academicJournals

Vol.6(1), pp. 1-8, October, 2016
DOI: 10.5897/ISABB-JFAS2016.0036
Article Number: 5DDCEC161368
ISSN 1937-3244

http://www.academicjournals.org/ISAB-JFAS

ISABB Journal Of Food And Agriculture Science

Full Length Research Paper

Effect of boiling, pressure cooking and germination on the nutritional and antinutrients content of cowpea (*Vigna unguiculata*)

Omenna E. C.[1]*, Olanipekun O. T.[1] and Kolade R. O.[2]

[1]Institute of Agricultural Research and Training, Obafemi Awolowo University, P.M.B. 5029, Moor Plantation, Ibadan, Oyo State, Nigeria.
[2]Federal College of Agriculture, Moor Plantation, Ibadan, Nigeria.

Received 8 January, 2016; Accepted 13 June, 2016

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 (*Vigna unguiculata*). The results showed that the germinated cowpeas (GMC) had the highest value of crude protein (22.89%), crude fat (3.81%) and crude fibre (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. 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. On the other hand, germination had increased the amount of methionine, lysine and tryptophan by 10.94, 18.89 and 20.90%, respectively, while the pressure cooking and boiling had mild losses of methionine, lysine and tryptophan. Similarly, germination had increased the amount of macro elements (0.0036 mg/kg for Na, 0.024 mg/kg for K, 0.021 mg/kg for Ca, 0.037 mg/kg for P, and 0.022 mg/kg for Mg) while boiling and pressure cooking had 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 that anti-nutrient factors in cowpeas but germination (sprouting) had excellent nutritional qualities. Future research work should be carried out on combination of heat treatment with germination to reduce the anti-nutrient factors in grain legumes.

Key words: Cowpea, antinutritional factors, nutritional composition.

INTRODUCTION

There is an 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 (Mune et al., 2013). This situation resulted from the constant increase of the human population and growing interest for protein to industry for application in food and non-food markets (Mune et al., 3013). Legumes are considered as poor man's meat. They are generally rich in protein (18 to 25%), and good sources of minerals and vitamins (Youseff et al., 1989). 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 (Udensi et al., 2007). Legume seeds have an average of twice as much protein as cereals and the nutritive value of the proteins are usually high (Vijayakumari et al., 1998). 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. This can be achieved by the consumption of the legumes whole and in various processed forms (condiments) (Arisa and Aworh, 2007). Therefore, the use of grain legumes for food is restricted by their beany flavor and the presence of anti-nutritional and toxic factors (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 (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 (Igile, 1996). Traditional processing techniques such as soaking, cooking, sprouting (germination) or roasting have limited effects on elimination of anti-nutritional factors, and sometimes could decreased protein quality and affected certain functional properties (Friedman, 1992; Yusuf et al., 2008). However, this work tends to investigate the effects some traditional processing methods on the nutritional composition and anti-nutritional constituent of cowpeas.

Description of cowpea (*Vigna unguiculata*)

Cowpea belongs to the family Leguminosae, other names commonly used include catjang, black-eyed bean or china pea (Udensi et al., 2008), southern pea, clossus, or crowther peas (Uzogara and Ofuya, 1992). In Sudan, it was known as lubia helo 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 (Obasi and Wogu, 2008). Its fresh or dried seeds, pods and leaves are commonly used in human food, since they are highly valuable as fodder (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 climatological constraints (Gomez, 2014).

Dual purpose varieties of cowpea have been developed in order to provide both grain and fodder while suiting the different cropping systems encountered in Africa (Tarawali et 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 (Ikechukwu, 2000). Though not as efficient as soybean as a protein sources, cowpea was capable of accumulating useful levels of protein and digestible dry matter under the variable growing conditions of the study (Koratkar and Rao,1997).

MATERIALS AND METHODS

Collection and preparation of cowpeas

Dry cowpeas were 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 samples were cooked in tap water at 100°C in the ratio 1:10 (w/v) on a kerosene stove for 65 min until it became soft when touched with finger. The boiled cowpea was tagged BDC.

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

Germination of samples: Cotton wad was laid on the plastic tray before the cowpea seeds were placed on it and then covered with cotton wool. And subsequently irrigated twice daily for three days until the seeds begin to sprout. The germinated cowpea was tagged GMC.

Sample analyses

The samples were analyzed chemically according to the official methods of analysis described by the Association of Official Chemist (AOAC, 1990) at the biochemistry laboratory of the Institute of Agriculture Research and Training, IAR&T, Moor Plantation, Ibadan.

*Corresponding author. E-mail: emmanuelomenna@gmail.com or omennaec@iart-ngonline.org. Tel: +2348039334092.

Determination of proximate composition

The samples were separately analyzed for proximate composition using the official standard methods. Moisture content of the sample was determined by 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 (Akubor, 1997). Protein content was estimated from the crude nitrogen content of the sample determined by the micro Kjeldhal method (N × 6.25). Carbohydrate was calculated by difference method. Crude fat, crude fiber and ash content of the samples were determined by the procedure given by Association of Official Analytical Chemists (2005).

Crude protein determination

The crude protein in the sample were determined by the routine semi-micro Kjeldahl, procedure/technique. This consists of three techniques of analysis, namely digestion, distillation and titration.

Apparatus: Analytical balance, digestion tubes, digestion block heaters, 50 ml burette, 5 ml pipette, 10 ml pipette, 10 ml measuring cylinder, 100 ml beakers, and fume cupboard.

Reagents: Concentrated H_2SO_4, 0.01 N HCl, 40% (W/V) NaOH, 2% boric acid solution, methyl red–bromocresol green mixed indicator, and Kjeldahl catalyst tablet.

Digestion: 0.5 g of each finely ground dried sample was weighed carefully into the Kjeldahl digestion tubes to ensure that all sample materials got to the bottom of the tubes. To this were added 1 Kjeldahl catalyst tablet and 10 ml of concentrated H_2SO_4. These were set in the appropriate hole of the digestion block heaters in a fume cupboard. The digestion was left on for 4 h, after which a clear colourless solution was left in the tube. The digest was cooled and carefully transferred into 100 ml volumetric flask, thoroughly rinsing the digestion tube with distilled water and the flask was made up to mark with distilled water.

Distillation: The distillation was done with Markham Distillation Apparatus which allows volatile substances such as ammonia to be steam distilled with complete collection of the distillate. The apparatus was steamed out for about 10 min. The steam generator is then removed from the heat source to the entire developing vacuum to remove condensed water. The steam generator is then placed on the heat source (that is, heating mantle) and each component of the apparatus was fixed up appropriately.

Determination: 5 ml portion of the digest above was pipetted into the body of the apparatus via the small funnel aperture. To this was added 5 ml of 40% (w/v) NaOH through the same opening with the 5 ml pipette. The mixture was steam-distilled for 2 min into a 50 ml conical flask containing 10 ml of 2% boric acid plus mixed indicator solution placed at the receiving tip of the condenser. The boric acid plus indicator solution changes colour from red to green showing that all the ammonia liberated have been trapped.

Titration: The green colour solution obtained was then titrated against 0.01 N HCl contained in a 50 ml burette. At the end point or equivalent point, the green colour turns to wine colour which indicates that all the nitrogen trapped as ammonium borate $[(NH_4)_2BO_3]$ have been removed as ammonium chloride (NH_4Cl). The percentage nitrogen in this analysis was calculated using the formula: %N = Titre value × Atomic mass of nitrogen × Normality of HCl used × 4 Or %N = Titre value × Normality/Molarity of HCl used × Atomic mass of N × Volume of flask containing the digest × 100 Weight of sample digested in milligram × Vol. of digest for steam distillation. The crude protein content is determined by multiplying percentage nitrogen by a constant factor of 6.25, that is, % CP = %N × 6.25.

Determination of ash

Apparatus: Porcelain crucibles, a dessicator, analytical balances and a furnace.

Determination: 2.0 g of the sample were weighed into a porcelain crucible. This was transferred into the muffle furnace set at 550°C and left for about 4 h. About this time it had turned to white ash. The crucible and its content were cooled to about 100°C in air, then room temperature in a dessicator and weighed. This was done in duplicate. The percentage of ash was calculated from the formula:

Ash content = Wt. of ash/Original wt. of sample × 100

Fibre determination

Apparatus: Heating mantle, crucibles, furnace, sieve cloth, fibre flask, funnel, analytical weighing balance, and a dessicator.

Reagents: 0.255 N H_2SO_4, 0.313 N NaOH, and acetone.

Determination: 2.0 g of the sample was accurately into the fibre flask and 100 ml of 0.255 N H_2SO_4 was added. The mixture was heated under reflux for 1 h with the heating mantle. The hot mixture was filtered through a fibre sieve cloth. The filtrate obtained was thrown off and the residue was returned to the fibre flask to which 100 ml of 0.313 N NaOH was added and heated under reflux for another 1 h. The mixture was filtered through a fibre sieve cloth and 10 ml of acetone added to dissolve any organic constituent. The residue was washed with about 50 ml hot water on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue were oven-dried at 105°C overnight to drive off moisture. The oven-dried crucible containing the residue was cooled in a dessicator and later weighed to obtain the weight W_1. Crucible with weight W_1 was transferred to the muffle furnace for ashing at 550°C for 4 h.

Crucible containing white or grey ash (free of carbonaceous material) was cooled in the dessicator and weight to obtain W_2. The difference $W_1 – W_2$ gives the weight of fibre. The percentage fibre was obtained by the formula:

%Fibre = $W_1 – W_2$ / Wt. of sample × 100

Crude fat or ether extracts determination

Apparatus: Soxhlet apparatus and accessories, oven, desiccator and analytical balance.

Reagents: Petroleum spirit or Ether (40 – 60°C boiling point).

Determination: 1 g of each dried sample was weighed into fat free extraction thimble and pug lightly with cotton wool. The thimble was placed in the extractor and fitted up with reflux condenser and a 250 ml Soxhlet flask which has been previously dried in the oven, cooled in the desiccator and weighed. The Soxhlet flask is then filled to $^3/_4$ of its volume with petroleum ether (b.pt. 40 – 60°C), and the Soxhlet flask. Extractor plus condenser set was placed on the heater. The heater was put on for 6 h with constant running water from the tap for condensation of ether vapour. The set is constantly

watched for ether leaks and the heat source is adjusted appropriately for the ether to boil gently. The ether is left to siphon over several times say over at least 10 to 12 times until it is short of siphoning. It is after this is noticed that any ether content of the extractor is carefully drained into the ether stock bottle. The thimble containing sample is then removed and dried on a clock glass on the bench top. The extractor, flask and condenser is replaced and the distillation continues until the flask is practically dry. The flask which now contains the fat or oil is detached, its exterior cleaned and dried to a constant weight in the oven. If the initial weight of dry Soxhlet flask is Wo and the final weight of oven dried flask + oil/fat is W_1, percentage fat/oil is obtained by the formula:

$W_1 – W_o$ / Wt. of Sample taken × 100

Dry matter and moisture determination

Apparatus: Oven, crucibles, dessicator and balance.

Reagents: Silical gel, grease.

Determination: 2 g of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into the oven set at 100°C to dry to a constant weight for 24 h overnight. At the end of the 24 h, the crucible plus sample was removed from the oven and transferred to dessicator, cooled for 10 min and weighed.

If the weight of empty crucible is Wo, weight of crucible plus sample is W_1, weight of crucible plus oven-dried sample W_3.

(%DM) %Dry Matter = $W_3 – W_o / W_1 – W_o \times 100$

%Moisture = $W_1 – W_3 / W_1 – W_o \times 100$

Or

%Moisture = 100 – % DM.

Determination of methionine (A Spectrophotometric method)

Lunder (1973) recommended an improved method for the colorimetric or spectrophometric determination of methionine in acid hydrolysates of biological products. Methionine was determined using the method described by Lunder (1973).

Sample (0.2 g) was weighed into a 30 ml test tube. 10 ml of Tris buffer reagent of pH 7.2 was added with 0.1 mg papain and incubated at 55°C for 18 h overnight. It was removed from incubator and 4 drops of orthophosphoric acid (88%) added to stop enzymatic hydrolysis. The mixture was transferred into a 25 ml volumetric flask and made up to volume with distilled water. The solution obtained was shaken thoroughly and filter through a Whatman No. 41 Filter paper into a test tube or conical flask. 5 ml aliquot was pipetted out of this into another test tube to which 1 ml of 5 N NaOH, 1 ml of 1% aqueous glycine and 1 ml of 1% sodium nitroprusside were added. The tubes were kept in the water bath at 40°C for 10 min after thorough shaking.

The contents were allowed to cool and 6 ml of 88% orthophosphoric acid added with thorough shaking to obtain a red colour. Methionine standards of range 0.5 to 2.5 mg/ml were prepared and treated as mention earlier. Absorbances of samples and standards were read using Spectronic 21D Spectrophotometer at a wavelength of 520 nm. % Methionine or Methionine in g/100 g protein can be calculated using the formula: %Methionine = Factor × Absorbance of sample/Sample weight ×% Protein in sample × 100%Protein in sample was determined by the Kjeldahl method.

Determination of lysine

Lysine was evaluated using the method prescribed by Jambunathan et al. (1983) in which the rapid methods was applied for estimating lysine and protein in sorghum.

Sample (1 g) was weighed into a 5 ml centrifuge tube. 40 ml of acid orange dye solution was added and shaked for 1 h. The mixture was filtered through a Whatman No. 41 filter paper into a 50 ml volumetric flask and made up to mark with distilled water and incubated at 60°C for 18 h overnight. Standards Lysine of range 0.35 to 0.85 mg/ml were prepared and treated with acid orange dye to develop colour. The absorbances of the standards as well as samples were read on a Spectronic 21D Spectrophotometer at a wavelength of 415 nm.

%Lysine or Lysine in g/100 g protein can be calculated using the formula:

Factor × Absorbance / Sample weight × % Protein in sample × 100

Chemical analysis of mineral elements concentration (Na, K, Mg, Ca, P {Fe, Zn, Cu})

Samples were analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist (AOAC, 2005). All analyses were carried out in triplicate.

Calcium, potassium and sodium determination

Apparatus: Heating mantle, crucible, glass rod, flame photometer, 100 ml volumetric flask, Whatman No. 1 Filter paper, wash bottle, 10 ml pipette, and funnel.

Reagent: 2 M HCl.

Determination: The ash of each sample obtained was digested by adding 5 ml of 2 M HCl to the ash in the crucible and heat to dryness on a heating mantle. 5 ml of 2 M HCl was added again, heat to boil, and filtered through a Whatman No. 1 filter paper into a 100 ml volumetric flask. The filtrate was made up to mark with distilled water stoppered and made ready for reading of concentration of calcium, potassium and sodium on the Jenway Digital Flame Photometer (DFP7 Model) using the filter corresponding to each mineral element.

The concentration of each of the element was calculated using the formula:

%Ca or %K or %Na = Meter Reading (MR) × Slope × Dilution factor / 1000

where MR × slope × dilution factor = Concentration in part per million (ppm or mg/kg). You get concentration in % when you divide by 10000.

Phosphorus determination (Spectrophotometric method)

Phosphorus was determined routinely by the vanado-molybdate colorimetric or spectrophotometric method.

Apparatus: Spectrophotometer or colorimeter, 50 ml volumetric flask, 10 ml pipette, filter paper, funnel, wash bottle, glass rod, heating mantle, and crucibles.

Reagents: Vanadate – Molybdate yellow solution, 2 M HCl.

Determination: The ash of each sample obtained was treated with 2 M HCl solution as described for calcium determination earlier. 10 ml of the filtrate solution was pipetted into 50 ml standard flask and 10 ml of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 min for full yellow development. The concentration of phosphorus was obtained by taking the optical density (OD) or absorbance of the solution on a Spectronic 20 spectrophotometer or colorimeter at a wavelength of 470 nm.

The percentage phosphorus was calculated from using the formula:

%Phosphorus = Absorbance × Slope × Dilution factor / 10000

Determination of Mg, Cu, Mn, Fe and Zn using BUCK200 AAS

The digest of the ash of each sample earlier as obtained in calcium and potassium determination was washed into 100 ml volumetric flask with deionised or distilled water and made up to mark. This diluent was aspirated into the Buck 200 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination (AOAC, 2005).

Determination of trypsin inhibitor activity (TIA)

The casein digestion method

Defatted ground sample (0.2 g) was weighed into a centrifuge tube. 10 ml of 0.1 M phosphate buffer was added and shaked on a shaker at room temperature for 1 h. The suspension was centrifuged at 5000 rpm in a centrifuge for 5 min. The content was later filtered through a Whatman No 42 filter paper into a 250 ml conical flask.

0.2, 0.4, 0.6, 0.8, and 1.0 ml of the filtrate were pipette into a set of triplicate set of test-tubes (one set for each level of extract). The final volume is adjusted to 2 ml by the addition of 0.1 M phosphate buffer. These test-tubes were arranged into a water bath maintained at 37°C. A blank was prepared by adding 6 ml of 5% TCA solution to one set of triplicate tubes. 2 ml of 2% casein solution was added to all the tubes which is previously kept at 37°C to incubate for 20 min. The reaction of casein will be stopped by the addition of 6 ml of 5% TCA solution and this was allowed to proceed for 1 h at room temperature. The mixture was later filtered at room temperature through a Whatman No. 42 filter paper into 100 ml conical flask. 0.2, 0.4, 0.6, 0.8, and 1.0 ml of stock trypsin solution were also pipetted into a triplicate set of test-tubes (one set for each level of trypsin) as mentioned earlier 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 280 nm. The actual absorbance of sample is 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 280 nm in 20 min per 10 ml of the reaction mixture under the conditions mentioned herein.

Trypsin Inhibitor Unit for each sample was calculated using the formula: Change in absorbance of sample extract/ 0.01 × mg protein in sample

Determination of phytate

Phytate was determined according to the method described by Maga (1983). 2 g of each sample was weighed into 250 ml conical flask. 100 ml of 2% hydrochloric acid was added to soak each sample in the conical flask for 3 h. This was filtered through a double layer of hardened filter paper. 50 ml of each filtrate was placed in 0.50 ml conical flask and 107 ml distilled water was added in each case to give proper acidity. 10 ml of 0.3% ammonium thiocyanate (NH_4SCN) solution was added into each solution as indicated. This was titrated with standard iron (III) chloride solution which contained 0.00195 g iron per ml. The end point was slightly brownish-yellow which persisted for 5 min. The % phytic acid was calculated using the formula:

%Phytic acid = Titre value × 0.00195 × 1.19 × 100 × 3.55 / Wt. of sample

Determination of tannin

Sample (0.20 g) was measured into a 50 ml beaker 20 ml of 50% methanol was added and covered with parafilm and placed in a water bath at 77 to 80°C for 1 h. It was thoroughly shakied to ensure a uniform mixing. The extract was quantitatively filtered using a double layered Whatman No 41 filter paper into a 100 ml volumetric flask, 20 ml water added, 2.5 ml folin-Denis reagent and 10 ml of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allow to stand for 20 min. Bluish-green color was develop at the end of range 0 to 10 ppm where similar treatment was given as 1 ml of the sample earlier.

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 760 nm. Percentage tannin was calculated using the formula.

%Tannin = Absorbance of sample × average gradient factor × dilution factor/ Wt. of sample × 10,000

Data analysis

Data obtained were analyzed by the one-way analysis of variance (ANOVA) and means were compared using the Least Significant Difference (t-Test). Significant difference between the treatments was accepted at 5% probability.

RESULTS AND DISCUSSION

Effect of treatment on the percentage of proximate composition of cowpea

The results of the proximate analysis in Table 1 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 role in food storage. Cowpea is not only a good source of

Table 1. Percentage of proximate composition of cowpea.

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

Mean values with the same superscript(s) in a column are not significantly different ($p<0.05$)

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

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

Mean values with the same superscript in a column are not significantly different ($p<0.05$).

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 the publication reported 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.

Effect of treatment on the anti-nutrients composition of cowpea

The result in Table 2 showed that there was significant difference among the anti-nutrient composition of treated cowpeas. However, the germinated cowpeas (GMC) had increased anti-nutrient content when compared with the raw cowpeas (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 Omoruyi et al. (2007) that boiling and roasting were effective in lowering the levels of anti-nutrient factors in Caribbean tuber crops. Also 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 Osman (2007) that germination significantly decreased the TIA activity in D.lablab by 19.3%.

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 cowpeas had 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 Hefnawy (2011) that cooking treatments decreased the concentration of lysine and tryptophan in lentils (*Lens culinaris*.) This finding contradicted with the report by Soetan and Oyewole (2009) that germination decreased the concentration of lysine and tryptophan in chickpeas (*Cicer arietinum L.*).

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 dec application. Germination had increased the amount of Na, K, Ca, P, Mg while boiling and pressure cooking

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

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

Mean values with the same superscript in a column are not significantly different ($p<0.05$)

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

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

Mean values with the same superscript in a column are not significantly different ($p<0.05$)

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

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

had increased the amount of this macro element content when compared with the raw sample. This result agreed with the report by Udensi et al. (2008) that boiling of *Mucuna flagellipes* resulted in products with lowest mineral contents. It was also in agreement with the report of Hefnawy (2011) that matured cowpea lost 23% mg when pressure cooked.

Effect of treatment on the micro elements content of cowpea

The result in Table 5 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 agreed with the report of Hefnawy (2011) that cooking in boiling water caused great losses of copper and iron. It was also reported by Hefnawy (2011) that pressure cooked mature cowpeas had 30% loss of copper. Mean values with the same superscript in a column are not significantly different ($p<0.05$).

CONCLUSION AND RECOMMENDATION

This research showed that heat processing amounted to 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 had 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.

Further research should be carried out to determine the effect of heat treatment in combination with germination on cowpea flour as to reduce the anti-nutrient factors.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

AOAC (1990). Official methods of Analysis(14th ed). Association of Official Analytical Chemists. INC IIII, North Nineteenth street, suite 210 Arlington V.A, USA. pp. 222-114.

AOAC (2005).Official methods of Analysis (18th ed). Association of Official Analytical Chemists. 18tth ed. Washington, DC USA.

Arisa, N U, Aworh, O C (2007). Production, Quality Assessment and Acceptability of African yam bean Sphenostylis stenoscarpa sauce. J Food Process Preserv 31:771-778.

Friedman M (1992). Dietary impact of food processing. Annu Rev Nutr. 12(1):119-137.

Gomez C, (2014). Cowpea: Post-Harvest Operations. In: Mejia (Ed.), Post Harvest compendium, AGST.

Hefnawy TH (2011).Effect of processing method on nutritional composition and antinutrient factors in lentils, Ann Agric. Sci. 56(2):57-61.

Igile GO, (1996).Phytochemical and Biological studies on some constituents of *Vernonia amygdalina* (compositae) leaves. Ph.D thesis, Department of Biochemistry, University of Ibadan, Nigeria.

Ikechukwu J, (2000). Cowpea hulls as potential feedstuff for broilers. Master of sciences, Anim Nutr Biochem Univ Niger.

Koratkar R, Rao AV, (1997). Effect of saponin on azoxymethane induced peneoplastic lesion in the colon of mice. Nutr cancer, 27:206-209.

Jambunathan R, Rao NS, Gurtu S (1983). Rapid methods for estimating Lysine and protein in Sorghum. Cereal Chem. 60:192-194.

Lunder TJ, (1973). An improved method for the colorimetric or spectrophotometric determination of methionine in acid hydrolysates of biological products. Ind. Aliment (Pinerlo, Italy) 12:94-98.

Maga JA, (1983). Phytate: Its chemistry, occurrence, food interaction, nutritional significance and methods of analysis. J Agric Food Chem. 30: 1-9.

Mune MA, Minka S R, Mbome L I, (2013). Response surface methodology for optimisation of protein concentrate preparation from cowpea Vigna unguiculata (L.) Walp Food Chem 110: 735-741.

Obasi NE, Wogu CO, (2008). Effect of soaking time on the proximate and mineral compositions and antinutritional factors of yellow maize (Zea mays). Nig. Food J. 26(2):69-77.

Omoruyi FO, Dilworth L, Asemota HN, (2007). Antinutritional factors, zinc, iron and calcium in some Caribbean tuber crops and the effect of boiling or roasting. Nutr. Food Sci. 37(1):8-15.

Osman AM, (2007). Effect of different processing methods on nutrient composition, anti-nutritional factors and in vitro protein digestibility on Dolichos lablab bean (*Lablab purpureus* (L) Sweet). Pak. J. Nutr. 6(4):299-303.

Soetan KO, Oyewole OE (2007) The needs of adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds. Afr. J. Food Sci. 3(9): 223-232.

Udensi EA, Ekwu FC, Isinguzo JN (2007). Antinutrient factors of vegetable cowpea (sequipedalis) seeds during thermal processing. Pak. J. Nutr. 6(2):194-197.

Udensi EA, Arisa NU, Maduka M, (2008). Effect of processing method on the level of antinutritional factors in Mucuna flagellipes Nig. Food J. 26(2): 53-59.

Vijayakumari P, Siddhuraju P, Pugalenthi M, Janardhanan K, (1998). Effect of soaking and heat processing on the levels of antinutrients and digestible protein in seeds of Vigna aconitifolia and Vigna sinesis. Food Chem.*63:*259-264.

Wang N, Lewis MJ, Brenman JG, Westby A, (1997). Effect of processing methods on nutrients and antinutrient factors in cowpea. Food Chem. 58:59-68.

Youseff MM, Abdal MA, Shekibs LAE, Ziena HM, (1989). Effects of dehulling, soaking and germination of chemical composition, mineral elements and protein patterns of feba beans (*Vicia feba* L). Food Chem. 23:129-136.

Yusuf AA, Ayedun H, Sanni LO, (2008). Chemical composition and functional properties of raw and roasted Nigerian bennisee *(Sesamum indicum)* and Bambara groundnut *(Vigna subterranean).* Food Chem. 111(2):277-282.