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Effect of Boiling, Pressure Cooking and Germination on the Nutrient and Antinutrients Content of Cowpea (Vigna unguiculata)

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Abstract

The study was conducted to investigate the effect of boiling, pressure cooking and germination on the proximate, nutrients, amino acids and anti-nutrients content of cowpea (Vignaunguiculata). The results showed that the germinated cowpeas (GMC) had the highest value of crude protein (22.89%), crude fat (3.81%) and crude fiber (2.10%) followed by raw cowpeas (RWC) and pressure cooked cowpeas (PCC) while boiled cowpeas (BDC) had the least. There was comparable value of ash content in all the samples except for BDC with the least. Boiling had significantly higher moisture content than others. The carbohydrate value ranged from 57.21 to 58.13% for GMC and BDC respectively and 59.69 to 59.74% for RWC and PCC respectively. Comparable calorific value of GMC and BDC was significantly higher than that of PCC and RWC. The decreasing order of anti-nutrient factors in treated cowpeas is: GMC > RWC > PCC > BDC. This result inferred that boiling is an adequate processing for drastic reduction of the anti-nutrient factors (phytate, tannin, Trypsin inhibitor and total phenol) in cowpeas. Germination increased the amount of Methionine, lysine and tryptophan by 10.94%, 18.89% and 20.90% respectively, while the pressure cooking and boiling caused mild losses of Methionine, lysine and tryptophan. Similarly, germination had increased the amount of macro elements (0.0036mg/kg for Na , 0.024mg/kg for K, 0.021mg/kg for Ca, 0.037mg/kg for P and 0.022mg/kg for Mg) while boiling and pressure cooking decreased the amount of these macro elements compared with the raw sample. Heat treatments (boiling and pressure cooking) recorded decreased level of micro elements (Fe, Zn, Cu, Mn) while germination had increased the micro elements by 4.66%, 3.78%, 13.85% and 6.38% for Fe, Zn, Cu and Mn respectively. Therefore, it could be concluded that the heat treatments (boiling and pressure cooking) had significantly reduced the anti-nutrient factors in cowpeas but germination (sprouting) had excellent nutritional qualities.

Keywords: Antipsychotics; Antidepressants; Cholesterol; Dyslipidemia; Schizophrenia; Metabolic Disorders

Introduction

There is increasing world demand of less expensive proteins with good nutritional and functional properties, particularly in developing and under-developed countries where the supply of food of animal origin is limited due to non-availability and high cost [1] (Muneet al., 2013). Legumes are considered as poor man's meat. They are generally rich in protein (18-25 %), and good sources of minerals and vitamins [2] (Tharanathan and Mahadevamma, 2003). Legumes are good sources of cheap and widely available proteins for human consumption. They are staple foods for many people in different parts of the world [3] (Udensiet al., 2010). Legume seeds have an average of twice as much protein as cereals and the nutritive value of the proteins are usually high [4] (Vijaykumarriet al., 1997). Studies have shown that the lesser known legumes together with other conventional legumes can be used for combating protein malnutrition prevalent in the third world. Therefore, use of grain legumes for food is restricted by their beany flavor and the presence of anti-nutritional and toxic factors [5] (Friedman, 1992; Yusuf et al., 2008).There is a wide distribution of biologically-active constituent throughout the plant kingdom, particularly in plants used as animal feeding stuff and in human nutrition [6] (Igile, 1996). The knowledge that these compounds elicit both toxic and advantageous biological responses has given rise to several investigations in recent times as to their possible physiological implications in various biological systems [6] (Igile, 1996). Traditional processing techniques such as soaking, cooking, sprouting (germination) and roasting have limited effects on elimination of anti-nutritional factors, and sometimes could decrease protein quality and affect certain functional properties [5](Friedman, 1992; Yusuf et al.,2008).

Cowpea belongs to the family leguminosae, other names commonly used include catjang, black-eyed bean or china pea [7] (Taiwo, 1998), southern pea, clossus, or crowther peas [8] (Uzogara and Ofuya, 1992). In Sudan, it is known as lubiahelo or white lubia. Cowpea is one of the most important food legumes crop widely grown in semi-arid tropics as an inexpensive source of protein in both human diet and animal feed [9] (Maheet al., 1994; Ofuya, 2001). Its fresh or dried seeds, pods and leaves are commonly used in human food, since they are highly valuable as fodder [10] (Gomez, 2014). Cowpea has great flexibility in use; farmers can choose to harvest them for grain or to harvest forage for the livestock, depending on economical or climatologically constraints [10] (Gomez, 2014).

Dual purpose varieties of cowpea have been developed in order to provide both grain and fodder while suiting the different cropping systems [11] (Tarawaliet al., 1997). Cowpea by-products such as cowpea seed waste and cowpea hulls (which result from the dehulling of the seeds for food) have been used to replace conventional feedstuff in some developing countries [12] (Ikechukwu, 2000). Cowpea is traditionally processed in different ways and the impacts of these traditional cooking methods on the nutritional composition of cowpea were yet unknown. This work tends to investigate the effects some traditional processing methods on the nutritional composition and anti-nutritional constituent of cowpea.

Materials and Methods

Collection and Preparation of Cowpea

Dry cowpea was collected from the seed processing unit of International Institute of Tropical Agricultural (IITA), Ibadan. The cowpeas had no foreign materials, wrinkled and mouldy seeds and were divided into four portions. Each portion contained 20 cowpea seeds. The first portion of the sample was without treatment (raw) and considered as control. The second, third and fourth portions were processed by boiling, pressure cooking and germination respectively.

Boiling of samples: The cowpea was cooked in tap water at 100oC in the ratio 1:10 (w/v) on a kerosene stove for 65minutes until it became soft when crushed between fingers. The boiled cowpea was tagged BDC.

Pressure cooking of samples: The cowpea were pressure cooked in tap water (1:10, w/v) with crown star pressure cooker at 20pscal pressure (122oC) for 55min.until it became soft when crushed between fingers. The cowpea processed under this treatment was labeled PCC

Germination of samples: wool was laid on the plastic tray before the cowpea seeds were placed on it and then covered with cotton. And subsequently sprinkled with water twice daily until the seeds began to sprout. The germinated cowpea was tagged GMC.

Sample Analyses

The samples of cowpea were analysed chemically according to the methods described by the Association of Official Analytical Chemists [13] (AOAC, 2005) at the Biochemistry laboratory of the Institute of Agriculture Research and Training, IAR&T, Moor Plantation, Ibadan.

Determination of proximate composition: Moisture content of the cowpea was determined using Association of Official Analytical Chemists method (AOAC, 2005). The gross energy values were estimated by multiplying the crude protein, fat and carbohydrate by their at water values of 4, 9 and 4 kcal/g respectively [14] (Akubor, 1997). Protein content was estimated from the crude nitrogen content of the sample determined using the micro Kjeldhal method (N × 6.25). Carbohydrate was calculated by difference. Crude fat, crude fibre and ash content of the samples were determined according to AOAC (2005).

Crude protein determination: The percentage nitrogen in this analysis was calculated using the formula: % N = Titre value x Atomic mass of Nitrogen x Normality of HCl used x 4 Weight of sample digested in milligram x Vol. of digest for steam distillation. The crude protein content is determined by multiplying percentage Nitrogen by a constant factor of 6.25 i.e. % CP = % N x 6.25. The crucible and its content were cooled to about 100ºC in air, then room temperature in a dessicator and weighed. This was done in duplicate. The percentage ash was calculated from the formula below:

$$\text{Ash content} = \frac{\text{wt. of ash}}{\text{Original wt. of sample}} \times \frac{100}{1}$$

Fibre determination: 2.0gm of the sample was accurately into the fibre flask and 100ml of 0.255N H_2SO_4 added. The mixture was heated under reflux for 1 hour with the heating mantle. The hot mixture was filtered through a fibre sieve cloth. The filterate obtained was thrown off and the residue was returned to the fibre flask to which 100ml of (0.313N NaOH) was added and heated under reflux for another 1 hour. The mixture was filtered through a fibre sieve cloth and 10ml of acetone added to dissolve any organic constituent. The residue was washed with about 50ml hot water on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue were oven-dried at 105ºC overnight to drive off moisture. The oven-dried crucible containing the residue was cooled in a dessicator and later weighed to obtain the weight W1. The crucible with weight W1 was transferred to the muffle furnace for Ashing at 550°C for 4 hours.
The crucible containing white or grey ash (free of carbonaceous material) was cooled in the dessicator and weight to obtain W2. The difference W1 – W2 gives the weight of fibre. The percentage fibre was obtained by the formula

Determination of methionine and lysine: Methionine was determined using the method described by [15] Lunder (1973).Lysine was evaluated using the method described by Jambunathanet al. (1987) in which the rapid methods were applied for estimating lysine and protein in sorghum.

Chemical analysis of Mineral elements concentration: Cowpea was analysed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist [14] (AOAC, 2005). All analyses were carried out in triplicate.

Determination of Trypsin Inhibitor Activity (TIA): 0.2g of ground cowpea was weighed into a centrifuge tube.10ml of 0.1MPhosphate buffer added and shaken on a shaker at room temperature for 1hr. The suspension was centrifuged at 5000rpm in a centrifuge for 5min.The content was later filtered through a Whatman No 42 filter paper into a 250ml conical flask. 0.2,0.4,0.6,0.8,and 1.0ml of the filtrate were pipette into a set of triplicate set of test-tubes(one set for each level of extract).The final volume was adjusted to 2ml by the addition of 0.1M phosphate buffer. These test-tubes were arranged into a water bath maintained at 37oC.A blank was prepared by adding 6ml of 5% tricarboxylic acid solution to one set of triplicate tubes.2ml of 2% casein solution was added to all the tubes were previously kept at 37oC to incubate for 20min.The reaction of casein was stopped by the addition of 6ml of 5% TCA solution and this was allowed to proceed for 1hr at room temperature .The mixture was later filtered at room temperature through a Whatman No 42 filter paper into 100ml conical flask. About 0.2, 0.4, 0.6, 0.8, and 1.0ml of stock trypsin solution were also pipetted into a triplicate set of test-tubes (one set for each level of trypsin) as above and treated similarly as sample to the point of filtration.The absorbance of the filtrates of both samples and standard trypsin solution were read on a Spectrophotometer at a wavelength of 280nm.The actual absorbance of sample was the difference between absorbance of stock trypsin filtrate and that of sample filtrate. The absorbance of blank was also read. One trypsin inhibitor unit (TIU) is arbitrarily defined as an

increase of 0.01 absorbance units at 280nm in 20min per 10ml of the reaction mixture under the conditions mentioned Trypsin Inhibitor Unit for each sample was calculated using the formula: Change in absorbance of sample extract 0.01 X mg protein in sample

Determination of Phytate: Phytate was determined according to the method described by [16] Maga (1983). 2g of each sample was weighed into 250ml conical flask. 100mls of 2% hydrochloric acid was added to soak each sample in the conical flask for 3 hours. This was filtered through a double layer of hardened filter paper. 50ml of each filtrate was placed in 0.50ml conical flask and 107mls distilled water was added in each case to give proper acidity. 10mls of 0.3% ammonium thiocyanate (NH4SCN) solution was added into each solution as indicated. This was titrated with standard iron (III) chloride solution which contained 0.00195g Iron per ml. The end point was slightly brownish-yellow which persisted for 5 minutes. The % phytic acid was calculated using the formula:

$$\% \text{ Phytic Acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100 \times 3.55}{\text{Wt. of Sample}}$$

Determination of Tannins: 0.20g of sample was measured into a 50ml beaker 20ml of 50% methanol was added and covered with parafilm and placed in a water bath at 77-80oC for 1 hour. It was shaken thoroughly to ensure a uniform mixing. The extract was quantitatively filtered using a double layered Whatman No 41 filter paper into a 100ml volumetric flask, 20ml water added, 2.5ml Folin-Denis reagent and 10ml of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20min. The absorbance of the Tannic acid standard solutions as well as samples was read after color development on a spectronic 21D spectrophotometer at a wavelength of 760nm. Percentage tannin was calculated [14] (AOAC, 2005).

Data Analysis: Data obtained were analysed and means were compared using the Least Significant Difference (t-Test). Significant difference between the treatments was accepted at 5% probabilityby the Statistical Analysis System (SAS) program (SAS Institute, Cary, N.C.).

Results and Discussion

Effect of treatment on the percentage of proximate composition of cowpea

The results of the proximate analysis in Table 1 above showed that there was significant difference among the proximate compositions of treated cowpeas. The germinated cowpeas (GMC) had the highest values of crude protein (22.89%), crude fat (3.81%) and crude fibre (2.10%) while boiled cowpeas (BDC) had the least values of crude protein (17.79%), crude fat (3.56%) and crude fibre (1.81%). There was comparable value in the ash content of raw sample and other treated samples except the boiled which had the least. The moisture content of boiled cowpeas (BDC) was significantly higher ($p<0.05$) than others. This result indicated higher level of water activity in boiled and pressure cooked samples which plays a vital role in food storage. Cowpea is not only a good source of protein but also offers substantial amount of carbohydrate and calorific values. Boiling and germination had comparable carbohydrate values which were significantly lower than that of raw and pressure cooked samples. Reversibly, the calorific values obtained from both boiled, (BDC) and germinated cowpea, (GMC) were comparable and significantly higher than that of raw, (RWC) and pressure cooked (PCC) samples. This finding agreed with reports by Soetan and Oyewole (2009) that cooking treatment caused significant ($p < 0.05$) decrease in fat and ash. Meanwhile, the effect of processing on the fat and ash was different from the publication reported by Soetan and Oyewole (2009) that germination caused significant ($p < 0.05$) decreased in fat and ash.

Treatment	C. Protein	C. Fat	C. Fibre	Ash	Moisture	CHO	Caloric Value
RWC	21.58±0.11b	3.71±0.02b	1.95±0.02b	3.80±0.02a	9.25±0.02d	59.69±0.04a	156.71c
PCC	19.93±0.05c	3.64±0.02c	1.91±0.02c	3.71±0.02a	11.07±0.02b	59.74±0.03a	156.76c
GMC	22.89±0.10a	3.81±0.02a	2.10±0.01a	4.17±1.49a	9.82±0.03c	57.21±0.33b	165.13a
BDC	17.79±0.10d	3.56±0.02d	1.81±0.02d	3.57±0.02b	15.14±0.02a	58.13±0.04b	163.76ab

Table 1: Percentage of proximate composition of cowpea.

Mean values with the same superscript(s) in a column are not significantly different ($p<0.05$). RWC represents raw cowpea, PCC = pressure cooked cowpea, GMC =germinated cowpea and BDC= boiled cowpea.

Effect of treatment on the anti-nutrients composition of cowpea

The result in Table 2 above showed that there was significant difference among the anti-nutrient content of treated cowpeas. However, the germinated cowpeas (GMC) had increased anti-nutrient content when compared with the raw cowpea (RWC). The decreasing order of anti-nutrient factors (phytate, tannin, total phenol and TIA) in treated cowpeas is: GMC > RWC > PCC > BDC. It implied that boiling drastically reduced the anti-nutrient factors (phytate, tannin, trypsin inhibitor and total phenol) in cowpeas. This result inferred that boiling is an adequate processing for the anti-nutrient reduction in legumes. This result was in agreement with the report by [17] Omoruyiet al. (2007) that boiling and roasting were effective in lowering the levels of anti-nutrient factors in Caribbean tuber crops. Also [18] Wang et al. (1997) reported that steam blanching of cowpea resulted in higher reduction in Trypsin inhibitor activity than using water blanching. Conversely, the effect of processing on the Trypsin inhibitor activities was in disagreement with the publication reported by [19] Osman (2007) that germination significantly decreased the TIA activity in D. lablab by 19.3%.

Treatment	Phytate	Total phenol	Tannin	Trypsin inhibitor
RWC	0.053±0.00b	0.886±0.01b	0.344±0.00b	19.81±0.03b
PCC	0.052±0.00c	0.828±0.00c	0.326±0.00c	6.77±0.02c
GMC	0.055±0.00a	0.922±0.41a	0.365±0.00a	20.00±0.04a
BDC	0.051±0.00d	0.810±0.00d	0.306±0.00d	0.33±0.02d

Table 2: Percentage of anti-nutrients composition of cowpea.

Mean values with the same superscript(s) in a column are not significantly different ($p<0.05$). RWC represents raw cowpea, PCC = pressure cooked cowpea, GMC =germinated cowpea and BDC= boiled cowpea.

Effect of treatment on the percentage composition of selected amino acids of cowpea

The percentage composition of selected essential amino acids in Table 3 showed that germination of cowpea increased the amount of methionine, lysine and tryptophan while the pressure cooking had slight decrease in methionine, lysine and tryptophan whereas boiling of cowpeas had the highest reduction in methionine, lysine and tryptophan. This result agreed with the report made by [20] Hefnawy (2011) that cooking treatments decreased the concentration of lysine and tryptophan in lentils (Lens culinaris.) This finding contradicts the report by [21] Soetan and Oyewole (2009) that germination decreased the concentration of lysine and tryptophan in chickpeas [22] (Cicerarietinum L.).

Treatment	Methionine	Lysine	Tryptophan
RWC	1.92±0.03b	0.90±0.03b	0.67±0.02b
PCC	1.75±0.02c	0.77±0.02c	0.56±0.02c
GRM	2.13±0.02a	1.07±0.04a	0.81±0.02a
BDC	1.57±0.02d	0.64±0.02d	0.40±0.40d

Table 3: Percentage composition of selected essential amino acids of cowpea.

Mean values with the same superscript(s) in a column are not significantly different ($p<0.05$). RWC represents raw cowpea, PCC = pressure cooked cowpea, GMC =germinated cowpea and BDC= boiled cowpea.

Effect of treatment on the macro elements content of cowpea

The micro elements composition of cowpeas in Table 4 showed that heat processing amounted to loss of nutrients; this may be due to leaching during heat application. Germination had increased the amount of Na, K, Ca, P, Mg while boiling and pressure cooking had decreased the amount of this macro element content when compared with the raw sample. This result agreed with the report by [23] Udensiet al. (2010) that boiling of Mucunaflagellipes resulted in products with lowest mineral contents. It was also in agreement with the report

of [20] Hefnawy (2011) that matured cowpea lost 23% Mg when pressure cooked.

Treatment	Na	K	Ca	P	Mg
RWC	0.0020±0.00b	0.0235±0.00b	0.0196±0.00b	0.0355±0.00b	0.0218±0.00b
PCC	0.0012±0.00c	0.0226±0.00c	0.0187±0.00c	0.0347±0.00c	0.0210±0.00c
GRM	0.0036±0.00a	0.0242±0.00a	0.0206±0.00a	0.0370±0.00a	0.0224±0.00a
BDC	0.0008±0.00d	0.0216±0.00d	0.0178±0.00d	0.0333±0.00d	0.0219±0.00d

Table 4: Macro elements content of cowpea (mg/kg).

Mean values with the same superscript(s) in a column are not significantly different ($p<0.05$). RWC represents raw cowpea, PCC = pressure cooked cowpea, GMC =germinated cowpea and BDC= boiled cowpea.

Effect of treatment on the micro elements content of cowpea

The result in Table 5 above showed that heat treatments (boiling and pressure cooking) recorded decreased level of micro elements (Fe, Zn, Cu, and Mn) while germination had increased the micro elements by 4.66%, 3.78%, 13.85% and 6.38% for Fe, Zn, Cu and Mn respectively. This result agrees with the report of [20] Hefnawy (2011) that cooking in boiling water caused great losses of copper and iron. It was also reported by [20] Hefnawy (2011) that pressure cooked mature cowpeas had 30% loss of copper.

Treatment	Fe	Zn	Cu	Mn
RWC	10.51±0.03b	3.44±0.02b	1.30±0.02b	2.35±0.02b
PCC	10.19±0.02c	3.23±0.02c	1.20±0.02c	2.20±0.03c
GMC	11.00±0.03a	3.57±0.02a	1.48±0.02a	2.50±0.02a
BDC	10.06±0.02d	3.15±0.02d	1.09±0.02d	2.11±0.02d

Table 5: Micro elements content of cowpea (mg/kg).

Mean values with the same superscript(s) in a column are not significantly different ($p<0.05$). RWC represents raw cowpea, PCC = pressure cooked cowpea, GMC =germinated cowpea and BDC= boiled cowpea.

Conclusion

This research showed that wet heat processing caused the loss of nutrients; this may be due to leaching during heat application. There was drastic reduction of anti-nutrients content of boiled and pressure cooked cowpeas and this probably is because the anti-nutrients are heat labile. On the other hand, germination had increased the nutrients and anti-nutrients composition of cowpeas. Germination as a biochemical process induced enzymatic reactions that resulted in the bioavailability of some nutrients. This result indicated that in addition to protein, cowpea is rich in macro and micro minerals and if properly processed, it can alleviate malnutrition.

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