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# Effect of Harvesting Stage on Cowpea Leaf Nutrient Composition

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**Abstract:** Cowpea leaves are enjoyed as vegetables in many parts of Africa as they contain a lot of antioxidants, micronutrients and nutraceuticals whose deficiency is prevalent among people in Sub-Saharan Africa. Cowpea leaves undergo several physiological and metabolic changes during their maturity stages which may affect their nutritional content. However, farmers lack knowledge on the best cowpea harvesting stage. This research therefore aimed at obtaining information on the right harvesting stage that would enhance cowpea utilization by farmers. Cowpeas variety M66 was planted in RCBD and the treatments which were replicated thrice included harvesting at 21, 35 and 49 DAS. Data was collected on chlorophyll content, iron, calcium, crude fibre, beta carotene, protein and moisture content. The data was subjected for variance using Statistical Analysis System 9.2 edition and significantly different means separated using LSD at 5%. The harvesting stage significantly ($p \leq 0.05$) influenced the chlorophyll content with 49 DAS recording the highest content at 51.39 nm followed by 35 DAS with 41.87 nm and the least at 21 DAS with 22. 05 nm. The moisture content decreased with the stage of harvest with highest moisture content being observed at 21 DAS and the least at 49 DAS in both trials. The iron content of cowpea leaves was significantly ($p \leq 0.05$) different at 49 DAS in both trials. The calcium content at 21 and 49 DAS in both trials was significantly ($p \leq 0.05$) different. The protein content was significant ($p \leq 0.05$) in all the stages of harvesting with the highest protein content in both trials being recorded at 21 DAS and the least being recorded at 49 DAS in both. Crude fibre content increased with the stage of harvesting in both trials. This research highlights the essence of harvesting cowpea leaves at the correct harvest stage for increased nutrient utilization.

**Keywords:** Vegetables, Cowpeas, Leaves, Harvesting Stage, Nutrient Content

## 1. Introduction

Cowpea (*Vigna Unguiculata*) is a highly adaptable plant resilient to a variety of stressors [1]. It is a warm-weather plant that can grow in dry places and where other legumes would not [2]. Cowpea is the third most popular leafy vegetable in Sub-Saharan Africa in terms of quantity, after amaranth, nightshade, pumpkin leaves and African spider plant, which are some of the most prominent traditional vegetables in the area [3]. Cowpea leaves are widely used as culinary ingredients in soups, stews, and sauces [4] and different populations around the nation consume the leaves fresh, fermented or even dried. Farmers use the nutritional advantages of both the grains and leaves that the cowpea, a dual-purpose legume produces [5].

However, the stage of harvesting cowpea leaves influences growth process and the nutrient composition. It has been reported that as plants grow older and mature, the amount of protein in their leaves declines while the number of indigestible structural polysaccharides, physiologically active substances, and antioxidants increase [6, 7]. Over maturity of the cowpea leaves result in excess fibers making the leaves less palatable and bitter due to the high anti-nutrient levels [8]. When plant leaves mature, their nutrient content decreases, which causes a rise in indigestible structural polysaccharides and a drop in protein levels [9].

Harvesting stage also influences the nutritional value and post-harvest quality of vegetable crops. Depending on the type of production, variety, and weather circumstances,

cowpea leaves can be harvested at various stages of maturity. Leaf picking can begin two to three weeks after planting and continue throughout the plant's growth cycle. The majority of leafy vegetables have a preferred growing stage when the flavor and palatability are good for human consumption [10]. Vegetables that are harvested at an immature growth stage wilt more quickly and are more susceptible to mechanical harm [11]. Adegbaju [12] reported that the developmental phases of plants have an impact on the quantities of nutrients in vegetables. Different organs and tissues have different nutrition and metabolite distributions throughout a plant's growth and development, causing major changes at critical phases of their growth cycle, such as flowering, vegetative and senescence and metabolites as nutrients [13]. Mijena [9] reported that when a plant's leaves mature, its nutritional quality degrades, causing protein levels to drop and indigestible structural carbohydrates to rise. Throughout the maturation phase, biologically active chemicals may also increase based on the many biosynthetic pathways and metabolic regulatory systems [7].

Consumption of cowpea leaves at their optimum stage will provide the required dietary intake and minimize food insecurity in Kenya. However, there is little information available on the nutrient value of cowpea leaves gathered at varying stages of harvesting. The purpose of this study was to determine the variation of nutrients in cowpea leaves at different growth stages and to advise on the stage that would provide optimum nutrients and minerals.

# 2. Materials and Methods

### *2.1. Experimental Site*

The cowpeas were planted in a farmers' field next to Chuka University horticultural demonstration farm in Tharaka Nithi County. The farm lies at 0°19` S, 37°38` E and 1535 m above sea level. The region receives roughly 1,200 mm of rainfall each year, which is distributed bimodally, with the long rains falling from March to June and the short rains from October to December. The predominant soil type is humic nitisol, which is deep, well-weathered, and has moderate to high natural fertility and the average annual temperature is about 20°C [13].

### *2.2. Experimental Design*

The field experiment was set up using a randomized complete block design. The treatments included three harvesting stages i.e., 21,35 and 49 Days after sowing.

### *2.3. Planting Materials, Land Preparation and Planting*

Land was cleared to remove all the perennial weeds and vegetation, after which ploughing was then done using a fork jembe followed by harrowing to break the large soil clods so as to attain a fine tilth and incorporate plant residue. Leveling and raising of the field was also done. Pegging was done to divide the area into blocks. Holes for planting were dug in the plots. Cowpea variety M66 seeds obtained from KALRO were planted. The variety has a fair tolerance to aphids, scab, and the yellow mottle virus. The cultivar flowers in around 55–60 days and matures completely in about 80–90 days [14]. The field plan consisted of a 2 m by 2 m common plot with 70 cm between rows and 20 cm within rows, respectively. To facilitate material movement and agronomic operations, the field was demarcated into blocks of well-known locations with alleyways connecting blocks. NPK 23:23:0 fertilizer was applied during planting at the approved rates. Direct sowing of the cowpea seeds into the holes was followed by daily morning and evening waterings while the soil was dry. The plots were manually kept weed-free. Pest and diseases were judiciously controlled using recommended management methods.

### *2.4. Data Collection*

The collection of data collection was done over two cultivations, January-March and April-June 2022. Data collection was done sequentially following different stages of harvesting.

### *2.5. Determination of Effects of Harvesting Stage on Nutrient Composition*

#### *2.5.1. Chlorophyll Content*

Every two weeks, the chlorophyll content of randomly chosen leaves from cowpea plants was measured using a chlorophyll content meter by remote sensing to avoid damaging the leaf tissue [15]. The chlorophyll content was taken at every harvest stage i.e., at 21, 35 and 49 days and the data recorded for both trials.

#### *2.5.2. Moisture Content*

Cowpea leaves (1 g) were weighed, placed in a crucible and dried for eight hours at 105°C to get a constant weight [16]. The following formula was used to calculate moisture content;

Moisture= (Weight of empty crucible+ weight of sample) _ (weight of oven dried sample) ×100\weight of sample.

#### *2.5.3. Iron Content*

The dried cowpeas leaf samples (0.2 g) were measured and put into microwave Teflon tubes and 6mls of nitric acid was measured and added to the tube and 2 mls of hydrogen peroxide and put in an advanced microwave digester for one hour. After being taken out of the digester, the samples were filtered and diluted with distilled water. The sample volume was topped up to 50 ml mark of the volumetric flask and then the lid /top of the flask was put in place. The flask was inverted several times to ensure proper mixing. The mixture was then transferred to sample bottles ready for machine analysis. After the analysis the mineral content was read in an atomic absorption spectrophotometer model PG-990. This was done as described using [17].

#### *2.5.4. Calcium Content*

Dried cowpea leaf samples (0.2 g) were measured and put into microwave Teflon tubes, mixed with 6mls of nitric acid

and 2 ml of hydrogen peroxide and put in an advanced microwave digester for one hour. After being taken out of the digester, the samples were diluted with distilled water and filtered. The volume of the sample was topped up to 50 ml mark of the volumetric flask and then the lid /top of the flask was put in place. The flask was inverted several times to ensure proper mixing. The mixture was then transferred to sample bottles ready for machine analysis. After the analysis the mineral content was read in an atomic absorption spectrophotometer model PG-990. This procedure was done as described in AOAC international [17].

#### *2.5.5. Protein Content*

The proteins were determined using the Kjeldahl method, which is described in AOAC [18]. Cowpea leaf sample (1 g) was measured and put in digestion tubes (250 ml). Two kjeldahl catalyst tablets (copper sulphate and potassium sulphate were added into the digestion tube. Twelve millitres of concentrated sulfuric acid was measured and poured into digestion tubes and then digested at 420°C for one hour until liquid became clear or blue green appearance. The digester's exhaust manifold and digestion tube rack were removed, and they were both placed within a fume hood to cool to room temperature. Automatic distillation was then done with distilled water, sodium hydroxide solution and boric acid. The condensed liquid was then collected in flask with an indicator solution. Using hydrochloric acid to titrate the solution, the trapped nitrogen in the boric acid produced a pink color. The protein content was calculated using the formula.

$$\text{Protein content (\%)} = \text{Titre value} \times \text{Normality of acid} \times 1.4007 \times 6.25 \times 100 / \text{Weight of sample taken}$$

Where;
*M*= Molarity of the acid.
*W*=Weight of test portion.
*6.25*=Conversion Factor.

#### *2.5.6. Beta-Carotene Content*

Beta carotene concentration was determined using UV Spectrophotometer and column chromatography. The cowpea leaf sample used weighed two grams (g). Using a mortar and pestle and little amounts of acetone, the color was removed until the residue was colorless. After being mixed into a 100 ml volumetric flask, all the extracts were dried in a rotary evaporator at a temperature of about 60°C. One milliliter of petroleum ether was used to dissolve the beta-carotene in the evaporated sample through the densely packed column and the beta carotene was extracted. The Eluet was placed in a volumetric flask measuring 25 ml, and the absorbance was measured at 450 nm. The beta-carotene standard curve was then used to determine the beta-carotene content.

#### *2.5.7. Crude Fibre*

The acid-alkali digestion method was used to estimate crude fiber. The digested residue was dried in a crucible, and weighed. In a muffle furnace, the dry residue was lit, and then weighed. The crude fibre weight was determined by dividing the two weights by their difference. This was done as described by AOAC International [19]. Cowpea leaf sample weighing one gm was placed in a beaker. 200 ml of Concentrated Sulphuric acid ($H_2SO_4$) was measured, poured into the beaker containing the sample, heated, and then allowed to boil for 30 minutes. The sample was heated to boil before being filtered with a glass funnel and a cotton cloth. After that, hot water was used to rinse the filtrate, neutralizing the pH and removing any remaining acid. 200 ml of Sodium hydroxide (NAOH) was measured and poured into the beaker with the above filtered sample and placed in a hot plate and boiled for exactly 30 minutes. The sample was boiled, and then filtered once more in a conical flask with a discard flask using a glass funnel and cotton towel. And the filtrate was once again rinsed with hot water to remove the acid residue to neutralize the pH. The filtrate emanating from the boiling in the alkali was put in a clean crucible. The crucible and the sample were placed on a hot plate to evaporate excess water. The sample was then dried for a further two hours at 130°C in a hot oven before being removed and cooled. The dried fiber was weighed on a balance and weight noted.

$$\text{Crude Fibre} = (W_1 - W_2)/SW \times 100$$

Where;
$W_1$=Weight of the crucible with fibre; $W_2$=Weight of empty crucible; S= Sample weight;

$$\text{Acid insoluble ash} = (W_1 - W_2)/S \times 100$$

Where;
$W_1$=Weight of crucible with ash; $W_2$=Weight of empty crucible.

### *2.6. Data Analysis*

The data on chlorophyll content, moisture content, calcium, iron, crude fibre, protein content, beta carotene was subjected to analysis of variance using the Statistical Analysis System version 9.3 at a 5% probability level. Significant means were separated using LSD at $\alpha = 0.05$ to determine the differences between the harvesting stages.

## 3. Results and Discussion

This study aimed to determine the relationship between the harvesting stage of cowpea leaves and nutrient compostion and the following results were obtained.

### *3.1. Chlorophyll Content*

The effect of harvest stages on chlorophyll content was significantly ($p \leq 0.05$) different in both trials (Table 1). In trial one, the highest chlorophyll content was recorded at 49 days after sowing with 51.39 nm followed by harvesting at 35 DAS with 41.87 nm, and the least chlorophyll content was recorded at 21 DAS at 22.05 nmol/cm. In trial two 49 DAS recorded the highest chlorophyll at 59.82 nmol/cm followed by 35 DAS at 43.00 nm and 21 DAS had the least with 23.44

nm. When chlorophyll content was compared for both trials, the highest chlorophyll content was recorded in 21 &35 DAS at trial two. However, 49 DAS, in trial one recorded a higher chlorophyll content compared to same harvest stage in trial two (Table 1).

The chlorophyll content in the cowpea leaves increased as the number of harvest days increased (Table 1). The differences in chlorophyll content from one harvest stage to the next in both trials could be as a result of the cowpea leaves structural changes as they grew. Harvest stage 49 DAS recorded the highest chlorophyll content in both trials. This was because at this harvest stage the cowpea leaves were fully developed. These findings are in agreement with Stessman [20], who reported that the photosynthetic rate initially increased during leaf expansion in several plant species, such as rice, soybean, and tobacco, before declining upon maturation and dropping significantly during senescence as a result of significant modifications to the photosynthetic apparatus.

***Table 1.*** *Effect of harvesting stage on chlorophyll content in nanometer per centimeter (nm/cm).*

| Days after Sowing (DAS) | Trial One | Trial Two |
|---|---|---|
| 21 | 22.05$^{c}$* | 23.44$^{c}$ |
| 35 | 41.87$^{b}$ | 43.00$^{b}$ |
| 49 | 51.39$^{a}$ | 59.82$^{a}$ |
| LSD | 6.634 | 4.2118 |
| CV | 17.52 | 18.468 |

*Means with different letters along the column are significantly different at $p \leq 0.05$. LSD is Least Significant Difference; CV is Coefficient of Variance.

The least chlorophyll content was recorded at 21 DAS in both trials. This could be due to the fact the cowpea leaves were still developing and the cowpea plant had not developed enough leaves for photosynthesis hence the low content. These results were in line with those of Oguchi [21], who reported that leaf anatomy affected the photosynthetic capacity by altering the thickness of the mesophyll and increasing the space required for chloroplasts at the cell surface for gas exchange, which in turn affected the chlorophyll content. Young, unexpanded leaves typically have low photosynthetic rates, which rise up to full leaf expansion or shortly thereafter [22]. Leaves are considered to be the main providers of carbon and contain high chlorophyll pigment more than stem and some parts of the flower [23].

The chlorophyll content also varied between the two trials. This variation in chlorophyll content in both trials could have been caused by changing environmental condition as well as edaphic factors. The low chlorophyll content at trial one could have been due to water stress which affected the stomatal conducting and the photosynthesis processes since trial one was done during a dry season unlike trial two which coincided with rainy season. These findings are similar to Sanchez [24], who discovered that water stress reduced photosynthetic and stomatal conductance in the two cultivars of *Zea mays* throughout the tasseling period. Chlorophyll has been attributed to nitrogen uptake by plants therefore, growing condition and environment such as water stress and nitrogen depletion results in chlorophyll degradation [25]. The high chlorophyll content observed in trial two could have been due to increased moisture availability and greater development of the cowpea leaves which increased the photosynthetic process.

Chlorophyll alters leaf photosynthetic activity, which changes photon flux density, temperature, $CO_2$ concentration, nutrient and water availability, and crop yields [26]. Research by Hokmalipour [27] reported that addition of nitrogenous fertilizer to a field of *Zea mays* had an effect on chlorophyll, leaf index, leaf area and yield. [26] also reported that variation in chlorophyll in plants could be as a result of genetic variation or increase non-laminar chlorophyll-containing tissues and or environmental condition. The age of the plant affects the shape and thickness of the cells that make up the developing leaves [28].

### *3.2. Moisture Content*

In trial one, the moisture content of cowpea leaves was not significantly ($p \leq 0.05$) different when harvested at 21 and 35 DAS, however, harvesting at 49 DAS significantly ($p \leq 0.05$) differed from 21 and 35 DAS. In trial two, all the three harvesting stages significantly ($p \leq 0.05$) differed in moisture content. In both trials, harvesting at 49 DAS recorded the least, and at 21 DAS the highest moisture content. Generally, trial one recorded the highest moisture content compared to trial two (Table 2).

***Table 2.*** *Effect of harvesting stage on moisture content in percentage (%).*

| Days after Sowing (DAS) | Trial One | Trial Two |
|---|---|---|
| 21 | 90.37$^{a}$* | 88.33$^{a}$ |
| 35 | 90.17$^{a}$ | 84.85$^{b}$ |
| 49 | 84.20$^{b}$ | 74.10$^{c}$ |
| LSD | 1.91 | 1.377 |
| CV | 3.2163 | 2.0497 |

* Means with different letters along the column are significantly different at $p \leq 0.05$. LSD is Least Significant Difference; CV is Coefficient of Variance.

In both trials the moisture content showed a reducing trend from harvesting at 21 to 49 DAS (Table 2). This pattern suggests that as plants grew from the vegetative to the maturing stage, moisture levels were decreasing. Harvesting at 21 DAS, the cowpea leaves were very young and succulent and contained fewer total solids compared to the cowpea leaves harvested at 49 DAS which were more mature. The high moisture content could also have been caused by the structural alterations in the cowpea leaves that lost more moisture content via transpiration. These findings were in agreement with Oduntan [29] who also reported high moisture content in fresh leaves of *Sesamum radiatum* which decreased from 4$^{th}$ week after planting at 91.73% to 10$^{th}$ week after planting 48.19%.

According to research by Getahun [30] on the impact of plant age on the chili pepper moisture sorption isotherm, young chili peppers had a high moisture content, whereas fully grown chili peppers had a low moisture content. The

high moisture content observed at the early growth stages assisted in preserving the cells' protoplasmic material and they render the cowpea leaves perishable and vulnerable to microbial deterioration while being stored [31]. Trivellini [32] reported an increase in fresh weight in hibiscus rosa-*sinensis* flower during flower opening and a decrease in senescent flowers in petals, style stamen and stigma contrary to an increase in ovary tissues. Prior to flower opening, the high concentration of hexose sugar reduced the petal water potential, which encouraged the expansion of the petals' cells and flower opening and this supported water input. Despite the active process of flower senescence that leads in the breakdown of macromolecules and cell membranes, it also allows for the recycling and remobilization of essential nutrients for use by other plant organs [32]. This phenomenon explains the reason for continued decrease in moisture as the plants matures.

The significant ($p \leq 0.05$) differences in moisture content observed in trial two among the three harvesting stages could be attributed to the rains that were experienced in trial two and also to the structural changes that occurred in the cowpea plants as they grew older. These results were consistent with Abugre [33] who reported that maximum water content of spider flower varied among the plant type and was influenced by cultivation conditions and structural difference. The differences in moisture content observed between trial one and trial two could have been as a result of the changing growing seasons (Table 2). Plant moisture has been reported to reduce as the plant matures, due to of onset of senescence and increase in fibre content. Maturation of cowpea plant indicates physiological and metabolic alterations that result to maximum accumulation of dry matter and therefore reduction in moisture content of cowpea leaves can therefore be utilized as a possible way to reduce chances of spoilage during storage, therefore enhancing a better shelf life for cowpea leaves. The differences in moisture content between the two trials could have been due to differences in transpiration process. During the dry seasons, plants close their stomata to limit the amount of water evaporating from their leaves resulting in increased water content in the leaves and this explains the high moisture content observed in trial one. In rainy season, plant stomata remain open therefore resulting to continued transpiration rates and this explains the low moisture content in trial two.

### *3.3. Iron Content*

In trial one, the iron content of cowpea leaves harvested at 21 and 35 DAS did not significantly ($p \leq 0.05$) differ but harvesting at 49 DAS was significantly ($p \leq 0.05$) different. In trial two, all harvest stages did not differ significantly ($p \leq 0.05$) in iron content. Iron content was highest when cowpea leaves were harvested at 21 DAS and least at 49 DAS in both trials. Harvesting at 21 and 35 DAS, in trial one recorded a higher iron content compared to trial two. However, harvesting at 49 DAS in trial one recorded lower at iron content compared to trial two (Table 3).

***Table 3.*** *Effect of harvesting stage on iron content in grams (g).*

| Days after Sowing (DAS) | Trial One | Trial Two |
|---|---|---|
| 21 | 825.75[a]* | 787.12[a] |
| 35 | 796.14[a] | 734.35[a] |
| 49 | 489.19[b] | 634.15[a] |
| LSD | 179.87 | 154.36 |
| CV | 17.06 | 19.55 |

* Means with different letters along the column are significantly different at $p \leq 0.05$. LSD is Least Significant Difference; CV is Coefficient of Variance.

Harvesting stage significantly ($p \leq 0.05$) influenced the iron content of the cowpea leaves (Table 3). Iron content decreased with increase in the harvest days. These results contradict the findings of Tiffin [34] who reported an increase of iron content with leaf age. The decrease in the iron content as the cowpea leaves matured in the present study could have been caused by the diversion of iron to flower development. Flyman [35], reported an increase in iron concentration in *M. balsamina* increased between stages one and two, leveled off between stages two and four, grew between stages four and five, and then decreased until the last stage. Such redistribution process of minerals explains the decrease of iron in the cowpea leaves where they were reclaimed and translocated to other parts where they are reused. The decrease in iron content with leaf age was reported by Adebiyi [36] in edible mushroom and Khader [6] in green leafy vegetables as they matured towards fruiting.

The high differences in iron content observed in cowpea leaves harvested at 21 and 49 DAS could have been caused by the low rate of iron absorption by the cowpea plant as it matured. Many plant parameters, such as the traits and behavior of a certain plant species or variety, have been observed to alter how certain chemical species in soil solutions are absorbed by plants, hence affecting the amounts of minerals that are absorbed from soils [37]. The iron content in cowpea leaves was high when harvested at 21 and 35 DAS than when the plant approached reproductive and senescence phase According to research done by [38] on *Cleome gynandra, Amaranthus cruentus and Solanum villosum* at various stages of maturity, the iron concentration in *A. cruetus* increased dramatically after sowing. The rising pattern of iron suggested that it might be in a dissociable ion and accumulates with aging [39].

According to a study by Trivellini [32] on the spatial and temporal distribution of carbohydrates and mineral nutrients during the course of a *Hibiscus rosa-sinensis L*. flower's life. At the senescence stage, the iron concentration increased in the petals of open flowers and remained constant. A period of nutrient remobilization was implicated in the extremely quick senescence process of ephemeral flowers. Iron is kept in the flower petals during senescence even though it is much less concentrated in the petals than it is in the leaves. The enzyme ACC oxidase, which requires iron as a cofactor, autocatalyzes the ethylene burst that the petals of plants emit when they reach senescence [32]. The retention of Fe by the petals during senescence may be necessary for ethylene generation.

During trial one which was the dry season, there was a higher iron concentration at 21 and 35 DAS than in trial two which was done during the rainy season. This could have been caused by high accumulation of the iron content during the dry season. The low contents during trial two could be due to increased dilution of the iron as a result of the increased rain. This finding is consistent with the results of Mondol [40] who reported an accumulation of iron contents in water and plant samples during the dry season compared to the wet season. The low iron content in trial one at 49 DAS, compared to trial two could have been due to increased remobilization and redistribution of iron to form flowers.

### *3.4. Calcium Content*

In both trials, harvesting at 21 differed significant ($p \leq 0.05$) with harvesting at 35 and 49 DAS in the amount of calcium content retained, however, harvesting at 35 and 49 DAS did not significantly ($p \leq 0.05$) differ in the calcium content (Table 4). Trial two recorded a higher calcium content than trial one (Table 4). The calcium content in cowpea leaves decreased as the plant senescence. This could have been attributed to the immobile nature of calcium and therefore calcium accumulated in the plant leaves. Due to the monocarpic nature of the cowpea plant, it passes through a predetermined cycle of organ deterioration and senescence [41]. Hence, the nutrients in the cowpea plant are remobilized to the reproductive organs or seeds before the whole plant degraded [42]. In the flower organs of numerous species, Okoli [43] discovered that calcium is present in large amounts as calcium oxalate crystals.

***Table 4.*** *Effect of harvesting stage on calcium content in grams (g).*

| Days after Sowing (DAS) | Trial One | Trial Two |
|---|---|---|
| 21 | 8837.06[a]* | 10007[a] |
| 35 | 6577.26[b] | 6873.22[b] |
| 49 | 5981.8[b] | 6093.48[b] |
| LSD | 1611.8 | 1059.8 |
| CV | 21.61. | 22.86 |

* Means with different letters along the column are significantly different at $p \leq 0.05$. LSD is Least Significant Difference; CV is Coefficient of Variance.

According to research by Trivellini [32] on the developmental stages of *Hibiscus rosa-sinensis L*. flowers, the ca concentrations in gynoecium tissues ranged from 0.14 to 0.25 mg per organ, while the concentration in petals was 1-fold greater than that of other tissues, at 0.4 mg per organ. This demonstrates the crucial function of calcium during flower development. Free calcium ions in the ovaries have a crucial role in pollen tube production, which suggests the notion that they help pollen tube growth or inhibit rejection reactions that may occur during incompatibility. It also serves as a component of the cell wall [44].

The resultant effect of calcium mobilization for floral development is low ca concentration in other plant tissues such as leaves as observed in cowpea leaves. Other studies have showed that a reduction in the transpiration rate of the leaves resulted in decline in store of all the mineral elements in leaves contrary to higher mineral content in fruit [45]. The decreasing trend in ca content in cowpea leaves harvested at 21 and 49 DAS can be as a result of nutrient mobilization and remobilization. The phloem continues to function during petal senescence and exports nutrients from the senescing petals [46]. A number of cations, such as potassium, magnesium and calcium, are loaded into the phloem for export during petal senescence. Remobilization of phosphorus (P) results in the formation of an inorganic anion [47].

Trial two recorded a higher calcium content compared to trial one (Table 4). This could have been due to increased uptake by the plant as a result of increased rainfall since calcium is an immobile nutrient. This finding however contradicts [48] who reported the mean calcium content in *Pennisetum pedicellatum* to be less in the rainy season and more in the dry season. Cool temperatures, cloudy weather and rain have been reported to create a high humidity situation that reduces transpiration and consequently reduces calcium uptake.

### *3.5. Protein Content*

Harvesting stage significantly ($p \leq 0.05$) differed in protein content in both trials (Table 5). The highest protein content was recorded when cowpeas leaves were harvested at 21 DAS at and least when harvested at 49 DAS in both trials. Trial two recorded low protein content compared to trial one (Table 5). Protein content was high during the early stages of harvesting at 21 and 35 DAS in both trials. This could be as a result of the high moisture content in the cowpea leaves. Low protein content was observed when cowpea leaves were harvested at 49 DAS in both trials. The decrease in protein content with the stage of harvesting from 21 to 49 DAS is consistent with Oduntan [29], who observed that the crude protein content of Sesanum radiatum leaves increased from the early to mid-stages of growth and decreased during the late stages of growth. This finding was also in agreement with Khawas [49], who observed that the Culinary *Musa ABB* variety plants', protein content reduced from stage one to stage five as they matured and at various developmental phases of inflorescence. The amount of crude protein in plants is influenced by the physiological age of the plant tissue. Therefore, since crude protein is needed to synthesize non-essential amino acids, the observed pattern in crude protein concentration highlighted the need of harvesting leaves at the early growth stages [50].

***Table 5.*** *Effect of harvesting stage on protein in percentage (%).*

| Days after Sowing (DAS) | Trial One | Trial Two |
|---|---|---|
| 21 | 29.41[a]* | 28.47[a] |
| 35 | 28.59[b] | 26.26[b] |
| 49 | 27.52[c] | 24.07[c] |
| LSD | 0.618 | 0.956 |
| CV | 3.2389 | 5.4142 |

* Means with different letters along the column are significantly different at $p \leq 0.05$. LSD is Least Significant Difference; CV is Coefficient of Variance.

The low protein content could have been due to utilization of stored proteins as the cowpea leaves matured. This explains the reduced overall protein content at each harvesting stage. The three harvesting stages can be potential sources of crude protein; however, the early-collected leaves (21 & 35 DAS) have the most potential to do so in comparison to the leaves that were harvested at 49 DAS. According to reports, as leaves mature, the samples' protein levels gradually decline, as shown by the amounts of protein [51]. The observed decrease was caused by alterations in the rates of protein production during leaf maturity. In comparison to their older leaves, Pal [51] reported that the rate of protein synthesis was at its highest in young leaves. This observation was in agreement with Price [52], who also reported that the amount of protein breakdown increased significantly as leaves aged. This study compared the senescence of leaves and petal senescence in wallflowers. In contrast to the 65% protein retention in senescent petals, the senescent leaves only kept 5% of their maximal protein levels.

Thomas [53] reported that this decline in protein content was concurrent with the deterioration of the chlorophyll, given that the degradation of chloroplast proteins caused the majority of the remobilization to occur in leaves. Studies by Zhao [54] reported that crude protein in forage rye decreased continuously with progressed maturity until dough development. Inherent soil fertility and or nutrient application especially high in nitrogen play a key role in protein content in plant as it is a constituent of amino acid. The increase in yield and increased protein content are due to the fact that nitrogen (N) is a critical nutrient that crops require for growth [55]. Due to the increased production of structural carbohydrates as plants grew older, total nitrogen concentration declines with plant growth [56]. Similar findings were reported in wild soyabean by Zhai [57]. This explains the reason for the lowest protein content in the cowpea leaves harvested 49 DAS. This is because the amount of protein content in cowpeas toward senescence reduces considerably.

Protein content was higher in trial one compared to trial two (Table 5). This was attributed to seasonal variations experienced in the two trials. In their assessment of the potential nutritional value of frequently occurring grasses collected in native pasture throughout the dry and rainy seasons. Evitayani [58] observed that the dry season had higher protein content than the rainy season. These results however contradict those of Sangu [59] who by comparing the seasonal differences in the fodder quality of the seven most grazed and four most browsed plant species, the wet season's forage quality, protein content was found to be higher than the dry season.

### *3.6. Beta Carotene*

Harvesting at 21 and 35 DAS did not show any significant ($p \leq 0.05$) difference on beta carotene content in both trials however, harvesting at 49 DAS, differed significantly ($p \leq 0.05$) from harvesting at 21 and 35 DAS in both trials. Harvesting at 49 DAS recorded the least and at 21 DAS the highest beta carotene content in both trials**.** Beta carotene content was higher in trial two compared to trial one (Table 6).

***Table 6.*** *Effect of harvesting stage on beta carotene in milligrams (mg).*

| Days after sowing (DAS) | Trial One | Trial Two |
|---|---|---|
| 21 | 10.81[a]* | 18.96[a] |
| 35 | 9.86[a] | 17.19[a] |
| 49 | 7.61[b] | 10.51[b] |
| LSD | 1.1656 | 2.7056 |
| CV | 2.98 | 2.58 |

* Means with different letters along the column are significantly different at $p \leq 0.05$. LSD is Least Significant Difference; CV is Coefficient of Variance.

The high beta carotene content recorded in cowpea leaves harvested at 21 DAS and low at 49 DAS in both trials was attributed to the cowpea leaves approaching senescence. Most cells and tissues begin to degenerate with age as the leaves begin to senesce. Biesiada [60] reported a decrease in vitamin levels in leek as the stage of growth increased. Adegbaju [12] also reported a decline in vitamin content of *Celosia argentea* as it approached maturity. Studies by Laya [61] reported the beta carotene of edible cassava leaves of variety *AD* to be highest at 6 months after planting as compared to other variety samples. Further findings showed that beta-carotene content reduced in all samples at 9 months after planting.

The high beta carotene reported in trial two could have been due to lower temperatures experienced due to the rainy season which lowered the synthesis rate of the beta carotene resulting in a higher beta carotene. Findings by [61] also reported a reduction in beta carotene content during the main dry season. A significant reduction in carotenoids with drought stress on African eggplant accessions was also reported by Mibei [62].

### *3.7. Crude Fibre*

In this study there were significant ($p \leq 0.05$) differences in the crude fibre content at different harvesting stages. In both trial one and two all the three harvesting stages differed significantly ($p \leq 0.05$) and the highest crude fiber was recorded at 49 DAS followed by 35 DAS and the lowest was at 21 DAS. When the crude fibre content was compared before trials trial one had a higher content compared to trial one (Table 7).

***Table 7.*** *Effect of harvesting stage on crude fiber in milligrams (mg).*

| Days after sowing (DAS) | Trial One | Trial Two |
|---|---|---|
| 21 | 2.21[c]* | 2.53[c] |
| 35 | 5.33[b] | 4.34[b] |
| 49 | 8.02[a] | 5.83[a] |
| LSD | 0.9322 | 0.4804 |
| CV | 2.67 | 2.43 |

*Means with different letters along the column are significantly different at $p \leq 0.05$. LSD is Least Significant Difference; CV is Coefficient of Variance.

The results show that as the cowpea leaves matured the concentration of crude fiber became more fibrous. The fiber content increase in crude fibre from harvesting at 21 DAS to 49 DAS, in cowpea leaves could have been caused by the decrease in moisture content as the plant matured. Studies by Oduntan [29] reported that crude fiber content in *Sesamum radiatum* Schum leaves increased from 1% in the 4$^{th}$ week after planting to 1.84% in the 10$^{th}$ week. Other studies by Adelanwa [63] reported crude fiber content in Cabbage at ten weeks being close to that of *Solanum americanum* with fiber content of 13.79%.

Crude fiber consists of cellulose, hemicelluloses and lignin which form structural framework of plant tissues therefore as plant matures it accumulates more crude fiber for tissue support [64]. Gurjeet [65] established that crude fiber increased in *Avena sativa* as plants matured. The results further showed that increased application of nitrogenous fertilizer reduces considerably the amount of crude fiber content thus increasing digestibility of the plants. This may be because nitrogen treatment reduced the quantity of pectin, cellulose, and hemicellulose, which are important components of fiber, while increasing nitrogen uptake, which is a component of amino acids and protein. The increase in crude fibre content with increasing age of maturity was also reported byKökten [66] and Oduntan [29]. However, these results are contrary to Bamishaiye [67] findings that no significant differences were observed in the crude fiber content of *Moringa oleifera* leaves at the three phases of growth.

The percentage of crude fiber is correlated with plant wetness. Higher water levels in a plant correspond to decreased crude fiber content. When a plant ages, it becomes more fibrous, as shown by the rise in crude fiber content, which results in an increase in the amount of fiber present [50]. Trial, one had a higher fibre content when cowpea leaves were harvested at 35 and 49 DAS compared to trial two.

# 4. Conclusion

Following the findings of this study, there were marked differences in nutrient content of cowpea leaves at the different stages of harvesting. Variation of proteins, beta-carotene, crude fibre, iron, calcium across the three harvesting stages shows the essence of harvesting vegetables at 21 and at 35 days for maximum nutrient utilization This suggests that consuming cowpea leaves at various stages of growth would be the best way to get all the important nutrients and minerals in adequate amounts needed to maintain normal body functions.

# 5. Recommendations

Cowpea leaves for consumption as vegetables should be harvested at 21 and at 35 days in order to successfully maximize on the nutrient's availability and palatability.

# Competing Interests

The authors have not declared any competing interests.

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