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Sustainable recycling of poultry eggshell waste for the synthesis of calcium oxide nanoparticles and evaluating its antibacterial potency against food-borne pathogens

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ABSTRACT

Nanoparticles are considered new antibacterial agents with a potential broad range of applications. Recently, the synthesis of bio-nanoparticles (NPs) from natural sources such as coral, Ostrea shell, and eggshell, has attracted considerable attention. Eggshells are organic waste, rich in calcium carbonate ($CaCO_3$), and it is an easy method to reduce it into powder of nano size. Utilization of waste materials as a precursor for NPs synthesis makes the entire process cheaper, greener, and more sustainable. Waste chicken eggshells were collected from the Specific Pathogen Free farm in Egypt. Eggshells were rinsed, dried, and finely ground to powder. The sol-gel chemical method was used for the synthesis of CaO-NPs from eggshell powder. The characteristics of eggshell NPs were visualized using a scanning electron microscope, transmission electron microscope, Fourier transform infrared spectroscopy, and ultraviolet–visible spectroscopy. Additionally, the minimum inhibitory concentration was applied to test the antibacterial efficacy of CaO-NPs at 1.00, 0.50, 0.25, 0.12, and 0.06% concentrations on Methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Escherichia coli*, and *Salmonella enteritidis*. The results of the characterization confirmed the conversion of $CaCO_3$ to CaO-NPs with an average diameter of 27.7 nm. Zones of inhibition started to appear from 0.25% concentration for *B. cereus*, 0.50% for MRSA and *E. coli*, and 1.0% for *S. enteritidis*. The concentration of CaO-NPs solution strongly correlated with the resulting zone of inhibition (r= 0.86 to 0.90). CaO-NPs showed a potent efficacy against gram-positive bacteria. Hence, eggshell wastes from poultry production could be a feasible organic source for the biosynthesis of CaO-NPs with promising efficient antibacterial properties.

Introduction

Poultry production generates huge volumes of hatchery solid waste which involves empty shells, infertile eggs, dead embryos, late hatchings, and dead chickens. Eggshells account for approximately 10% of the eggs' weight and are typical solid waste in the food industry (Waheed *et al.*, 2020; Wu *et al.*, 2023). In 2018, global eggshell production reached 7.67 million tons (Aditya *et al.*, 2021). Solid hatchery wastes are traditionally disposed of through landfills, composting, rendering, or incineration, which costs the poultry industry millions of dollars annually. The improper disposal of waste takes up valuable land resources and pollutes the soil, while also serving as a medium for the transmission of bacteria (Grzeszczyk *et al.*, 2022). Hence, the accumulation of eggshell waste needs to be managed efficiently by developing a cost-effective and eco-friendly process to utilize eggshell waste (Cree and Pliya, 2019).

Eggshells consist of 96% $CaCO_3$ and 1% $MgCO_3$ and organic matter (Wu *et al.*, 2023). The shell material can be completely separated from other wastes and shell membranes, which raises the value of the resulting product (Das *et al.*, 2002; Glatz *et al.*, 2011). Eggshell waste is a valuable natural substance, rich in a highly purified form of calcium carbonate (more than 96% $CaCO_3$) as mineral calcite which is characterized by fewer impurities and has alkaline properties (Nys and Gautron, 2007; Cree and Rutter, 2015). The European Parliament and the Council have considered eggshell waste as a low-risk material, with no risk of transmitting diseases to humans, and can be directly used without the need for any pretreatment (Quina *et al.*, 2017). As the concept of the circular economy encourages the efficient reuse/recycling of wastes into beneficial resources with economic and environmental profits, numerous attempts have been made to investigate the various applications of eggshell wastes (Ferraz *et al.*, 2018; Lee *et al.*, 2020).

Calcium oxide (CaO) is a significant inorganic compound that has antimicrobial properties and can be prepared from $CaCO_3$ by decomposition above 900°C. Additionally, CaO nanoparticles could be prepared through a variety of methods, each resulting in nano-CaO with varied physical and chemical properties (Khine *et al.*, 2022). Metal oxide nanoparticles like CaO are safe, eco-friendly, stable, cost-effective, easy to produce, and possess unique structural and optical properties. Nanoparticles act like atoms as they have a larger surface-to-volume ratio, exhibit superior properties due to their size and morphology, are more chemically reactive, and fasten the diffusion compared to bulk materials (Bano and Pillai, 2020). The sol-gel chemical method is commonly used to prepare nanomaterials on a large scale, as it is characterized by its low cost, energy efficiency, high productivity, and rapid yield of fine homogeneous powder (Habte *et al.*, 2019).

Nanoparticles are considered novel antibacterial substances, especially metal oxide nanoparticles that with their unique physical and chemical properties could act in multiple modes of action against bacteria. In addition to changing bacterial cell membrane permeability, they interfere with sulfur and phosphorus-containing compounds, in a way that makes bacteria could not develop resistance against them (Marc Najjar *et al.*, 2019; Zhang *et al.*, 2022). Recently, the synthesis of nano-metal oxides through green methods exhibited lower cytotoxicity than that of the chemically produced NPs, making them more widely applied in food industries, biomedicine, and environmental applications (Zhang *et al.*,

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2022). Previous studies proved that CaO-NPs showed high antibacterial activity against *Bacillus*, *Pseudomonas aeruginosa*, *E. coli*, and biofilm formation, but lower antimicrobial action toward *Staphylococcus aureus*, *Salmonella typhi*, and *Vibrio cholerae* (Kumari *et al*., 2022). CaO-NPs mainly affect bacteria by generating superoxide radicals, which function through three key mechanisms; mechanically damaging the bacterial cell wall, initiating oxidative stress, and disrupting protein and cell structures (Kumari *et al*., 2022).

The aim of this study was to reuse the eggshell waste of chicken hatcheries for the preparation of organic (green) CaO nanoparticles (CaO-NPs) and testing the antibacterial capacity of the synthesized CaO-NPs through the minimum inhibitory concentration technique (agar diffusion method).

Materials and methods

Preparation of eggshell powder

Chicken eggshell wastes were collected from the Specific Pathogen Free (SPF) farm; located in Fayoum governorate, Egypt. The eggshells were thoroughly rinsed several times in a hot water bath to remove impurities and interfering materials. Afterwards, eggshells were sterilized in a hot water bath for 15 min, then dried at 120°C for 2 h in the hot air oven. The shells were ground into a fine powder with a grinder and sieved with a 100 µm sieve size (Park *et al*., 2007; Habte *et al*., 2019; Nikhil *et al*., 2020).

Synthesis of calcium oxide nanoparticles (CaO-NPs)

Calcium oxide nanoparticles were synthesized by the sol-gel method, which includes four successive steps: 1) preparation of homogeneous $CaCl_2$ solution, 2) formation of 'sol' by hydrolysis, 3) formation of 'gel' by condensation; and 4) drying of the formed gel (Park *et al*., 2007). For preparing calcium chloride ($CaCl_2$) solution, 12.5 g of powdered eggshell was dissolved in 250 mL of 1 M hydrochloric acid (HCl). For 'sol' formation, 250 mL of 1 M sodium hydroxide (NaOH) was added slowly (drop by drop) to convert the homogeneous $CaCl_2$ solution formed into 'sol' at ambient temperature. 'Sol' is a stable dispersion of colloidal particles of precursors in a solvent due to a hydrolysis reaction. For 'gel' formation, NaOH was slowly added to precipitate Ca $(OH)_2$ one over another producing a highly crystalline gel. A rigid and highly crystalline inorganic network was formed from the interconnection between the tiny particles within the liquid because of the condensation reaction. Ca $(OH)_2$ gel-containing solution was aged for one night at ambient temperature (Habte *et al*., 2019).

Afterwards, filtration was done by cleaning the filtrate with distilled water to remove adsorbed impurities in the precipitate then the gel was dried. Significant shrinkage and densification occurred during this process because the solvent (liquid phase) was removed. The powder was dried at 60°C for 24 h in an oven and calcined in a muffle furnace at 900°C for 1 h (Habte *et al*., 2019).

Characterization of CaO nanoparticles

The synthesized CaO nanoparticles were characterized through various analytical techniques (Mirghiasi *et al*., 2014; Mourdikoudis *et al*., 2018; Habte *et al*., 2019). The surface morphology, shape, aggregation state, and average size of the produced nanoparticles were examined through scanning electron microscope (SEM) and transmission electron microscopy (TEM). The Fourier transform infrared spectroscopy (FTIR) was used to determine the different functional groups present in the synthesized nanoparticle. The spectrum was measured in the range of 4000–400 cm^{-1} (the mid-infrared region) with a resolution of 4 cm^{-1}. An average of 16 scans were performed for each spectrum. Spectrums provide information about molecular structures and interactions by determining the positions of bands related to bond strength and nature.

Ultraviolet–visible (UV-Vis) spectroscopy was used to analyze the optical properties of the CaO-NPs and determine their magnitude, peak wavelength, and spectral bandwidth. UV-Vis is a relatively facile and low-cost characterization method often used for studying nanoscale materials. It compares the light reflected from a sample with that reflected from a reference material (George *et al*., 2017).

Preparation of the tested bacterial strains

Antimicrobial activity was assessed using Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus cereus* as gram-positive bacteria, while *Escherichia coli* and *Salmonella enteritidis* as Gram-negative bacteria. The bacterial strains were previously isolated and identified from dairy cattle origin. The pure culture of each bacterial strain was grown overnight in Brain Heart Infusion broth (HiMedia) containing 0.3% yeast extract at 37°C; the concentration was adjusted using sterile BHI broth until a 0.5 McFarland turbidity was attained. The inoculum was standardized to obtain the desired concentration of 10^8 CFU/ml.

Preparation of eggshell CaO-NPs concentrations

Five different concentrations of the eggshell CaO-NPs powder were prepared in sterile distilled water (1, 0.5, 0.25, 0.12, and 0.06 %).

Antibacterial potency (Minimum Inhibitory Concentration)

The Minimum Inhibitory Concentration (MIC) values of CaO-NPs against the selected bacterial strains were performed using the agar diffusion method according to Burt (2004). One hundred microliters of the standardized cell suspensions of each bacterial strain were spread on a Mueller-Hinton agar (HiMedia) using a sterile cotton swab. The agar medium was punched with six millimeters diameter wells and filled with different concentrations of nanoparticle solutions in equal amounts. The plates were then observed for zones of inhibition after 24 h incubation at 37°C. Control agar plates (at least 2 control plates) were prepared without adding CaO-NPs. The experimental plates were replicated as duplicates.

Statistical analysis

The R Project for Statistical Computing (Version 4.3.1) was used for data visualization and graphical analysis. Boxplot was built by the 'ggplot2' package (Wickham, 2016), clustered heatmap was generated through the 'pheatmap' package (Kolde, 2018), correlation matrix was created by the 'corrplot' package (Wei and Simko, 2021), and the principal component analysis (PCA) was done via the 'FactoMineR' and 'factoextra' packages (Lê *et al*., 2008; Kassambara and Mundt, 2020).

Results

Characterization

The synthesized nano-size CaO from eggshells was characterized by using SEM, TEM, FTIR, and UV-Vis. spectroscopy. The shape and size of the CaO-NPs were established by SEM and TEM results and measurements as shown in Figures 1 and 2. The SEM and TEM images showed that the synthesized CaO-NPs are uniformly dispersed nano-spherical-shaped particles. CaO-NPs with various diameters ranging from 18.8 to 44.9 nm (avg. diameter of 27.7 nm, median 27.2 nm) were observed, as plotted in Figure 3.

The FTIR spectrum of the synthesized CaO NP material is displayed in Figure 4. A narrow peak was plotted at 3637.61 cm^{-1} spectrum representing O–H stretching, while additional wide peaks were displayed at 1041.64, 1094.28, 1458.43, and 1637.33 cm^{-1} spectra, indicating C–O bond. Another tiny peak at 2926.69 cm⁻¹ represented the C–O bond. The absence of a sharp absorption in the regions at 1418.04-1415.52 cm^{-1} implied that the $CaCO_3$ as the main component of eggshell was not there anymore as it was converted to CaO. The strong peak at 435.07 cm^{-1} defines the Ca–O bond (Mostafa *et al*., 2023).

The UV-Vis spectrum of the synthesized CaO-NPs was presented in Figure 5, and it displayed a start wavelength at 200.00 nm and a stop wavelength of 900.00 nm, where CaO-NPs demonstrated a single sharp band with the maximum absorption at 291.50 nm, which registered 0.984 absorbance value.

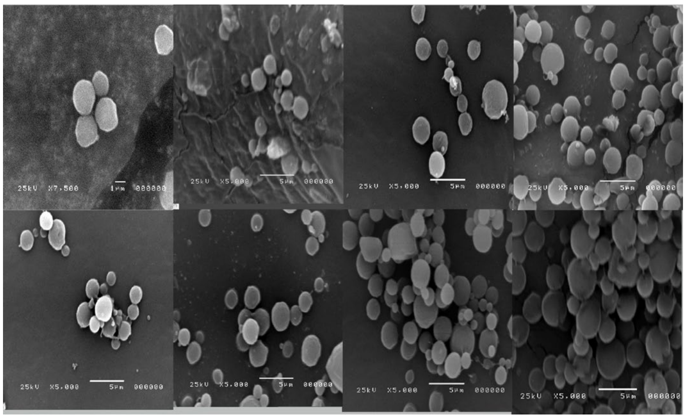

Fig. 1. Scanning electron microscopy (SEM) images of the synthesized CaO-NPs and particle size distribution.

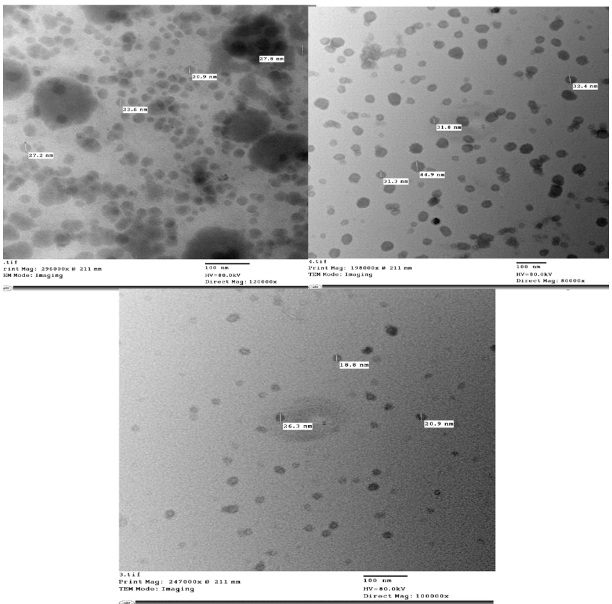

Fig. 2. Transmission electron microscopy (TEM) images of the synthesized CaO-NPs displaying particle size distribution.

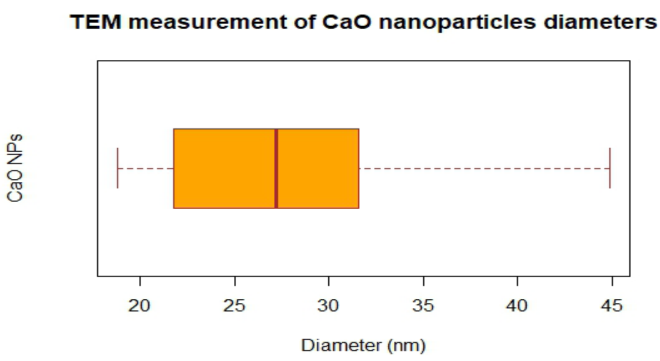

Fig. 3. Diameter range of the synthesized CaO-NPs measured by TEM.

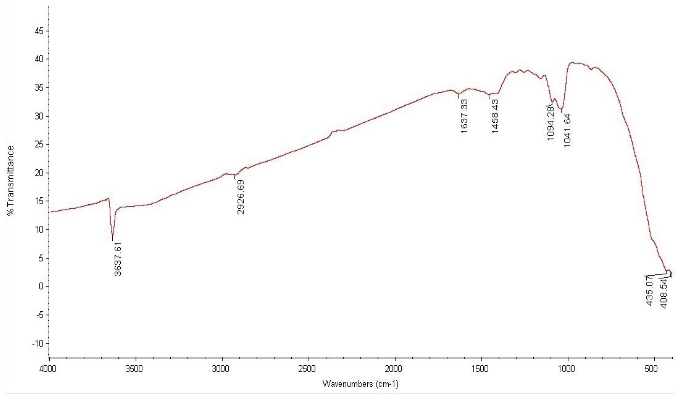

Fig. 4. Fourier transform infrared spectroscopy (FTIR) spectrum of synthesized CaO-NPs. The mid-infrared spectrum (wavenumbers cm^{-1}) were plotted on the horizontal axis between 4,000 and 400 cm^{-1}, while the peaks (absorbance bands) represent the different vibrations of the material's atoms. The vertical axis identifies the quantity of infrared light transmitted by the analyzed nanoparticle material.

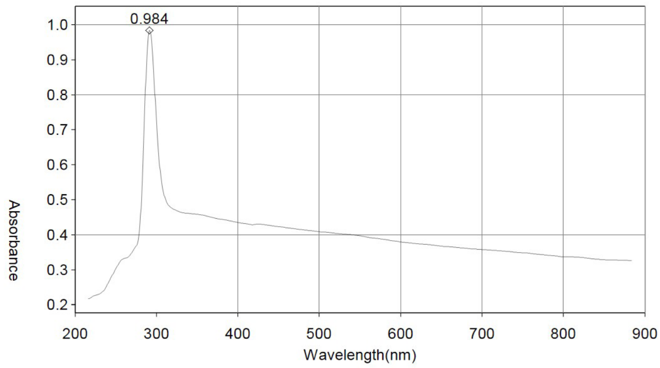

Fig. 5. Ultraviolet–visible (UV-Vis) spectroscopy spectrum of the synthesized CaO NPs.

Antibacterial potency of CaO-NPs by MIC test

Results of MIC through agar gel diffusion were demonstrated in Figures 6, 7, 8. Diameters of zones of inhibition revealed moderate antibacterial potency of CaO-NPs at the concentrations of 0.25%, 0.50%, and 1.0%. The highest antibacterial potency was recorded against gram-positive bacteria. Zones of inhibition for MRSA were 12.5 mm at 0.50% CaO-NPs solution, and 14.0 mm at 1.0% concentration. Also, *B. cereus* recorded 10 mm at 0.25% CaO-NPs solution, 11 mm at 0.50% solution, and 14.5 mm at 1.0% concentration. On the other hand, CaO-NPs exhibited antibacterial activity against gram-negative bacteria. Zones of inhibition for *E. coli* were 12.0 mm at 0.50% CaO-NPs solution, and 13.0 mm at 1.0% concentration. Besides, *S. enteritidis* recorded 11 mm at 1.0% concentration (Figures 6 and 7).

The heatmap in Figure 7 displayed that CaO-NPs at 1.00% and 0.50% concentrations posed the most potent antibacterial efficacy, as they were categorized in a separate cluster. Also, the heatmap showed that *S. enteritidis* was the least sensitive bacteria while *B. cereus* showed the most sensitive bacteria to CaO-NPs. Additionally, Figure 8 showed the correlation between different tested bacteria, where the correlation matrix indicated the strong positive correlations between the concentration of CaO-NPs solution and the resulting zone of inhibition (r = 0.86 to 0.90). Also, it showed a strong positive correlation between *B. cereus*, MRSA, and *E. coli* results (r = 0.78). The PCA confirmed the findings by demonstrating the correlation between MRSA and *E. coli* results, and the strong variation between *B. cereus* and *S. enteritidis*.

Discussion

In the current study, eggshell waste from the specific pathogen-free farm was used as a green resource to produce organic CaO nanoparticles (NPs). In this study, the Sol-gel method was applied for CaO NP synthesis

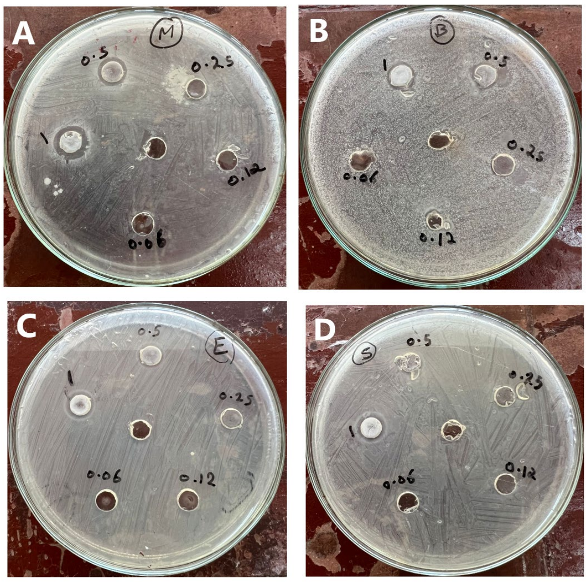

Fig. 6. The antibacterial efficacy of CaO-NPs (A) MRSA, (B) *B. cereus*, (C) *E. coli*, and (D) *S. enteritidis* at various concentrations by MIC.

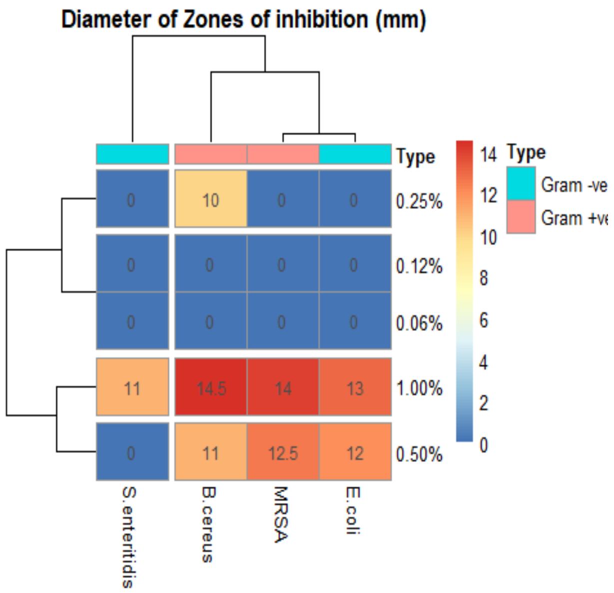

Fig. 7. Clustered heatmap of MIC zones of inhibition in relation to the varied dilutions of CaO nanoparticles solution.

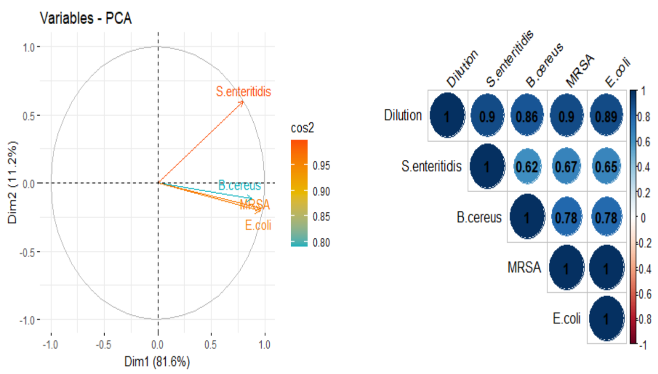

Fig. 8. Correlation matrix (the right plot) of the zones of inhibition of different bacteria and CaO-NPs dilution; showing the values of Pearson correlation coefficient (r). Blue circles represent positive correlations. The degree of color implies the intensity of the correlation. Principal component analysis (PCA) (the left plot) of the zones of inhibition clustering the bacterial strains. The color scale indicates the quality representation of the variables (cos2) between 0 and 1. The two principal components (PC) explained 92.8% of the total variation. The first PC (Dim1) represented the direction in which the data varied the most which explained 81.6% of the variance and had positive associations with MRSA and *E. coli.* The second PC (Dim2) represented the direction in which the data varied the second most which accounted for 11.2% of the variance and showed a large positive association with *S. enteritidis*. The third component (7.2%) showed a large positive association with *B. cereus.*

from eggshell powder. The eggshells are organic wastes, and the hardness of the shells is attributed to the presence of calcium carbonate compound, which made it simple to reduce it into nano-sized powder. NPs have attracted the attention of scientists due to their unique characteristics (like antimicrobial, shape, and size depending optical and electrical properties). NPs could be prepared by a variety of techniques, like sol-gel, chemical reduction, microwave processing, and more (Nikhil *et al*., 2020). In comparison to nano-production techniques, the sol-gel technique has several significant advantages, including its simplicity, economy, ambient temperature, not needing expensive equipment, and lack of pressure (Habte *et al*., 2019). Furthermore, using waste materials as a precursor for the synthesis of nanoparticles makes the entire process less expensive, greener (eco-friendly), and more sustainable.

The current experiment investigated the properties and particle size of the produced CaO NPs with the help of several analyzing methods (the scanning electron microscope (SEM), Transmission electron microscope (TEM), Fourier transform (FTIR), and Ultraviolet-visible spectroscopy (UV-Vis)). SEM and TEM images shown in Figures 1 and 2 demonstrated a spherical morphology of the produced CaO nanoparticles which were agglomerated with each other. Other studies have supported the spherical shape of CaO NPs (Mirghiasi *et al*., 2014; Habte *et al*., 2019). The size of calcium oxide nanoparticles was decreased after the drying of $Ca(OH)_2$ gel due to the release of CO_2 and H_2O. The calcination temperature was set at 900°C, which resulted in the vaporization of absorbed water and the decomposition of $Ca(OH)_2$ and $CaCO_3$ to CaO. Furthermore, TEM revealed the particle size range of CaO nanoparticles with an average of around 27.7 nm, which agreed with a previous report (Mirghiasi *et al*., 2014).

The obtained FTIR results were attributed to the C–O bond, signifying the carbonation of calcium oxide nanoparticles, which are consistent with Gedda *et al*. (2015) and Habte *et al*. (2019) findings. The displayed FTIR spectra for the synthesized CaO NPs showed a sharp narrow peak at 3637.61 cm^{-1}, representing the O-H bond found on the CaO surface, and is characteristic of standard CaO, as reported previously (Prayitno *et al*., 2020; Mostafa *et al*., 2023). Additionally, the tiny peak at 2926.69 cm^{-1} and wide peaks at 1637.33, 1458.43, 1094.28, and 1041.64 cm^{-1}, indicated the C–O bond, as recorded by Mostafa *et al*. (2023). The absence of a sharp absorption in the regions at 1418.04-1415.52 cm^{-1} implied that the $CaCO_3$ as the main component of eggshell was not there anymore as it was converted to CaO, as explained by Mostafa *et al*. (2023). The strong peak at 435.07 cm^{-1} is defining the Ca–O bond (Mostafa *et al*., 2023). FTIR technique is a vibrational spectroscopy and is usually used to confirm the identification and purity of compounds through the measurement of the spectrum of transmittance of infrared radiation (IR) by the sample material versus wavelength (Nikhil *et al*., 2020). Frequencies of functional groups like OH and C=O bonds are noticed at infrared spectra greater than 1,500 cm^{-1}. On the other hand, fingerprint frequencies, which are highly characteristic of the molecule as a whole; are found at infrared spectra less than 500 cm^{-1}.

Characterization of CaO-NPs by UV–Vis in the range of 200 to 800 nm revealed the maximum absorbance between 200–400 nm, which corresponds to previous studies (Jadhav *et al*., 2022; Tabrizi Hafez Moghaddas *et al*., 2022). UV-Vis. is a low-cost, simple, and quick technique frequently used in the study of nanoscale materials. It calculates the light reflection intensity from a sample and compares it with the light reflection intensity from a reference material. UV-Vis. spectroscopy is a significant tool for characterizing, classifying, and investigating NPs, as well as evaluating the stability of NP colloidal solutions because NPs have optical properties that are sensitive to shape, size, concentration, agglomeration state, and refractive index near the NP surface (Mourdikoudis *et al*., 2018).

The produced CaO NPs were tested for their antibacterial potency against Gram-negative and Gram-positive bacterial strains. The antibacterial action was assessed using the minimum inhibitory concentration (MIC) test by agar diffusion method. The CaO NPs displayed inhibition zones of diameters ranging from 10 to 14.5 mm, indicating diverse degrees of microbial sensitivity. This could happen because of the different structures in the bacterial cell wall since the cell wall of Gram-positive bacteria is approximately composed of 90-95% peptidoglycan, which permits molecules to enter the cell and act on the cell wall and the cytoplasm. The Gram-negative bacterial cell wall is more complicated, as the peptidoglycan layer is thicker by 2-3 nm than in Gram-positive bacteria and is covered with an outer membrane containing several proteins as well as lipopolysaccharides, making Gram-negative bacteria more resistant to CaO-NPs and other natural antimicrobials (Ahmed *et al*., 2021).

B. cereus was the most sensitive strain with a minimum inhibitory concentration of CaO-NPs at 0.25%, while *S. enteritidis* was the most resistant strain as it showed an inhibition zone of 11 mm with CaO-NPs concentration of 1%. The antibacterial activity of the CaO NPs against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) was studied previously by Gedda *et al*. (2015) and demonstrated that increasing the concentration of CaO NPs reduced the bacterial growth rate; and that the minimum inhibitory concentration (MIC) of CaO NPs was found to be 10 μg/ml for both bacteria, which agreed with our result. Moreover, Gedda *et al*. (2015) discovered that, in the presence of CaO NPs, there was a clear formation of an inhibition zone, as there was no significant bacterial growth present around the well. They recorded inhibition zone diameters for the CaO NPs against *E. coli* and *S. aureus* were 19.0±2.0 mm and 17.0±2.0 mm, respectively. This clearly shows that the higher the concentration of CaO NPs the more antibacterial activity against pathogenic bacteria is.

CaO NPs exhibit sufficient antibacterial activity because of their high surface area to volume ratios, which allow for good interaction with bacteria and increase the release of reactive oxygen species (ROS) on their surface (Bae *et al*., 2006; Roy *et al*., 2013; Gedda *et al*., 2015). Sawai (2003) and Hewitt *et al*. (2001) examined the production of ROS, which is increased in alkaline pH because of the dehydration effect of CaO NPs. The bacterial cell wall is destroyed by the ROS's influence on protein degradation when they interact with the carbonyl groups found in the peptide linkages and polyunsaturated phospholipids that make up the bacterial cell wall. Additionally, NPs accumulate inside the bacterial cells and around the cell membranes, as NPs enter the cells via cell membrane disruption (Gedda *et al*., 2015).

Also, previous studies have reported that the size of nanoparticles impacted the antibacterial activity, as the size range of 20-95 nm copper oxide NPs exerted antibacterial effectiveness against *E. coli* and MRSA (Zhang *et al*., 2022). The smaller the particle size the higher the binding capacity and the antibacterial activity. In a former study, green CaO-NPs were produced from waste chicken eggshells and tested for their antimicrobial activity in 100 μg/ml concentration. They recorded zones of inhibition against *B. subtilis* (14 mm), *S. aureus* (13 mm), and *E. coli* (16 mm), (Pasupathy and Rajamanickam, 2019).

Conclusion

The CaO nanoparticles synthesized from eggshell powder demonstrated distinct structural and optical properties, as well as antibacterial activity against a wide range of bacteria. The agar diffusion method (MIC) revealed the antimicrobial potency of nano-egg shell particles with a higher antimicrobial effect against gram-positive bacteria (MRSA and *B. cereus*) than against gram-negative bacteria (*E. coli* and *S. enteritidis*). As a result, the large-scale production of organic CaO NPs from eggshell waste generated from egg-producing poultry sectors is a possible way to recycle solid wastes and produce green nanoparticles with potential antimicrobial applications in food industries and biomedicine.

Conflict of interest

The authors have no conflict of interests to disclose.

References

Aditya, S., Stephen, J., Radhakrishnan, M., 2021. Utilization of eggshell waste in calcium-fortified foods and other industrial applications: A review. Trends in Food Science and Technology 115, 422-432.

Ahmed, L.I., Ibrahim, N., Abdel-Salam, A.B., Fahim, K.M., 2021. Potential application of ginger, clove and thyme essential oils to improve soft cheese microbial safety and sensory characteristics. Food Bioscience 42, 101177.

Bae, D.H., Yeon, J.H., Park, S.Y., Lee, D.H., Ha, S.D., 2006. Bactericidal effects of CaO (scallop-shell powder) on foodborne pathogenic bacteria. Archives of Pharmacal Research 29, 298-301.

Bano, S., Pillai, S., 2020. Green synthesis of calcium oxide nanoparticles at different calcination temperatures. World Journal of Science, Technology and Sustainable Development 17, 283-295.

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology 94, 223-253.

Cree, D., Pliya, P., 2019. Effect of elevated temperature on eggshell, eggshell powder and eggshell powder mortars for masonry applications. Journal of Building Engineering 26, 100852.

Cree, D., Rutter, A., 2015. Sustainable bio-inspired limestone eggshell powder for potential industrialized applications. ACS Sustainable Chemistry and Engineering 3, 941-949.

Das, K.C., Minkara, M.Y., Melear, N.D., Tollner, E.W., 2002. Effect of poultry litter amendment on hatchery waste composting. Journal of Applied Poultry Research 11, 282-290.

Ferraz, E., Gamelas, J.A., Coroado, J., Monteiro, C., Rocha, F., 2018. Eggshell waste to produce building lime: calcium oxide reactivity, industrial, environmental and economic implications. Materials and Structures 51, 115.

Gedda, G., Pandey, S., Lin, Y.C., Wu, H.F., 2015. Antibacterial effect of calcium oxide nano-plates fabricated from shrimp shells. Green Chemistry 17, 3276-3280.

George, G., Wilson, R., Joy, J., 2017. Ultraviolet Spectroscopy: A Facile Approach for the Characterization of Nanomaterials. In: Spectroscopic Methods for Nanomaterials Characterization. Elsevier. pp. 55-72

Glatz, P., Miao, Z., Rodda, B., 2011. Handling and treatment of poultry hatchery waste: A review. Sustainability 3, 216-237.

Grzeszczyk, S., Kupka, T., Kałamarz, A., Sudoł, A., Jurowski, K., Makieieva, N., Oleksowicz, K., Wrzalik, R., 2022. Characterization of eggshell as limestone replacement and its influence on properties of modified cement. Construction and Building Materials 319, 126006.

Habte, L., Shiferaw, N., Mulatu, D., Thenepalli, T., Chilakala, R., Ahn, J.W., 2019. Synthesis of nano-calcium oxide from waste eggshell by sol-gel method. Sustainability 11, 3196.

Hewitt, C.J., Bellara, S.R., Andreani, A., Nebe-von-Caron, G., McFarlane, C.M., 2001. An evaluation of the anti-bacterial action of ceramic powder slurries using multi-parameter flow cytometry. Biotechnology letters 23, 667-675.

Jadhav, V., Bhagare, A., Wahab, S., Lokhande, D., Vaidya, C., Dhayagude, A., Khalid, M., Aher, J., Mezni, A., Dutta, M., 2022. Green synthesized calcium oxide nanoparticles (CaO NPs) using leaves aqueous extract of moringa oleifera and evaluation of their antibacterial activities. Journal of Nanomaterials 2022, 1-7.

Kassambara, A., Mundt, F., 2020. factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R package version 1.0.7, https://CRAN.R-project.org/package=factoextra

Khine, E.E., Koncz-Horvath, D., Kristaly, F., Ferenczi, T., Karacs, G., Baumli, P., Kaptay, G., 2022. Synthesis and characterization of calcium oxide nanoparticles for CO2 capture. Journal of Nanoparticle Research 24, 139.

Kolde, R., 2018. Package 'pheatmap': Pretty Heatmaps. Version 1.0.12. R Package, 1. https://CRAN.R-project.org/package=pheatmap

Kumari, M., Sarkar, B., Mukherjee, K., 2022. Nanoscale calcium oxide and its biomedical applications: A comprehensive review. Biocatalysis and Agricultural Biotechnology 102506.

Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. Journal of Statistical Software 25, 1-18. Doi: 10.18637/jss.v025.i01

Lee, M., Tsai, W.S., Chen, S.T., 2020. Reusing shell waste as a soil conditioner alternative? A comparative study of eggshell and oyster shell using a life cycle assessment approach. Journal of Cleaner Production 265, 121845.

Mirghiasi, Z., Bakhtiari, F., Darezereshki, E., Esmaeilzadeh, E., 2014. Preparation and characterization of CaO nanoparticles from Ca (OH) 2 by direct thermal decomposition method. Journal of Industrial and Engineering Chemistry 20, 113-117.

Mostafa, F.A., Gad, A.N., Gaber, A.A.M., Abdel-Wahab, A.M.A., 2023. Preparation, characterization and application of calcium oxide nanoparticles from waste carbonation mud in clarification of raw sugar melt. Sugar Tech. 25, 331-338.

Mourdikoudis, S., Pallares, R.M., Thanh, N.T., 2018. Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. Nanoscale 10, 12871-12934.

Marc Najjar, M.D., Ross, M., Srivastava, A., Robyn Gartrell, M.D., Emanuelle Rizk, B.A., Perez, O., Lieberman, E., Drake, C., 2019. 34th Annual Meeting & Pre-Conference Programs of the Society for Immunotherapy of Cancer (SITC 2019): part 1. Journal for ImmunoTherapy of Cancer 7, 282.

Nikhil, P., Sudheer, P., Joy, P.J., Sruthi, T., Vangalpati, M., 2020. Synthesis and characterization of nanoparticles using egg shell. Materials Today: Proceedings 26, 3246-3249.

Nys, Y., Gautron, J., 2007. Structure and formation of the eggshell. In: Huopalahti, R., Lopez-Fandino, R., Anton, M., Schade, R. (Eds.), Bioactive Egg Compounds. Springer, Berlin, Heidelberg.

Park, H.J., Jeong, S.W., Yang, J.K., Kim, B.G., Lee, S.M., 2007. Removal of heavy metals using waste eggshell. Journal of Environmental Sciences 19, 1436-1441.

Pasupathy, S., Rajamanickam, M., 2019. Synthesis of pure and bio modified calcium oxide (CaO) nanoparticles using waste chicken egg shells and evaluation of its antibacterial activity. Int. J. Pharma Sci. Res. 1, 230.

Prayitno, A.H., Prasetyo, B., Sutirtoadi, A., 2020. Synthesis and characteristics of nano calcium oxide from duck eggshells by precipitation method. In IOP Conference Series: Earth and Environmental Science, 411, 012033. IOP Publishing.

Quina, M.J., Soares, M.A., Quinta-Ferreira, R., 2017. Applications of industrial eggshell as a valuable anthropogenic resource. Resources, Conservation and Recycling 123, 176-186.

Roy, A., Gauri, S.S., Bhattacharya, M., Bhattacharya, J., 2013. Antimicrobial activity of CaO nanoparticles. Journal of Biomedical Nanotechnology 9, 1570-1578.

Sawai, J., 2003. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. Journal of Microbiological Methods 54, 177-182.

Tabrizi Hafez Moghaddas, S.S., Samareh Moosavi, S., Kazemi Oskuee, R., 2022. Green synthesis of calcium oxide nanoparticles in Linum usitatissimum extract and investigation of their photocatalytic and cytotoxicity effects. Biomass Conversion and Biorefinery 1, 1-10.

Waheed, M., Yousaf, M., Shehzad, A., Inam-Ur-Raheem, M., Khan, M.K.I., Khan, M.R., Ahmad, N., Aadil, R.M., 2020. Channelling eggshell waste to valuable and utilizable products: a comprehensive review. Trends in Food Science and Technology 106, 78-90.

Wei, T., Simko, V., 2021. R package 'corrplot': Visualization of a Correlation Matrix (Version 0.92). Available from https://github.com/taiyun/corrplot

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Wu, S., Sun, T., Xu, H., 2023. A new way to efficient utilization of eggshell waste: As green dephosphorization agent and accelerator for reduction roasting of high-phosphorus oolitic iron ore. Process Safety and Environmental Protection 173, 702-714.

Zhang, S., Lin, L., Huang, X., Lu, Y.G., Zheng, D.L., Feng, Y., 2022. Antimicrobial properties of metal nanoparticles and their oxide materials and their applications in oral biology. Journal of Nanomaterials 2022, 2063265.