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Study of Potential Effect and Health Benefits of Soaking Time on the Nutritional Composition and Some Anti-Nutrient Factors in Cowpea *(Vigna unguiculata)*

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Abstract: *The study of the potential effect and health benefits of water soaking time on the nutritional composition and some anti-nutrient factors (phytate, hydrocyanic acid, oxalate, saponin, flavonoid, alkaloid and tannins) in cowpea (Vigna unguiculata) was determined. Cowpea seeds were divided into three batches of 100g each. Two batches were soaked in clean tap water for one and two hours respectively. The result revealed that the moisture contents of cowpeas increased with soaking time from (7.87% at 0hour, 50.52% at 1 hour to 53.35% at 2 hours); fat from (1.45% at 0hour, 1.57 at 1 hour to 2.25% at 2 hours), also increasing with soaking time were carbohydrate and caloric contents of the cowpea from (70.25% at 0hour, 70.96% at 1hour to 74.00% at 2hours) and (383.67% at 0hour, 384.76% at 1hour to 390. 43 at 2hours%) respectively. It was also observed that soaking of cowpea for one and two hours independently decreased the protein, fibre & ash contents of the cowpea ranging from (22.40% to 18.55% protein), (2.36% to 2.08% fibre) and (3.5398% to 3.1204% ash). All the anti-nutrients studied recorded slight reductions except oxalates and alkaloids which were significantly (P<0.05) reduced with soaking time. This indicates that longer water soaking time is an effective way of lowering toxic substances in legume-based foods and enhancing nutrient bioavailability.*

Key words*: Vigna unguiculata, anti-nutrients, potential effect, water soaking.*

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1.0 Introduction

For the vast majority of the world's population, plants constitute the primary source of food and medicine. They are the primary elements in traditional healing methods and have served as inspiration for some key pharmaceutical treatments (Okokon *et al*., 2012). In the rural areas especially in developing country, the popularly eaten food is mainly carbohydrate, indigenous vegetables and legumes play useful role in producing food quality like proteins, minerals, vitamins and fats, also roughage part of the vegetable aids in digestion (Ekpo *et. al*., 2012). Lack of essential nutrients in the body can cause injurious impact on health leading to deficiency diseases such as blindness, anemia, and kwashiorkor (Udofia *et. al.*, 2023). Foods that contain essential amino acids are mostly supplied in the diet from animal source such as milk, meat or from cereals and legumes. Deficiency in some essential nutrients also weaken the immune system leading to an increased risk of infection (Sillwaggon *et. al.*, 2008). Uffia *et. al.*, (2021), explained that plants contain a wide variety of compounds that exhibit some medicinal and nutritive properties which are used as spices, food or medicinal plants for the maintenance of good health. Legumes are staple foods for many people in different parts of the world. The seeds have an average of twice as much protein as cereals by percentage and usually contain a more balanced profile of essential amino acids (Vijayakumari *et. al.*, 1997; Awak *et al*., 2017).

According to Onyenuga (1968), cowpea is the most widely grown and distributed legume in Nigeria and parts of West Africa. Cowpea fixes atmospheric nitrogen through its root nodules. It grows well in poor soils with more than 85% sand and with less than 0.2 organic matter and a low level of phosphorus (Singh *et al.*, 2003). This crop is widely grown and consumed as a source of plant protein throughout the world especially for essential nutrients. It is regarded as vegetable meat due to grain high protein (25%) content of its grains. According to Ishiwu (2004), cowpea is eaten in various forms either alone or in combination with cereal grains like rice, maize or tuber. Cowpea is low in anti-nutrients and high in calories as well as minerals and vitamins. It contains about 24% protein, 62% carbohydrate and little amount of other nutrients (Rangel *et al*., 2003). Various types of products are produced from cowpeas through soaking, dehulling, grinding, boiling or frying (Nnayelugo *et al.*, 1995). These cowpea-based delicacies provide an overall amino acid balance that compares favourably well with that of animal protein sources (Ihekoronye and Ngoddy, 1985). The majority of diets in developing countries are cereal-based, it is then very imperative to switch to a legume-based diet to cope with the persisting protein and micronutrient malnutrition majorly faced by children (Minde *et al*., 2020). According to Gemede & Ratta (2014), anti-nutrient factors (ANFs) are termed secondary metabolites of plants that are biologically active and act as defence mechanisms through the metabolism of plants. They could be classified as protein and non-protein. Protein ANFs include trypsin inhibitors, lectins and chymotrypsin inhibitors that hinder protein absorption and are heat sensitive while non-protein ANFs include phytic acid, saponins, alkaloids, tannins and phenolics that are heat resistant and are difficult to eliminate only by heat (Fereidoon, 2014). The nutrients and anti-nutrients both are affected by different processing methods like cooking, soaking, fermentation and germination. These processing methods enhance the utilization, palatability, digestibility and bioavailability of the nutrients (Ramakrishna *et al.*, 2008). The presence of anti-nutrients such as phytate, polyphenols, and oxalate cause low bioavailability of nutrients in plant-based diets (Holst *et. al.*, 2008). Some studies have been conducted on the effect of processing on the chemical composition of some food materials (Eddy *et* al., 2004; Eddy and Ukpong, 2005)

For example, Olaniran *et al*. (2020), investigated the influence of cowpea soaking time on the nutritional, antinutritional and antioxidant properties of cassava cowpea-orange -flesshed potato blends. The study indicated a significant influence of the soaking tome on the protein, fat and fibre content of the product. Ogun *et al*., (2006) however found that the combined effect of soaking time and temperature can also account for the variation in composition. They reported that the inhibitory activities of trypsin and stachyose were reduced due to a combination of soaking time and temperature. In addition to other studies, food processing embracing soaking in water can have some impacts on the nutritional and antinutritional factors of the plant. Much of the reported studies on the impact of soaking on cowpeas are concentrated on the nutritional values while less attention is paid to the antinutritional factors, which are essential factors when considering the health benefits that can be derived from a given food materials. Therefore, in this study, we aimed to increase the research database by evaluating the effect of soaking on the antinutritional factors of cowpeas.

2.0 Materials and Methods

2.1 Sample collection

Healthy and matured dried seeds of cowpea (*Vigna unguiculata*) were bought from a local market in Akwa-Ibom State, Nigeria.

2.2 Preparation of samples

The cowpea seeds were manually sorted to free them from debris and stones. About 300g of resulting seeds were weighed and divided into three portions of 100g each. Two portions were soaked in 200 ml clean tap water separately for cleaning for 1 hour and two hours respectively. The remaining portion was left dry and served as the control. Treated and untreated bean samples were pulverized and subjected to proximate analysis.

2.3 Proximate analysis

Proximate analysis of each prepared sample was carried out in triplicate. The crude protein was obtained by the Kjeldahl method as described by (James, 1995). Fat content was determined by extracting the food samples with n-hexane using a soxhlet apparatus. Moisture and ash contents were determined according to the methods described by the methods of (Association of Analytical Chemists [AOAC], 2012)

2.4 Determination of anti-nutrient

Titrimetric methods of Harbone (1983) were used for the quantitative determination of tannins and hydrocyanic acid while spectrophotometric methods of the Association of Analytical Chemists (AOAC) (2012) were used for the quantitative determination of saponins, alkaloids, flavonoid oxalates and phytic acid.

2.5 Statistical analysis

All experiments were carried out in triplicate and the final results were expressed as means ± standard deviation (S.D.). One-way ANOVA was used to analyze data using SPSS (version 20). The probability values were considered to be statistically significant differences if $p<0.005$.

3.0 Results and Discussion

The effect of water soaking time on the proximate composition and potential health benefits and effects of water soaking time on some anti-nutrient factors in cowpea *(vigna unguiculata)* was investigated and the results are shown in Tables 1 and 2.

Table 1: Effect of soaking time on proximate composition of the cowpea seed *(Vigna unguiculata)*

Treatment/duration	Proximate composition (%)/calorie (Kcal)						
	Moisture	**Protein**	**Fat**	**Ash**	**Crude fibre**	**Carbohydrate**	**Calorie**
Unsoaked seeds	7.87^{c}	22.40^{a}	1.45^{c}	3.53^{a}	2.36^{a}	70.24^{c}	383.67^{c}
Soaked for I hour	50.52^{b}	21.70^{b}	1.57^{b}	3.46^{a}	2.31^{a}	70.96^{b}	384.76^{b}
Soaked for 2 hours	53.35^{a}	18.55^{c}	2.25^{a}	3.12^{b}	2.08^{b}	74.00^{a}	390.43^{a}
LSD	0.11						

*****Values are means of three determinations. Values with similar superscripts are not significantly different (P<0.05).***

Table 2: Effect of soaking time on anti-nutrients composition of the cowpea seed *(Vigna unguiculata)*

Treatment	Anti-nutrients (mg/100g)						
	Phytate	**Oxalate**	**Tannins**	**Hydrogen -cyanide**	**Flavonoids**	**Alkaloid**	**Saponin**
Unsoaked seeds	9.34^{a}	79.20^{a}	19.90^{a}	1.61^{a}	11.44^{a}	28.73^{a}	12.80^{a}
Soaked for I hour	8.60^{a}	61.60^{b}	14.86^{a}	1.41^{a}	9.46^{a}	25.02^{a}	11.20^{a}
Soaked for 2 hours	8.40^{a}	44.00^{c}	14.48^{a}	0.26^{a}	8.30^{a}	21.51^{b}	11.20^{a}
LSD	5.99						

*****Values are means of three determinations. Values with similar superscripts are not significantly different ($P<0.05$).***

The analysis for the percentage moisture content of cowpea seeds *Vigna unguiculata* revealed that the seeds soaked for two hours had the highest moisture content of 53.35%, followed by cowpea seeds soaked for one hour with 21.70% and the untreated cowpea (0 hour) with 7.87% (Table 1). From the results obtained, it was observed that the percentage moisture content of *Vigna unguiculata* increased with soaking time. An increase in moisture content could be due to the imbibition of water by the cowpea seeds. This is in agreement with the report by Oladunmoye, (2007) on a similar research on locust bean. The moisture content of a food is indicative of the level of dry matter content of the food (Adebowale *et al*., 2012). The level of crude protein significantly ($P<0.05$) declined in the cowpea seeds with soaking time. The crude protein values reduced from the initial value of 21.70% to 18.55% after two hours of soaking. The result was not in line with the work of Pujolà *et al.*, (2007) who reported that protein content increased after soaking and cooking the beans without using the soaking medium. This reduction could be attributed to the leaching of the protein from the cowpea seeds into the water as some proteins are soluble in water (Lehninger *et al.*, 2002). It was observed that the ash content of cowpea seeds decreased with soaking time though the difference was not significant ($P<0.05$) between the control (unsoaked), 3.53% and the value obtained after the first hour of soaking (3.46%). However, within 2 hours significant decrease in ash content (3.12%) was recorded. The reduction in ash content after soaking may be due to the leaching out of minerals and antinutrients into the water (Shah *et al*., 2011). These results are in line with the work of Qureshi (2020) that, soaking significantly decreased the ash content. The fat content increased with soaking time: 0 hours (1.45%), 1 hour (1.57%), 2 hours 2.25%. This study affirmed the earlier report of Effiong & Umoren (2011) who conducted a similar study on horse eye beans which significantly increased crude fat after water. With increased bioavailability of fat, absorption of fat-soluble vitamins is enhanced. Absorption of fat-soluble vitamins such as vitamins A and E is known to be enhanced by the presence of fats (Osborne *et al*., 1978). The slight reduction in fibre content from 2.36% to2.31% after 1 hour soaking time was not however, significant ($P<0.05$), but two hours thereafter, the 2.08% value recorded differed significantly ($P<0.05$) from values obtained for samples soaked for 1 hour and the unsoaked samples. This result was not in line with the work of Qureshi (2020), that soaking significantly increased the fibre content. This reduction could be due to erosion of some fibres by water. The carbohydrate content of cowpea seed powd er was also found to increase with soaking time from 70.25 to 74.00% (Table 1). These results are in line with the work of Qureshi (2020) which reported that soaking followed by boiling improves the carbohydrate content of legumes due to the breakdown of complex carbohydrates that were otherwise bound in the raw sample by boiling. This also confirms an earlier report by Agiang *et al*., (2010) that suggested that processing causes the starch granules to break down, softens the cellulose, and makes the starch more available. These values were found to be lower than those reported for some vegetables (Deshpande, 2002). The result obtained for the caloric value of all the samples shows that soaking led to increases in the caloric value of the cowpea. The increment recorded from 383.6673% in unsoaked samples to 390.4331% in seeds soaked for 2 hours was significant ($P<0.05$).

Table 2 shows that phytate was reduced from 9.34mg/100g to 8.40mg/100g after two hours of soaking. This might be due to leaching of the compound into the water. Previous studies by Desphande, (2002) and Vijayakumari *et al.* (2007) reported that soaking the legume seeds in water for a longer time resulted in maximum reduction of phytic acid. This could be due to the availability of phytic acid as a water-soluble salt (probably potassium phytate) in raw or dried legumes and general foodstuffs. Oxalate was also reduced from 79.20mg/100g to 44.00mg/100g after two hours of soaking. This shows that the soaked sample decreased in oxalate content significantly with a longer duration of soaking. There is the likelihood that soaking seeds for durations longer than 2 hours may lead to reductions in oxalate to a tolerated limit (Sagketkit, *et al*., 1999). The slight reduction in values of hydro-cyanide in cowpea seed with soaking time from 1.61mg/100g at 0hr to 0.26mg/100g after 2 hours was however, not significant ($P<0.05$). This corroborates the findings of Offor, *et al.*, (2011) in yam and potatoes. Catachew (2000) reported that a single dose of 2-5g of cyanide for human beings is usually fatal, also regular exposure to cyanide is injurious to humans owing to the high toxicity nature of the compound. Reduction in the levels of tannin was not significant within the duration of soaking (0hr; 19.90mg/100g, 1hr; 14.85mg/100g, 2hrs; 14.48mg/100g). This reduction is attributed to the leaching of soluble tannin in soaking solution (Makkar, 1993: Khandelwal *et al.,* 2010). Saponin and flavonoid contents of all the samples soaked reduced slightly and did not differ statistically ($P<0.05$) within the 2-hour soaking time. This reduction could be attributed to the diffusion of Saponin and flavonoid into the soaking water (Shi *et al.,* 2009). Alkaloids decreased significantly ($P<0.05$) following soaking from 28.73% to 21.51% within the 2-hour soaking period. Qureshi (2020), affirmed that simple boiling, cooking and soaking can reduce the concentration of anti-nutrients in food stuffs. From the data obtained in this study, it is evident that the processing method employed (soaking) had a significant effect ($P<0.05$) on all the anti-nutritional factors assessed. Ekpo *et. al.* (2012) and Adebayo (2014) affirmed that anti-nutrients leach out in water when soaked and that high concentration of anti-nutrients in vegetables and legumes inhibits the activities of some enzymes and also interfere with dietary iron, and calcium absorption. There was a clear reduction in the levels of the anti-nutritional factors and an increment in some nutrients in cowpea seeds due to soaking observed in this study. This indicates that longer water soaking time is an effective way of lowering toxic substances in legume-based foods and enhancing nutrient bioavailability.

4.0 Conclusion

This study investigated the effect of water soaking time on the proximate composition and anti-nutrient content of cowpea (Vigna unguiculata) seeds. Soaking significantly affected ($p<0.05$) both nutrient and anti-nutrient levels. Soaking time increased moisture content, fat content, carbohydrate content, and caloric value, while decreasing protein, ash, and fiber content. Soaking also significantly reduced ($p<0.05$) the levels of most anti-nutrients (phytate, oxalate, alkaloids) with some exceptions (tannins, saponins, flavonoids). These findings suggest that soaking cowpea seeds is an effective and simple household processing method to improve nutrient bioavailability and reduce the content of anti-nutritional factors.

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Compliance with Ethical Standards

Declarations:

The authors declare that they have no conflict of interest.

Data availability: All data used in this study will be readily available to the public.

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5.0 Author Contributions

Uffia, I. D. designed the experiments, conducted laboratory analyses, interpreted the results and wrote the manuscript; Udofia, O.

E. conducted the stastiscally analyses and Udofia, C. S contributed reagents and materials, revised the article for technical merits.