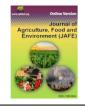


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Original Article



Effects of Processing Techniques on the Nutritional Quality of Cowpea (Vigna unguiculata L.) Varieties

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ABSTRACT

Cowpea is a widely consumed food crop produced in the Savanna zone of Ghana. Anti-nutrients/bio-active compounds in it limit the biological availability of important nutrients/minerals (proteins, carbohydrates, fat, sodium, zinc, calcium, iron e.t.c). This study employed soaking to investigate the nutritional value of three cowpea varieties (Wang Kae, Kirkhouse Benga and Padi-Tuya). The soaking was in two forms; soaking in water and soaking with 1% each of NaHCO3 and NaCl solutions. Standard chemical analytical procedures were carried out to measure proximate parameters (Fat, protein, carbohydrate, ash, moisture and crude fibre), anti-nutrients/bioactive compounds (Tannins, phytates, oxalate and flavonoids), and minerals (Sodium, iron, calcium and zinc) contents of the cowpea varieties. Significant differences ($p \le 0.05$) in proximate composition, anti-nutrients/bioactive compounds and minerals among the cowpea varieties were obtained. Moisture content, ash, crude protein, crude fat, carbohydrates and crude fibre varied among the soaking regimes for the samples in the ranges of 7.47-19.90%, 2.35-6.11%, 23.35-26.33%, 29.23-35.33%, 21.70-31.36% and 2.24-4.78%, respectively. Values for iron, zinc, calcium and sodium ranged between 24.86-214.46mg/kg, 45.02-216.93mg/kg, 31.12-56.59mg/kg and 34.82-136.13mg/kg, respectively. Tannins, phytate, flavonoids and oxalate values also ranged between 1.35-6.74mg/g, 4.18-10.70mg/g, 15.50-91.39mg/100g and 13.64-24.63mg/g, respectively. These results indicate that, soaking with water and (NaHCO₃ + NaCl) solution have potentialities for enhancing nutritional value in the cowpea varieties, which could be a means of combating nutritional deficiencies and food insecurity in Ghana and other countries in West Africa.

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Introduction

Cowpea is an important food for many people, particularly in developing countries in the tropical zones, since it is their fundamental source of protein and carbohydrates (<u>Baptista *et al.*, 2017</u>; <u>Shetty *et al.*, 2013</u>; <u>Trinidad *et al.*, 2010; <u>Phillips *et al.*, 2003</u>). Water-soluble vitamins and elements like calcium, iron, zinc, and potassium are also abundant in them. They have a high carbohydrate content, low in fat, and do not include cholesterol, while its dietary fibre level is quite high. They contain indigestible compounds such as raffinose and stachyose, which produce flatulence after consumption (Uzogara and Ofuya, 1992).</u>

Cowpea plants can thrive well under water-stress condition, grow well in different forms of soil, and when the roots

decay after harvest, they replenish low-fertility soils (<u>Dugie</u> <u>et al.</u>, 2009). When diets are made from cowpea, they deliver high-quality proteins that are comparable to soybeans in terms of protein content (<u>Aguirre et al.</u>, 2003; <u>Obatuli et al.</u>, 2003).

According to <u>IITA (2010)</u>, 52% of cowpea produced in Africa is consumed as food, 13% for animal feed, 10% for seeds, 9% for other purposes, and 16% become less useful. In most resource-poor households where traditional meals are mainly maize based foods, incorporating cowpea into their dishes can address nutritional insecurity. Malnutrition is ubiquitous in Sub-Saharan African poor households, particularly among infants and children, and diet-induced metabolic diseases have become prominent among resourcepoor households. Stunted growth and obesity, attributable to low quality diets were frequent in children of school-going age in impoverished communities in Western Kenya, according to a survey, and it affected up to 70% of the children (Abdulkadir *et al.*, 2009).

Cowpea is a food crop that can assist disadvantaged families meet their daily protein requirements. It is less expensive than animal protein; however, there are little studies on how to improve the nutritional significance of cowpea. It has been proven that soaking beans before cooking is necessary to remove harmful components in them and shorten their cooking time (Yildirim *et al.*, 2013).

Soaked cowpea can be consumed without any health concerns due to a reduction in toxic chemicals, which improves their nutritional characteristics (Abiodun & Adeleke, 2011). Anti-nutrients were leached and reduced after soaking cowpea varieties (Chipurura *et al.*, 2018). 56% of tannin was removed in red kidney beans after soaking (Pathak and Kulshrestha, 2017). Soaking is a frequent domestic technology for creating supplementary foods at home, and this has been found to improve nutritional value (Elmaki *et al.*, 2007).

The main objective of this research was to establish the effect of soaking with water and NaHCO₃ + NaCl on the nutritional enhancement of cowpea varieties. The specific objectives were; first, to determine the proximate components of the cowpea varieties; secondly, to determine some mineral contents of the cowpea varieties and thirdly, to identify some anti-nutritional/ bioactive compounds in the cowpea varieties.

Materials and Methods

Experimental location and material

The present study was conducted to determine the effects of varieties and different soaking regimes on the nutritional quality of cowpea. Sample preparation and chemical analysis were done at the Food Science Technology Laboratory of the University for Development Studies (UDS) and the Central Laboratory of the Kwame Nkrumah University of Science and Technology (KNUST). The cowpea varieties were obtained from the Savannah Agricultural Research Institute, Nyankpala and stored at room temperature prior to treatment.

Treatments and experimental design

This was a two-factor (3 x 3 factorial design) experiment comprising three cowpea varieties namely; (i) Wang kae, (ii) Kirkhouse-Benga, (iii) Padi-Tuya, and three soaking regimes namely; (i) Control, (ii) Soaking with water (iii) Soaking with (1% NaCl + 1% NaHCO₃) solution. The experiment was in a randomized complete design with three (3) replicates.

Treatment of cowpea varieties Control

200g of cowpea grains for each variety was put in airtight plastic bags at room temperature. They were later dried under sunlight for 16 h. A blender was used to mill the cowpea grains into gritty flour after drying, and 100g each were weighed into airtight plastic bags and sent for laboratory analysis.

Soaking with water

200g each of the three cowpea varieties were soaked individually in 200 ml of distilled water for 24 h. After 24 h, decantation was done to separate the soaked cowpea from

the resultant solution. Finally, the grains were rinsed in distilled water and dried under sunlight for 16 h. A blender was used to reduce the cowpea into gritty flour after drying.

100g each of the flour were weighed into airtight plastic bags and sent for laboratory analysis.

Soaking with 1% each of NaCl and NaHCO₃

200g each of the three cowpea varieties were soaked in 200 ml of $(1\% \text{ NaCl} + 1\% \text{ NaHCO}_3)$ solution for 24 h. After the time was due, decantation was done to separate the soaked cowpea from the resultant solution. Finally, the grains were rinsed in distilled water and dried under sunlight for 16 h. A blender was used to reduce the cowpea into gritty flour after drying and 100g each were weighed into airtight plastic bags and sent for laboratory analysis.

Proximate composition

Official methods of analysis by the Association of Analytical Chemists (AOAC, 1990) were used.

Moisture content

A sample of about 5g was dried, weighed, and placed in a dish. The sample was put in an oven for 5 h at 105 °C. The contents of the dish were removed and cool to room temperature before being weighed. The content of the dish was dried in an oven for 30mins, then cooled and weighed. Moisture content was determined using the formula:

% Moisture (w/w) =
$$\frac{\text{Weight of wet sample} - \text{Weight of dry sample}}{\text{Weight of wet sample}} \times 100$$

Ash content determination

A tarred crucible containing about 5g of sample was weighed and placed in a muffle furnace at 600 °C. The muffle furnace was heated for 2 h and turned off. The temperature was allowed to drop to 250 °C. Ash concentration was calculated using the formula:

% $\mathbf{Ash} = \frac{(\text{Weight of crucible+ ash}) - (\text{Weight of empty crucible})}{\text{Weight of crucible+ sample - Weight of empty crucible}} \times 100$

Fat content: Soxhlet extraction

A folded filter paper was used to weigh about 5g of the dry sample. To prevent the sample from being lost, a little piece of cotton wool was placed in the thimble, and roughly 150 ml of petroleum spirit was added into a round bottom flask. To evaporate the solvent, the flask and fat/oil were heated in an oven at around 103 °C. Fat was determined using the formula:

% Fat (dry basis) =
$$\frac{(\text{Weight of flask} + \text{oil} - \text{Weight of flask})}{\text{Weight of sample}} \times 100$$

Crude fibre determination

About 2g of sample was weighed from crude fat determination into a 750ml Erlenmeyer flask. About 200 ml of 1.25% sulphuric acid was added and the mixture was boiled for exactly 30 mins. After 30 mins, the contents were immediately filtered and washed with water. The filtrate was filtered through Fischer's crucible and carefully rinsed with water before adding 15 ml of 96% alcohol. The crucible and its contents were dried at 105 °C for 2 h. The crucible was ignited in a furnace for 30 mins, then cooled and reweighed. Crude fibre was determined by the formula:

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% Crude fibre = \frac{\text{weight of crucible + sample (before - after) ashing}}{\text{Weight of sample}} \times 100
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Protein determination

A digestion flask was filled with 2g of the sample, half of a selenium-base catalyst tablet, and a few anti-bumping chemicals. 25 ml concentrated sulphuric acid was added, and the flask was shaken thoroughly. The flask was slowly heated until the solution turned clear. The sample was put into a 100ml volumetric flask and made up to the mark.

A 250 ml conical flask was filled with 25 ml of 2% boric acid and 2 drops of mixed indicator. The conical flask and its contents were put beneath the condenser. 10ml of the digested sample solution was measured into a Kjedahl unit decomposition flask, fixed, and a 40% NaOH excess (approximately 15-20 ml) was added to it. Ammonia was created and distilled into a collection flask until it reached a volume of 150–200 ml and was collected. The distillate was titrated with a 0.1N HCL solution until a colourless solution was produced.

% Nitrogen = $\frac{100 \text{ x} (\text{Va-Vb}) \text{x} \text{ NA x} 0.01401 \text{ x} 100}{\text{W} \text{ X} 10}$

Va- volume in ml of standard acid used in titration Vb- volume in ml of standard acid used in blank NA- normality of acid W- weight of sample taken

% Crude Protein = %N x 6.25 (AOAC, 1990)

Carbohydrate determination

% Carbohydrate =100 - (% moisture +% fat +% protein +% ash)

(AOAC official methods 942.05)

Phytochemical /Anti- nutritional factor analysis Oxalate

Total oxalate was determined using the procedure of <u>Day</u> and <u>Underwood (1986)</u>. To 1g of the sample, 20 ml of 0.1M HCl was added in a 50 ml beaker. The solution was carefully stirred intermittently with magnetic stirrer for 1 h and filtered using whatman No 1 filter paper. The filtrate was titrated against 0.1M KMnO4 solution at 60 °C for at least 15s until a light pink hue was seen.

Phytate

Phytate was determined using the method of <u>Reddy and</u> <u>Love (1999)</u>. About 4g of the sample was soaked for 3 h in 100 ml of 2% HCL and then filtered using Whatman filter paper. To 25 ml of the filtrate, 5 ml of 0.3% ammonium thiocyanate solution was added as an indicator. The resultant solution was titrated with Fe III chloride solution until a brownish yellow colour that persisted for 5 min was obtained.

Tannin

Tannin was determined by the method of <u>Trease and Evans</u> (1978). 2g of the sample was soaked in 10 ml of 70% acetone and placed in an ice bath. The solution was filtered and 0.5 ml of the supernatant, followed by 0.5 ml of distilled water, 0.5 ml of Folins' reagent, and 2.5 ml of 20% Na₂CO₃ solution were added in a test tube. The test tube was vortexed and incubated for 40 min at room temperature. The absorbance of the reaction mixture of each sample was measured at 725nm using spectrophotometer.

Flavonoid

Total flavonoid was determined according to the method of Ordonez et al. (2006). The sample was extracted in 80%



ethanol and kept for overnight. To 0.5 ml of sample extract, 0.5 mL of 2% AlCl₃ in ethanol was added and allowed to stand for 1 hr. Absorbance of golden yellow color taken at 420 nm using a UV-Vis Spectrophotometer.

Mineral analysis

Digestion

0.5g of the sample was weighed into a digestion tube. 2.5 ml of concentrated H₂SO₄, 2.5 ml nitric acid (HNO₃) and 1 ml perchloric acid were added to the digestion tube. The sample and the acid were digested until solution turns colourless. After digestion, sample was allowed to cool and then transferred into a 50 ml volumetric flask containing about 25 ml of distilled water and later topped up with more distilled water to the 50 ml mark.

Mineral determination

The sample was assayed for the presence of the various metals on a Varian A240FS Atomic Absorption Spectrophotometer with the acclaimed instrument parameters comprising detection limits for each metal under study. The specified detection limits for each analyte were; Sodium (0.124 mg/L), Calcium (0.098 mg/L), Iron (0.051 mg/L) and Zinc (0.069 mg/L).

Statistical analysis

Data was subjected to analysis of variance (ANOVA) using Genstat statistical software (18th edition). Means separation was done using Fisher's protected method with least significance difference at 95% confidence interval (<u>Gomez and Gomez, 1984</u>).

Results and Discussion

Proximate composition

Moisture content of the cowpea varieties across the soaking regimes ranged between 7.47%-19.90%. The control samples recorded moisture that is in line with the standard moisture limit (0-13%) according to James (1995). The increment in moisture of the processed cowpeas is similar to the finding of Abdulsalami & Sheriff (2010), who recorded an increment from 8.7% in control flour to 9.59% after soaking. Processed sample with the lower moisture may have a longer shelf life than the ones with higher moisture content as reported in previous studies. Ash content ranged between 2.35%-6.11%. The reductions in ash of most of the processed cowpea varieties are similar to the documentations of Abdulsalami & Sheriff (2010), where they had initial levels of 5.37 % which reduced to 2.89% after processing. The reduction may be attributed to leaching of the soluble ash components, which may influence the mineral composition of the cowpea varieties due to the volatility of minerals as confirmed in previous studies. Crude fat yield of cowpea varieties in the soaking regimes, varied between 29.23%-35.33%. The content of crude fat falls out of the range (1%-7%) as indicated in the studies conducted by (Worthinton et al., 1972), (Giami et al., 2001) and (Saharan et al., 2002). Also, Adebowale et al. (2013) reported 5.6%-9.4% as fat content observed in their studies. Findings from this study do not conform to some previous studies. These cowpea varieties show good fat content and could be considered as a good source of crude fat for industrial purposes. Protein content ranged between 23.35%-26.33%. The yield of crude protein for all the samples fall in the range (17%-34%) as reported by Sgabieri and Whitaker (1982), whiles Dovlo et al. (1976) reported 25%. The crude protein for the processed samples is

less than the raw samples, which is in agreement with the findings of Adegunwa et al. (2012) after thermal processing of Sesamum indicum. Environmental and genetic variations could also account for the observed variations in protein content. Considering the protein yield of all the processed varieties, they could be beneficial for industrial application, especially in the production of protein-enriched foods. Carbohydrate content was recorded in the range between 21.70%-31.36%. This study contradicts the results obtained by Agiang et al. (2010), which suggest that processing causes granules to breakdown, softens cellulose and making starch available. The soaked samples may help regulate blood sugar, reduce the risk of obesity, cardiovascular diseases due to a reduction of glycemic index and this has been confirmed in the study conducted by (Du et al., 2014). Crude fibre content varied between 2.24%-4.78%. The yield of crude fibre in all the samples falls within the range (1.98%-7.22%) as estimated by Farinde (1990) in a study of underutilized legumes. In addition, crude fibre of raw Wang Kae in this study falls within the range (4.74 - 6.85g/100g) as reported by Abdulsalami & Sheriff (2010). The reduction in crude fibre content for most of the samples after soaking has been confirmed in a study by (Ndidi et al., 2014) where boiled and roasted yam bean samples yielded a relatively lower crude fibre than untreated samples. Diets that have little fibre are undesirable since they could lead to difficulty in easing bowels when consumed. Such diets have been linked with diseases of colon like piles, appendicitis, and cancer (Okon, 1983). Therefore, it would be important to recommend processed cowpea varieties with considerably good fibre content in order to address the above-mentioned diseases.

Table 1. Proximate composition of cowpea varieties in soaking regimes.

Treatment	Moisture	Ash	Fat	Fibre	Protein	Carboh
Combination	(%)	(%)	(%)	(%)	(%)	ydrate
						(%)
V_1T_0	7.48	3.87	31.04	2.24	26.33	31.36
V_1T_1	13.80	3.73	32.23	3.08	24.39	27.21
V_1T_2	18.91	2.35	30.82	2.24	25.02	26.49
V_2T_0	7.52	3.95	29.23	4.33	26.27	30.89
V_2T_1	19.90	2.37	31.87	3.85	24.60	23.75
V_2T_2	15.20	4.11	35.33	3.49	24.68	22.56
V_3T_0	7.73	4.09	31.38	4.78	24.09	30.36
V_3T_1	17.18	6.11	33.20	3.34	24.16	21.71
V_3T_2	14.18	3.72	31.67	3.52	23.34	28.06
LSD _{0.05}	0.23	0.11	2.34	0.15	0.28	1.91
Level of	**	**	**	**	**	**
significance						

LSD = least significance difference, ** = Significant at 5% level of probability, V_1 = Padi-Tuya, V_2 = Kirkhouse Benga, V_3 = Wang Kae, T_0 = Control, T_1 = Soaking with water, and T_2 = Soaking with (1% NaCl + 1% NaHCO₃) solution

Mineral composition Iron and Zinc

The contents of iron and zinc of the cowpea varieties in the soaking regimes varied between 24.86-214.46mg/kg and 45.02-216.93mg/kg respectively. The loss of iron content for Kirkhouse Benga were 49.82% and 76.9% after soaking in NaHCO₃ + NaCl solution and water respectively. Also, there was a loss of 77.21 % and 71.93% iron after soaking Wang Kae in NaHCO₃ + NaCl solution and water respectively. The above losses in iron content for Kirkhouse



benga and Wang Kae far exceeds the 40% loss of iron content recorded in sorghum grain after soaking in distilled water (Lestienne *et al.*, 2005). Therefore, consuming Padi-Tuya soaked in NaHCO₃ + NaCl solution will likely help to mitigate anemia due to its high iron yielding capability as reported by (WHO, 2008).

The loss of zinc content in Kirkhouse Benga was 73.50% and 29.39 % after soaking in NaHCO₃ + NaCl solution and water respectively. In addition, there was a loss of 75.29% zinc in Wang Kae after soaking in water. With the exception of Kirhouse Benga soaked in water, the remaining losses in zinc content exceeds the 30% in sorghum grain after soaking in distilled water (Lestienne *et al.*, 2005). Reduction after soaking medium. Zinc is found in a huge variety of enzymes and other proteins, where it serves as a structural component (Lestienne *et al.*, 2005).

Calcium and Sodium

The yield of calcium and sodium of the cowpea varieties in the soaking regimes ranged between 31.12-56.59mg/kg and 34.82-136.13mg/kg. Kirkhouse Benga soaked in water will likely ensure enough calcium bioavailability than the other samples due to its low phytate content as this has been reported by (Bora, 2014; Grases *et al.*, 2017). Also, Padi-Tuya soaked in NaHCO₃ + NaCl solution may have a potential of more calcium absorption due to the lower amount of oxalate in it, and hence may reduce the incidence of kidney stones in the urinary tract of humans as reported by (Nachbar *et al.*, 2000).

Padi-Tuya soaked in NaHCO₃ + NaCl solution may have a potential of more sodium absorption due to the lower amount of oxalate in it which will likely improve the bioavailability of sodium (Nachbar *et al.*, 2000).

Table 2. Mineral composition of cowpea varieties insoaking regimes.

Treatment	Sodium	Calcium	Iron	Zinc
Combination	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
V_1T_0	87.45	35.19	80.89	189.09
V_1T_1	34.82	31.12	144.04	216.93
V_1T_2	127.15	36.97	214.46	195.27
V_2T_0	86.53	39.82	206.04	172.09
V_2T_1	85.51	34.38	47.59	121.52
V_2T_2	52.85	38.79	104.50	45.62
V_3T_0	72.91	43.23	109.10	182.15
V_3T_1	136.13	56.59	30.62	45.02
V_3T_2	41.27	39.97	24.86	196.63
LSD _{0.05}	0.01	0.68	0.23	0.04
Level of	**	**	**	**
significance				

LSD = least significance difference, ** = Significant at 5% level of probability, V_1 = Padi-Tuya, V_2 = Kirkhouse Benga, V_3 = Wang Kae, T_0 = Control, T_1 = Soaking with water, and T_2 = Soaking with (1% NaCl + 1% NaHCO₃) solution

Anti-Nutritional Factors/ Bioactive compounds Oxalate and Phytate

Oxalate content for the cowpea varieties in the soaking regimes ranged between 13.64-24.63mg/g, whiles phytate varied between 4.18-10.70mg/g. The oxalate content of all the cowpea varieties were significantly (p < 0.05) reduced across the soaking regimes. This is similar to the findings of (<u>Chipurura *et al.*, 2018</u>) who found out that, soaking of legumes reduced anti-nutritional elements. Also, the

reduction in oxalate of the processed cowpea varieties in this study, have been reported by (<u>Kadam and Salunkhe, 1985</u>) in their study of the processing of horse gram and moth bean. Iron and zinc bioavailability are likely to enhance because of reduced oxalate in the processed samples, since oxalate may no longer chelate these micronutrients. Iron bioavailability will enhance metabolism of proteins, lipids, carbohydrates etc.

The phytate content of all the cowpea varieties were significantly reduced (p< 0.05) across the soaking regimes. The reduction in phytate as observed by most of the processed cowpea varieties in this study could improve starch digestibility as reported in a study conducted by (Siqueira *et al.*, 2013) in which the researchers found that, pequi peel flour had improved starch digestibility after reduction in phytic acid. Another study by (Sinha and Kawatra, 2003) investigated the effect of soaking and dehulling on cowpea (*Vigna unguiculata*) and found that the phytic acid levels of soaked and dehulled pulses fell by 16.3% and 30.1% respectively, and this study has confirmed phytic acid reduction.

Tannins and Flavonoids

The yield of tannin for Kirkhouse Benga soaked in NaHCO₃ + NaCl solution may lead to more nutrient digestibility, since tannin content in it has been significantly (p < 0.05) reduced, compared with the other processed samples. Soaking of cowpea varieties with sodium salts solution (NaHCO₃ + NaCl solution) resulted in a reduction of tannin, which is similar to the findings of (Ogun *et al.*, 1989) in their study of anti-nutrients of selected legumes. Also, (Pathak and Kulshrestha, 2017) have reported the reduction for tannins after soaking, in their study of red kidney bean.

The yield of flavonoid for all the cowpea varieties were significantly (p < 0.05) decreased across the soaking regimes. The reduction in anti-nutrients, and flavonoids in the processed cowpea varieties in this study is similar to what was reported by (Yildirim *et al.*, 2013) who found out that, soaking legumes prior to cooking reduces toxic factors present in the legumes. The reduction in flavonoids in the processed cowpea varieties has been confirmed in the study of bioactive compounds in food by (Kris-Etherton *et al.*, 2002).

Table 3. Anti-nutritional/Bioactive composition ofcowpea varieties in soaking regimes.

Treatment Combination	Oxalate (mg/g)	Tannin (mg/g)	Phytate (mg/g)	Flavonoid (mg/100g)
V ₁ T ₀	22.38	6.18	8.59	94.16
V_1T_1	20.80	6.74	6.07	15.50
V_1T_2	13.64	4.44	6.53	34.62
V_2T_0	23.59	2.44	4.22	88.12
V_2T_1	21.11	3.07	4.18	42.66
V_2T_2	19.18	1.72	5.05	46.62
V_3T_0	24.63	1.35	10.70	91.39
V_3T_1	20.48	4.01	6.73	22.39
V_3T_2	20.71	1.75	10.01	55.15
LSD _{0.05}	0.08	0.54	0.02	0.84
Level of	**	**	**	**
significance				

LSD = least significance difference, ** = Significant at 5% level of probability, V_1 = Padi-Tuya, V_2 = Kirkhouse Benga, V_3 = Wang Kae, T_0 = Control, T_1 = Soaking with water, and T_2 = Soaking with (1% NaCl + 1% NaHCO₃) solution

Conclusion

This study indicates that, soaking with water and $NaHCO_3 + NaCl$ solution has a potential of improving the nutritional quality of cowpea varieties, which could be a means of combating nutritional deficiencies and food insecurity in Ghana and other West African countries. Anti-nutrients/bioactive compounds in particular could be exploited more for use in the development of nutraceuticals using appropriate separation techniques.

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