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Effect of cooking on the Nutritional Contents and Phytoconstituents of *Vigna Subterranea [L.]Verdc.*

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ABSTRACT

This research was carried out to assess the differences in the nutritional content and phytoconstituent of Bambara nuts(*Vigna subterranea [L.] Verdc*.) before and after cooking. Samples were collected and analysed according to the standard methods. The proximate composition of the raw sample revealed the percentage of moisture content, 7.93 %; crude protein, 22.49 %; crude fiber, 7.51 %; crude lipid, 8.45 %; ash content, 5.68 % and nitrogen- free extract, 47.94 %. While the cooked sample had moisture content, 10.72 %; crude protein, 20.88%; crude fiber, 3.97 %; crude lipid, 15.13 %; ash content, 3.28 % and nitrogen- free extract, 42.86 %. The lower value of crude protein in the cooked sample as compared to the raw could be as a result of leaching of soluble proteins into the cooking water indicating that food processing may influence the availability of nutrients either positively or negatively. From the phytoconstituent analysis, it was observed that the values of tannins, flavonoids, saponins, steroids, phytates, alkaloids and oxalates reduced significantly ($P< 0.05$) by cooking. Furthermore, the correlation coefficient were carried out on the data generated and it was found that the nutritional contents were negatively correlated ($p < 0.05$, $r = -0.9$) with most of the phytoconstituents which indicated that cooking makes the nuts safe for eating.

Keywords: Bambara nut, cooked sample, nutrients, phytoconstituents, raw sample

INTRODUCTION

Vigna subterranea (L.)Verdc. is a leguminous plant which belongs to the Fabaceae family. The plant grows well in the sub-Saharan Africa's warm tropics as such it is widely cultivated in the northern parts of Nigeria. Similar to the peanut plant, *V. subterranea* bears pods which ripens underground to provide edible nutritious seeds which are used in food and beverage preparations. The common name of *V. Subterranean* nut is Bambara nut, but locally in Nigeria, it goes by different names among the three major ethnic groups, viz. Okpa (Igbo), Epa-Roro (Yoruba), Kwaruru or Gurjiya (Hausa) (Adeleke *et al.*, 2018).

Bambara nut play a major role in the fight against malnutrition to a large proportion of the populace in developing countries like Nigeria due to its high protein contents as well as cheap availability (Halimi *et al.*, 2019). Due to its high protein value, it is a very important food eaten by peasants in Africa who cannot afford the expensive animal protein (Useh *et al.*, 2017). Although, Bambara nut did not assume the importance of a staple food as did the cereal crops like rice, wheat, beans, maize, or barley. Yet, it plays an important role like other staple foods both in meeting nutritional requirements and soil improvement because of nitrogen fixation (Abdulrashid and Hassan, 2021).

Generally, Bambara nuts are processed through various techniques like boiling, roasting, and frying. It can be eaten fresh or boiled after drying, and can be ground either fresh or dry to make puddings (Khan *et al.*, 2021). In some parts of Nigeria, the nuts are often crushed into flour, to prepare local dishes like "*alele*," "*alleleganye*," "*danwake*," "*gauda*," "*kosai*," "*kunu*," "*tuwo*," and "*waina*". In other parts, dried bambara nuts are made into paste and used in the preparation of various fried or steamed products, such as "*akara*" and "*moi-moi*" while the fresh immature nuts are also eaten raw (Tan *et al.*, 2020). Notwithstanding the aforementioned various uses of the nuts, very few people in the forest zones of tropical Africa are aware of its nutritional benefits. Additionally, a greater number of people are still fixed to a monoculture of limited crops for nourishment in their daily diets which is tantamount to nutrient insecurity. Therefore, in furtherance of efforts to eliminate poverty and malnutrition and to achieve the UN Sustainable Development Goals by 2030, it is essential to increase the rate of consumption of nutritious foods, especially among the low income countries (Khan *et al.*, 2021).

Bambara nuts is high in protein, carbohydrate and dietary fiber, mostly low in fat, while being a good source of vitamins and minerals (Uche *et al.*, 2014).Its protein have been found to be subjugated by essential amino acids like lysine

and leucine (Igbabul *et al.*, 2013; Anhwange and Atoo, 2015). Lysine enables the synthesis of carnitine, which converts fatty acids into energy and also plays an important role in the production of hormones, antibodies and enzymes (Abiodun and Adepeju, 2011; Okafor *et al.*, 2014). While a deficiency in lysine can lead to niacin deficiency and cause a health condition called pellagra. Leucine on the other hand aid muscular mass increase and it also helps muscular recovery after exercise. It regulates blood sugar and supplies the body with energy. It can be used in place of glucose in 'fasting' states (Anhwange and Atoo, 2015).

Considering that the different processing methods often utilized for Bambara nuts have wide-ranging effects on the bioavailability of nutrients because the degree at which nutrients are lost during processing differs from one processing method to the other (Okafor *et al*., 2014). Hence, *Vigna subterranea* is a promising commodity that should attract more research works to be carried out both on its raw and cooked states. Regarding the numerous health and nutritional benefits of *V. subterranea* and its ability to resist harsh weather conditions, it is s absolutely important to investigate the differences in the nutritional contents and phytoconstituents of raw and cooked Bambara nuts to add more literature on the nutritional integrity of this widely consumed nuts.

MATERIALS AND METHODS

Reagents

All chemicals used were of analytical grade and of highest purity possible. They were supplied by BDH Labs (UK), BDH Chemicals Limited Poole England.

Sample Collection and Preparation

Bambara nuts (1000 g) were purchased at Kwali and Kubwa markets, Abuja, Nigeria. The flour was prepared according to the method described by Igbabul *et al.*,(2013). Thus, the nuts were crushed to obtain the seeds which were winnowed to remove all foreign materials such as dust, debris and immature seeds. The fresh seeds were divided into two batches: unprocessed (raw) and processed (cooked).Then 200 g clean seeds were soaked in cold water for 8 hours, after which it was dehulled using plate mill with 6mm clearance between the plates and dried at 650^{o}C for 48 hours in air draught drier (Mermmet, Germany). The dry seeds were pulverized into powder using a hammer mill and sieved through 0.8mm mesh. The sample was packaged in moisture-proof, air-tight polyethylene containers and kept at 4°C prior to analyses.

Processing Techniques: This was carried out by the method of Abdulsalami and Sheriff, (2010) with slight modification. A second portion of the raw seeds was soaked for 8 hours in distilled water in the ratio of 1/3 (w/v). After the seeds were soaked, they were dehulled as stated above and excess solution was drained off. For the cooking process, the soaked seeds were cooked by boiling in distilled water (100^{o}C) for 3 hours at a seed to water ratio of 1/10 (w/v). This is the established time frame known for it to be ready as practiced by the locals. The cooking water was drained off and the seeds were air-dried for 3 days, ground and sieved into fine powder and packaged in moisture-proof, air-tight polyethylene containers and kept at 4°C in the refrigerator prior to analyses.

Proximate Analysis

The above samples were subjected to proximate analysis to determine the moisture content, crude protein, crude fibre, crude lipid, ash content and nitrogen – free extract (N.F.E) using the standard methods of the Association of Official Analytical Chemists (AOAC, 2010). All determinations were carried out in triplicate and reported in percentage.

The moisture content was determined by the measurement of weight loss due to evaporation of moisture in hot air oven and the weight loss after drying at 105^{o}C to a constant weight. Micro-Kjeldahl's method was employed in determining the crude protein from the total nitrogen content of the sample, which is mainly from the protein and other non-protein nitrogenous compounds such as amides and ammonium compounds. Crude fibre was determined according to the method outlined by Anhwange and Atoo, (2015). Crude lipid was determined by subjecting the dried sample to extraction with petroleum ether using the Soxhlet apparatus. The organic soluble substances thus removed were collected in a flask, dried and weighed. Ash content was determined by igniting a known amount of moisture free sample in a muffle furnace at 550 ^{o}C for 4 hours and then weighed after cooling to room temperature. Nitrogen – free extract (N.F.E) was determined based on methods outlined in AOAC, (2010).

PHYTOCHEMICAL CONSTITUENTS

Qualitative Phytochemical Constituents: Qualitative phytochemical screening of tannis, flavonoids, anthraquinones, saponins, steroids, cardenolides, cardiac glycosides was carried out using standard methods (Useh *et al.*, 2017; Abdulrashid and Hassan, 2021) as follows;

Tannins (Braymer's Test): 0.5 g of the extract was stirred with 10 ml of distilled water and then filtered. 2 ml of the filtrate was treated with 1% alcoholic ferric chloride solution and observed for formation of blue-black or greenish colour solution.

Flavonoids: 2 ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicated the presence of flavonoids.

Anthraquinones: 1 ml of the extract was treatedwith a few drops of 10 % ammonia solution and the formation of a pink precipitate indicated the presence of anthraquinone.

Saponin (foam test): 2 ml of the extract was added to 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirmed the presence of saponins.

Steroids (Libermann-Burchard test): To the 2 ml of the test solution, a few drops of chloroform, 3 - 4 drops of acetic anhydride and one drop of concentrate sulphuric acid were added. Appearance of purple colour, which changes to blue or green colour, showed the presence of steroid.

Cardenolides: 2 ml of benzene was added to 1 ml of the sample extract. The formation of a turbid brown colour indicated the presence of cardenolides.

Cardiac Glycosides (Keller Kelliani's Test): 5ml of each extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1ml concentrated sulphuric acid. A brown ring formation at the interface indicated the presence of deoxysugar characteristics of cardiac glycosides.

Quantitative Analysis: The quantitative analysis for tannins, flavonoids, saponins, phytates and oxalates were done according to the methods described by Useh et al., (2017).

STATISTICAL ANALYSIS

In order to quantitatively analyse and confirm the relationship among the parameters determined, Pearson correlation analysis was applied to the dataset. All the statistical analyses were performed using statistical software SPSS Windows version 25.0

RESULTS AND DISCUSSION

The results of the proximate composition of the raw and processed samples are presented in Table 1. From the results, it was revealed that the moisture content of the raw sample was 7.93±0.3% which was lower than that of the cooked sample, 10.72±0.08 %. The low moisture content of the raw sample indicated that it would have an enhanced shelf-life of the flour in terms of storage than the cooked one. The percentage crude protein (22.49±0.1%) recorded in the raw seed was greatly reduced after processing with a value of 20.88±0.05 %. The observed reduction of crude protein in the cooked sample as compared to the raw form might be as a result of leaching of soluble proteins during cooking or due to the pretreatment which indicates the susceptibility to protein denaturation and leaching of these nutrients. A similar observation was made by Uche *et al.,* (2014) but it is in contrast to the work of Abdulsalami and Sheriff, 2010 who reported a higher value of crude protein in the cooked sample. Percentage crude fibre ranged from 3.97±0.3 % to 7.51±0.06 % in the cooked and raw samples respectively which showed that crude fibre was significantly reduced ($P< 0.05$) by cooking. There was a significant increase ($P < 0.05$) of crude lipid in the cooked sample with a value of 15.13±0.07 % as compared to the raw sample which recorded 8.45±0.1 %.This may be due to concentration of the endosperm by the processing method adopted. This corresponds with a previous work carried out by Okafor *et al.,* (2014) which stated that dehulling of legumes significantly concentrates major components like oil. Ash content greatly reduced from 5.68±0.4 % in the raw sample to 3.28±0.1% in the processed sample. The significant reduction in ash content of seeds with cooking is in agreement with results of Adeyanju and Abimbola, (2015) who recorded decreased in ash from 2.80 % in the raw seeds to 1.79 % in the boiled seeds. Nitrogen-free extract (NFE) values ranged from 42.86±0.05 % to 47.94±0.09 % for the cooked and raw samples respectively. The reduction in the NFE content could be due to hydrolysis of starch to simple sugars during the cooking period. Hydrophilic groups in carbohydrate molecules caused it to take up moisture in proportion to the relative humidity of the environment. This characteristic behaviour encouraged moisture uptake and apparent reduction in percentage of NFE.

Table 1: Proximate composition of Bambara Nut

Parameters	Raw Sample (soaked & dehulled) (%)	Cooked Sample (soaked, dehulled & cooked) (%)
Moisture Content	7.93±0.3	10.72±1.99
Crude Protein	22.49±0.1	20.88±1.47
Crude Fibre	7.51±0.06	3.97±0.51
Crude Lipid	8.45±0.1	15.13±1.24
Ash Content	5.68±0.4	3.28±0.1
Nitrogen-Free Extract	47.94±0.09	42.86±1.4

Values are triplicate of mean ± standard deviation

The results of the phytoconstituents are recorded in Tables 2 and 3. It was found that out of the total phytochemicals determined, only five were found present in both the raw and cooked sets of samples concerned. The preliminary phytochemical screening indicated the presence of cardiac glycosides, steroids, saponins, flavonoids and tannins in both the raw and cooked samples. The findings are similar to research of Alhassan *et al.* (2018) which showed most of the phytochemicals existing in *Balanites aegyptiaca* kernels, hence, Bambara nuts are potential source of useful active compounds. Although these phytochemicals are known to play a vital role in the lives of plants and animals, it is noteworthy that they could also have some adverse effects on animals (anti-nutritional) especially when consumed in large quantities, which could affect the availability of nutrients and interfere with the metabolic processes leading to retard growth and development. These anti-nutritional contents can be reduced easily by proper processing techniques such as cooking and roasting to acceptable limits (Abdulrashid and Hassan, 2021).

Table 2: Qualitative Phytochemical composition of Bambara Nut

Parameters	Raw Sample	Cooked Sample
Tannins	+	+
Flavonoids	+	+
Anthraquinone	-	-
Saponin	+	+
Steroids	+	+
Cardenolides	-	-
Cardiac Glycosides	+	+

Key: (+) = present, (-) = absent

Table 3: Quantitative Analysis of Phytochemical Constituents of Bambara Nut

Parameters	Raw Sample (mg/100 g)	Cooked Sample (mg/100 g)
Tannins	0.50±0.02	0.22±0.01
Flavonoids	17.46±1.4	8.73±1.2
Saponins	21.09±0.7	13.14±0.7
Phytates	15.28±2.1	6.52±0.8
Oxalates	1.26±0.05	0.14±0.02

Note: Values are triplicate of mean ± standard deviation

The phytochemical composition of Bambara nuts are presented in Table 3. Tannin contents ranged from 0.22±0.01 mg/100 g in cooked sample to 0.50±0.02 mg/100 g in raw sample. Tannin contents were found to reduce drastically after cooking and to this effect, its nutritional value will improve since tannins are known to form complexes with proteins and reduce their digestibility and palatability. This is in agreement with the work of Abiodun and Adepeju, (2011) who reported that dehulling and cooking reduced tannin contents in Bambara nut. The flavonoids and saponins contents likewise reduced from 17.46±1.4 mg/100 g to 8.73±0.5 mg/100 g and from 21.09±0.7 mg/100 g to 13.14±0.5 mg/100 g for the raw and cooked samples respectively. Similarly, the phytate and oxalate contents also reduced drastically after cooking from 15.28±2.1 mg/100 g to 6.52±0.8 mg/100 g and from 1.26±0.05 mg/100 g to 0.14±0.02 mg/100 g for the raw and cooked samples in that order. It has been reported that the hulls of *Vigna subterranea [L.] Verdc.* are the optimum source of these phytochemicals (Harris *et al.,* 2018) which are being denatured and removed during the cooking process. Cooking cause considerable skin (epidermal) ruptures and facilitates the leakage of soluble phytochemicals into cooking water (Abiodun and Adepeju, 2011). Therefore, the reduction in the levels of these chemical compounds in the cooked sample may be due to their solubility in hot water.

Table 4: Pearson Correlation Matrix of Raw Sample

	Moisture	Protein	Fibre	Lipid	Ash	NFE	Tannins	Flavonoids	Saponins	Phytate	Oxalate
Moisture	1										
Protein	0.895	1									
Fibre	-0.84	-0.994	1								
Lipid	0.889	0.593	-0.499	1							
Ash	0.111	0.542	-0.633	-0.355	1						
NFE	-0.356	-0.735	0.807	0.11	-0.968	1					
Tannins	-0.979	-0.967	0.933	-0.778	-0.311	0.539	1				
Flavonoids	0.911	0.631	-0.54	.999*	-0.309	0.061	-0.808	1			
Saponins	-0.596	-0.891	0.937	-0.163	-0.864	0.963	0.747	-0.211	1		
Phytate	-0.936	-0.995	0.977	-0.671	-0.455	0.663	0.988	-0.706	0.841	1	
Oxalate	-0.896	-1.000**	0.993	-0.595	-0.54	0.734	0.968	-0.633	0.89	0.995	1
**. Correlation is significant at the 0.01 level (2-tailed).											
*. Correlation is significant at the 0.05 level (2-tailed).											

Table 5: Pearson Correlation Matrix of Cooked Sample

	Moisture	Protein	Fibre	Lipid	Ash	NFE	Tannins	Flavonoids	Saponins	Phytate	Oxalate
Moisture	1										
Protein	-0.558	1									
Fibre	0.256	0.66	1								
Lipid	-0.008	0.835	0.965	1							
Ash	-0.069	-0.79	-0.982	-.997*	1						
NFE	-0.435	0.99	0.759	0.904	-0.868	1					
Tannins	0.986	-0.413	0.412	0.157	-0.233	-0.28	1				
Flavonoids	-0.18	-0.716	-.997*	-0.982	0.994	-0.807	-0.34	1			
Saponins	0.959	-0.77	-0.029	-0.292	0.217	-0.673	0.899	0.107	1		
Phytate	0.443	0.497	0.98	0.893	-0.925	0.614	0.585	-0.961	0.171	1	
Oxalate	0.996	-0.627	0.171	-0.095	0.017	-0.511	0.968	-0.094	0.98	0.364	1
*. Correlation is significant at the 0.05 level (2-tailed).											

ASSESSMENT OF RELATIONSHIPS OF THE ANALYSED PARAMETERS

Tables 4 and 5 present the results of the correlation analysis of the examined Bambara nut parameters. This analysis was carried out in order to know the relationships that exist between the different parameters. The result obtained showed that positive and negative correlations existed between the examined parameters. Statistically, a high positive correlation (> +0.65) indicates that a change in one parameter will cause a similar change in the other parameter and a high negative correlation (< -0.65) indicates that a change in one parameter will cause a change in the other parameter but in the opposite direction. From Table 4, a strong negative significant relationship was observed between Protein versus Oxalate (r = -1.000, p< 0.01), and a moderately positive significant correlation was seen between Lipid versus Flavonoids in the correlation matrix (r = 0.999, p< 0.05). Also, from Table 5, a moderately negative significant correlation was observed between Fibre versus Flavonoids (r = -0.997, p< 0.05) and Lipid versus Ash (r = -0.997, p< 0.05) (2-tailed). Many other relationships between various quantitative variables were also seen with the least correlation values. Generally, it was revealed that the nutritional contents were negatively correlated (r = -0.9, p < 0.05,) with most of the phytoconstituents which indicated that cooking makes the nuts safe for eating. These results of correlation can prove useful in understanding the relationships between the nutritional contents and phytoconstituents of Bambara nuts.

CONCLUSION

This study revealed that Bambara nuts are a good source of protein, crude fibre, lipid and carbohydrate. They also contain phytoconstituents most of which are reduced during the process of cooking and therefore, enhancing the nutritional value of Bambara nut. It could therefore be deduced that the consumption of processed Bambara nuts makes the available nutrients more digestible, absorbable and available for growth and development.

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