLUSIONS AND RECOMMENDATIONS

This research concluded that soaking temperature and pH had significant effect on the proximate and mineral composition of bambara nut flour. The reduction in crude fibre, moisture content and increase in crude protein are desirable attributes in the bambara nut flour. This research concluded that the optimum soaking temperature (67.67 ^{0}C) and pH (3.1) had significant effect on the proximate composition and the mineral composition of bambara nut flour. Hence, this will enhance the utilization and nutritional qualities of bambara nut seeds.

Its therefore recommended that for optimum nutritional qualities bambara nut should be processed at soaking temperature (67.67 ^{0}C) and pH (3.1). However, more research is recommended on the effect of soaking temperature and pH on the amino acid profile of bambara nut flour.

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