

The Effect of Silver Nanoparticles on Growth, Bioactive Compounds, Antioxidants, Scanning Electron Microscope and Functional Properties on Germination of Cowpea Beans (*Vigna unguiculata* (L.) Walp)

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Abstract

The motive of the study was to find the effect of various concentrations of silver nanoparticles (20, 40, 60, 80, and 100 ppm) on the germination of cowpea beans. The process involved applying silver nanoparticles to cowpea beans for 2 h followed by germination process for 5-days via jute bag. The results demonstrated the concentration of 60 ppm exhibited the longest root length and highest percentage of germination in contrast to the other various samples. moisture content of germinated cowpea treated with 100 ppm silver nanoparticles exhibited 17.32% whereas both non-germinated cowpea flour (C) and germinated cowpea flour (C0) beans had showed a reduction in of ash content and fat content in comparison with other remaining samples. The fiber content of cowpea beans increased up to 80 ppm but decreased to 100 ppm. Moreover, the microstructural investigation of germinated cowpea beans was conducted using scanning electron microscope (SEM). The addition of 60 ppm silver nanoparticles resulted in the highest total phenolic content (148.51 mg GAE/100 g), flavonoids (452.52 mg catechin/100 g) and ferric reducing antioxidant power (FRAP) (9.27 mmol Fe(II) Eq/g). The antioxidant profile was the highest in germinated cowpea beans treated with 80 ppm (85%). Functional properties of germinated sample treated with 60 ppm of silver nanoparticles resulted in improved fat absorption capacity (FAC), Carr's index and Hausner ratio. Thus, the study revealed that the incorporation of silver nanoparticles into cowpea beans at a concentration of 60 ppm significantly enhanced germination, antioxidant activity, total phenolic content (TPC), germination percentage as compared to other samples.

Keywords

Cowpea beans, Germination, Silver nanoparticles, Bioactive compounds, Functional properties, Scanning electron microscope

Introduction

The cowpea (*Vigna unguiculata* (L.) Walp) is a leguminous plant crop that is the most nutritious, adaptive, and versatile species. This species is an important component of dry-land productivity and a vital sustenance source. Cowpea is referred to as black eyed pea [1], herbaceous Latin America [2]. It is consumed globally as a high-quality plant protein source and has low fat content as well as favorable amino acid sequence to grain in short it is a vital source of nutrients [3]. It contributes to soil enrichment by means of nutrient recycling via fixation of nitrogen in conjunction with modulating microorganisms [4]. Furthermore, cowpea possess certain quantities of anti-nutritional elements that must be eliminated or reduced to enhance their nutritional quality and sensory attractiveness [3].

The utilization of cowpea in daily diet significantly limited by several factors including deficiency of certain phenolic compounds, such as haemagglutinins,

dihydroxyphenylalanine, oxalic acid, cyanogenic glucosides, proanthocyanidins, phytic acid, tannins, and saponins that are also known to be nutrient-wise unfavorable to human beings because of their tendency to bind proteins [3].

Germination of cowpea is an efficient method to reduce anti-nutrients [1]. It involves soaking the seeds in desired solution for an extended period typically for 6 - 12 h [5]. During the process of seed germination, numerous metabolic processes occur within the seed that lead to physicochemical transformations that convert stored carbohydrates and proteins into simpler compounds that provide nourishment for the developing embryo. Germination increases carbohydrates and protein digestibility, improves vitamin bioavailability, increases antioxidants, decreases anti-nutritional factors. Previously mentioned variations are conditioned upon germination parameters including temperature, time of germination, light, and humidity [6]. Germination is a biological essential process that induces advantageous physiological transformations in cowpea beans, thereby increasing the nutritional content of protein through increases in digestibility and free amino acids content [7].

From recent years production of nanoparticles is increased and producing as it's an emerging technology that may play a vital role to enhance the components of food. Nanoparticles has the properties to investigate their effect on germination and efficiency of food components [8]. Silver nanoparticles are recognized as a highly effective antibacterial agent capable of effectively combating bacterial infections both in laboratory settings (*in vitro*) and in living organisms (*in vivo*). The antimicrobial efficacy of silver nanoparticles encompasses both Gram-negative and Gram-positive bacteria, as well as strain resistance multiple drugs [9]. Silver nanoparticles exhibit antifungal properties, making them valuable in agriculture for safeguarding plants from fungal infections. The research findings provide additional evidence to support the efficacy of these compounds as anti-phytopathogenic substances since they have been shown to exert a useful impact on the growth, also on the developmental processes of plants grown in cultivation. The presence of silver nanoparticles is an enhancement in the germination of seed parameters and growth of plants [10].

Thus, the aim of this study is to enquire about the impact of various concentration of silver nanoparticles in sprouting of cowpea beans. This research identified the best concentration of silver nanoparticles based on proximate concentration, color profile, antioxidant activity, TPC, flavonoids, functional properties and to investigate the morphological study of cowpea beans treated with different concentration (20, 40, 60, 80, and 100 ppm).

Materials and Methods

Method for the preparation of silver suspension

Sigma Aldrich Inc: a company based in the United States provided the silver nanoparticles in powder form with an appropriate size under 100 nm. These nanoparticles were stabilized using polyvinyl pyrrolidone. Silver nanoparticles were

dispersed into water that was distilled by using a sonication method to mitigate agglomeration. Several approaches were employed in the characterization of silver nanoparticles for the present investigation. Impact of silver nanoparticles on the seed germination was managed by using various concentration such as 0 (control), 100, 200, 500, 1000, 2000, and 4,000 mgL⁻¹) [11].

Method for germination

Cowpea seeds underneath sterilization through treated with a 4% solution of sodium hypochlorite then rinse with water that was double distilled. Hundred grams of cowpea beans were soaked in silver nanoparticles solution at different concentrations such as 0 (control), 100, 200, 500, 1000, 2000, and 4000 mgL⁻¹). After two hours of soaking, beans were spread on jute bag for five days at 25 ± 2 °C at room temperature. After 5 days, germination was observed. The root lengths and percentages of germination of sprouted beans were evaluated. For further analysis, these beans were collected, dried, and milled. Flour samples were sealed in polyethylene bags for further evaluation.

Determination of proximate composition of sprouted cowpea beans treated with silver nanoparticles

The proximate nutrient analysis of the raw samples was conducted using established protocols for determining fiber (protocol no. 32-10), fat (protocol no. 920.85), moisture (protocol no. 925.10), protein (protocol no. 920.87) and crude ash (protocol no. 923.03) [12].

Determination of color profile of sprouted cowpea beans treated with silver nanoparticles

The color profile was determined through the utilization of a colorimeter, namely the Hunter lab color quest colorimeter. The variables a* (indicating greenness/redness), L* (representing darkness/lightness), and b* (denoting blueness/yellowness) were employed to define the color profile [13].

Determination of bioactive compounds of sprouted cowpea beans treated with silver nanoparticles

Preparation of extract for determining the bioactive compounds

Ten-gram cowpea seeds flour was added to 100 ml of 100% v/v ethanol. Utilizing the orbital shaker, mixture was homogenized for a period of 8 h. The extract was distinguished through filtration with the use of Whatman no.1 filter paper. Residual solvent of ethanolic extract was removed with the help of rotary vacuum evaporator (specifically, the EYELA N.N. series) at 40 °C temperature at reduced pressure. Following the process, collected extract was utilized for determining bioactive compounds [14].

Determination of free radical scavenging activity via DPPH

A precise quantity of 2.9 ml extract was dissolved in 0.1 ml of 0.0 mM DPPH solution which was left in darkness for 30 min at room temperature of 23 °C. after incubation period absorbance at 517 nm by spectrophotometer (UV-Vis 3000, ORI, Germany) the sample was filtered after 30 min. To produce the control solution 2.9 ml DPPH solution was com-

combined with 0.1 ml of methanol [15]. The activity of DPPH was determined as:

$$DPPH\% = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

Determination of TPC

The precise quantity of extract of sample 1 ml dissolved in 1 ml of sodium carbonate solution (7.5%) and 1 ml of Folin-Ciocalteus reagent. After allowing the solution to stand for 30 min, the absorbance at 765 nm was determined using a spectrophotometer (UV-Vis 3000, ORI, Germany) which is expressed as mg GAE/g [16].

Determination of flavanol content

A precise quantity of 200 μ l sample is mixed with 2000 μ l of AlCl_3 (2% w/v) solution and 3000 μ l (50 g/L) of sodium acetic acid solution. After vigorously shaking the solution, it was left to rest at 20 °C for 2.5 min. After measuring absorbance at 440 nm against a blank solution following the stay time. This was expressed as mg of quercetin equivalents (QE) per gram of dried weight (mg QE/g extract) [17].

Determination of total flavonoids content

A precise quantity of 1 ml sample extract was dissolved in a solution containing 5.6 ml of distilled water, 0.2 ml of AlCl_3 (10% solution prepared in methanol), and 0.2 ml of potassium acetate. The mixture was allowed to stand for 30 min at room temperature. A spectrophotometer (UV-Vis 3000, ORI, Germany) was used to detect absorbance at 415 nm. For preparation of solution quercetin ranging from 0 to 500 μ l dissolved in ethanol [18].

Determination of FRAP

The analysis of FRAP was conducted using the methodology described by the method of Sutharut and Sudarat [19]. The FRAP reagent was formulated by combining 10 mm TPTZ in 40 mm HCl, 0.3 M acetate buffer (PH 3.6), and 20 mm FeCl_3 in a volumetric ratio 1:10:1, a volume of 2 μ l of the sample was combined with 1.3 ml of FRAP reagent. The mixture was then kept at a temperature of 37 °C for 30 min. The measurement of absorption was conducted at a 595 nm wavelength (UV-Vis spectrophotometer). The FRAP levels were quantified as millimoles of Fe(II) equivalent per gram of flour.

Determination of functional properties of sprouted cowpea beans treated with silver nanoparticles

Water solubility index (WSI) and water absorption capacity (WAC)

Two grams of sample were collected in pre-weighed centrifuge tubes. 20 ml of distilled water was added to the sample. The suspension was maintained for 2 h at room temperature with occasional stirring followed by centrifugation at a speed of 3000 rpm for a time of 10 min. The liquid portion was delicately transferred into a Petri dish that had been previously weighed and it was retained for the purpose of determining the WAC [20]. The weight of the sediment in its wet state was measured. The WAC was calculated by following equation:

$$WAC \text{ (g/g)} = \frac{\text{wet sediment, s weight (g)}}{\text{weight of dry flour (g)}}$$

Supernatant that was used to figure out the WAC was dried at 105 °C overnight and then weighted [20]. The WSI was calculated by following equation:

$$WSI (\%) = \frac{\text{weight of dry supernatant (g)}}{\text{dry weight of flour (g)}} \times 100$$

Oil absorption capacity (OAC)

2.5 g of sample was combined with the 25 ml ground nut oil in a centrifuge tube that had been pre-weighed. After one min of stirring, the mixture underwent centrifugation at 4000 rpm for twenty min. Subsequently, the surplus oil was decanted, and tube's contents were weighed [20]. The oil absorption capacity was determined by following equation:

$$OAC \text{ (g/g)} = \frac{\text{weight of oil absorbed (g)}}{\text{weight of sample (g)}}$$

Tapped density and bulk density

The determination of bulk density (ρ_b) for the cowpea flour powder samples involved filling a pre-weighed 10 ml of graduated cylinder (with a least count of 0.5 ml) of known volume (V_o). The volume and weight of the contents were then recorded. The expression of bulk density was in units of kilograms per cubic meter (kg/m^3) [20]. Bulk density is calculated by following equation:

$$\text{Bulk density} = \frac{M}{V_o}$$

The tapped volume (V_f) was determined by tapping the measuring cylinder twenty times on a flat tabletop surface while containing a known amount of sample [20]. Tap density calculated as following:

$$\text{Tap density} = \frac{M}{V_f}$$

Swelling index (SI) and swelling capacity (SC)

The sample was added to 100 ml pre-weighed measuring cylinder until it reached the 10 ml mark. The weight of sample was obtained by weighing the cylinder. Using a vortex mixer, distilled water was added to the sample up to 50 ml mark and thoroughly mixed to ensure homogeneity. For 3 h, the mixture had been left to stand. The SI was measured by using following equation:

$$SI = \frac{\text{volume of soaked sample} - \text{volume of sample prior to soaking}}{\text{weight of sample}}$$

Hydrated sediment that was collected during the determination of the SI was utilized in the calculation of the SC with using following equation:

$$SC (\%) = \frac{\text{weight of wet sediment (g)}}{\text{weight of sample}} \times 100$$

Determination of foaming capacity (FC)

The FC was determined as 1 g sample placed in 250 ml beaker and added 50 ml distilled water. The mixture was agitated for 1 min using household blender. The content was then promptly transferred to a graduated measuring cylinder with 100 ml volume [20]. The recorded volume of foam in milliliters was documented, subsequently FC of sample was calculated.

$$FC = \frac{\text{volume of foam after whipping} - \text{volume of foam before whipping}}{\text{volume of foam before whipping}} \times 100$$

Determination of morphological study of sprouted cowpea beans treated with silver nanoparticles

SEM were employed to inspect the surface morphology and structural composition of cowpea legumes treated with silver nanoparticles (Musashino, Tokyo Metropolitan, JEOL, Japan).

Statistical analysis

The statistics presented in all tables are the mean values obtained from three separate analyses. Significant distinctions for multiple comparisons were established using one way analysis of variance following the Duncan test by use of SPSS 16.0 statistics software. The result was reported as mean \pm SD. The result was considered statistically significant with a $p < 0.05$.

Results and Discussion

Effect of silver nanoparticles on percentage germination and root length of cowpea bean

Percentage germination of cowpea bean

The process of seed germination is typically regarded as the pivotal stage in the establishment of seedlings as it plays a crucial role in finding the outcome of crop production. The process of seed germination is a multifaceted phenomenon that is inclined by various genetic and environmental factors including salinity, light, and temperature [21]. Nowadays, the use of nanomaterials to enhance the efficacy of micronutrients has emerged as a novel strategy to augment seedling vigor and promote the germination percentage of seeds. The utilization of nano-emulsion holds considerable importance in enhancing the nutritional components within plants, a field commonly referred to as agricultural nanotechnology [22]. Research conducted on the germination of cowpea showed that impact of silver nanoparticles in figure 1a. The results demonstrated the percentage of germination of cowpea legumes treated with silver nanoparticles at different concentrations as compared to the control group (distilled water). germination percentage exhibited a generally significant increase following 2 h immersion in various concentrations of silver nanoparticles, beginning at low concentrations (upto 60 ppm) and subsequently declining as the concentration increased. Positive outcomes were observed when seedlings were submerged in solutions containing 60 ppm silver nanoparticles, germination percentage exhibited an improvement in contrast to the control group. However, the highest concentration (100 ppm) and least concentration (20 ppm) negatively on germination power to 79.85% and 80.66% as compared to alternative treatment.

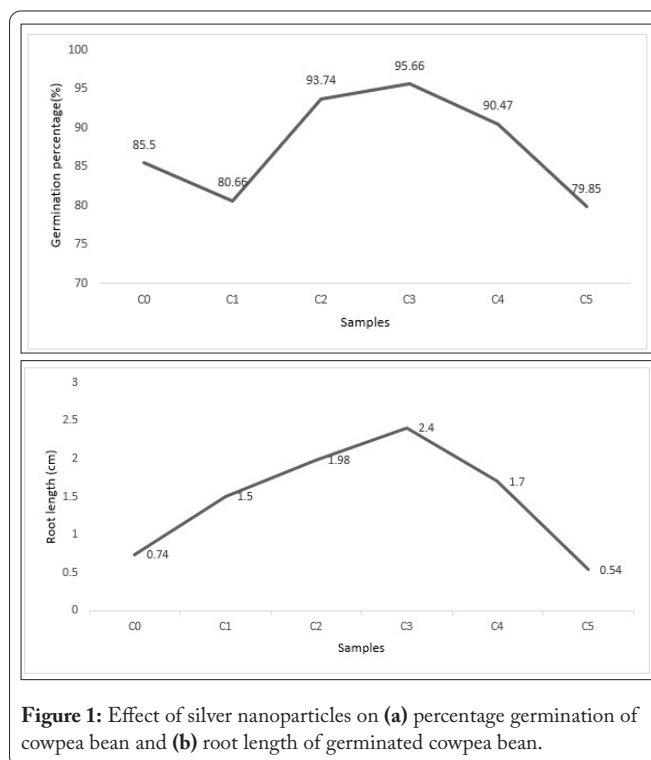


Figure 1: Effect of silver nanoparticles on (a) percentage germination of cowpea bean and (b) root length of germinated cowpea bean.

Root length of germinated cowpea beans

The experimental results indicated that cowpea treated with silver nanoparticles with a concentration of 60 ppm resulted in enhance the length of radicals. Conversely, the highest concentration of 100 ppm was shown to cause a reduction in radical length. In this regard, the optimal performance was achieved at a concentration of 60 ppm, resulting in the most substantial and statistically significant increase in the length of seedling radicals (2.4 cm) compared to the control group. Moreover, it has been observed that silver nanoparticles exhibited enhanced efficacy in promoting the growth parameters of cowpea beans when used at lower concentrations.as shown in figure 1b.

The effect of silver nanoparticles on proximate composition

The proximate composition of raw, sprouted, and silver nanoparticles treated cowpea beans is shown in table 1. The results obtained on the nutritional structure of cowpea beans showed a significant ($p < 0.05$) impact on moisture, content of protein, ash and fiber which increased from 11.06 to 17.32%, 13.32 to 17.15%, 3.17 to 2.40% and 23.25 to 50.70% respectively. Study also showed statistically significant decrease ($p < 0.05$) in the percentage of carbohydrate and fat content from 46.36 to 15.04% and 2.02 to 1.63% correspondingly by increase the concentrations of silver nanoparticles during the processes of germination of cowpea beans. In contrast, control cowpea beans observed decreased proportion in ash (4.04%), moisture (11.08%), fat (2.11%), fiber (22.12%), carbohydrate (45.21%) and protein (12.32%). The uppermost ash, crude fiber, and protein contents were detected after the exposure to 60 ppm silver nanoparticles wherein relatively carbohydrate and fat percentage was significant ($p < 0.05$) lower in other silver nanoparticles-treated germinated cowpea beans. During the method of germination, enzymes break down the fibers,

Table 1: Effect of silver nanoparticles on proximate composition of germinated cowpea beans^a.

Sample	Moisture content (%)	Ash (%)	Fat (%)	Protein (%)	Crude fiber (%)	Carbohydrate (%)
C	11.18 ± 0.06 ^a	4.04 ± 0.05 ^c	2.11 ± 0.02 ^c	12.32 ± 0.20 ^a	22.12 ± 0.57 ^a	45.21 ± 0.41 ^b
C0	11.06 ± 0.80 ^a	3.66 ± 0.25 ^d	1.96 ± 0.25 ^c	13.70 ± 0.48 ^b	23.25 ± 0.55 ^b	46.36 ± 1.04 ^d
C1	13.80 ± 0.66 ^b	3.17 ± 0.15 ^c	1.55 ± 0.20 ^a	13.69 ± 0.39 ^b	25.03 ± 0.02 ^c	42.73 ± 0.68 ^c
C2	14.37 ± 0.92 ^b	3.33 ± 0.11 ^c	2.02 ± 0.01 ^c	15.00 ± 0.76 ^c	29.07 ± 0.07 ^d	35.64 ± 1.45 ^a
C3	14.92 ± 0.59 ^b	2.79 ± 0.08 ^b	1.63 ± 0.02 ^{ab}	13.32 ± 0.30 ^b	38.67 ± 0.36 ^c	29.20 ± 0.89 ^a
C4	15.15 ± 1.23 ^b	2.82 ± 0.03 ^b	1.78 ± 0.05 ^b	14.48 ± 0.15 ^c	50.70 ± 1.10 ^e	15.04 ± 2.45 ^a
C5	17.32 ± 0.16 ^c	2.40 ± 0.02 ^a	1.67 ± 0.21 ^{ab}	17.15 ± 0.15 ^d	43.69 ± 0.64 ^f	17.74 ± 1.14 ^a

Note: ^aAll values are means of triplicate determinations. Means within a column with different superscripts are significantly different at $p < 0.05$. C: non-germinated cowpea flour; C0: germinated cowpea flour; C1: 20 ppm silver nanoparticles germinated cowpea flour; C2: 40 ppm silver nanoparticles germinated cowpea flour; C3: 60 ppm silver nanoparticles germinated cowpea flour; C4: 80 ppm silver nanoparticles germinated cowpea flour; C5: 100 ppm silver nanoparticles germinated cowpea flour.

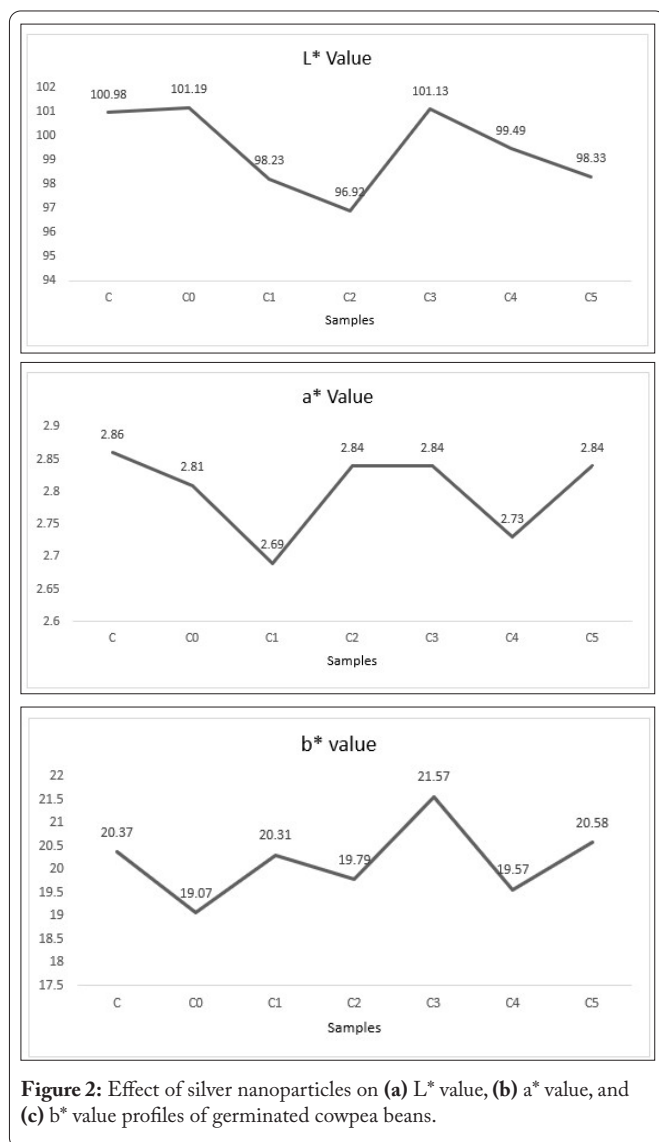
proteins, and carbohydrates into their constituent molecules [23]. The moisture content of cowpea bean considerably increased after 48 h of germination as compared to that of the raw seed flour. The moisture content is an essential characteristic for food samples [24]. The rise in protein levels could be attributed to the decrease in the dry matter content, specifically carbohydrates caused by respiration during germination [24]. Masood et al. [25] also stated that the weight of the plant material decreases, and the amount of protein increases during germination. Devisetti and Prakash [26] reported a comparable discovery for non-germinated samples where researchers observed non-germinated cowpea beans exhibited 2.14% fat, 4.21% ash and 21.66% fiber available. Previously research has reported the increment in protein content during germination could be linked to the modifications in the metabolic pathways within the seeds. The consumption of carbohydrates and later release of carbon dioxide and water has resulted in a slight decrease in the amount of solid material, hence increasing the proportion of protein in relation to the dry matter (protein percentage based on the absence of water [27]). Carbohydrates are vital compounds for all living creatures and constitute the most common biological substances. Solanki et al. [28] stated that fatty acids undergo oxidation to generate carbon dioxide and water, which in turn produces energy for the synthesis of various structural components in young seedlings. Previous studies demonstrated the increase in moisture levels could enhance the process of germination of seed and the emergence of many types of beans and other crops [2]. Boukar et al. [2] stated the presence of a hydrophilic polymer seed coating had a negative impact on the sprouting and development of cowpea beans in fields. Cowpea has a high protein quality due to its abundance of essential amino acids. The ratio of non-essential and essential amino acids is 1.03 and 0.99, respectively. This makes cowpea an affordable and concentrated source of proteins with essential amino acids [26]. Röhlig and Engel [29] stated that germination enhances the nutritional value of protein by enzymatically breaking it down into smaller pieces through the action of proteases. Fiber contributes to reducing the time it takes foods to pass through the digestive tract, promoting a healthy and varied population of gut bacteria, lowering overall blood cholesterol levels, and decreasing blood glucose levels after meals [30].

The effect of silver nanoparticles on color profile of cowpea beans

The color profile of cowpea beans is well thought-out as significant quality parameters that increased the consumers acceptability and enhanced customer appeal. Silver nanoparticles results in a significantly ($p < 0.05$) effects on the color profile of cowpea beans with interrelated with non germinated cowpea beans. The L^* value of germinated cowpea beans significant ($p < 0.05$) lessened from 101.13 to 96.92 on silver nanoparticles exposure from 20 ppm to 100 ppm, respectively. The maximum luminosity level was detected at a concentration of 60 ppm of silver nanoparticles exposure that is 101.13. The observed decline in the L^* value of sprouted cowpea beans indicated a reduction in luminosity that could be related to the occurrence of silver nanoparticles and carotenoids in cowpea beans. However, a^* value of germinated cowpea beans non-significant ($p < 0.05$) increased from 2.69 to 2.84 on silver nanoparticles exposure on 20 ppm to 100 ppm. Where in b^* value of germinated cowpea significantly ($p < 0.05$) increased in 21.57 to 29.57 on treated with the silver nanoparticles ranges from 20 ppm to 100 ppm. Where the higher value of a^* and b^* were observed at 60 ppm silver nanoparticles that is 2.84 and 21.57, respectively, as shown in figure 2. Control germinated cowpea showed higher a^* value that is 2.86 were having lower L^* and b^* value 100.98 and 20.37, respectively. The change in L^* , a^* and b^* , that is hunter lab parameters during germination could be attributed to the migration of chromatic pigments from the outer layer of the grain to the inner endosperm as well as non-enzymatic browning [31].

The effect of silver nanoparticles on SEM analysis of germinated cowpea beans

As exposed in figure 3, the SEM was used to analyze the link between the cell wall microstructures and cell wall structure of sprouted cowpea beans under different treatment settings. The size of granules was ranging heterogeneously in 0 to 10 μm . The attention of this study was to evaluate the structural modifications in cotyledon cells of sprouted cowpea seeds specifically examining the cellular composition after germination of 2 days. The middle lamella separating single cells and cell walls was observable [32]. As the germination time

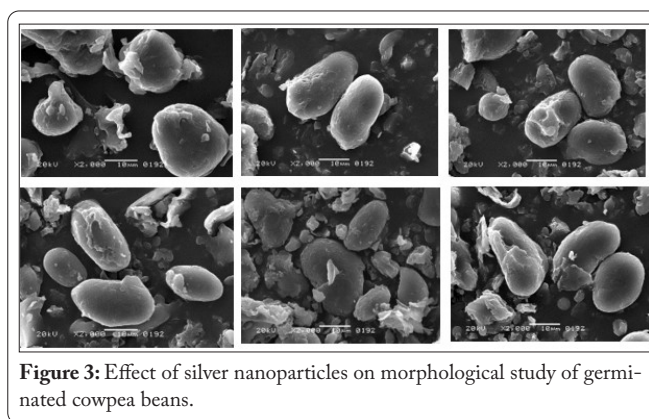


rose, the movement of endogenous enzymes also rose resulting in noticeable alterations in the microstructure. Nevertheless, both categories of starch granules had a uniform surface and were distinct from proteins with a few minor alterations that could be considered as subtle indications of amylolysis [32].

The effect of silver nanoparticles on bioactive compounds of germinated cowpea beans

TPC analysis

The outcomes showed a considerable improvement in phenolic compounds with an increase from 123.51 to 148.51 mg GAE/100 g upon the addition of silver nanoparticles. This improvement was statistically significantly ($p < 0.05$) as showed in table 2. A current study showed that TPC of sprouted cowpea beans is influenced by the quantity of silver nanoparticles and the situations under which they are sprouted [33]. A concentration-dependent increase in TPC was observed with the addition of silver nanoparticles. The highest TPC was noted in 60 ppm at 148.51 mg GAE/100 g. The lowest TPC was seen in germinated cowpea beans treated with a concentration of 100 ppm of silver nanoparticles. These variations observed may be related to the enhanced en-



zymatic activities linked with the oxidation of the phenolic compounds. During process of germination, enzymes break down carbohydrates, protein, and fiber into digestible forms. Hence, the difference could be attributed to the variability in germination situations, extraction methodology, the activation of nanoparticles, and bean cultivars. Our result for non-germinated cowpea beans showed 113.37 mg GAE/100 g.

Previous studies showed that due to preventing the bio accessibility of nutrients in legumes and cereals, phenolic compounds are generally seen as anti-nutrient. Though, phenolic compounds grip health encouraging capacity [34]. Significant variation was seen in the TPC across different cowpea varieties with a recorded value of 119.61 ± 2.48 mg GAE/100 g. In addition, whole cowpeas consist of around 30% bound phenolics and 70% free phenolics. The seed coat contains a TPC that is 5 to 10 times higher than seeds [3]. This decrease in TPC during germination contrasts with a few findings, who reported a rise in TPC in sprouted legumes. Glucose serves as the main starting point to produce phenolic compounds. Various crucial molecular signaling pathways, such as phenylpropanoid pathway, the oxidative shikimate pathway, pentose phosphate pathway, acetate/malonate pathway, hydrolysable tannin, and glycolysis pathway, participate in creation and conversion of diverse phenolic compounds. At beginning of germination, phenolic compounds that attached to cell wall complexes might be released because of the breakdown of their connections inside the cell walls such as proteins and carbohydrates [35].

Total flavonoid content

In the same way the addition of nanoparticles to sprouted cowpea beans resulted to a significantly ($p < 0.05$) rise in the concentration of total flavonoids contents in the flour made from germinated cowpea beans. In comparison with non-germinated cowpea beans, sprouted cowpea beans treated with silver nanoparticles exhibited a significant rise in flavonoids content ranging from 236.26 to 425.52 mg gallic acid/100 g. the flavonoid content in control cowpea (C0) was 236.26 ± 3.64 mg catechin/100 g, while the germinated cowpea samples (C) exhibited lower values (104.00 ± 2.00 to 153.55 ± 3.78 mg catechin/100 g). Flavonoid content demonstrated a varying trend with increasing concentrations of silver nanoparticles. The highest flavonoid content was observed in C3 (60 ppm) at $425.52 \pm$ mg catechin/100 g. Flavonoids were present in

Table 2: Effect of silver nanoparticles on bioactive compound of germinated cowpea beans^a.

Sample	TPC (mg GAE/100 g)	Flavonoids (mg catechin/100 g)	DPPH (%)	FRAP (mmol Fe(II) Eq/g)
C	113.37 ± 0.64 ^c	104.00 ± 2.00 ^a	47.42 ± 0.42 ^b	4.57 ± 0.27 ^a
C0	135.57 ± 0.26 ^c	236.26 ± 3.64 ^c	65.12 ± 4.48 ^d	7.57 ± 0.50 ^c
C1	109.79 ± 6.81 ^c	349.42 ± 4.44 ^d	46.65 ± 3.14 ^b	7.94 ± 0.46 ^c
C2	123.51 ± 1.45 ^d	387.56 ± 9.04 ^c	57.86 ± 2.70 ^c	8.81 ± 0.18 ^d
C3	148.51 ± 3.37 ^f	425.52 ± 8.65 ^e	75.40 ± 4.59 ^e	9.27 ± 0.22 ^d
C4	101.72 ± 0.50 ^b	402.10 ± 3.05 ^f	85.01 ± 4.51 ^f	7.38 ± 0.09 ^c
C5	83.72 ± 1.87 ^a	153.55 ± 3.78 ^b	35.73 ± 4.98 ^a	5.81 ± 0.59 ^b

Note: ^aAll values are means of triplicate determinations.

almost every plant. They constitute secondary metabolites that are mostly responsible for the pigmentation of leaves, flowers, and seeds, excluding chlorophyll. Flavonoids contained health advantages associated with their indirect and direct effects on oxidative stress and inflammation, as well as their interactions with different receptors and disease-preventing enzymes [36]. The total flavonoid content of bean fractions exhibited a consistent correlation with rises in melatonin, total phenols, and radicle length during the entire sprouting period [37].

Antioxidant activity

The impact of silver nanoparticles concentration on antioxidant activity of sprouted cowpea beans is exposed in **table 2**. The findings demonstrated that the presence of silver nanoparticles at different concentrations had significantly ($p < 0.05$) on the free radical scavenging activity of cowpea beans. DPPH scavenging activity in raw cowpea (C) was measured at 47.42%. This value serves as a baseline for the antioxidant potential of non-germinated cowpea. Sample with only germination exhibited 65.12% antioxidant activity indicating a notable increase compared to raw cowpea. As the concentration of silver nanoparticles increased from 20 ppm to 100 ppm a concentration-dependent trend was observed. Notably, 80 ppm displayed the highest DPPH activity at 85.01%, surpassing even the germination control. The overall increase in DPPH scavenging activity during germination and further enhancement with silver nanoparticles underscore the dynamic nature of antioxidant responses during plant development. The results suggested that silver nanoparticles especially at certain concentrations could contribute to the antioxidant potential of germinating cowpea noteworthy rise in DPPH radical scavenging observed when concentration of silver nanoparticles increase but rapid decrease in DPPH on 100 ppm. Previous studies show that the germination process leads to an increase in the percentage of DPPH inhibition indicating that the antioxidants present in sprouts become more effective in neutralizing free radicals. This highlights the important physiological significance of sprouts in reducing degenerative diseases by eliminating free radicals. These findings aligned with the work of Khyade and Jagtap [38] who reported enhanced antioxidant activity in plants and same result. **Table 2** showed the results of FRAP during the germination of cowpea with different concentrations of silver nanoparticles. The FRAP activity in raw cowpea (C) was measured at 4.57 mmol

Fe(II) Eq/g, providing a baseline for the antioxidant potential of non-germinated cowpea. The germination control (C0) exhibited a notable increase in FRAP activity at 7.57 mmol Fe(II) Eq/g, suggesting an enhancement in antioxidant potential during the sprouting process. As concentration of silver nanoparticles increases from 20 ppm (C1) to 100 ppm (C5), a concentration-dependent trend was observed. The highest FRAP activity was noted in C3 (60 ppm) at 9.27 mmol Fe(II) Eq/g. The concentration-dependent increase in FRAP activity with silver nanoparticles treatment suggests a positive relationship between nanoparticles concentration and antioxidant potential. The overall increase in FRAP activity during germination and further enhancement with silver nanoparticles highlights the dynamic nature of antioxidant responses during the early stages of plant development. The results suggest that silver nanoparticles, particularly at certain concentrations, can contribute to the enhancement of the antioxidant potential of germinating cowpea. Further investigations into the molecular mechanisms behind this observed effect and potential impacts on nutritional quality would provide valuable insights for agricultural and food science applications. The FRAP test revealed that the extracts obtained from germinated cowpea exhibited the highest ability to provide hydrogen and transfer electrons to facilitate the oxidation process [39]. The increased presence of antioxidants in germinated sample resulted in a greater reducing power as compared to the non-germinated sample [40].

The effect of silver nanoparticles concentration on functional properties of cowpea beans

Functional properties for raw, germinated, and silver nanoparticles treated cowpea beans are shown in **table 3**. The results obtained on the nutritional composition of germinated cowpea beans showed a significant ($p < 0.05$) impact on WSI, WAC (g/g), SC, SI(%), FAC, and FC which increased from 10.26 to 16.16 (g/g), 2.34 to 3.72 (g/g), 10.26 to 16.16 (g/g), and 3.82 to 4.43 (ml/g), respectively. The WAC of C0 is somewhat lower in contrast with C. The difference could result from alterations in the composition and structure of the flour during the germination process. The WAC is enhanced when silver nanoparticles are added during the germination period, increasing from 20 ppm to 100 ppm. There is a clear relationship between the amount of silver nanoparticles present and the level of water absorption where higher concentra-

Table 3: Effect of silver nanoparticles on hydration properties and FAC of germinated cowpea beans^a.

Samples	WAC (g/g)	WSI (g/g)	SC (ml/g)	SI (%)	FAC (g/g)	FC (ml/ 100 ml)
C	2.43 ± 0.01 ^b	8.50 ± 0.40 ^b	2.86 ± 10.65 ^a	2.34 ± 0.11 ^a	2.32 ± 0.10 ^{cd}	29.53 ± 0.56 ^a
C0	2.34 ± 0.01 ^a	11.10 ± 0.10 ^d	3.82 ± 0.57 ^b	3.39 ± 0.21 ^d	2.27 ± 0.02 ^{bc}	31.00 ± 1.00 ^{ab}
C1	3.4367 ± 0.02 ^d	10.26 ± 0.25 ^c	4.43 ± 1.08 ^f	2.63 ± 0.17 ^c	2.38 ± 0.01 ^{de}	52.00 ± 1.00 ^c
C2	3.2200 ± 0.02 ^c	11.10 ± 0.10 ^d	3.95 ± 1.23 ^c	3.18 ± 0.08 ^{cd}	1.77 ± 0.02 ^a	51.66 ± 2.08 ^c
C3	3.5800 ± 0.01 ^f	15.13 ± 0.15 ^e	4.23 ± 0.83 ^c	2.54 ± 0.09 ^{ab}	2.22 ± 0.02 ^b	41.00 ± 1.0 ^d
C4	3.5267 ± 0.02 ^c	16.16 ± 0.20 ^f	4.43 ± 0.97 ^f	3.04 ± 0.08 ^c	2.41 ± 0.02 ^c	37.33 ± 1.52 ^c
C5	3.7233 ± 0.01 ^g	8.10 ± 0.10 ^a	4.05 ± 1.23 ^d	3.27 ± .14 ^{cd}	2.26 ± 0.01 ^{bc}	32.00 ± 1.0 ^b

Note: ^aAll values are means of triplicate determinations.

tions of silver nanoparticles result in more water absorption. Among the samples treated with silver nanoparticles, 100 ppm silver nanoparticles C0 has the highest WAC i.e., 3.723 (g/g). The WSI results showed control germination resulted in a significant improvement in water solubility when compared to non-germination cowpea flour. These findings indicated that the sprouting process had a beneficial result on the WSI. Higher concentration water solubility was significantly higher ($p < 0.05$) at concentrations of 60 ppm (15.13 g/g) and 80 ppm (16.16 g/g) compared to lower values and the control group. The WSI reached its highest point at a concentration of 80 ppm silver nanoparticles suggesting that this concentration was suitable to achieve maximum solubility. Significantly, the WSI of 100 ppm silver nanoparticles that is 8.10 g/g was found to be lower indicating a possible saturation or negative impact at higher doses. The findings demonstrated that had significantly ($p < 0.05$) on the results on swelling capacity (ml/g). The swelling capacity of controlled germinated cowpea increased as compared to C that suggested that the germination process positively influenced the ability of the flour to absorb water. The greatest swelling capacity was observed at a concentration of 80 ppm of silver nanoparticles that is 4.43 ml/g. The SI of cowpea flour is much higher in the control group when it is germinated (3.39%), compared to C (2.34). Among the germinated samples there is a trend of increasing SI with higher concentrations of silver nanoparticles silver nanoparticles, peaking at 100 ppm (3.27%). The findings demonstrated that had significantly ($p < 0.05$) on the results on FAC (g/g) that when we compare C to controlled C0, C0 is slightly better at absorbing fat (2.27) than C (2.32). This suggested that germination may influence the fat absorption properties of cowpea flour. At 80 ppm silver nanoparticles, the capacity for fat absorption is reduced by 2.41, suggesting that higher nanoparticles concentrations may have an adverse effect on fat absorption due to saturation. Cowpea flour that has been germinated with silver nanoparticles produces greater FC than C. 20 ppm and 40 ppm show the highest FC, while 100 ppm has the lowest among the nanoparticles treatments. These findings suggested that the presence and concentration of silver nanoparticles impact FC.

The effect of silver nanoparticles various concentration on flowing properties of cowpea

Bulk density, tap density, Hausner ratio and Carr's index

Table 4: Effect of silver nanoparticles on flow properties of germinated cowpea beans^a.

Sample	Tap density (g/cm ³)	Bulk density (g/cm ³)	Carr's index	Hausner ratio
C	0.74 ± 0.03 ^a	0.55 ± 0.01 ^a	25.81 ± 2.89 ^b	1.34 ± 0.05 ^b
C0	0.80 ± 0.01 ^b	0.64 ± 0.01 ^c	20.00 ± 0.25 ^a	1.25 ± 0.00 ^a
C1	0.82 ± 0.01 ^b	0.61 ± 0.01 ^b	25.60 ± 1.10 ^b	1.34 ± 0.02 ^b
C2	0.91 ± 0.005 ^d	0.72 ± 0.01 ^d	21.13 ± 1.17 ^a	1.27 ± 0.01 ^a
C3	0.82 ± 0.01 ^b	0.61 ± 0.01 ^b	25.61 ± 0.31 ^b	1.34 ± 0.005 ^b
C4	0.86 ± 0.01 ^c	0.66 ± 0.01 ^c	23.23 ± 2.05 ^{ab}	1.30 ± 0.03 ^{ab}
C5	0.82 ± 0.01 ^b	0.61 ± 0.01 ^b	25.18 ± 2.76 ^b	1.33 ± 0.04 ^b

Note: ^aAll values are means of triplicate determinations.

have been given in table 4. Tap density of C is 0.74 g/cm³ but it increased in germinated cowpea beans treated with silver nanoparticles. Tap density measures the volume occupied by a given mass of powder after tapping or vibration. Germinated samples treated with silver nanoparticles observed increased in tap density it could be due to the particles within the powder have undergone rearrangement or compaction, resulting in a reduction in void spaces between particles and an increase in overall density. Increased tap density is important in various industries, including pharmaceuticals, food processing, and powder metallurgy, as it can affect the flowability, compressibility, and handling characteristics of powders. It is often desirable to achieve higher tap densities to improve the efficiency of powder processing operations, such as tablet manufacturing, blending, or packaging. The highest bulk density was observed in C2, that is 0.7 g/cm³ while the least was found in non-germinated 0.55 g/cm³. Increased bulk density referred to a situation where the mass of a material occupies a smaller volume resulting in a higher density. Increased bulk density signified higher mass-to-volume ratio and could be implicated for material properties, processing efficiency, product quality, and environmental sustainability. Hausner ratio and Carr's index of C, C1, C3 and C6 observed insignificant difference while they were decreased in the rest of the samples. The Hausner ratio measures the flowability of a powder or granular material. A higher Hausner ratio indicated poorer flowability and it is suggested that the powder is more prone to compaction and cohesive forces leading to increased resistance to flow. Thus, the samples treated with 40 ppm silver nanoparticles observed

better Hausner ratio and Carr's Index. Previous studies noted that germination enhanced functioning characteristics except SI and bulk density [41]. Sakare et al. [41] also observed an inverse relation between germination time and bulk density. Ghavidel and Prakash [42] also reported inverse relationship for cowpea, green gram, and lentil. The rise in WAC during germination can be described to the breaking of the polysaccharide molecules, a rise in content of protein, and alterations in the quality of proteins. These changes lead to an increase in the number of sites available for interaction with water, ultimately resulting in an enhanced water holding capacity [41]. The increased water holding capacity of protein isolates is caused by the greater abundance of polar amino acids. Similarly, the high oil absorption capacity can be attributed to presence of significant fraction of hydrophilic chains and polar amino acids on the surface of protein molecules [43]. The interaction between polar amino acid water molecules and water molecules residues of the proteins leads to variations in the WAC of flour [44].

Conclusion

This study highlighted the impact of five different concentrations such as 20, 40, 60, 80, and 100 ppm of silver nanoparticles on the germination of cowpea beans. Out of all the possibilities tested, a concentration of 60 ppm was found to be the most effective treatment for enhancing both root length and germination percentage. Furthermore, the inclusion of 60 ppm silver nanoparticles resulted in enhanced phytochemical and physicochemical qualities. The level of ash and fat decreased, however the level of protein and fiber increased. The incorporation of both lower and greater doses of nanoparticles adversely affected the bioactive components and germination percentage of cowpea beans. The presence of silver nanoparticles significantly affects the color profile of cowpea beans, resulting in a decrease in L^* and b^* values. This indicates the degradation of chlorophyll content and carotenoids following seed germination. The functional properties led to a drop in FAC, Carr's index, and Hausner ratio, whereas there was an increase in WSC, WAC and FC. outcomes of this study have practical significance for assessing the compatibility of silver nanoparticles with living organisms and for determining their possible usage in enhancing the characteristics of different agricultural products.

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None.

Conflict of Interest

None.

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