

Effect of soaking, dehulling and boiling on protein, polyphenolic and antinutrient content of cowpeas (*Vigna unguiculata* L. Walp)

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ABSTRACT Legumes are good sources of protein and are rich in lysine and tryptophan, which are limiting amino acids in cereals. Common legumes include sugar beans, soya beans and cowpeas. Although soya beans are an important commercial legume, cowpea (*Vigna unguiculata* L. Walp) varieties grown by some communities in developing countries have many food and non-food uses. The objective of this study was to determine the effect of soaking, dehulling and boiling on the protein, polyphenolic and antinutrient content of cowpea varieties, namely CBC2 and CBC4. Phenols, tannins, flavonoids, alkaloids and saponins were present in the cowpea varieties. Boiling caused a significant ($p \leq 0.05$) decrease in the protein content of both cowpea varieties. Soaking for 24 hours resulted in an insignificant ($p > 0.05$) change in the protein content of CBC2, but significantly ($p \leq 0.05$) decreased the protein content of CBC4. On the other hand, dehulling seeds soaked for 6 hours resulted in a significant increase ($p \leq 0.05$) in the protein content of both varieties. Boiling caused a significant decrease ($p \leq 0.05$) in total phenolic content (TPC), total flavonoid content (TFC), and content of condensed tannins and saponins in both cowpea varieties. Also, soaking for 6, 12, 18 and 24 hours significantly ($p \leq 0.05$) decreased the content of these phytochemicals. The greatest losses in TPC, TFC, condensed tannins and saponins for both varieties were observed in seeds soaked for 6 hours and dehulled samples. The results of this study show that boiling, soaking and dehulling reduced the protein, polyphenolic and antinutrient content of the cowpea varieties.

Keywords

Traditional processing
Legumes
Total phenolic content
Tannins
Saponins

Introduction

Legumes are good sources of protein and are rich in lysine and tryptophan, which are limiting amino acids in cereals [1]. Although soya bean is an important commercial legume, cowpea (*Vigna unguiculata* L. Walp) varieties have many food and non-food uses. The crop is used for human consumption, feed for livestock and to improve soil fertility through nitrogen fixation [2]. The green leaves can be prepared into a relish, while the seeds are boiled and eaten as a fresh vegetable. Mature and dried cowpea seeds are boiled for immediate consumption and can also be canned. In Ghana, cowpeas are consumed whole, split or milled into

flour which can be used to make food products that include cakes, pies, chips and biscuits [3].

Cowpeas are low in fat and high in protein. Thus, the legume can prevent cardiovascular and metabolic diseases associated with high fat diets. Furthermore, cowpeas are rich in thiamine, riboflavin, vitamins A and C, niacin, calcium, potassium, magnesium and carbohydrates [1]. In addition to their superior nutritional composition, cowpeas have been reported to contain significant levels of phenolic compounds such as phenolic acids and polyphenolics that include flavonoids and tannins. Cai *et al* identified protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, 2,4-dimethoxybenzoic acid and cinnamic acid in 17 cowpea varieties grown in Arkansas [4]. Duenas *et al* determined the phenolic composition of the Carilla cowpea variety and found ferulic acid, *p*-coumaric acid, gallic acid, vanillic acid, *p*-hydroxybenzoic acid, protocatechuic acid, myricetin glucoside, mono- and diglycosides of quercetin, and a quercetin diglycoside acylated with ferulic acid [5]. These phenolic compounds have antioxidant properties due to their free radical scavenging and metal chelating activities. Therefore, foods that are rich in phenolic compounds

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can protect against the oxidative damage of macromolecules such as proteins, carbohydrates and DNA. This oxidative damage is caused by free radicals. Ndhlala *et al* reported that in the absence of antioxidants, free radicals can cause conditions such as cardiovascular diseases, atherosclerosis, cancer, Parkinson's, Alzheimer's and inflammatory diseases [6]. However, high concentrations of polyphenolic antioxidants such as tannins are considered to be antinutritional because they decrease starch and protein digestibility as well as mineral absorption [7]. Trypsin inhibitors, protease inhibitors, saponins and phytic acids are other antinutrients found in legumes that lower the absorption of minerals and inhibit proteases and amylases in the human body [8].

Dehulling, soaking, germination, fermentation and thermal treatments are some traditional processing methods which have been reported to reduce antinutrients in food. Therefore, the objective of this study was to determine the effect of boiling, soaking and dehulling on the protein, phenolic composition and antinutrient content of two cowpea varieties grown in Zimbabwe.

Materials and methods

Sample procurement

Dried cowpea samples (5 kg) of each variety, namely CBC2 and CBC4, were obtained from the Crop Research and Specialist Services Department, Ministry of Agriculture, Harare, Zimbabwe. The seeds were cleaned, placed in polythene bags and stored at room temperature ranging from 20°C to 25°C in earthenware pots.

Preparation of soaked cowpeas

Raw cowpea seeds (100 g) of each variety were soaked in 1 litre of deionised water at room temperature for 6, 12, 18 or 24 hours. After soaking, the samples were dried at 65°C in a cabinet drier for 24 hours and ground into a fine powder.

Preparation of soaked and dehulled cowpeas

Raw cowpea seeds (100 g) of each variety were soaked in 1 litre of deionised water at room temperature for 6 hours and dehulled manually using a pestle and mortar. The dehulled seeds were dried in a cabinet drier at 65°C for 24 hours and ground into a fine powder.

Preparation of boiled cowpeas

Raw cowpea seeds (150 g) of each variety were placed in a stainless-steel pot and cooked in deionised water until soft as felt by pressing with two fingers. After cooking, the cow-

pea seeds were dried in a cabinet drier at 65°C for 24 hours and ground into a fine powder.

Moisture content

The moisture content of the cowpea samples was analysed according to AOAC methods [10]. The results were used to calculate the protein, total phenolic content (TPC), total flavonoid content (TFC), condensed tannins and saponins on a dry matter basis.

Protein content

Crude protein content was estimated using the rapid Biuret method described by Johnson *et al* [11].

Extraction of phytochemicals

Before extraction of the phenolic compounds, the cowpeas (50 g) were defatted using hexane (60 ml) with overnight stirring to minimise subsequent oxidation of the extracts. The mixture was filtered and the residue was extracted twice with hexane for 1 hour. Defatted samples (0.5 g) were mixed with 5 ml of 50% methanol, vortexed for 1 min and ultrasonicated for 10 min. The ultrasonicated mixture was centrifuged at 3,000 rpm for 10 min and the supernatant produced was used for phytochemical screening, and determination of total phenolic, total flavonoid and condensed tannin content of the cowpea varieties.

Phytochemical screening

Flavonoids were determined by the alkaline reagent test [12]. The presence of tannins was determined using ferric chloride [12]. Alkaloids were determined using Dragendorff's test [13]. Saponins and phenols were qualitatively determined using methods described by Chidewe *et al* [13].

Total phenolic content

The extract (50 µl) was mixed with distilled water (950 µl), followed by Folin Ciocalteu reagent (1N, 500 µl) and sodium carbonate (2%, 500 µl). The mixture was incubated at room temperature for 40 min, and then a 20 Genesys spectrophotometer at 725 nm was used to measure the absorbance of the mixture against a blank of 50% methanol. The total content of phenolic compounds was expressed as milligrams gallic acid equivalents per 100 g of cowpea sample (mg GAE/100 g).

Total flavonoid content

The extract (4 ml) was mixed with 2% methanolic $AlCl_3 \cdot 6H_2O$ (2 ml) and the mixture was incubated in a dark room for 15

min. A Spectronic 20 Genesys spectrophotometer at 430 nm was used to measure the absorbance of the extracts against a blank of 50% methanol. Catechin was used as the standard and the total content of flavonoids was expressed as mg of catechin equivalents (CE) per 100 g of cowpea sample (mg CE/100 g).

Condensed tannins

The extract (0.5 ml) was added to butanol-HCl reagent (butanol:HCl, 95:5 v/v). The mixture was added to 0.1 ml of ferric reagent (2% of ferric ammonium sulphate dissolved in 100 ml of 2N HCl), vortexed for 2 min and heated at 90°C in a water bath for 1 hour. The mixture was cooled and a Spectronic 20 Genesys spectrophotometer at 550 nm was used to measure the absorbance of the mixture against a blank of 50% methanol. The total content of condensed tannins was expressed as grams of leucocyanidin equivalents (LE) per 100 g of cowpea sample (g LE/100 g).

Saponins

Determination of saponins followed the double extraction gravimetric method described by Harbone.

Statistical analysis

Data collected were analysed using the GraphPad Prism statistical package (Version 5, GraphPad Software, La Jolla, CA, USA). The results reported in this study are given as the mean±standard deviation. One-way analysis of variance (ANOVA) was used to determine if there were significant differences in protein, phenolic compounds and antinutrients between the soaked, soaked and dehulled, and boiled cowpeas. Means were separated by the Tukey HSD test for post-ANOVA pairwise comparisons with $p \leq 0.05$ considered significant.

Results and discussion

Moisture and protein content

The moisture content of the dried cowpea samples ranged from 8.39±0.29 g/100 g to 15.71±0.72 g/100 g, as shown in Table 1. The moisture levels obtained are comparable to the recommended maximum permissible levels of 15% for legumes [14]. The results imply that the seeds were unlikely to be spoiled by microbes due to moisture content as it was below permissible levels. Boiling raw dried seeds of both cowpea varieties significantly reduced their protein content (Table 2).

Sample	CBC2 (g/100 g)	CBC4 (g/100 g)
Raw	13.46±0.39 ^a	15.71±0.72 ^u
Boiled	12.03±0.25 ^b	11.82±0.45 ^v
Soaked and dehulled	9.90±0.30 ^c	8.39±0.29 ^w
Soaked for 6 hours	10.17±0.38 ^d	8.48±0.30 ^w
Soaked for 12 hours	11.92±0.28 ^e	12.97±0.23 ^x
Soaked for 18 hours	10.76±0.27 ^d	11.74±0.52 ^v
Soaked for 24 hours	11.00±0.20 ^d	10.54±0.56 ^y

Results are the mean±standard deviation for three replicates. Means with different superscript letters in the same column are significantly different at ($p \leq 0.05$)

Table 1 - Moisture content of cowpea samples

Similarly, Osman reported a decrease in the protein content of lablab beans due to boiling [15]. In contrast, Wang *et al* observed that boiling beans and chickpeas in water significantly ($p \leq 0.05$) increased their protein content [16]. The losses observed in this study and those in the literature are possibly caused by thermal degradation and subsequent leaching of soluble proteins and amino acids into the cooking water [17]. On the other hand, soaking caused an insignificant ($p > 0.05$) change in the protein content of both cowpea varieties except for CBC4 seeds soaked for 24 hours. Dehulling of raw dried seeds soaked for 6 hours resulted in a significant increase ($p \leq 0.05$) in the protein content of both cowpea varieties. Similarly, Ghavidel *et al* [18] found that the protein content of chickpea, lentil, cowpea and green gram samples increased after the seeds were dehulled due to the fact that the endosperm is richer in protein than the husk [19].

Phytochemical screening

The CBC2 and CBC4 cowpea varieties were positive for phenols, tannins, flavonoids, alkaloids and saponins (Table 3). Methanol (100%) extracts of both CBC2 and CBC4 were negative for phenols and alkaloids. There were no flavonoids in the aqueous extracts of either variety. However, all the phytochemicals tested were present in 50% methanol extracts.

Sample	CBC2 g/100 g	% Change	CBC4 g/100 g	% Change
Raw	24.31±0.14 ^a	0.00	26.49±0.34 ^{uv}	0.00
Boiled	18.95±0.32 ^b	-22.04	21.06±0.13 ^v	-20.50
Soaked and dehulled	30.99±0.16 ^c	27.48	32.38±0.32 ^w	22.40
Soaked for 6 hours	24.30±0.32 ^a	0.00	25.75±0.13 ^u	-0.03
Soaked for 12 hours	24.79±0.65 ^a	0.02	26.62±0.33 ^w	0.00
Soaked for 18 hours	24.46±0.32 ^a	0.01	25.58±0.33 ^u	0.03
Soaked for 24 hours	24.05±0.09 ^a	-0.01	24.96±0.32 ^z	-0.06

Results are the mean±standard deviation for three replicates. Means with different superscript letters in the same column are significantly different at ($p = 0.05$)

Table 2 - Protein content on a dry matter basis and corresponding % decrease after processing

Sample	CBC2			CBC4		
	Water	50% Methanol	100% Methanol	Water	50% Methanol	100% Methanol
Phenols	+	+	-	+	+	-
Tannins	+	+	+	+	+	+
Flavonoids	-	+	+	-	+	+
Alkaloids	+	+	-	+	+	-
Saponins	+	+	+	+	+	+

+ Indicates positive, - indicates negative

Table 3 - Phytochemical screening of CBC4 and CBC2 cowpea cultivars

The presence of saponins, alkaloids, flavonoids, tannins and phenols suggests that both cowpea varieties contain health-promoting and anti-nutritional factors. Flavonoids and tannins are antioxidant phenolic compounds, which protect against human conditions such as cancer, diabetes and cardiovascular disease. Saponins are bioactive compounds associated with antifungal, antiviral, immunostimulant, anticarcinogenic, molluscicidal, antiplatelet aggregation, antidiabetic and hypocholesterolaemic properties [20]. It has been found that alkaloids such as pyrrolizidine, tropane, piperidine, quinolizidine, quinolone, ergot and glycoalkaloids can block carcinogenic substances and can repair DNA damage in cells [21]. Tannins, saponins and alkaloids have both health benefits and adverse effects. In humans and animals, tannins and saponins can interfere with the metabolism and absorption of starch, proteins and minerals [22]. On the other hand, alkaloids have been reported to disrupt the transmission of nerve impulses, and can react with cellular components causing cellular toxicity, genotoxicity and eventually carcinogenesis [23].

Total phenolic content

The TPC of the cowpea varieties was significantly ($p \leq 0.05$) reduced with an increase in the soaking time, as shown in Table 4. The highest loss was observed in CBC4 (76.80%) after soaking for 24 hours, while CBC2 (63.17%) had the lowest. Also, boiling resulted in a significant ($p \leq 0.05$) reduction in the TPC of both cowpea varieties with the loss being higher for CBC2 (36.97%) compared with CBC4 (15.24%). The TPC of boiled samples of both varieties was higher than that of soaked samples. Dehulling after soaking the dried seeds for 6 hours resulted in the greatest loss of TPC. From the study, it was also observed that for both varieties, soaking for 6 hours and for 12 hours caused an insignificant ($p > 0.05$) difference in the TPC of both CBC2 and CBC4 cowpea varieties.

Sample	CBC2 mg GAE/100 g	% Decrease	CBC4 mg GAE/100 g	% Decrease
Raw	144.66±1.48 ^a	0.00	34.05±0.30 ^u	0.00
Boiled	91.17±0.87 ^b	36.97	28.86±0.38 ^u	15.24
Soaked and dehulled	29.02±0.86 ^c	79.93	3.68±0.16 ^v	89.19
Soaked for 6 hours	75.55±2.00 ^d	47.77	15.29±0.56 ^w	55.10
Soaked for 12 hours	69.54±1.02 ^d	51.93	15.24±1.18 ^w	55.24
Soaked for 18 hours	58.85±6.21 ^e	59.32	10.21±0.148 ^x	70.01
Soaked for 24 hours	53.28±0.01 ^f	63.17	7.90±0.06 ^x	76.80

Results are the mean±standard deviation for three replicates. Means with different superscript letters in the same column are significantly different (at $p=0.05$)

Table 4 - Total phenolic content on a dry matter basis and corresponding % decrease after treatment

The significant decrease in TPC after soaking observed in this study is similar to the findings of Yasmin *et al* [24] who reported a significant ($p \leq 0.05$) decrease in the TPC of red kidney beans due to soaking. Also, Siah *et al* reported a significant decrease in the phenolic content of faba beans as a result of soaking [25]. In contrast, Chutipanyaporn *et al* reported a significant ($p \leq 0.05$) increase in the TPC of different bean varieties on soaking [26]. Akillioglu *et al* found a significant increase in the TPC of common beans and pinto beans after boiling [27]. The researchers attributed this increase in TPC to thermal disruption of the cell walls of the legumes leading to the release of extractable phenolic compounds [26]. In this study, the decrease in the TPC of the cowpeas during boiling may be a result of chemical modifications [28] and leaching of phenolics into the boiling water [29]. However, the extent to which the phenolic compounds were lost or retained in CBC2 and CBC4 seeds varied. This may be due to the differences in physicochemical properties such as thickness, size, shape and hardness of the seeds [30].

Flavonoids

The TFC of the cowpeas decreased significantly ($p \leq 0.05$) with an increase in the soaking time, as shown in Table 5. Soaking followed by dehulling caused the highest decrease in the TFC of both CBC2 and CBC4 cowpea varieties. Also, a significant ($p \leq 0.05$) decrease in TFC was observed after both

Sample	CBC2 mg QE/100 g	% Decrease	CBC4 mg QE/100 g	% Decrease
Raw	15.57±1.74 ^a	0.00	13.08±1.45 ^t	0.00
Boiled	12.80±1.24 ^b	17.80	11.05±0.95 ^u	15.53
Soaked and dehulled	9.21±0.92 ^c	40.79	9.07±1.65 ^v	30.59
Soaked for 6 hours	13.30±0.89 ^d	14.58	10.41±0.12 ^w	20.37
Soaked for 12 hours	13.13±1.41 ^d	15.63	9.98±0.51 ^x	23.67
Soaked for 18 hours	11.75±0.35 ^e	24.51	9.32±1.21 ^v	28.68
Soaked for 24 hours	10.81±0.81 ^f	30.57	9.09±0.72 ^v	30.49

Results are the mean±standard deviation for three replicates. Means with different super-script letters in the same column are significantly different (at $p=0.05$)

Table 5 - Flavonoid content on a dry matter basis and corresponding % decrease after treatment

varieties were boiled. For CBC4, boiling caused a smaller decrease in TFC compared with all soaking times. Dehulling caused significant ($p\leq 0.05$) reductions in the flavonoid content of the cowpeas because the seed coat containing these compounds was removed during dehulling [5].

Adeniyani *et al* reported that flavonoids and other bioactive compounds of beniseed (*Sesamum indicum* L.) increased after boiling [31]. However, Brend *et al* reported insignificant ($p>0.05$) changes in the TFC during boiling of quinoa seeds [32]. Soobrattee *et al* reported that the TFC of quinoa seeds decreased significantly ($p\leq 0.05$) after boiling [33]. These variations in flavonoid content may be due to differences in the methods used to process the legumes. For example, Brend *et al* allowed the boiled quinoa seeds to reabsorb the cooking water before analysis [32]. However, in this study, the cooking water containing the leached flavonoids was discarded. Xu *et al* observed that other cooking methods such as steaming are better at retaining flavonoids compared with boiling [34].

Condensed tannins

The condensed tannins of both cowpea varieties decreased with an increase in the soaking time, as shown in Table 6. Soaking for 24 hours was more effective in reducing the condensed tannins of CBC2 than of CBC4. Soaking for 6 hours

Sample	CBC2%	% Decrease	CBC4 %	% Decrease
Raw	0.276±0.002 ^a	0.00	0.284±0.001 ^u	0.00
Boiled	0.111±0.001 ^b	59.78	0.129±0.002 ^v	54.58
Soaked and dehulled	0.096±0.001 ^c	65.22	0.104±0.001 ^w	63.38
Soaked for 6 hours	0.166±0.001 ^d	39.86	0.222±0.001 ^x	21.83
Soaked for 12 hours	0.160±0.002 ^e	42.03	0.208±0.001 ^y	26.76
Soaked for 18 hours	0.156±0.002 ^f	43.48	0.207±0.001 ^z	27.11
Soaked for 24 hours	0.152±0.001 ^g	44.93	0.193±0.001 ^z	32.04

Results are the mean±standard deviation for three replicates. Means with different super-script letters in the same column are significantly different (at $p=0.05$)

Table 6 - Percentage of condensed tannin content per sample (dry matter basis) and the corresponding % decrease after each treatment

followed by dehulling resulted in the highest loss in condensed tannins for CBC2 (65.22%) and CBC4 (63.38%). The boiled samples of both varieties had significantly ($p\leq 0.05$) lower condensed tannin content than raw dried samples.

Wang *et al* reported that cooking decreased tannins in black grams and kidney beans [16]. Similarly, Mubarak recorded a decrease in the tannins in mung beans after boiling [35]. The decrease in condensed tannin content observed in this study and reported in the literature

may be due to changes in the chemical reactivity and polymerisation of the tannins due to heat [36]. Nakitto *et al* reported that soaking followed by dehulling sugar beans effectively reduced tannins [37]. Other researchers also found that dehulling effectively reduced the tannin levels in beans [38]. This decrease may be explained by the fact that most polyphenolic compounds are found in the seed coat [39]. Thus in this study, removal of the seed coat caused a significant ($p\leq 0.05$) decrease in condensed tannins. Khandelwa *et al* observed a significant decrease in the tannin content of Indian pulses with an increase in soaking time [40]. The reduction in tannin content was possibly due to leaching of the tannins into the soaking water [41]. Therefore, soaking may reduce the levels of condensed tannins and consequently increase the bioavailability of nutrients in the cowpeas.

Saponins

The loss of saponins in CBC2 seeds due to boiling was not significantly ($p\leq 0.05$) different from that caused by soaking for 6–24 hours (Table 7).

Soaking for 6 hours followed by dehulling resulted in the highest loss in the saponin content of both varieties. In this study, the saponin content of the samples was lower than the value of 4.8% reported by Agugo *et al* [42] for 'Akidi'. Also, the authors found that dehulling decreased the saponin content by 25% in the 'Akidi' samples, which decrease is significantly ($p\leq 0.05$) lower than the values obtained for cowpeas used in the current study. The decrease in saponin content may be attributed to soaking of the seeds and dehulling. Saponins are polar [43], thus during soaking and blanching they are leached into the soaking, washing and blanching liquors. Consequently, soaking, dehulling and boiling processes can be used to reduce saponin levels in cowpeas.

Sample	CBC2%	% Decrease	CBC4 %	% Decrease
Raw	2.75±0.08 ^a	0.00	3.57±0.42 ^u	0.00
Boiled	1.67±0.23 ^{bd}	39.27	2.86±0.13 ^v	19.89
Soaked and dehulled	0.97±0.14 ^c	64.73	1.27±0.12 ^w	64.43
Soaked for 6 hours	2.25±0.20 ^b	18.18	2.65±0.13 ^v	25.77
Soaked for 12 hours	1.90±0.09 ^b	30.90	2.61±0.21 ^v	26.89
Soaked for 18 hours	1.65±0.13 ^b	40.00	2.03±0.05 ^x	43.13
Soaked for 24 hours	1.62±0.11 ^d	41.09	1.50±0.26 ^{wx}	57.98

Results are the mean±standard deviation for three replicates. Means with different superscript letters in the same column are significantly different (at $p < 0.05$)

Table 7 - Percentage of saponin content per sample (dry matter basis) and corresponding % decrease after each treatment

Conclusion and recommendations

Boiling, soaking and dehulling reduced the protein, polyphenolic and antinutrient content of the cowpea varieties. Removal of the seed coat, heat-induced degradation, polymerisation reactions as well as leaching of these compounds into the soaking or boiling water probably contributed to the observed losses. Therefore, it is important that the cooking water containing the leached phytochemicals is consumed or used to prepare other dishes. Furthermore, pre-processing steps such as dehulling should be discouraged if people are to fully benefit from the high content of polyphenolics found in the seed coat. However, if antinutrients are of major concern, soaking and dehulling can be used to reduce their subsequent effects in the human body. Soaking followed by dehulling was found to concentrate proteins and reduce antinutrients in cowpeas. Thus, these combined pre-processing steps are recommended in the manufacture of protein-rich foods for special diets.

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Human and Animal Rights

This article does not contain any studies with human or animal subjects performed by the any of the authors.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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