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Characterisation of Nano-Calcium Citrate from Waste Broiler Chicken Bones Synthesized Using Lime as a Novel Food Supplement

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ABSTRACT

Broiler chicken bone calcium oxide is 5.6 g mixed with 20 mL of distilled water. A mixture of 50 mL of lime juice and 30 mL of distilled water was added and then stirred using a hotplate magnetic stirrer at a temperature of 75°C for 30 min at a speed of 500 rpm/min. Ethanol 50% was added to as much as 20 mL (v/v), dried in the sun for 5 d, and then ground using a blender. Parameters observed were particle-size analysis (PSA), scanning electron microscopy (SEM), energy dispersive x-ray (EDX), fourier transform infrared (FTIR), and color. Data from PSA, SEM, EDX, FTIR, and color test results were analyzed descriptively. The research results show that green synthesis with lime can change the particle size of broiler chicken bone calcium into nanoparticles. Lime can be used to synthesize broiler chicken bone calcium into nano-calcium citrate with a particle size of around 524.1 nm. Colorimetric evaluation showed excellent brightness (L^* 72.60), minimal redness (a^* 3.20), moderate yellowness (b^* 15.60), and acceptable whiteness (68.31), demonstrating lime's dual function as both a synthesizing agent and natural purifier. These findings indicate that the lime-mediated synthesis produces high-quality nano-calcium citrate from poultry byproducts, making it a promising candidate for novel food supplement applications.

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Introduction

Indonesia, with a population of around 280 million, has a large consumption of animal food. One of the most consumed foods of animal origin is chicken meat. Effendi et al. (2023) noted that broiler meat production in 2021 reached 3,426,042 tons. The consumption of broiler meat shows an increasing trend every year. Every consumption of chicken meat will produce bio-waste, one of which is chicken bones. The resulting chicken bones have low economic value and are in large quantities. Chicken bones are mainly produced from the poultry slaughterhouse industry, poultry meat processing industry, restaurants, and households. Chicken bones have not been used optimally. The utilization of chicken bones changed only in the form of flour.

The percentage of chicken bones is around 22.49-30.27% of the chicken's slaughter weight (Patriani and Hafid, 2019). Ningsih et al. (2022) the percentage of chicken bones is around 12.28-21.96% in breast meat, 23.29-26.60% in thigh meat, 31.96-37.14% in wing meat. Chicken bones have a chemical composition of 21% collagen, 9% water, 69% calcium phosphate, and

1% other ingredients based on the percentage of weight. The high calcium content in chicken bones has the potential to be used as an alternative source of calcium (First et al., 2019). Calcium, as the main mineral constituent of bones and teeth, is needed by the body. If the calcium needed by the body is not met, the density of bones and teeth will also decrease, resulting in brittleness. The International Osteoporosis Foundation (IOF) reports that the prevalence of osteoporosis in women in Indonesia at the age of 50-70 years and over 70 years is 23% and 53% (Widarsa et al., 2018). Men after the age of 55 have a higher risk of osteoporosis when compared to women (Jahari and Prihatini, 2014).

Calcium is consumed in general in micro-sized amounts. This size is related to the amount of calcium absorbed by the body, which is usually only 50%, so it often causes deficiency (Aminingsih et al., 2018; Zufadhillah et al., 2018; Ranjan et al., 2019). Optimization of the use of waste chicken bone can be done by converting it into a nanometer-sized particle. Minerals in nano size are included in advanced materials that have high economic value and have much better characteristics when compared to macro-sized

minerals. The application of nanotechnology can change the calcium source particles from 13,229 nm to 347 nm (Prayitno *et al.*, 2020). The performance of nano-sized particles is better due to the increased surface area (Habte *et al.*, 2019).

One type of calcium that is widely produced is nano-calcium citrate (NCaS). Nano-calcium can be used as a food supplement (Prayitno *et al.*, 2021) and as a drug delivery system that can be almost 100% absorbed by the body (Suptijah, 2019). Chicken bone waste can be used as a food supplement ingredient with nanotechnology applications. Green synthesis is one method for synthesizing nanoparticles. Green synthesis is considered easy to do and environmentally friendly. This is because the resulting product is low in toxicity (Khan *et al.*, 2022). The green synthesis process for producing nanoparticles has the advantage of reducing waste and pollutants. The process of synthesizing calcium nanoparticles can use citric acid. Citric acid is an acidic solvent that can be used for nanoparticle synthesis (Li *et al.*, 2013).

Citric acid is found in many lime fruits (*Citrus aurantiifolia*). Lime contains about 7-7.6% citric acid and has a pH value of around 2-3 (Seftiono *et al.*, 2020). The application of nanotechnology can change the size of calcium particles from broiler chicken bone waste to nano-sized ones through the green synthesis method. Nanotechnology is the process of engineering materials from something that may not have any benefits into very valuable materials and products that are very expensive. Therefore, this research needs to be carried out to synthesize nano-calcium from broiler chicken bone waste as a novel food supplement ingredient through green synthesis with lime.

Materials and Methods

Broiler Chicken Bone Flour Preparation

Preparation of broiler chicken bones into flour was made as described by Okfrianti *et al.* (2011) which has been modified. Broiler bones are washed and then boiled for 1 h. Broiler chicken bones are mixed with water in a ratio of 1:6 (w/v), then cooked in a press cooker for 2 h. Broiler chicken bones are then baked at a temperature of 105°C for 24 h and then mashed with a sample mill.

Broiler Chicken Bone Calcium Oxide Preparation

The preparation of broiler chicken bone meal into calcium oxide (CaO) was made as described by Prayitno *et al.* (2021) which has been modified. Chicken bone meal is calcined at 600°C for 2 h to obtain CaO powder.

Chicken Bone Nano-Calcium Preparation

The preparation of nano-calcium green synthesis from broiler bone with lime was prepared as described by Li *et al.*, (2016) which has been modified. Broiler chicken bone calcium oxide is 5.6 g mixed with 20 mL of distilled water. A mixture of

50 mL of lime juice and 30 mL of distilled water was added, then stirred using a hotplate magnetic stirrer at 75°C for 30 min at a speed of 500 rpm per min. Ethanol 50% was added to as much as 20 mL (v/v), dried in the sun for 5 d, and then pulverized using a blender to produce nano-calcium powder for broiler chicken bones.

Particle-Size Analysis

Broiler chicken bone nano-calcium powder produced through the green synthesis process with lime was tested for particle size using a particle-size analyzer (Dai *et al.*, 2018).

Scanning Electron Microscopy Analysis

The morphology of the nano-calcium powder ultrastructure of broiler chicken bones produced through the synthesis of greenery with lime was tested using a scanning electron microscope (Prayitno *et al.*, 2020).

Energy Dispersive X-Ray Analysis

The material composition of broiler chicken bone nano-calcium powder produced through a green synthesis process with lime was tested using SEM-EDX (Prayitno *et al.*, 2020).

Fourier Transform Infrared Analysis

Functional groups of broiler chicken bone nano-calcium powder produced through a green synthesis process with lime were tested using Fourier transform infrared spectroscopy (Dheyab *et al.*, 2020).

Color Analysis

The color of the broiler chicken bone nano-calcium powder was tested using a color reader (Park *et al.*, 2021).

Data Analysis

The results of research on green synthesis and nano-calcium characterization of broiler chicken bones using lime were analyzed descriptively by looking at the data obtained from the results of nano-calcium characterization, which included particle size using a particle-size analyzer, particle morphology using a scanning electron microscope, material composition using energy dispersive x-ray spectroscopy, material functional groups using fourier transform infrared spectroscopy, and color (Prayitno *et al.*, 2020).

Results and Discussion

Particle-Size Analysis

The particle-size analysis test results of broiler chicken bone nano-calcium through green synthesis with lime are presented in Figure 1. The green synthesis process of broiler chicken bone nano-calcium using lime has a particle size of around 524.1 nm based on particle size analysis. Nanometer-scale particles can be obtained using the precipitation method (Balaganesh *et al.* 2018; Siswoyo and Gunawan 2018; Prayitno *et al.* 2021).

The results of this study are in accordance with the results of research conducted by Li *et al.* (2016), who reported that the synthesized nano-calcium citrate has a particle size of around 500 nm.. Clogston *et al.* (2020) reported that

nanotechnology focuses on nanoparticle-based products with nanometer sizes ranging from 1–1,000 nm. The particle sizes obtained in this study include nanoparticles because they have sizes below 1,000 nm.

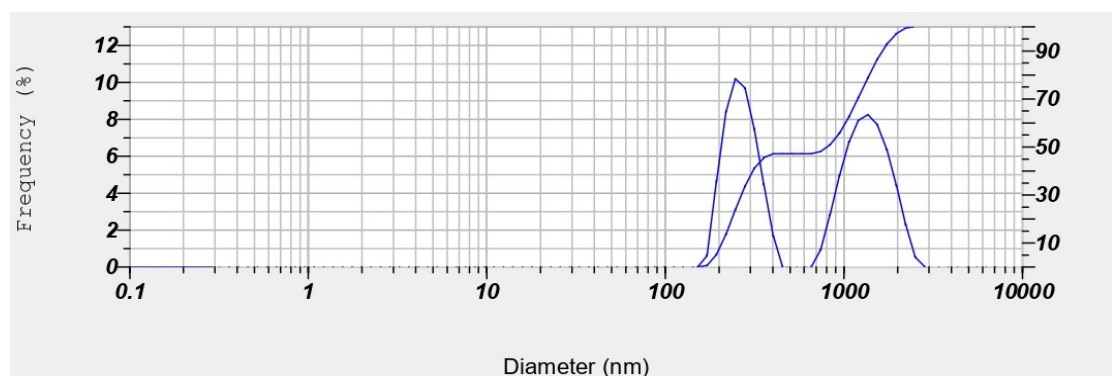


Figure 1. PSA test results showing the particle size of nano-calcium broiler chicken bones from green synthesis using lime.

Fourier Transform Infrared

Fourier transform infrared spectroscopy test results from nano-calcium broiler chicken bones through green synthesis with lime are presented in Figure 2. Fourier transform infrared spectroscopy was analyzed at a wavelength of 4000–400 cm^{-1} (Prayitno *et al.* 2021). Fourier transform infrared spectroscopy test results can change calcium particles from broiler chicken bones into nano-calcium citrate (NCaS). The results of the Fourier transform infrared spectroscopy test showed that through the green synthesis process with lime, broiler chicken bone calcium can be transformed into calcium citrate. The spectrum that appears around wave number 3,384.78 cm^{-1} shows the OH

group band. Calcium citrate is shown with a sharp peak in the OH group band. The green synthesis process of broiler chicken bones using lime obtained FTIR spectra at wave numbers 1540.62 cm^{-1} , 1464.43 cm^{-1} , and 1428.05 cm^{-1} .

Li *et al.* (2016) reported that calcium citrate through FTIR was shown at wave numbers of 3,479 cm^{-1} and 3,176 cm^{-1} , which showed OH bonds. Apart from that, calcium citrate is also directed at the carboxy group, which is related to the calcium ion, which forms the carboxylate, which is shown at a wave number between 1,578–1,443 cm^{-1} . Souza *et al.* (2013) reported that calcium citrate has FTIR spectra at wave numbers 3,482 cm^{-1} , 1,582 cm^{-1} , and 1,441 cm^{-1} .

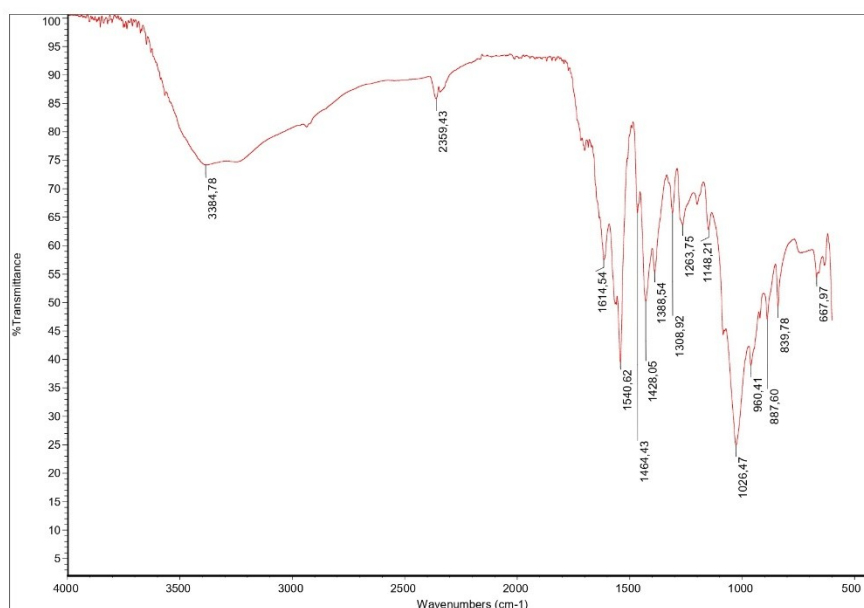


Figure 2. FTIR test results of nano-calcium citrate from green synthesis using lime.

Scanning Electron Microscopy

The results of the scanning electron microscopy test of nano-calcium broiler chicken bone through green synthesis with lime are presented in Figure 3. The results of the scanning electron microscopy test are used to determine the morphology and ultrastructure of a material

(Prayitno *et al.*, 2020). SEM test results with 5,000x magnification show the morphological form of calcium citrate crystals in broiler chicken bones through green synthesis with lime. Calcium citrate obtained from the synthesis of green with lime has a morphology with an irregular shape and non-uniform crystal size, and tends to clump.

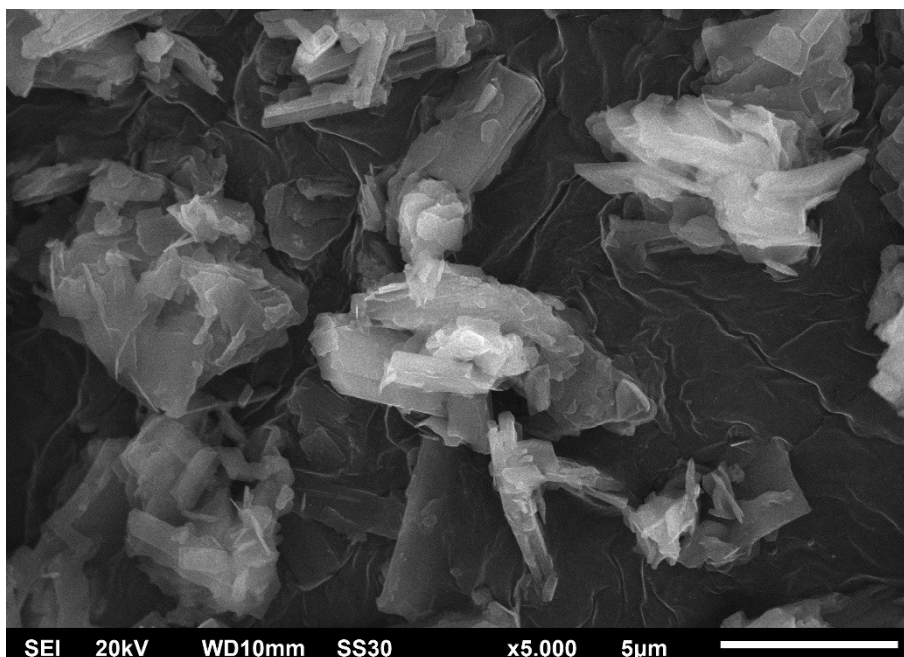


Figure 3. SEM test results of nano-calcium citrate from green synthesis using lime.

Li *et al.* (2016) reported that nano-calcium citrate particles have a morphology that tends to clump. This indicates that the nano-calcium citrate prepared with lime has a higher surface energy and potentially better biological activity.

Energy Dispersive X-Ray

The results of the energy dispersive x-ray test of nano-calcium broiler chicken bone through green synthesis with lime are presented in Figure 4. The results of the energy dispersive x-ray test are used to determine the material composition of a material (Prayitno *et al.*, 2020).

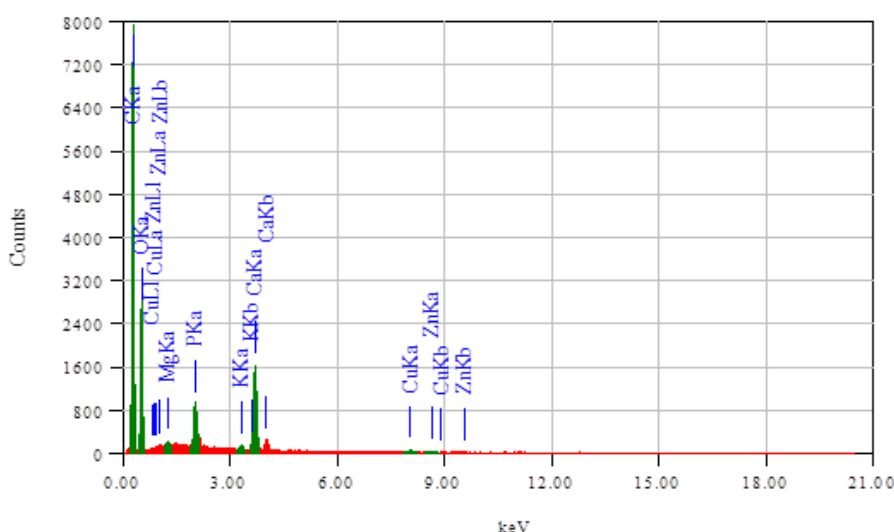


Figure 4. EDX test results of nano-calcium citrate from green synthesis using lime.

The results of the EDX test showed that the most abundant elements contained in the green synthesis of broiler chicken bones using lime were

C (85.81%), O (5.05%), Mg (0.18%), P (1.78%), K (0.24%), Ca (5.63%), Cu (0.81%), Zn (0.49%), MgO (0.31%), P₂O₅ (4.09%), K₂O (0.29%), CaO (7.88%), CuO (1.02%), and ZnO (0.61%). Elements C, O, P, and Ca are the most abundant chemical elements produced from the green synthesis of broiler chicken bones using lime. This is in accordance with the results of a study conducted by Bitire *et al.* (2021) who reported that the most abundant elements in chicken bones were

C, O, Ca, and P. Chicken bones contained elements C (74.3%), Ca (13.1%), O (9.8%), and P (0.9%).

Color Analysis

The color test results of the synthesized broiler bone calcium with lime are presented in Table 1, while the broiler chicken bone calcium powder synthesized with lime is shown in Figure 5.



Figure 5. Broiler chicken bone calcium powder synthesized with lime.

The color test results of broiler chicken bone calcium synthesized with lime showed an *L** value of 72.60, an *a** value of 3.20, a *b** value of 15.60, and a whiteness degree of 68.31. The high *L** value indicates a significant level of brightness, reflecting that the calcium powder has a light and visually clean color. The low *a** value suggests a slight reddish tendency, while the relatively high *b** value indicates a yellowish hue in the sample. The

whiteness degree of 68.31 demonstrates that the calcium powder has a relatively bright visual appearance, though it has not yet reached the perfect whiteness level of pharmaceutical-grade calcium. These results indicate that lime, as a natural source of citric acid, can provide a bleaching and purifying effect on broiler chicken bone calcium, resulting in calcium citrate with a visually favorable color.

Table 1. Color test results of broiler chicken bone calcium citrate

Color Parameter	Lime
<i>L*</i>	72.60±0.42
<i>a*</i>	3.20±0.14
<i>b*</i>	15.60±0.14
Whiteness	68.31±0.45

According to Anggraeni *et al.* (2024), the bone purification process can produce calcium with a cleaner and brighter appearance. Additionally, research by Wijayanti *et al.* (2024) showed that the use of organic acids, such as citric acid, in the calcium synthesis process from bones can enhance color brightness and reduce protein content and other organic compounds that may lower the whiteness degree. This suggests that citric acid from lime acts as a bleaching and purifying agent, thereby increasing the *L** value and whiteness degree of broiler chicken bone calcium. The use of lime as a natural citric acid source, along with other bioactive compounds such as flavonoids and ascorbic acid, may indirectly support the decolorization process of bones during synthesis. Therefore, lime has potential as a

natural agent in broiler bone calcium synthesis, as it can produce calcium with a sufficiently bright color.

Conclusion

Broiler chicken bones, when treated with lime, can produce nano-calcium citrate. The resulting nano-calcium citrate particle size is around 524.1 nm, and the most produced elements are C, Ca, O, and P, respectively. Colorimetric evaluation showed excellent brightness (*L** 72.60), minimal redness (*a** 3.20), moderate yellowness (*b** 15.60), and acceptable whiteness (68.31), demonstrating lime's dual function as both a synthesizing agent and natural purifier. These

findings indicate that the lime-mediated synthesis produces high-quality nano-calcium citrate from poultry byproducts, making it a promising candidate for novel food supplement applications.

Conflict of interest

The authors declared that there is no potential conflict of interest regarding this manuscript.

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Author's contribution

MSU as study conception and design, MRR as data collection, ARS and NAR as analysis and interpretation of results, and AHP as writing the manuscript.

Ethics approval

The conducted study was not related to either human or animal use.

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