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Research Article

Comparative Effect of Silver Nanoparticles on Germinated, Cooked, Autoclaved and Microwaved Red Kidney Beans

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Abstract

The study investigated the impact of 60ppm silver nanoparticles (AgNPs) on red kidney beans using various techniques such as germination, cooking, autoclaving, and microwaving. Samples treated with silver nanoparticles displayed alterations in composition compared to raw and untreated samples with notable modifications in protein, fat, and carbohydrate content. The highest total phenolic content 1.59 mg gallic acid/g, flavonoid content 445.2 mg catechin and antioxidant activity 89.0%, was observed in germinated beans treated with silver nanoparticles. Trypsin inhibitor content ranged from 0.04 to 2.83 mg/g, with the highest value observed in raw beans and the least was observed in germinated beans treated with silver nanoparticles. Tannin content varied from 0.40 to 1.26 mg/g and the phytic acid content ranged from 1.09 to 4.18 mg/g, with the least found in GA-treated beans. The highest level was observed in raw beans. Also, imaging analysis revealed distinct alterations in bean surface structure treated with AgNPs. Germinated beans exhibited AgNPs adhering to or penetrating the seed coat, modifying surface morphology. Cooked beans displayed AgNPs aggregates on the surface, indicating altered distribution upon heating. Microwaved beans showed microwave-induced effects, potentially leading to uneven distribution and cluster formation of AgNPs due to localized heating. Autoclaving induces structural changes in beans with AgNPs interacting with the surface forming aggregates or deposition. While, the treatment with AgNPs on beans caused modifications in FTIR spectrum profile such as shifting peak positions or intensities or the emergence or absence of certain bands.

Keywords: Silver Nanoparticles, Kidney Beans, Germination, Cooking, Autoclaving, Microwaving.

Introduction

Red kidney bean *(Phaseolus vulgaris)* is herbaceous annual plant in the Leguminosae family. Although commonly planted in hot climates across the world, it was domesticated separately in ancient Mesoamerica and the Andes (Pathania 2014). Legumes are regarded as a significant source of protein, particularly in under developed nations where it is challenging for people with poor incomes to rely only on animal proteins (Shaban 2019). Kidney beans are high in vegetable protein, carbohydrate, soluble and insoluble fiber, vitamins (especially B group), and minerals (notably potassium, iron, zinc, magnesium, and manganese) (Sathe 1984). However,

they have variable concentrations of non-nutritive phytochemicals like flavonoids and polyphenols, which offer several health advantages against a variety of illnesses like cancer, heart disease, and immune system disruption (Van der Poel 1990). Various methods could increase the nutritional property and reduce antinutrients of legumes to using different methods such germination, cooking, autoclaving, and microwaving. Germination starts when the seed absorbs water and concludes when the test ruptures and the radicle elongates (Avezum 2023). Cooking means legumes mainly pulses heated to the point at which it is suitable for consumption. It would suggest that when it has the right texture, flavor, and scent and are sufficiently soft and simple to chew (Wood 1017). Autoclaving is the procedure that involves applying high pressure and steam to beans. The disruption of starch granules occurs when the autoclave reaches temperatures above 100 °C, hastening the retrogradation process (Dupuis 2014). Microwave involves radiation to cook beans. The principle of microwave cooking relies on the interaction between the microwaves and the water molecules within the food (Sutivisedsak 2010). However, the most synergistic effect to improve the quality is seed priming (Rastin 2013). Seed priming is the process of treating seeds with natural and artificial substances prior techniques in order to induce a certain physiological condition in plants. The primed state of a plant is the physiological condition in which it may activate defense responses more quickly, more effectively, or both (Abebe 2009). Seed priming is conducted by using some techniques such as hydro-priming, osmo-priming, hormonal priming, nutri-priming, on-farm priming, and bio-priming. Among all, nano-priming is noticeably more successful method for seed priming. Recently, research in nanotechnology has got a lot of attention because of its applications that generates from agri-food to biotechnology, cosmetic and textile industry with its beneficial uses (Pérez-Esteve 2013). Focusing our interest on silver nanoparticles (AgNPs), these have been widely used in medicine and biotechnology fields due to their properties as antimicrobials. Numerous research studies have also confirmed the effectiveness of AgNPs to inhibit the growth of pathogenic bacteria such as *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Escherichia coli* and *Proteus vulgaris (*Abbaszadegan 2015).

Moreover, many studies have documented that high dose of nanoparticles could have negative impact on plants and beans (Sharma 2012). Therefore, 60ppm AgNPs as seed priming for further processing such as germination, cooking, autoclaving, and microwaving is investigated. Furthermore, we aimed to explore the variation based on proximate composition, bioactive compounds, and antinutrients were determined. Moreover, scanning electron microscopy and FTIR were studied.

Materials And Methods

Collection of samples

Red kidney beans were bought from the local market. Seeds were cleaned and the extraneous particles were removed by hand sorting. They were then divided into four parts for further processing with silver nanoparticles such as germination, cooking, autoclave, and microwave.

Silver Suspensions Preparation

Silver nanoparticles (powder form, < 100 nm) stabilized by polyvinyl pyrrolidone (PVP) were procured from Sigma Aldrich Inc., USA. Silver nanoparticles were dissolved in the distilled water by sonication process to prevent aggregation. Influence of AgNO3 on treatment of seed was conducted by utilization of 60ppm concentrations such as 500 mg L−1 (Islam 2023).

Germination

Three-hundred grams kidney beans were soaked in 60ppm solution of silver nano particles for 2hrs. Samples were taken out, washed, and kept on jute bag for five days at room temperature (25±°C) (Islam 2023).

Cooking

Three-hundred grams kidney beans were soaked in 60ppm solution of silver nano particles for 2hrs. Samples were taken out, washed, and kept for boiling in 3000mL water for 90 mins at 100°C. Boiled red kidney beans were dried at 45°C in oven and ground to form flour (Luo 2013).

Autoclaving

Three-hundred grams seeds were soaked in 60 ppm solution of silver nano particles for 2hrs. Then they were rinsed, washed with distilled water, and autoclaved using vertical autoclave at 15 lb pressure (121°C) until they became soft when felt between the fingers (35 min). The autoclaved seeds were dried at 45°C in oven and ground to form flour (Luo 2013).

Microwaving

Three-hundred grams seeds were soaked in 60ppm solution of silver nano particles for 2hrs. Then they were rinsed, washed with distilled water, and placed in a glass beaker with water (1:10 w/v), then were cooked in a microwave oven on 1200watt for 15 min until they became soft when felt between the fingers. The cooked seeds were dried at 45^0C in oven and ground to form flour (Luo 2013).

Proximate analyses

The proximate analyses of samples for moisture, crude fat, crude fiber, total ash, and carbohydrate were performed in triplicates using the procedures described by Association of Official Analytical Chemists (AOAC, 2000).

Preparation of extract for determining the bioactive compounds

Extracts for bioactive compounds were prepared by the method described in Aryal, S., et al (2019).

Determination of Total Phenolic Content

Total Phenolic Content was determined by the method described in Abbaszadegan, A., et al. (2015).

Determination of antioxidant activity by DPPH

Antioxidant Activity was determined by following the method

of Aryal, S., et al (2019). DPPH activity was measured by using the following equation:

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

Determination of Total Flavonoid Content

Total Flavonoid Content was measured by using the method described in Aryal, S., et al (2019).

Determination of Trypsin inhibitors

Trypsin inhibitory activity (TIA) was determined colorimetrically using UV/visible spectrophotometer in accordance with AACC International method 22-40.01 (AACC International 2000), with some modifications. Trypsin inhibitor activity was defined as the amount of trypsin units inhibited per mg of sample (Shi 2017).

Determination of tannin

Tannins was determined colorimetrically using UV/visible spectrophotometer in accordance with AACC International method 22-40.01 (Emmanuel 2018).

Determination of phytic acid

Phytic acid was determined by using a UV-Vis spectrophotometer (UV–Vis 3000, ORI, Germany). Following equation was used to determine Phytic Acid (Chaudhry 2011).

Phytic Acid (mg/g): 0.02*Absorbance+0.01 (mg)/ 0.5g

Colour profile

Colour parameters of different accessions (grains) and their flours were measured using Ultra Scan VIS Hunter Lab (Hunter Associates Laboratory Inc., U.S.A.). Colour parameters were *L** (darkness/lightness), *a** (greenness/redness) and *b** (blueness/yellowness).

Scanning electron microscopy

Microstructure of flours was studied using a scanning electron microscope (Merlin, Carl Zeiss, Germany). Flours were adhered to stubs using a double-sided carbon tape and viewed at 5 kV and 1400X after being coated with gold using a sputter coater (Quorum Technologies, Lewes, UK).

FTIR (Fourier Transform Infrared)

Samples were pressed by the ATR accessories straight onto the diamond ATR crystal. All seed samples attenuated total reflectance spectra were scanned with a resolution of $4cm^{-1}$, and 4 scans were accumulated per spectrum. All spectra were recorded in triplicates, and the average of three measurements was calculated for each. Prior to each ATR experiment, an air background spectrum was acquired. Spectral data was collected by compressing the sample with the ATR accessory.

Statistical analysis

All analyses were performed in triplicates. Data was statistically analyzed using Analysis of Variance (ANOVA) techniques using Statistix 8.1 software. The significant level among the mean at $p < 0.05$ was measured by analyzing the least significant difference.

Results and Discussion

Proximate analyses

The proximate composition of raw, germinated, cooked, autoclaved, and microwaved kidney beans treated with 60ppm AgNPs are shown in Table 1.

Effect of silver nanoparticles on raw, germinated, cooked, autoclaved and microwaved red kidney beans on proximate composition[a]

Table 1: [a]All values are means of triplicate determinations. Means within a column with different superscripts are significantly different at P < 0.05.

Samples	Moisture Content (%)	Ash (%)	Fat (%)	Protein (%)	Crude Fibre (%)	Carbohydrate (%)
Raw	8.86±0.15^{e}	4.60±0.26^{c}	1.33±0.11^{d}	26.2±0.20^{g}	6.20±0.20^{g}	52.81±0.89^{a}
G_0	8.83±0.05^{e}	3.61±0.10^{b}	1.19±0.10^{f}	23.6±0.10^{d}	3.60±0.10^{c}	59.17±0.10^{c}
G_A	8.13±0.05^{c}	2.61±0.10^{a}	0.80±0.10^{a}	23.6±0.10^{d}	3.60±0.10^{c}	61.26±0.10^{d}
$_C0$	8.63±0.11^{d}	3.26±0.10^{b}	1.15±0.01^{c}	25.1±0.10^{f}	5.10±0.10^{f}	56.76±0.01^{b}
C_A	8.13±0.05^{c}	2.56±0.35^{a}	0.82±0.01^{a}	24.1±0.10^{e}	4.80±0.10^{e}	59.59±0.49^{c}
$_A0$	7.86±0.05^{b}	3.60±0.10^{b}	1.19±0.01^{e}	21.7±0.10^{b}	3.20±0.10^{b}	62.45±0.21^{d}
A_A	7.36±0.05^{a}	2.90±0.10^{a}	1.13±0.01^{c}	20.8±0.10^{a}	2.80±0.10^{a}	65.01±0.26^{e}
$_M0$	7.99±0.05^{b}	4.40±0.10^{c}	1.13±0.06^{b}	23.1±0.10^{d}	4.20±0.10^{d}	59.18±0.17^{c}
M_A	7.00±0.27^{a}	2.10±0.10^{b}	1.08±0.01^{f}	22.7±0.10^{c}	3.50±0.10^{c}	63.62±0.11^{e}

Abbreviations: G_0: Germinated kidney beans, G_A: 60ppm AgNPs germinated kidney beans, C_0: Cooked Kidney bean, C_A: 60ppm AgNPs Cooked kidney bean flour, A_0: Autoclaved kidney bean flour, A_A: 60ppm AgNPs Autoclaved Kidney bean flour, M_0: Microwaved Kidney bean, M_A :60ppm AgNPs Microwaved Kidney bean flour

Moisture

Significant difference ($p< 0.05$) in moisture content of raw KB (8.86%), G_0 germinated KB (8.83%), C_0 cooked KB (8.63%), A_0 autoclaved KB (7.46%), and M_0 microwaved KB (7.99%) were observed. The moisture content of samples with 60ppm AgNPs decreased with their respective samples as shown in Table 1. It is because the treatment with 60ppm AgNPs could alter the structure of beans making an increase in porosity that could facilitate in evaporation process (Bamal 2021). Also, an increase in heat transfer could lead to more rapid evaporation of water from the beans, thus reducing their moisture content. The low moisture content of the KB (*Phaseolus vulgaris* L.) remains an asset in storage and preservation of the nutrients. Higher moisture content could lead to food spoilage through increasing microbial action (Pitt 2022). Thus, food containing higher moisture contents are more susceptible to spoilage as they create the ideal conditions for microbial growth. These microbes causes the breakdown of food's nutrients resulting in the development of toxic substances. As a result, shelf life of the food and the quality are reduced (Smith 2023).

Ash content

Difference in ash content of raw KB (4.60%), G_0 germinated KB (3.61%), C_0 cooked KB (3.26%), A_0 autoclaved KB (3.60%), and M_0 microwaved KB (4.40%) were observed when seeds were not treated with AgNPs. Table 1 indicated that samples treated with 60 ppm AgNPs had a considerably lower ash content. Due to their antibacterial qualities, AgNPs can stop the growth of bacteria and fungus, among other microbes. These microbes could aid in the deterioration of organic materials, including the disintegration of cellular constituents that raise the ash content. The ash level of the beans may drop because of slower organic material decomposition brought on by decreased microbial activity using AgNPs (Bruna 2021).The breakdown of organic materials, including the minerals and other elements that raise the ash concentration, is mostly dependent on microorganisms. The breakdown process slows down when microbial activity is reduced, maybe as a result of adverse circumstances like decreasing moisture or temperature. Consequently, a longer time of preservation for the organic material results in a lower conversion of these elements into ash (Coulibaly 2012).

Fat content

Fat content of raw KB (1.33%), G_0 germinated KB (1.19%), C_0 cooked KB (1.15%), A_0 autoclaved KB (1.19%), and M_0 microwaved KB (1.13%) were observed as shown in Table 1. Upon, treatment with AgNPs, fat content in kidney beans were decreased such as G_A germinated kidney beans (0.80%), C_A cooked KB (0.82%), A_A autoclaved KB (1.13%), and M_A microwaved KB (1.08%). Enzymes involved in lipid metabolism may become less active when exposed to AgNPs. For instance, the enzymes known as lipases are in charge of converting lipids into glycerol and fatty acids. Reduced fat breakdown inside the beans due to inhibition of these enzymes may result in a decreased fat content (Holt 2005). Catalyzing the breakdown of fats into fatty acids and glycerol is the function of enzymes like lipases. The breakdown of lipids is greatly reduced when these enzymes' activity is impeded whether by the presence of enzyme inhibitors or environmental variables like low pH or temperature. Because of this, the beans total fat content stays higher than it would have if regular enzymatic activity had taken place, which eventually results in a slower rate of fat removal (Villeneuve 2000).

Protein content

Variation in protein content of raw KB (26.2%), G_0 germinated KB (23.6%), C_0 cooked KB (25.1%), A_0 autoclaved KB (21.7%), and M_0 microwaved KB (23.1%) were observed (Table1). Protein content decreased following treatment with 60 ppm AgNPs, with reductions observed in GA germinated kidney beans (23.6%), CA cooked kidney beans (24.1%), AA autoclaved kidney beans (20.8%), and MA microwaved kidney beans (22.7%). Raw kidney beans were having high protein content while all other treatments had reduced protein levels (Table 1). AgNPs might interfere with enzymatic processes involved in protein synthesis or stability, resulting in reduced protein production or accelerated degradation. Antimicrobial silver ions are released when AgNPs dissolve; these ions can interact with cell wall proteins that contain thiols and affect their activities. AgNPs can attach to proteins and form complexes with electronic donors that include atoms of oxygen, phosphorus, nitrogen, or sulfur when they come in contact with the outer membrane. The literature provides the greatest description of the interaction with thiol groups. Therefore, by their interaction with disulfide bonds and blockage of active sites, AgNPs cause membrane-bound enzymes and proteins to become inactive (Salama 2021). Thus, the interaction of silver nanoparticles with thiol groups, which are sulfur-containing functional groups present in many biological molecules, has been extensively studied in the literature. Because of their strong attraction for thiol groups, these nanoparticles interact with the disulfide bonds found in proteins and enzymes. Proteins, especially those that are membrane-bound, can lose their ability to fold normally and maintain their structural integrity when silver nanoparticles attach to these disulfide linkages (Rai 2009). Enzyme active sites may get blocked as a result of this interaction, making the enzymes inactive. This results in the loss of function of the impacted proteins and enzymes, which can disrupt vital biological functions, such as those that preserve the integrity and functionality of cell membranes (Rizzello 2014).

Crude fiber content

Difference in fiber content raw KB (6.20%), G_0 germinated KB (3.60%), C_0 cooked KB (5.10%), A_0 autoclaved KB (3.20%), and M_0 microwaved KB (4.20%) were observed (Table1). Crude fiber content decreased significantly following treatment with 60 ppm AgNPs, with reductions noted in G_A germinated kidney beans (3.60%), C_A cooked kidney beans (4.80%), A_A autoclaved kidney beans (2.80%), and M_A microwaved kidney beans (3.50%). AgNPs may disrupt the function of enzymes involved in the formation or degradation of fibers. This interference may slow down the synthesis of cellulose and other fiber constituents or hasten their breakdown, which would lower the amount of crude fiber (Komatsu 2022). Plant cells' ability to generate and break down fibrous materials depends on the activity of certain enzymes, which silver nanoparticles may inhibit. These enzymes, which include hemicellulases and

cellulases, are in charge of breaking down and synthesizing cellulose and other structural polysaccharides that comprise the cell walls (Kumar 2011). When these enzymes come into contact with silver nanoparticles, they may bind to the active site of the enzyme or cause structural disruptions that limit the activity of the enzyme. This inhibition may cause cellulose and other fiber constituents to biosynthesize more slowly, which would decrease the amount of these crucial structural elements produced (Qian 2013).

Carbohydrates content

Variations in carbohydrates content of raw KB (52.81%), G_0 germinated KB (59.17%), C_0 cooked KB (56.76%), A_0 autoclaved KB (62.45%), and M_0 microwaved KB (59.18%) were observed (Table1). Upon treatment with 60ppm AgNPs, carbohydrate content significantly increased such as G_A germinated kidney beans (61.26%), C_A cooked KB (59.59%), A_A autoclaved KB (65.01%), and M_A microwaved KB (63.62%). Treated seeds might undergo stress reactions in response to AgNPs exposure, resulting in modifications to their metabolism intended to mitigate the stress. A greater carbohydrate content might occur from changes in the metabolism of carbohydrates, such as increased synthesis or decreased breakdown (Vannini 2014). Due to their exposure to silver nanoparticles, seeds treated with these particles may undergo stress responses, which set off a cascade of metabolic changes intended to alleviate the stress. The regular metabolic pathways, especially those involved in the metabolism of carbohydrates, may change as a result of this stress response. For example, in reaction to stress, the seeds may enhance the synthesis of carbohydrates as a defense mechanism to produce more energy or store surplus carbon. As an alternative, less carbs may be broken down, saving energy and resources to assist the seeds survive the harsh environment. The seeds' total glucose content may rise as a result of these metabolic changes (Dueñas 2015).

Bioactive compounds

Total phenolic content

The results indicated an increase in phenolic compounds from 1.32 mg/g in raw KB to 1.55 mg/g in germinated ones. The highest was observed in G_A i.e. 1.59 mg/g. The increment is due to the incorporation of AgNPs as shown in Table 2. Increase in phenolic content of germinated kidney beans at 60ppm AgNPs could be due to the release of ferulic acids during the sprouting of beans (Pedrosa 2021). Decrease in total phenolic content in C_o (1.35 mg/g), A_o (1.12 mg/g) and M_o (0.91 mg/g) were observed. It is due to thermal processing because phenolic compounds are susceptible to breakdown at high temperatures. Reduced content of phenolic compounds can occur during cooking, particularly when using techniques like autoclaving and boiling that expose beans to high temperatures for extended periods of time (Paucar-Menacho 2017). However, with the addition of 60ppm AgNPs value of phenolic content improved as it showed positive results such as C_A (1.52 mg/g), A_A (1.15 mg/g) and M_A (1.21 mg/g). AgNPs used the activation of enzymes to manufacture phenolic compounds from glucose and aromatic amino acids. Through the oxidative pentose phosphate, shikimic acid, and glycolytic routes, enzymes generate aromatic amino acids, such as phenylalanine, and then transform them into phenolic compounds. Afterwards, in the endoplasmic reticulum, these phenolic molecules transform into flavonoids, coumarin, and stilbene (Demirbas 2016).

Effect of silver nanoparticles on raw, germinated, cooked, autoclaved and microwaved red kidney beans on antinutritional compounds[a]

Table 2: [ea]All values are means of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$.

Samples	Total Phenolic Content	Flavonoids	DPPH
	(mg gallic acid/g)	(mg catechin/100g)	(%)
Raw	1.32±0.01[c]	154.1±0.65[b]	47.0±2.74[d]
G_0	1.55±0.01[d]	162.3±0.65[c]	67.0±2.74[e]
G_A	1.59±0.01[e]	445.2±0.65[f]	89.0±2.74[f]
C_0	1.35±0.00[c]	156.1±0.29[b]	48.9±0.75[d]
C_A	1.52±0.02[d]	243.3±2.94[e]	87.6±0.07[f]
A_0	1.12±0.00[a]	217.1±6.18[d]	10.3±1.04[a]
A_A	1.15±0.00[ab]	227.4±5.63[e]	23.9±1.15[b]
M_0	0.91±0.00[a]	133.1±1.06[a]	24.0±0.00[b]
M_A	1.21±0.00[b]	186.9±4.69[c]	39.5±1.24[c]

Abbreviations: G_0: Germinated kidney beans, G_A: 60ppm AgNPs germinated kidney beans, C_0: Cooked Kidney bean, C_A: 60ppm AgNPs Cooked kidney bean flour, A_0: Autoclaved kidney bean flour, A_A: 60ppm AgNPs Autoclaved Kidney bean flour, M_0: Microwaved Kidney bean, M_A :60ppm AgNPs Microwaved Kidney bean flour

Flavonoids

Flavonoids are important secondary metabolites and active biomolecules in beans. Total flavonoids contents of raw KB (1.54 mg catechin/100g), G_0 KB (162.3 mg catechin/100g), C_0 KB (156.1 mg catechin/100g), A_0 KB (217 mg catechin/100g), and M_0 KB (133.1 mg catechin/100g) were observed in Table 2. According to our findings it showed that there is an increase in flavonoids content when the seeds are exposed to AgNPs such as G_A KB (445.2 mg

catechin/100g), C_A KB (243.3 mg catechin/100g), A_A KB (227.4 mg catechin/100g), and M_A KB (186.9 mg catechin/100g). Sample with only microwave showed the least value because of the high radiation that reduced flavonoid content. However, AgNPs improved the values because silver ions and flavonoids may act as antioxidants by transferring a single electron and a hydrogen atom, respectively (Poljsak 2021).

Antioxidant activity

Antioxidants of raw KB (47%), G_0 KB (67%), C_0 KB (48.9%), A_0 KB (10.3%), and M_0 KB (24%) were shown in Table 2. According to our results the least was observed in Autoclave and microwave treatment i.e. 10 and 24 % respectively. This reason for this could be that antioxidants may oxidize when subjected to higher temperatures, such as those seen during autoclaving or microwave cooking. This process can reduce their antioxidant capacity, leading to a decrease in overall activity (Elemike 2017). However, these activities are increased when they are exposed to AgNPs such as G_A (89%), C_A (87%), A_A (23%), and M_A (39.5%). The reason for this could be that AgNPs naturally possess antioxidant properties. Applying these agents to beans may help reduce oxidative stress, which may help preserve flavonoids. In addition to flavonoids, phenolics also have antioxidant properties. Antioxidant properties of samples introduced with AgNPs are increased due to the simultaneous activity of polyphenols and AgNPs as a catalyst. The enhanced antioxidant ability with AgNPs is caused by the presence of phenolic compounds, terpenoids, and flavonoids in plants which allow nanoparticles to act as singlet oxygen quenchers, hydrogen donors, and reducing agents (Riaz 2019). The synergistic interaction between polyphenols and silver nanoparticles (AgNPs) enhances the antioxidant capabilities of samples treated with AgNPs. By serving as catalysts, silver nanoparticles can enhance the antioxidant activity of plant samples through several processes. The combined effects of AgNPs and several bioactive substances found in the plants, including flavonoids, terpenoids, and phenolic compounds, are responsible for this improvement. Due to their interaction with the AgNPs contained in the seeds, these compounds' natural antioxidant qualities are strengthened (Savelkoul 1992).

Antinutritional compounds

Trypsin inhibitors

Trypsin inhibitors are low molecular weight proteins also known as serine protease inhibitors and are present in a variety of dietary sources, such as raw legumes and beans. They could bind lysine and arginine residues in trypsin, which is a proteolytic enzyme secreted by the pancreas. Thus, reducing the digestion of protein and making unsuitable for human consumption (Shi 2017). The effect of processing such as germination, cooking, autoclaving, and microwaving on trypsin inhibitor in red kidney beans as shown in Table 3. According to our findings it is shown that the raw kidney beans contained the highest trypsin inhibitor (2.83 TIU/mg) which is significantly decreasing in all samples that were processed. It is due to the fact that these are present in the cotyledons fraction in pulses. As these were processed during germination; trypsin inhibitor declined significantly G_0 (0.11 TIU/mg). Germination decreases trypsin inhibitor levels in kidney beans through a combination of enzymatic breakdown, activation of endogenous proteases, synthesis of new proteins, and leaching. This decrease contributes to the improved digestibility and nutritional quality of germinated beans compared to raw beans (Mikhailova 2020). Samples C_0 KB (0.16 TIU/mg), A_0 KB (0.18 TIU/mg), and M_0 KB (0.18 TIU/mg) also showed significantly decreased in values because trypsin inhibitor is heat sensitive and therefore inactivated by thermal methods due to denaturation (Pitt 2022). However, the treatments with AgNPs showed rapid decrease in trypsin inhibitor that gives positive results such as G_A KB (0.04 TIU/mg), C_A KB (0.08 TIU/mg), A_A KB (0.07 TIU/mg), and M_A KB (0.70 TIU/mg). AgNPs could interact with enzymes present in beans or induce the activation of enzymes that could degrade trypsin inhibitors. This enzymatic degradation could lead to a reduction in the concentration of trypsin inhibitors in treated beans (Gupta 2018).

Effect of silver nanoparticles on raw, germinated, cooked, autoclaved and microwaved red kidney beans on bioactive compounds[a]

Table 3: [a]All values are means of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$.

Samples	Trypsin Inhibitor	Tannins	Phytic Acid
		mg/g	
Raw	2.83±0.03^{e}	1.26±0.03^{f}	4.18±0.00^{f}
G_0	0.11±0.01^{b}	0.62±0.05^{b}	1.10±0.74^{b}
G_A	0.04±0.01^{a}	0.40±0.15^{a}	1.09±0.14^{a}
C_0	0.16±0.01^{bc}	0.90±0.00^{e}	2.66±0.05^{e}
C_A	0.08±0.00^{b}	0.73±0.04^{c}	1.31±0.07^{c}
A_0	0.18±0.00^{c}	0.83±0.00^{d}	2.43±0.05^{e}
A_A	0.07±0.01^{a}	0.74±0.03^{c}	1.99±0.01^{d}
M_0	0.18±0.00^{c}	0.81±0.02^{d}	2.46±0.05^{c}
M_A	0.70±0.00^{d}	0.76±0.00^{c}	1.91±0.05^{d}

Abbreviations: G_0: Germinated kidney beans, G_A: 60ppm AgNPs germinated kidney beans, C_0: Cooked Kidney bean, C_A: 60ppm AgNPs Cooked kidney bean flour, A_0: Autoclaved kidney bean flour, A_A: 60ppm AgNPs Autoclaved Kidney bean flour, M_0: Microwaved Kidney bean, M_A :60ppm AgNPs Microwaved Kidney bean flour

Tannins

Tannins are the complex form of polyphenols. It provides meals an astringent taste and hinders the absorption of iron, glucose, and vitamin B12 (Chaudhry 2011). The level of tannin was the highest in raw kidney beans 1.26mg/g as shown in Table 3. However, it decreased to the lowest level in germinated kidney beans treated with AgNPs (0.40 mg/g), followed by untreated germinated kidney beans. The observed reduction in tannin content after germination was a result of activated various enzymes as part of their metabolic processes. These enzymes catalyze the breakdown of complex compounds like tannins into simpler forms (Chaudhry 2011). AgNPs have the potential effects on plant metabolism and biochemical pathways as it could influence enzyme activity, gene expression, and cellular processes in plants (Dhull 2022).

Phytic Acids

Phytic acid, a saturated cyclic acid with six reactive phosphate groups acts as a chelating agent. It has the ability to bind positively charged functional groups or minerals thus reducing their bioavailability during food metabolism (Gupta 2015). The content of phytic acid in raw kidney beans is around 4.18 mg/100 g on a dry basis. Table 3 showed the changes in phytic acid concentration in different treatments. The highest decline was observed in G_A KB 1.09mg/100gm. Treatment of germinated kidney beans with AgNPs could affect the activity of enzymes involved in phytic acid metabolism or influence the expression of genes related to phytic acid synthesis. The loss is associated to the increase in phytase activity of the germinating grains that hydrolyses phytic acid (Chaudhry 2011) therefore sample G_0 also showed decrease in phytic acid i.e. 1.10mg/100g. Whereas, samples of cooking, autoclaving and microwaving also showed decrease in phytic acid because heat could break down the molecules of phytic acid leading to its degradation. Phytic acid is relatively heat-sensitive, and prolonged exposure to high temperatures can lead to its hydrolysis, resulting in decreased level (Chaudhry 2011).

Colour Profiles

Colour profile have three components such as L, a and b that describes the lightness of color. Figure 1(a-c) showed the color profile of samples. Samples exhibited decreased lightness (L*) and increased values for the color parameters (a* and b*) indicating a distinctive shift in color tones.

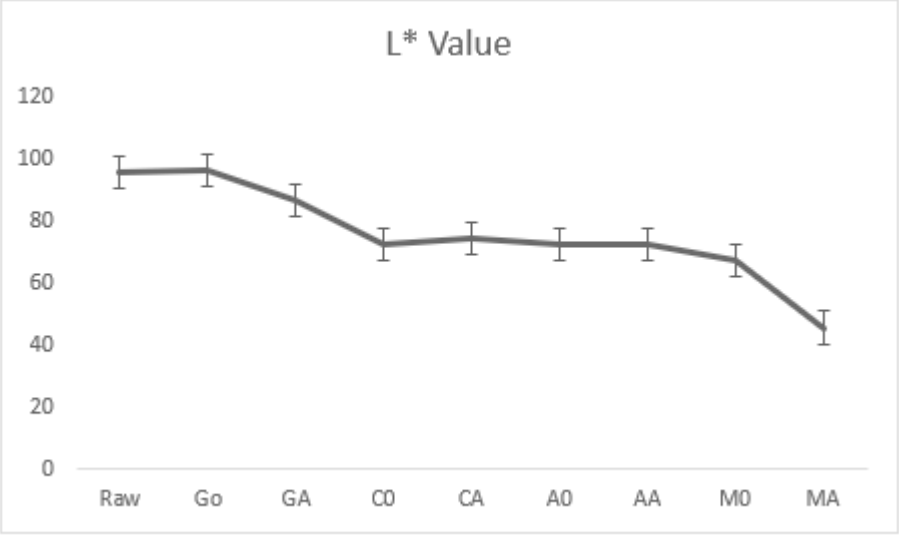

Figure 1(a): Effect of silver nanoparticles on raw, germinated, cooked, autoclaved and microwaved on L* Profile of kidney beans [a]All values are means of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$.

Abbreviations: G_0: Germinated kidney beans, G_A: 60ppm AgNPs germinated kidney beans, C_0: Cooked Kidney bean, C_A: 60ppm AgNPs Cooked kidney bean flour, A_0: Autoclaved kidney bean flour, A_A: 60ppm AgNPs Autoclaved Kidney bean flour, M_0: Microwaved Kidney bean, M_A :60ppm AgNPs Microwaved Kidney bean flour

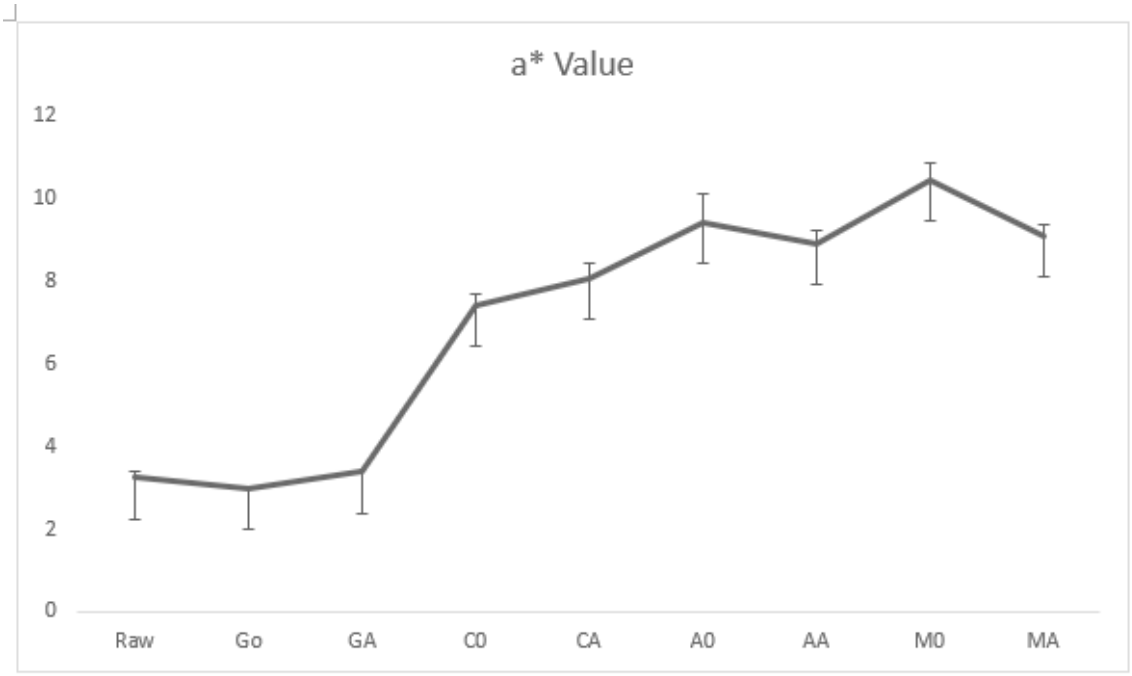

Figure 1(b): Effect of silver nanoparticles on raw, germinated, cooked, autoclaved and microwaved on a* Profile of kidney beans. [a]All values are means of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$.

Abbreviations: G_0: Germinated kidney beans, G_A: 60ppm AgNPs germinated kidney beans, C_0: Cooked Kidney bean, C_A: 60ppm AgNPs Cooked kidney bean flour, A_0: Autoclaved kidney bean flour, A_A: 60ppm AgNPs Autoclaved Kidney bean flour, M_0: Microwaved Kidney bean, M_A :60ppm AgNPs Microwaved Kidney bean flour

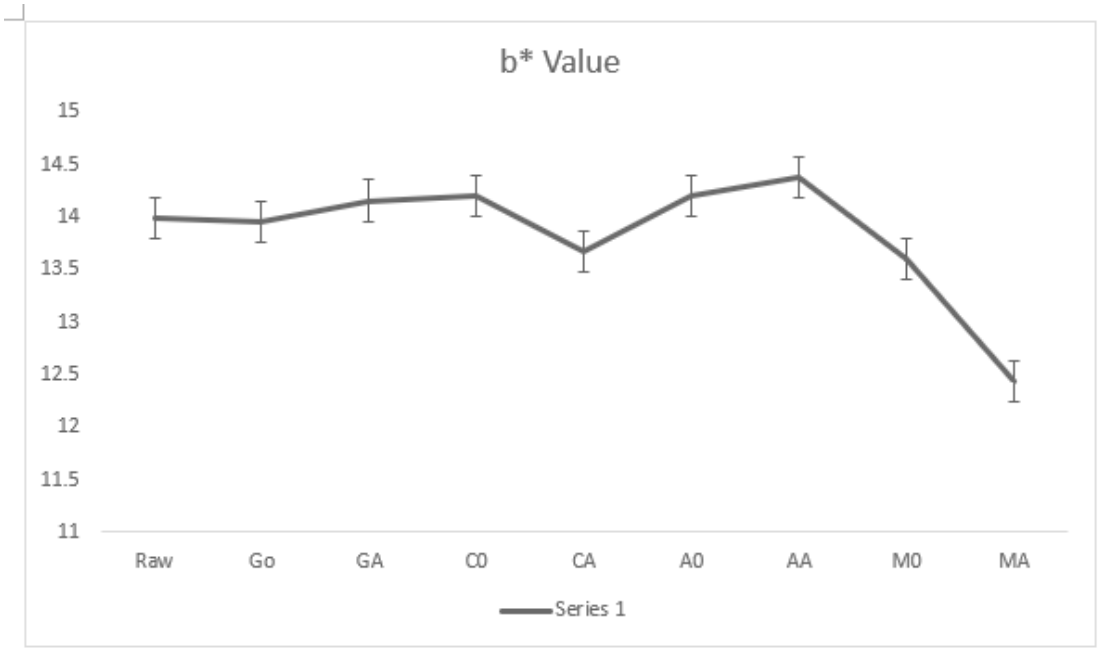

Figure 1(c): Effect of silver nanoparticles on raw, germinated, cooked, autoclaved and microwaved on b* Profile of kidney beans. [a]All values are means of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$.

Abbreviations: G_0: Germinated kidney beans, G_A: 60ppm AgNPs germinated kidney beans, C_0: Cooked Kidney bean, C_A: 60ppm AgNPs Cooked kidney bean flour, A_0: Autoclaved kidney bean flour, A_A: 60ppm AgNPs Autoclaved Kidney bean flour, M_0: Microwaved Kidney bean, M_A :60ppm AgNPs Microwaved Kidney bean flour

Scanning electron microscopy

Scanning electron microscopy (SEM) analysis highlighted distinct morphological changes in Red Kidney beans subjected to various treatments as shown in Figure (a-h). It is observed that imaging of germinated beans treated with AgNPs showed alterations in the surface morphology compared to untreated beans. AgNPs adhere to the surface of the beans or penetrate into the seed coat, affecting the overall surface structure. Whereas cooked beans leading to the formation of AgNP aggregates on the bean surface. SEM of microwaved beans treated with AgNPs may show microwave-induced effects on bean structure, such as tissue heating or expansion. AgNPs may distribute unevenly on the surface of microwaved beans, potentially forming clusters or agglomerates due to localized heating effects. Autoclaving may cause structural alterations in beans, such as cell wall disruption or protein denaturation. AgNPs interact with the surface of autoclaved beans, leading to the formation of nanoparticle aggregates or deposition on bean surfaces.

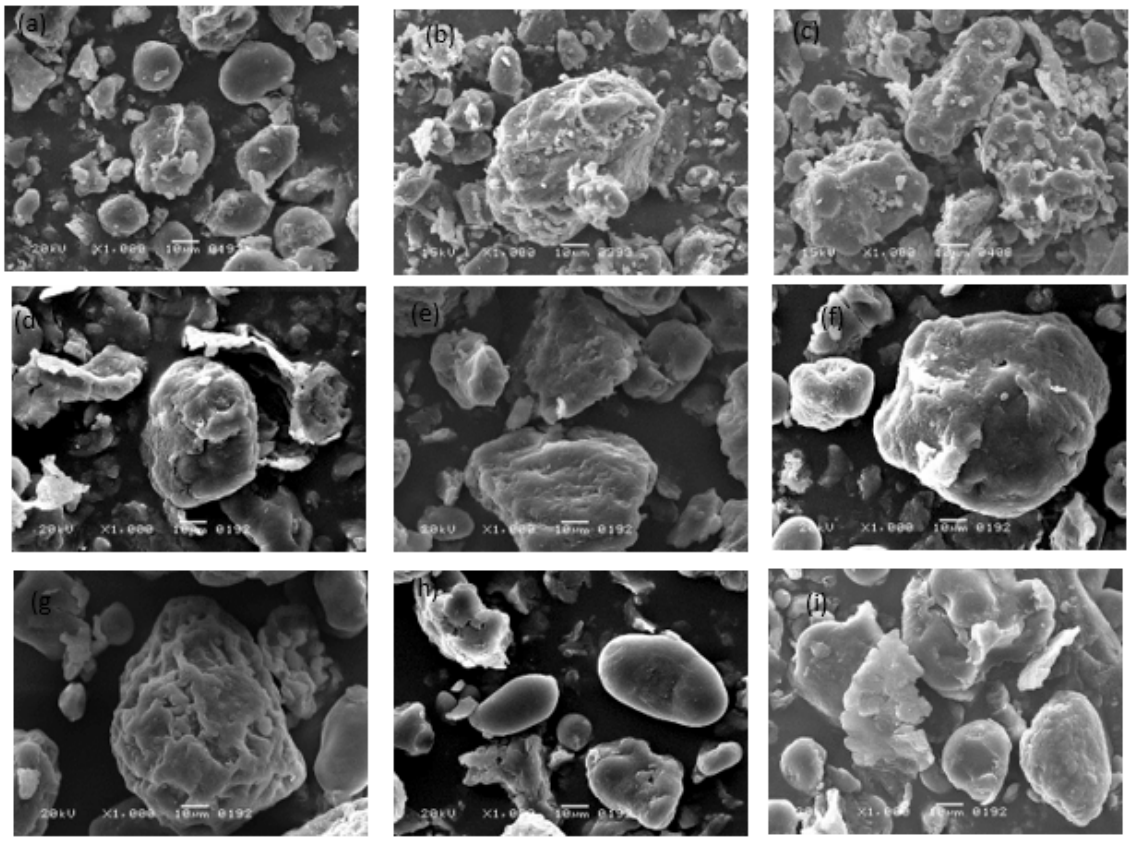

Figure 2(i-a): Scanning Electron Microscopy micrograph of AgNO3 tested in this study on raw, germinated, cooked, autoclaved and microwaved kidney beans: (a) Raw Kidney beans (b) Germinated kidney beans, (c) 60ppm AgNPs germinated kidney beans, (d) Cooked Kidney bean, (e) 60ppm AgNPs Cooked kidney bean flour, (f) Autoclaved kidney bean flour,(g) 60ppm AgNPs Autoclaved Kidney bean flour, (h) Microwaved Kidney bean, (i) 60ppm AgNPs Microwaved Kidney bean flour

FTIR

The FTIR (Fourier Transform Infrared) analysis of germinated, cooked, autoclaved and microwaved red kidney beans treated with 60ppm silver nanoparticles are presented in Figure 3 (a-h). The peaks are categorized based on their frequency range in wavenumbers (cm^-1) and associated chemical functionalities. Notable peaks include those representing various organic compounds and functional groups. Peaks in the range of 733-858 cm^-1 correspond to (C-H) stretching vibrations, while peaks in the range of 805-835 cm^-1 are indicative of aromatic compounds. Other significant peaks include those associated with specific functional groups such as C–O–C groups (911-1150 cm^-1), polysaccharides (1035-1149 cm^-1), ester carbonyls (1150-1270 cm^-1), and phenol rings (1458-1591 cm^-1). Additionally, peaks corresponding to hydroxyl compounds (3200-3400 cm^-1) and carboxyl acids (2500-3300 cm^-1) are also identified. These peak frequencies and their corresponding functional groups serve as valuable indicators for characterizing the chemical composition and structure of the analyzed sample using FTIR spectroscopy (Thummajitsakul 2023). According to our findings, protein amide bonding is commonly represented by peaks in FTIR spectra (e.g., amide I and amide II bands). The

intensity of these peaks may alter in response to AgNP treatment suggesting possible interactions between AgNPs and proteins or variations in protein structure brought on by various processing techniques. AgNP treatment and processing techniques may have an impact on peaks that are associated with carbohydrates, such as those that show glycosidic connections or C-O stretching vibrations in polysaccharides. Changes in bands related to these components, such as C-O or C-H stretching vibrations also occurred. Peak intensity or position variations could be the result of interactions with AgNPs during germination, microwaving, or autoclaving, or they could be due to structural changes in the carbohydrates. AgNPs treatment and processing methods may induce changes in the overall FTIR spectral profile of beans, including shifts in peak positions, changes in peak intensities, or the appearance/disappearance of specific bands. These changes can provide valuable information about the combined effects of AgNPs treatment and processing on the molecular composition of beans.

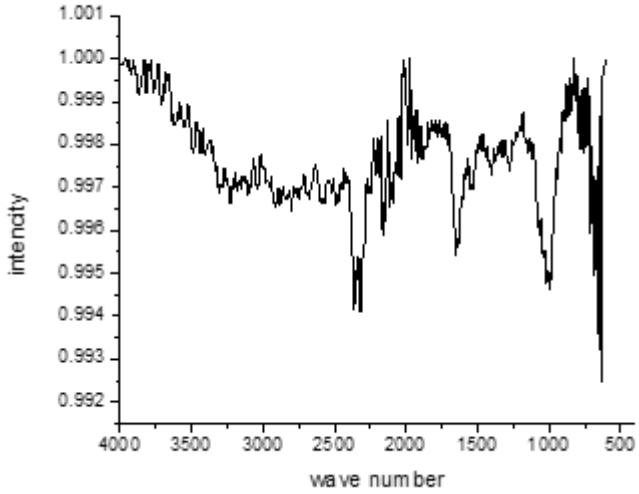

Figure 3(a)

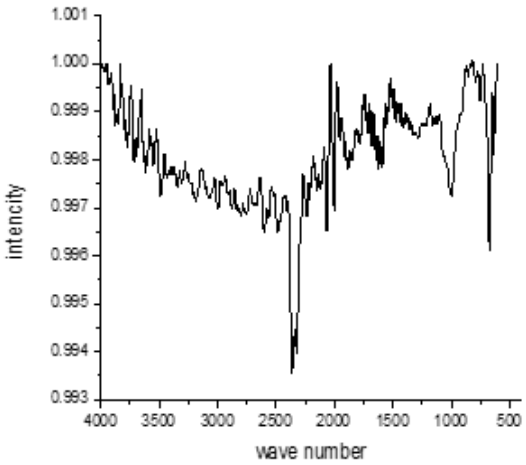

Figure 3(b)

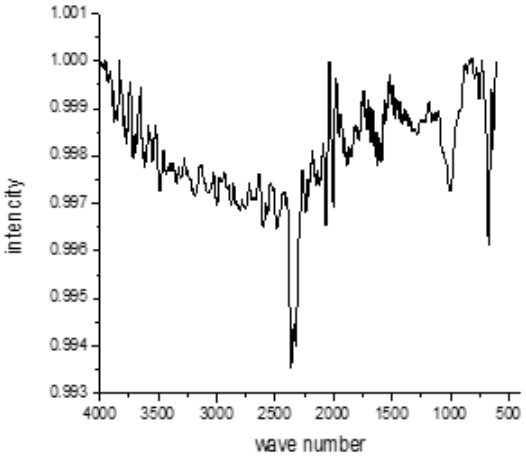

Figure 3(b)

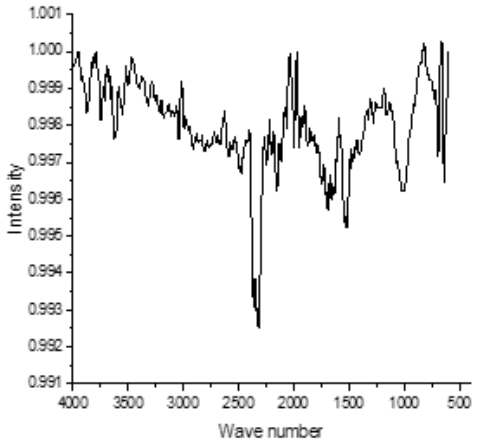

Figure 3(d)

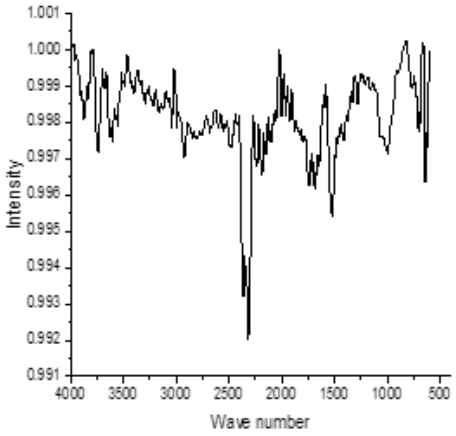

Figure 3(e)

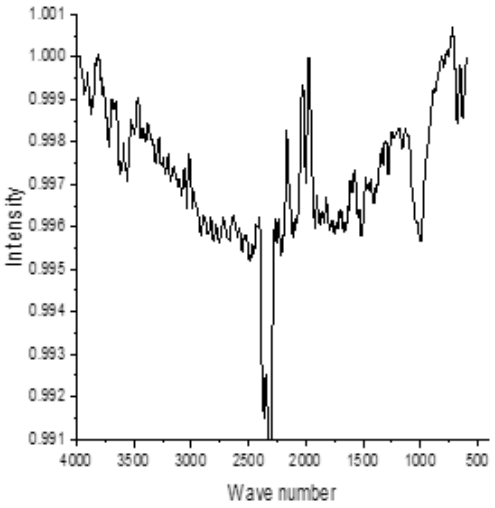

Figure 3(f)

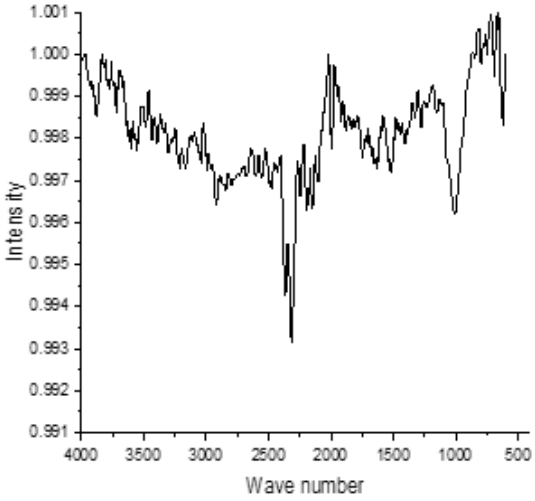

Figure 3(g)

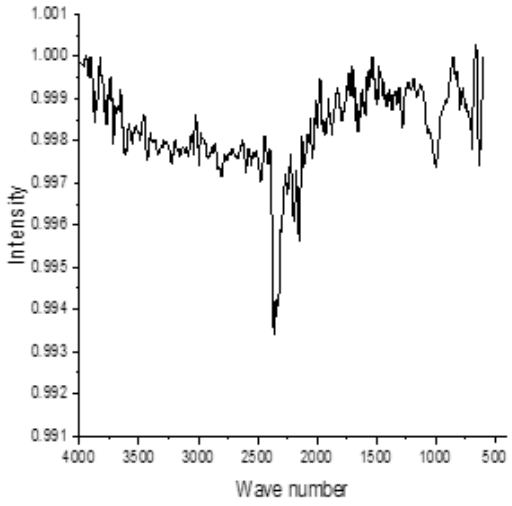

Figure 3(h)

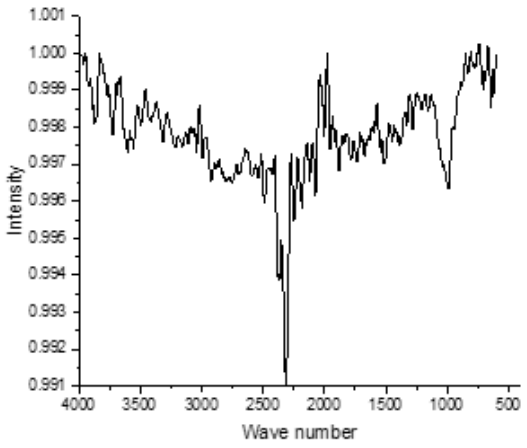

Figure 3(i)

Figure 3 (a-i)

Fourier transform infrared spectroscopy (FTIR) analysis of AgNO3 tested in this study on raw, germinated, cooked, autoclaved and microwaved kidney beans: (a) Raw Kidney beans (b) Germinated kidney beans, (c) 60ppm AgNPs germinated kidney beans, (d) Cooked Kidney bean, (e) 60ppm AgNPs Cooked kidney bean flour, (f) Autoclaved kidney bean flour,(g) 60ppm AgNPs Autoclaved Kidney bean flour, (h) Microwaved Kidney bean, (i) 60ppm AgNPs Microwaved Kidney bean flour

Conclusion

The aim of this study was to investigate the effects of AgNPs on the proximate composition, bioactive compounds, antinutrients, colour profile and imaging profile of beans subjected to different processing methods. Through comparative analysis, the research elucidated that the seed priming with 60ppm AgNPs in each process showed positive results as compared with their counterparts. Notably, germinated beans treated with AgNPs exhibited the highest levels of total phenolic content (1.59 mg gallic acid/g), flavonoid content (445.2 mg catechin/g), and antioxidant activity (89.0%). The study also investigated variations in antinutrient content, with trypsin inhibitor levels ranging from 0.04 to 2.83 mg/g, showing the highest in raw beans and the lowest in germinated beans treated with AgNPs. Tannin content varied from 0.40 to 1.26 mg/g, and phytic acid content ranged from 1.09 to 4.18 mg/g, with the lowest levels observed in germinated beans treated with AgNPs and the highest in raw beans. Overall, treatment with AgNPs in various processing methods significantly increased in developing functional foods with added health benefits. Also, it could lead to improved processing protocols for the production of high-quality beans. These findings underscored the diverse impacts of AgNPs on red kidney beans across various processing techniques, highlighting their potential in enhancing nutritional value and food safety.

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Declaration of Interest Statement

It is declared that we authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding declaration

The authors did not receive support from any organization for the submitted work.

Disclosure statement

The authors report there are no competing interests to declare.

Data availability statement

It includes both original data generated in my research and could be shared on your command.

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