Effect of Sprouting Time on the Nutrient and Anti-Nutrient Properties of Cowpea (Vigna Unguiculata)

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Abstract: In this study the effect of sprouting time (0h, 24h, 48h, 72h, 96h and 120h) on the nutritional, and anti-nutritional properties of cowpea was investigated. Cowpea seeds were sprouted in the laboratory under room conditions $(25^{\circ}C)$. The germinated seeds were removed, sun-dried, weighed and milled for proximate, minerals, functional and anti-nutritional factors analysis using standard methods. Sprouting resulted in increase in most of the nutrients except fat and carbohydrate which were reduced. The protein ranged from 20.16-34.05%, Ash 3.07-4.77%, fat 1.51-3.02%, fibre 4.14-5.08%, moisture 5.64-6.72% and carbohydrate 47.87-64.01%. The minerals (potassium, sodium, calcium, magnesium and phosphous) in the sprouts increased as the germination time increased. All the determined anti-nutrients (alkaloids, flavonoids and phytates) decreased with increase in germinating time. It can be concluded that sprouting improved the nutritional quality of the cowpea in terms of higher concentration of nutrients, and reduced antinutrients.

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I. Introduction

Cowpea (Vigna uniguiculata), an important legume is a versatile crop, Cowpea grain contains, an average of 23-25% protein and 50-67% carbohydrate. Presence of significant amounts of protein, calories and some water soluble vitamins, makes cowpea a promising food ingredients (Chingakham et al., 2015). It is a good source of protein in the tropics with the seed containing appreciable amounts of lysine and tryptophan but is deficient in methionine and cystine when compare to animal protein world, it is a major source of dietary protein that nutritionally complements staple low-protein cereal and tuber crops. It is also a valuable and dependable commodity that produces income for farmers and traders (Singh et al., 2005; Langyintuo et al., 2003).

Legumes, however contains 40 to 60% carbohydrates which are mainly in the form of starch and oligosaccharides (raffinose, starchyose or verbascose). Oligosaccharides being indigestible by mammalian enzymes induce flatulence among susceptible individuals (Udensi et al., 2007). This may cause extreme discomfort like rectal elimination of socially unacceptable odorous flatus, gases, increased belching and heartburn (Akporhonor et al., 2006). This unwelcome components has resulted to restricted use and consumer apathy for legume. Legumes also contains toxic components and anti-nutrients which interfere with digestive processes, consequently preventing efficient utilization of the legumes protein and other nutrients. The toxic component and anti-nutients found in legumes are trysin inhibitors, tannins, phytates, hemaglutinins and anthocyanins (Betsheh et al., 2005). Other constraints to the use of legumes include unacceptable flavor and the long and tedious cooking time.

Most of the toxins and anti-nutrients in legumes can be reduced to tolerable levels by sprouting or germination (Ene-Obong and Obizoba, 1996; Oluwole and Taiwo, 2009). Sprouting remarkably improve the nutritional quality of legumes by breaking down nutritionally undesirable constituents (Chavan and Kadam 2001; Henry and Massey 2001).

This study aims at investigating the effect of sprouting time on the nutrient, and anti-nutrient factors of cowpea (Vigna unguiculata). With a view to widening its utilization in food systems

2.1 materials collection

II. Materials and methods

The cowpea (Vigna uniguiculata)seeds were purchased from a market (Eke Awka) in Anambra state. The chemicals used were obtained from Food Science & Technology Laboratory, Imo State University Owerri.

2.2 Sample Preparation

The cowpea seeds were soaked in three times their volume of water for 12 hours. The soaked seeds were then washed and placed on a moist plane wood and covered with moist cloth. Then, the moist seeds were divided into five portion of 500g each and were allowed to germinate in dark at room temperature (26-28[°]c) for 24, 48, 72, 96, and 120 hours respectively. During the germination period, the seeds were sprinkled with water daily. At the end of the germination periods, ungerminated seeds were carefully sorted out. The sprout were thoroughly washed and plumules and radicles were carefully removed. The seeds were sun dried for 3-4 days and milled into flour using a domestic grinder and sieved with 1.0mm mesh sieve before packaging in polyethylene bags for further studies.

2.3 Proximate Analysis of Flour Samples

2.3.1 Determination of Moisture Content

A measured sample (5g) were weighed into a previously weighed crucible. The crucible and its content were dried in an oven at 105° C for 3 hrs, then cooled in a desiccator and reweighed. Drying, cooling and weighing were repeatedly carried out at one hour intervals until no further diminution in weight was observed (i.e. constant weight is achieved). The weight of moisture that got lost during the drying was calculated and expressed as a percentage of the weight of sample analyzed

% moisture content =
$$\frac{W2 - W3}{W2 - W1}$$
 $\frac{x \cdot 100}{1}$

Where

 $W_1 = Weight of crucible$

 W_2 = Weight of empty crucible + sample before drying

 $W_3 = Weight of empty crucible + sample dried to constant weight.$

2.3.2 Determination of Crude Protein

The total nitrogen content of diet was determined using kjeldahl method and a conversion factor of 6.25. 2g of sample, 0.1g of copper sulphate, 2.5g anhydrous sodium sulphate granules as catalyst, 25mls of concentrated sulphuric acid and 10 anti-bumping glass bead were added into the kjedahl flask . The flask and its content were swirled gently and digested by heating at 90°C for 3 hrs, until a light green colour was obtained. The heating was stopped and after sometime, the content of the flask changed from green to colourless. The flask was allowed to cool before distillation. The distillation unit was set-up adding four drops of methyl red indicator into a conical flask containing 50ml of 2% boric acid and the conical flask connected to condenser such that the condenser tips dipped into the liquid. A measured volume of 8ml of 50% NaOH and 50ml of distilled water where added through the funnel into the digesting flasks already mounted on the distiller. Cooling water was connected and the distiller were switched on. The flasks contents were distilled into the basic acid solution until 150mls of distillates were collected. The distillate was titrated with 0.1M hydrochloric acid solution. The percentage total Nitrogen for rice bran enriched yam was calculated thus % crude protein = % total nitrogen X 6.25

Total Nitrogen (%)

$$\frac{V_2 - V_1 \ x \ 1.4 \ x \ M}{W}$$

Where ; W = weight of sample

 V_1 = volume of HCl acid required for blank test.

 V_2 = Volume of HCl acid solutionrequired for sample test

M = Molarity of HCl acid

=

2.3.3 Determination of Crude Fat

A measured weight (2g) of sample was wrapped with filter paper and placed in the soxhlet extraction unit with a reflux condenser and a 250ml round bottom flask which was previously weighed. One hundred and twenty (120) milliliters of petroleum ether as solvent was added to the flask and heated. The samples were extracted of its fats continuously for five hours. At the end of the 5 hours, the sample was removed and the solvent was recovered. The flask containing crude fat was dried in the Oven at 50° C for two minutes, cooled in the desicators and reweighed. The percentage fat was calculated as follows. % crude fat = Weight of fat x 100

2.3.4. Determination of Ash

Two gram (2g) each of the samples were weighed into a clean crucible and were charred over a hot plate until no more smoke were given off. The samples were transferred into a muffle furnaces at 550° C using a pair of thongs and were left at this temperature for two hours until a white or light gray ash resulted. With the

aid of the thongs, the crucible with the charred samples were removed from the furnance and placed in the desicator to cool. The samples were weighed and the percentage ash content calculated as shown below.

Percentage ash (dry weight basis) =
$$\frac{\text{weight of ash}}{\text{Weight of sample}} = \frac{100}{1}$$

Where W_1 = initial weight of empty crucible W_2 = weight of crucible + sample before drying W_3 = Weight of crucible + ash.

2.3.5. Determination of Crude Fibre

Two grams (2g) each of the blend samples were weighed into a beaker containing 200ml of a solution (1.25g of H_2SO_4 per 100ml of solution) and boiled under reflux for 30 mins. The solution was filtered through a linen cloth on a fluted funnel and washed with boiled water until the washings were no longer acidic. The residue was transferred to a beaker and boiled for 30mins with 200ml of a solution containing 1.25g of carbonate – free NaOH per 100ml. The final residue was filtered through a thin but closed pad of washed and ignited asbestors in a Gooch crucible. The residue was washed with boiled water until the washings were no longer basic, oven dried and weighed (W₁). It was then incinerated, cooled and weighed (W₂).

The loss in weight after incineration $(W_1 - W_2) \ge 100$ is the percentage of crude fibre.

Where W_1 = weight of oven dried sample

 W_2 = weight of macerated sample

2.3.6 Determination of Carbohydrate Content

The carbohydrate was calculated by difference as the nitrogen free extractive (NFE) using the method of AOAC (2006).

Calculation: % CHO (NFE) = 100 - % (A + B + C + D + E) Where:

A = protein content, B = ash content, C = crude Fat content, D = moisture content, E = crude Fibre content

2.4 Determination of Antinutrient/Phytochemical Content of Flour Samples

2.4.1 Determination of Flavonoid

Percentage flavonoid was determined with Boham and kalipai (1994) method. Ten (10) grams of a sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed. The formula below was used to calculated percentage flavonoid.

% flavonoid =
$$\frac{W_2 - W_1}{10}$$
 x 100

Where

$$W_2$$
 =weight of sample + flavonoid extract.

W₁=weight of empty filter paper.

2.4.2 Determination of Alkaloid

This was determined using method described by Obadoni and Ochuko (2001). Five grams (5g) of the sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered to stand for 4 hours. This was filtered and concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the filtrate until the precipitation was completed. The whole solution was allowed to settle and the precipitate were collected by filtration and weighed. The formula below was used to calculate the percentage alkaloid

% Alkaloid =
$$\frac{W_2 - W_1}{W}$$
 $\frac{x \ 100}{1}$

Where;

W = Weight of sample $W_1 = weight of empty filter paper$

 W_2 = Weight of paper + precipitate

2.4.3 Determination of Phytate

The phytate content will be determined using the method of described by Haugh and Lantzsch (1993). Two grams (2g) of each sample was extrac ted with 20ml 0f 2% HCl for 3 hours. To 1ml of the extract will be added 1ml of 0.3% ferric ammonium sulphate solution in a test tube and stoppered. The mixture will then be boiled for 30 minutes in water bath. The tubes will be cooled in ice for 15 min and allowed to adjust to room

temperature. The contents of the test tubes was then centrifuged at 3000rpm for 30 minutes. One milliliter (1.0 ml) of the supernatant was mixed with 1.5ml of 0.02M, 2,2 dipyridine solution and the absorbance will be read at 519nm against a blank (distilled water) in a spectrophotometer (Atomic Absorption Spectrophotometer- AAS model SP9). The experiment was carried out in triplicates. Phytate was expressed in percentage and calculated as shown below

% phytate
$$Au$$
 Au $X C$ $X Vf$ $X 100$
As W Va 1

Where: Au =Absorbance of test sample As =Absorbance of standard solution C = Concentration of standard solution W = Weight of sample used

Vf = Total volume of sample

Va = Volume of extract used

2.5 Determination of Minerals

The minerals content of the samples was determined by the dry ash acid extraction method described by James (1996). A measured weight of each sample (5g) was burnt to ashes (as in ash determination) in a muffle furnance at 550° C. The resulting ash was dissolved in 10ml of 2M HCL solution and diluted to 100ml in a volumetric flask using distilled water. It was filtered and the filtrate used for the mineral analysis. All the experiment carried out for minerals were in triplicates.

2.5.1 Determination of Calcium and Magnesium

Calcium and magnesium contents of the samples extract were determined using versunate EDTA comflexiometric filtration method as described by James (1996). A measured volume of each extract (20mls) was dispersed into a conical flask, pinch doses of the masking agents (Potassium cyanide, potassium ferocyanide, hydroxylamine) were added to it. Then 20mls of ammonia buffer was added to adjust the pH to 10.0 A pinch of the indicator Erichrom black T was added and the mixture was shaken very well. Then it was titrated against 0.02N EDTA solution until the colour changed from mauve to a permanent deep blue colour. This titration gave a reading for combined concentration of Ca and Mg ions. This is as a result of Ca²⁺ and Mg²⁺ forming complexes with EDTA at a pH of 10.00. A second titration was conducted to determine Ca alone. This was a repeat of the previous one with a slight change in that 10% NaOH solution was used to raise the pH of the digest to 12.0 and then titrated with 0.2N EDTA using selechrome dark blue as indicator in place of Erichrome black T. At pH 12.0, Ca²⁺ complexes with EDTA. A reagent blank was titrated to serve as control. The experiment was repeated two more times. The calcium and magnesium contents were calculated separately using the formula.

% calcium or magnesium = $100 \text{ X} = \frac{\text{EW}}{\text{W}} \text{X} = \frac{\text{Vf}}{\text{Va}} \text{X} = \frac{\text{Vf}}{\text{Va}} \text{X} = \frac{100 \text{ Vf}}{\text{Va}} \text{V} = \frac{100 \text{ V}}{\text{Va}} \text{V} = \frac{100 \text{ V}}{\text{V}} = \frac{100 \text{ V}}{\text{V}} = \frac{100 \text{ V}}{\text{V} = \frac{100 \text{ V}}{\text{V}} = \frac{100 \text$

Where;

W	=	Weight of sample, EW =	Equivalent weight
Ν	=	Normality of EDTA, $Vf =$	Total volume of extract
Va	=	Volume of extract titrated, $T =$	Titre value of sample
В	=	Titre value of blank	

The experiment was replicated for three times and the mean value recorded.

2.5.2 Determination of Potassium and Sodium

Flame photometry was used to determine the concentrations of potassium and sodium as described by James (1996). The instrument (Jenway 600i flame photometer) was set up according to the manufacturers instructions. The equipment was switched on and allowed to stay for about 10 minutes. The gas and air inlets were opened and the start knob was turned on as the equipment is self igniting. After ignition the flame was adjusted to a non luminous (blue) flame. Meanwhile, standard potassium and sodium solution were prepared separately and each was diluted to concentration of 2,4,6,8 and 10ppm. The appropriate filter was selected i.e. for potassium and sodium. The highest concentrated standard solution (10ppm) was aspirated and its emission intensity adjusted to 100 units. Thereafter, starting with the least concentrated (2ppm), each standard solution was aspirated and caused to spray over the non-luminous butane flame. The emission intensity read directly on the instrument and the reading were recorded. Then, the sample digests were also aspirated and their readings recorded. The emission intensities of the standards were plotted against their concentrations to obtain a standard curve (calibration graph) for each element. Subsequently the optical density emission recorded from each of the samples were matched against those in the curve, thus using the curve to extrapolate the quantity of each

potassium and sodium ions in the sample. The experiment was repeated three more times to get a mean concentration. The concentration of the test minerals were calculated as follows

K (mg/100g) or Na (mg/100g) =
$$100$$
 X 1 X 1 Va Vf X D
W Va

Where;

W = weight of sample used

 $\mathbf{Y} =$ concentration (in ppm) from curve

Vf = total volume of extract

Va = volume of extract (digest) flamed

D = dilution factor where applicable.

2.5.3 Determination of Phosphorus

The method described by AOAC (2006) was used. Two grams (2g) of sample was ashed for 4hrs at 550° C. It was cooled, and 5ml of 6N HCL and several drops of nitric acid were added. It was heated to dissolve the ash completely. The solution was cooled and transferred to a 100-ml vol. flask and diluted to volume with distilled H₂0. Aliquot was pipetted, which were expected to contain, 0.5 - 1.5mg of phosphorus into a 100ml vol. flask. Twenty mililitre (20ml) of molybdovanadate reagent was added (this reagent was prepared by dissolving 20g of ammonium molybdate in 200ml of hot water. Then 1g of ammonium meta-vanadate was dissolved in 125ml of hot water and cooled, then 140ml of conc nitric acid was added. The cooled molydate and vanadate solutions were then combined and diluted to 1 litre). The sample and the molybdovanadate reagent were diluted to 100ml and was allowed to develop colour for 10min. the absorbance was read at 400nm against a phosphorus standard curve.

Calculation

 $P(mg/dl) = \underline{absorbance of test x conc of Std (5mg/dl)}$ Absorbance of std.

Preparation of Standard Curve

Stock standard solution of 2mg P/ml was made by weighing 8.7874g of $KH_2 PO_4$ that has been dried at $105^{0}C$ for 2hr. It was transferred quantitatively to a 1litre vol. flask and about 750ml of distilled water was added to dissolve it. It was diluted to volume with water and refrigerated until use. A working standard solution containing 0.1mg phosphorus/ml was made by diluting 50ml of the stock solution to one litre with distilled water. Aliquots of the working standard solution of 0,5,8, 10 and 15ml was transferred to a freshly rinsed 100ml vol. flasks. Twenty milliliter (20ml) of the molydovandate reagent was added to each flask containing the standards, and was diluted to volume with water and mixed well. The flask was kept to stand for 10min to complete colour development. The absorbance was read at 400nm 0.0 standard (blank) was used to zero the spectrophotometer.

3.1 Proximate Analysis

III. Results and Discussion

Effect of sprouting on the proximate composition of cowpea sprouts is shown in Table 3. 1. The results showed that the crude protein increased from 20.92% to 34.05% as the time of sprouting increased. Mehta et al. (2007) have found that there is increase in protein content of cowpea after 28h of sprouting. Uppal and Bains (2012) observed crude protein increase from 8 to 11% after sprouting. This increase in protein content may be attributed to loss in dry matter, particularly carbohydrate through respiration (Uppal and Bains, 2012). Also, Bau et al. (1997) assumed that the increased was due to synthesis of enzyme proteins (for example, proteases) by germinating seed or other constituents. A further explanation was done by Norogaki et al. (2010) where they noted that protein synthesis occurred during inhibitions and that hormonal changes play an important role in achieving the completion of germination.

Table 3.1 Mean values for proximate composition (%) of sprouted cowpea at different time intervals

Sprouting time	Protein	Ash	Crude fat	Crude fibre	Moisture	Carbohydrate	
(h)							
0	20.16 ^{de} ±2.87	$3.07^{a} \pm 1.79$	3.02 ^a ±0.08	$4.14^{\circ} \pm .09$	5.63 ^b ±0.08	64.01 ^a ±6.20	
24	21.92 ^{de} ±2.18	3.73 ^a ±1.55	$2.84^{ab}\pm0.12$	$4.28^{bc} \pm 0.09$	5.63 ^b ±0.08	$61.60^{ab} \pm 3.60$	
48	24.54 ^{cd} ±2.29	$3.97^{a}\pm0.06$	$2.58^{abc} \pm 0.11$	$4.49^{abc} \pm 0.06$	5.65 ^b ±0.05	58.77 ^{abc} ±1.47	
72	27.71 ^{bc} ±1.57	4.43 ^a ±0.75	2.10 ^{bc} ±0.12	$4.47^{abc} \pm 0.15$	$5.66^{b} \pm 0.04$	55.72 ^{bc} ±3.26	
96	$30.66^{ab} \pm 2.94$	4.63 ^a ±0.55	1.93°±0.16	$4.92^{ab}\pm0.08$	$5.68^{b} \pm 0.09$	52.72 ^{cd} ±2.96	
120	34.05 ^a ±1.93	$4.77^{a}\pm0.68$	1.51 ^{cd} ±0.13	$5.08^{a}\pm0.07$	6.72 ^a ±0.12	47.87 ^d ±2.43	
LSD	5.11	2.34	0.84	0.65	0.53	7.89	

Values are means \pm SD of three replications. Means with different superscript letters in the same columns are significantly different at 5% level of significance.

The crude fat concentration decreased with sprouting time. It decreased from 3.02% to 1.51%. This result was in line with Badshah et al. (1991) and chung et al. (1998) who noted significant losses in lipid content during canola sprouting. The decrease in fat content of seed could be due to total solid loss during soaking prior to germination (Wang et al. 1997) or use fat as an energy source in sprouting process (El-Adawy, 2002).

Crude fibre content was significantly increased (p<0.05). The values increased from 4.14% to 5.08%. This increment may be due to emergence of plumule and radical during germination (Berry et al., 1988). In another study, Chung et al. (1998) reported that in barley, sprouting was associated with significant increase in crude fiber from 3.75% in unsprouted barley to 6% in 5 days sprout due to synthesis of structural carbohydrates such as cellulose and hemicelluloses, a major constituent of cell walls.

Ash contents, calculated on moisture free basis, increased with increase in sprouting time. The ash content slightly increased but were not significantly difference (p<0.05). After 120h germination the ash content reaches maximum level. El-Adawy (2002) reported significant increase in ash content during sprouting in mungbean, pea and lentil seed. The decrease in crude fat and carbohydrate content during sprouting may be have led to the apparent increase observe in ash and other chemical components.

The moisture content of cowpea significantly increased in all the sample from 5.63 to 6.72%. After entry of water in seed coat, seed swelling starts and initiates sprouting. During sprouting, the water intake of seed varies. The percent increase was highest in 120h. Murugkar and Jha (2009) also observed an increase in moisture 5.4 to 56.1 % after 48 h of sprouting in soybean. Uwaegbute et al. (2000) observed that the moisture increased from 15.6 to 17.6 % after sprouting cowpea. Usually during sprouting, seeds absorb water by a process called imbibitions (Nonogaki et al., 2010, Sampath et al., 2008).

Carbohydrate content of sprouted cowpea decreased from 64.01 to 47.87%. Uppal and Bains (2012) reported 5.6 % decrease and Jirapa et al. (2001) reported 2.34 % decrease in carbohydrate content after 24 h of sprouting in cowpea. Vidal-Valverde et al. (2002) explained that during sprouting, carbohydrate was used as source of energy for embryonic growth which could explain the changes of carbohydrate content after sprouting. Additionally, β -amylase activity that hydrolyzes the starch into simple carbohydrate was increased (Suda et al.,1986). Starch in cotyledon was broken down into smaller molecules such as glucose and fructose to provide energy for cell division while the seeds mature and grow (Nonogaki et al. 2010, Vidal-Valverde et al., 2002).

3.2 Mineral Composition

The result of the mineral composition determination in Table 3.2 showed increases in Potassium, Sodium, Calcium, Magnessium and Phosphorus content of the sprouted cowpea seeds as the sprouted time was increased. The increased from 27.00 to 35.98mg/100g, 2.02 to 2.60mg/100g, 30.3 to 45.52mg/100g, 0.93 to 1.70mg/100g and 9.23 to 12.20mg/100g respectively. The mineral content of the sprouted seeds flour were comparatively higher than the raw seeds flour. This corroborated with earlier report of Inyang and Zakari (2008). The desirable changes in the nutrient contents may be due to the breakdown of complex compounds into simpler forms. The metabolic activity of resting seeds increase as soon as they are hydrated during soaking. Complex biochemical changes occur during hydration and subsequent sprouting. The reserved chemical constituents that are used to make new compounds.

Sprouting	time	Potassium	Sodium	Calcium	Magnessium	Phosphorus
(h)		(mg/100g)	(mg/100g)	(mg/100g)	(mg/100g)	(mg/100g)
0		27.00°±2.86	$2.02^{a}\pm0.08$	30.30 ^e ±2.22	0.93 ^{bc} ±0.13	9.23 ^d ±0.45
24		28.14°±3.04	2.15 ^a ±0.05	32.21 ^{de} ±1.69	1.15 ^{bc} ±0.04	$9.50^{d}\pm0.10$
48		30.21 ^{bc} ±3.19	2.23 ^a ±0.11	34.56 ^{cd} ±1.36	$1.22^{bc} \pm 0.07$	10.57°±0.40
72		33.00 ^{ab} ±1.15	$2.32^{a}\pm0.13$	37.98 ^{bc} ±2.56	1.34 ^{ab} ±0.31	11.24 ^{bc} ±0.76
96		34.67 ^{ab} ±1.12	$2.46^{a}\pm0.14$	$40.12^{b} \pm 1.90$	1.50 ^{ab} ±0.34	11.98 ^{ab} ±0.42
120		$35.98^{a} \pm 1.02$	$2.60^{a}\pm0.12$	45.52 ^a ±1.55	$1.70^{a}\pm0.05$	12.20 ^a ±0.05
LSD		4.96	0.75	4.18	0.43	0.95

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Values are means \pm SD of three replication. Means with different superscript letters in the columns are significantly different at 5% level of significance.

3.4 Anti-Nutritional Factors

Significant reduction in anti-nutrients content was observed after sprouting, which is shown in Table 3.4. The phytate content decreased from 2.98% to 1.50%. Uppal and Bains (2012) observed 43.19% decrease in phytate in cowpea after sprouting for 24h. During soaking, phytate ions get leached in soaking water due to concentration gradient (Modgil et al., 2009).

The alkaloid content decreased slowly as germination time increased. In each of the treatments tested, a reduced content of these compounds was observed. The germination process did not completely remove all the alkaloids contents present in the seeds, this is possible due to chemical structure (Villacies et al., 2015).

		inter vais		
Sprouting time (h)	Alkaloids	Flavonoids	Phytate	
	(%)	(%)	(%)	
0	$1.20^{a}\pm0.09$	$1.00^{a}\pm0.12$	$2.98^{a}\pm0.32$	
24	1.01 ^a ±0.15	$0.90^{a}\pm0.07$	$2.70^{ab} \pm 0.21$	
48	$0.92^{ab} \pm 0.08$	$0.81^{ab} \pm 0.04$	$2.56^{ab} \pm 0.15$	
72	0.65 ^{ab} ±0.07	0.58 ^{abc} ±0.14	$2.21^{ab} \pm 0.05$	
96	0.33 ^{bc} ±0.09	0.25 ^{bc} ±0.07	$1.98^{ab} \pm 0.16$	
120	0.15°±0.06	0.09°±0.04	$1.50^{b}\pm0.18$	
LSD	0.65	0.62	1.34	

Table 3.4	Mean values for A	Anti-nutritional	factors 0	f sprouted	and unsprouted	cowpea at	different time
				- a la			

Values are means \pm SD of three replication. Means with different superscript letters in the columns are significantly different at 5% level of significance.

Flavonoid content of the germinated flour were higher than the ungerminated flour. The values decreases with increase in the germination time. This findings were attributed to the biochemical metabolism of seeds during germination which might produce some secondary plant metabolites such as anthocyanins and flavonoids (Randhr et al., 2004). Researches

have shown that intake of foods rich in flavonoid protects human against diseases associated with oxidative stress. The mechanisms of action of flavonoids are through free radicals scavenging or chelating process and protection against oxidative stress (Shahidi and Naczk, 1995).

IV. Conclusion

Sprouting improves the nutritional worth of the cowpea in terms of higher concentration of protein, fat and mineral contents. It also reduces the anti-nutritional factors (Alkaloids, Flavonoids and phytates) studied. Also Sample (120h) should be consumed more than other samples (0h, 24h, 48h, 72h and 96h because sprouting time improved the nutritional worth of cowpea. In general, sprouted cowpea should be consumed more than unsprouted ones due to its enhanced nutritional worth and reduction in the anti-nutritional factors.

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