BUKTI KORESPONDENSI

ARTIKEL JURNAL INTERNASIONAL BEREPUTASI (TERINDEKS PADA DATABASE INTERNASIONAL BEREPUTASI DAN BERDAMPAK FAKTOR)

JudulArtikel	: The Effect of Extraction Time on the Physicochemical Characteristics of Nanocalcium Powder From Chiken and Duck Eggshells
Jurnal	: Potravinarstvo, Slovak Journal of Food Sciences, Vol.15 Hal. 712-722, 2021. e-ISSN : 1337-0960, p-ISSN : 1338-0230.
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1. Bukti Submission
2. Bukti Review, Corrected, dan Edited
3. Bukti Artikel di Publish pada Potravinarstvo, Slovak Journal of Food Sciences

1. Bukti Submission

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Potravinarstvo Slovak Journal ofFoodSciences

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Slovak Journal of
Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. XX, XXXX, p. XX-XX
https://doi.org/10.5219/XXX
Received: XXXXXXXXXXXX. Accepted: XXXXXXXXXXXX.
Available online: XXXXXXXXXXXX at www.potravinarstvo.com

ISSN 1337-0960 (online)

EFFECT OF EXTRACTION TIME ON THE PHYSICOCHEMICAL CHARACTERISTICS OF NANOCALCIUM POWDER FROM CHICKEN AND DUCK EGGSHELLS

Nura Malahayati, Tri Wardhani Widowati, Nurul Saniah Alsoyuna

ABSTRACT
Calcium deficiency is associated with the risks of bone fracture and osteoporosis. This type of malnutrition has been a concern of governments and the World Health Organization for decades, and extensive efforts have been made to address it. There are several solutions to increase calcium intake.One is to take calcium in the form of nanocalcium. The objective of the present research was to determine the effect of extraction time on the physicochemical characteristics of nanocalcium powder extracted from chicken and duck eggshells through precipitation. This research was conducted by using a completely randomized factorial design with two factors, and each treatment analysis was repeated three times. The first factor was the type of eggshell (chicken and duck), and the second was the extraction time (1,1.5, and 2 hours). The observed parameters were physical (yield and color) and chemical characteristics (moisture, ash, calcium content, and crystalline structure). The results showed that the type of eggshell had a significant effect ($p<0.05$) on yield, color (lightness), and moisture content and that extraction time had significant effect ($p<0.05$) on yield, color (chroma and hue), moisture, and ash content. The highest content of the crystalline structure of nanocalcium formation (100%) was nanocalcium powder from chicken and duck eggshells, with 1 hour of extraction time. The particle size of the crystalline structure of nanocalcium from chicken and duck eggshells were 41.54 nm and 24.90 nm, respectively.

Keywords: nanocalcium; chicken; duck; eggshells; extraction

INTRODUCTION
Calcium is the main mineral in the human body, with as much as 1.5%–2% of adult body weight or about 1 kg **(Ariyanti, 2012)**. Calcium plays an important role in stiffness, bone strength, and most metabolic processes, including blood clots, muscle contractions, hormonal systems, glycogen metabolism, cell proliferation, and differentiation **(WHO, 2006)**. People's calcium consumption varies according to age and sex.

Indonesian people's dietary intakes of calcium, 254 mg/day, are far below the requirement to build proper bone mass. It is widely accepted in the literature that a low calcium intake over the years contributes to the development of bone fracture and osteoporosis.The factors that lead to calcium deficiency among Indonesians are poor dietary habits, low intake of natural sources of calcium (e.g., dairy products), low intake of other calcium sources that must be consumed in large amounts to meet the recommended dietary intake for calcium, and low bioavailability (e.g., cereals, nuts, and green leafy vegetables). Another factor is consumer behavior, such as purchasing power, food preferences, and special conditions (e.g., teen years, young adult years, childbearing years, pregnancy, breastfeeding, later adult years, lactose intolerance, and a vegetarian diet).

There are several solutions to increase calcium intake.One of these is consuming calcium-fortified foods. However, calcium in these food products is more available in the form of microcalcium, so the absorption of calcium in the body (only about 50%) is not optimal **(Lekahena et al., 2014)**.

Current technological advances,particularly nanotechnology,can overcome the problem of eggshell waste by extracting nanocalcium powder with particle sizes of 10–100 nm in diameter. This process increases the economic value of abundant eggshell waste in line with the concept of zero waste products.It also reduces calcium deficiency by converting calcium from the eggshells into absorbable forms **(Suptijah, Jacoeb and Deviyanti, 2012)**.

Using nanotechnology in the fractionation process of the precipitation method converts calcium carbonate into calcium oxide so that it produces nanocalcium powder with good physical and chemical characteristics. This method controls the solubility of the material in the solution through pH and temperature changes by adding certain chemicals to convert soluble compounds into insoluble solids or by mixing acidic bases,producing solids and water **(Purwasasmita and Gultom, 2008)**. This method is very effective because it produces nanoparticles through a simple and low-cost process **(Gulsun, Gursoy and Oner, 2009)**.

Eggs are the main protein source in Indonesia. They are popular with the community because they are highly nutritious, relatively cheap compared to other animal protein sources, delicious, and easily digested in the body. In Indonesia, the production of chicken eggs in 2018 reached 1,644,460 tons, with an average consumption per capita as much as 302.71 grams per day **(Central Statistics Agency, 2016)**.

Increasing the value of egg production is in response to the increasing demand for eggs.Thus, the potential for eggshell waste in Indonesia is quite large but has not been used optimally. People generally dispose of eggshells without using them first even though 96% of their calcium content (94% calcium carbonate, 1% magnesium carbonate, and 1% calcium phosphate) can be additional material extracted for food minerals. The main composition of calcium carbonate in eggshells can cause environmental pollution because soil microbes can't degrade it easily**(Trilaksani, Salamah and Nabil, 2006)**.

Several studies confirmed that nanosizing increases the bioavailability of calcium **(Park et al., 2008; Seo et al., 2009; Hilty et al., 2011).** Nanocalcium powder can be used in various products and food fortification as a form of functional food that benefitspeople's health. Therefore, research was conducted on the effect of eggshell type (chicken and duck) and extraction time on the physicochemical characteristics of nanocalcium powder.

Scientific Hypothesis

It is hypothesized that different types of eggshells and extraction time had significant effects on the physicochemical characterictics of eggshells' nanocalcium powder.

MATERIAL AND METHODOLOGY

Samples

Chicken and duck eggshells were obtained from the cake home industry in Palembang City, South Sumatera Province, Indonesia.

Chemicals

The main chemicals used in this study were lanthanum, chloric acid, potassium hydroxide, and demineralized water.

Instruments

The instruments used in this study were an atomic absorption spectrophotometer (AAS, Shimadzu AA-7000, Japan), a chromameter(CR-410 Konica Minolta, Japan), andX-raydiffraction(XRD, RigakuBenchtop XRDMiniflex 60, Japan).

Laboratory Method

Eggshell powder preparation (Rahmawati and Nisa, 2015)

Chicken and duck eggshells (500 g) were washed with water until clean. The cleaned eggshells were boiled at 100ºC for 10 minutes to kill pathogenic microbes.They were drained andthen dried in an oven for 2 hours at 60ºC.Then, the eggshells were placed at room temperature. They were ground to powder and sieved with a 100 mesh sieve. The eggshell powder was included in the oriented polystyrene) plastic and stored at 4ºC for analysis.

Eggshell nanocalcium powder preparation (Khoerunnisa, 2011)

Eggshell powder was immersed in 1N HCl solvent (1:5) for 48 hours and then extracted at 90ºC for 1, 1.5, and 2 hours. The extracts were then filtered with filter paper to obtain filtrates and sediments. The filtrate was precipitated by adding 3N NaOH.It was stirred and left until the precipitate was formed. The precipitate was then neutralized by using aquabidest to pH neutral. The solution was separated from the sediment by pouring it slowly so that the precipitate was not wasted. The sediment was dried in an oven for 3 hours at 105ºC.It was ashed in a muffle furnace at 600ºC for 5 hours and refined with a mortar. The nanocalcium powder was packed in airtight plastic bags and stored at 4°C until it was used.

Yield determination

The nanocalcium yield was calculated usingthe following formula:

$$\text{Yield (\%)} = \frac{\text{weight of nanocalcium powder}}{\text{weight of eggshell powder}} \text{ x } 100\%$$

Color determination of nanocalcium

Nanocalcium powder's color was measured using Munsell (1977).The values were L (lighteness), C (chroma), and H (hue) scales.

Moisture content (AOAC, 2005) of nanocalcium

A sample of approximately 1 g (W_1) was placed on a dish and dried at 105°C for 18 hours. After drying, a dish containing samples was transferred to a desiccator for 15 minutes. Then, the dish was reweighted until its weight was constant (W_2). Nanocalcium's moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{(W_1 - W_2)}{W_2} \text{ x } 100\%$$

W_1 = weight (g) of sample before drying
W_2 = weight (g) of sample after drying

Ash content (AOAC, 2005) of nanocalcium

A sample of about 1 g sample (W_1) was placed on a porcelain crucible. Then, it wasput in a muffle furnace at 550°C until the samples turned whitish gray. The ash content of the nanocalcium was calculated using the following formula:

$$\text{Ash content (\%)} = \frac{\text{weight of ash}}{\text{weight of the sample}} \times 100\%$$

Calcium determination (AOAC, 2005) of nanocalcium

The calciumcontent of samples was determined using an AAS (Shimadzu AA-7000, Japan) according to the AOAC (Association of Official Analytical Chemists) method (AOAC, 2005). The ground sample (5 g) was placed in an ashing vessel, charred in a muffle furnace, and then ashed at 500°C overnight. The completely ashed sample was dissolved in 10 mL of concentrated hydrochloric acid. The solution was boiled and evaporated nearly to dryness. The residue was redissolved in 20 mL of 2N hydrochloric acid and boiled gently. The solution was cooled and diluted to 100 mL with distilled deionized water. Its absorbance was then measured using the AAS at 422.7 nm.The measurements were calibrated using a commercial standard solution (Merck KGaA, Germany). To eliminate phosphorus interference in the measurements, lanthanum was added to the test ash and standard solutions so that the final solution contained 1% lanthanum.

Crystalline structure of nanocalcium powder

XRD was used to measure the crystal structure of samples.

Statistical Analysis

The research was conducted using a completely randomized factorial design with two factors, and each treatment analysis was repeated three times. The first factor (A) was the type of eggshell (A_1 = chicken and A_2 = duck), and the second factor (B) was the extraction time (B_1 = 1hour, B_2 = 1.5 hour, and B3 = 2 hours). All analyses were performed in triplicates. The data were subjected to the analysis of variance followed by Fisher's least significant difference (LSD)test to compare treatment means.Differences were considered at a significant level of 95% (p<0.05) by using SPSS v.19 software.

RESULTS AND DISCUSSION

Physical Characteristics of Nanocalcium Powder

Yield

The yield of nanocalcium powder ranged from 11.62% to 15.27% for all samples. The lowest average yield was found in the A_1B_1 treatment (chicken egg shells, 1 hour extraction time), while the highest yield value was found in the A_2B_2 treatment (duck eggshell, extraction time of 1.5 hours). The results showed that the type of eggshell and the extraction time had a significant effect on the yield of nanocalcium powder (p<0.05). Table 1 shows the value of the average yield of nanocalcium powder.

The yield of duck nanocalcium powder was significantly higher (p<0.05) than that of chicken nanocalcium powder due to the main components forming the eggshell, calcium carbonate ($CaCO_3$). Calcium carbonate is a major component of an eggshell **(Shwetha et al., 2018).** Duck eggshells have a higher $CaCO_3$ content (96.48%) **(Sari, 2013)** than chicken eggshells (90.90%) (**Warsy et al., 2016)**. However, the result of research conducted by **Ajayan et. al. (2020)** showed thatthe average percentage of calcium carbonatein eight varieties of chickens (89.05%) was higher than that in six varieties of ducks (84.63). **Adeyeye (2009)** stated that hen eggshells contain more calcium than duck eggshells. This is because the differences in calcium carbonate content between shellsare due to differences in chicken or duck varieties. Also, calcium carbonate content depends on the ration of the dietary calcium of poultry feed **(Lestari,Riyanti, and Wanniatie, 2015)**.

During the extraction process, a higher $CaCO_3$ content and a longer extraction time caused a higher solventpenetration into the eggshell powder, resulting in more compounds to diffuse out of the shell and giving a higher yield. However, a yield of 2-hour extraction time was not significantly different from that of 1.5 hours. This is because the calcium carbonate cycle has a reversible reaction so that it can allow the return of products to reactants, where carbonic acid can react again with calcium carbonate to form calcium bicarbonate **(Dewi et al., 2015)**. **Risnojatiningsih (2012)** stated that the formation of $Ca(HCO_3)_2$ occurs when the formed $CaCO_3$ continues to react with water containing CO_2 gas. The result also agreed with the findings of **Khoerunnisa (2011)**, showing that the yield of local mussel shell nanocalcium with 1N HCl extraction significantly increased from 1-hour extraction (5.02%) to 1.5 hour (8.53%) and decreased insignificantlyfrom 1.5 hours (8.53%) to 2 hours of extraction (7.89%).

Color

The color of nanocalcium powder from chicken and duck eggshellswas white. This indicates that calcium oxide (CaO) has formed. This is in accordance with the statement of **Sing et al. (2011)** and **Tangboriboon et al. (2012)** that the eggshells' change in color to white during calcinations indicates that a complete chemical transformation from calcium carbonate to calcium oxide has been achieved.

The lightness value of nanocalcium powder ranged from 92.00% to 94.03%. The lowest average lightness value was found in A_1B_1 (chicken eggshell, extraction time of 1 hour), while the highest lightness value was found in treatment A_2B_2 (duck eggshell, extraction time of 1.5 hours). Chroma value of nanocalcium powder ranged from 6.73% to 7.07%. The lowest average chroma value was found in A_2B_2 (duck eggshell, extraction time of 1.5 hours), while the highest chroma value was found in A_1B_1 (chicken eggshell, 1 hour extraction time). The hue value of nanocalcium powder ranged from 26.30° to 28.87°. The lowest hue value was found in the A_1B_1 treatment (race chicken eggshell, extraction time of 1 hour), while the highest hue value was found in the A_2B_2 treatment (duck eggshell, extraction time of 1.5 hours). Table 1 shows the average lightness, chroma, and hue values of nanocalcium powder.

The value of lightness is the degree of brightness of a product. Chicken and duck eggshells produced high lightness values of nanocalcium powder. This was becausethe preparation of nanocalcium powder through HCl caused pigment deposition in each eggshell so it degraded easily and the color became brighter. Duck eggshells contain biliverdin pigments,hence their greenish blue color.On the other hand, chicken eggshells contain porphyrin pigments,hence their brownish shells **(Mushawwir and Latipudin, 2013; Yonata et al., 2017)**. The immersion process with HCl and extraction can cause the greenish color of the duck eggshells to degradeeasily, resulting in a whiter color, while the brownish color of the chicken eggshell produces a darker color than the duck egg nanocalciumpowder. The difference in the components of the constituent minerals in the eggshell also affects the lightness value of the nanocalcium powder. The main component of the constituent minerals of nanocalcium powder is calcium, which generally has a white color.Therefore, the lightness value of nanocalcium powder was also high.

Chroma is a parameter used to determine the color intensity of a product. The chroma value is inversely proportional to the lightness value because if a product has a dark color, the intensity of the resulting color will be stronger.If the lightness value is high, then the chroma value produced from a product will be lower. The longer the extraction, the more $CaCO_3$ hydrolyzed by HCl will produce a whiter color, resulting in a decreased chroma value.

Hue is a value to determine the dominant wavelength of the color in a product. The hue of nanocalcium powder was red (R), with a range of 18°–54°. The longer the extraction, the more the hue of nanocalcium powder turned yellowish red. This was because the longer the extraction, the more calcium was extracted from the eggshell. The constituent elements of calcium from the flame color test are brick red **(Permata et al., 2018)**. Therefore, the more calcium in eggshell powder, the more the hue of the nanocalcium powder turned yellowish red.

Chemical Characteristics of Nanocalcium Powder

Moisture content

The moisture content of nanocalcium powder ranged from 0.16% to 0.33% for all samples. These values were very small due to drying in the preparation of nanocalcium powder. The moisture content of nanocalcium of all samples is shown in Table 1. That of nanocalcium powder of chicken eggshells was significantly higher than that of duck eggshells (p<0.05). This must be becausethe initial moisture content of duck eggshells (1.43±0.04%) was significantly lower than that of chicken eggshells (1.99±0.01%), decreasingthe water content in the calcination stage ofthe nanocalcium preparation.

The extraction time significantlyaffected the moisture content of nanocalcium powder (p<0.05). The longer the extraction time, the lower the moisture content of nanocalcium powder. This was due to the evaporation of water in the filtrate so that the water content of the nanoparticle powder became free water. The decrease in water content was also due to the protein content in eggshell powder being hydrolyzed when it is mixed with chloric acid and the heating process resulting in decreased water binding. This observation was in agreement with the result found by **Trilaksani et al. (2006)** and **Agustini et al. (2011)**,who stated that the low water content in tuna bone and clam shell powder was due to protein hydrolysis during heating in powder preparation.

Ash content

The ash content of nanocalcium powder ranged from 96.80% to 98.69% for all samples. Table 1 shows the ash content of all samples' nanocalcium. The extraction time significantlyaffectedthe ash content of nanocalcium powder (p<0.05). The ash content increased as the extraction time increased. This may because the longer the extraction time,the longer the contact of nanoparticle powder and solvent (chloric acid). This, in turn, increased the chance of hydrolysis reaction. This result was supported by the findings by **Widyastuti et al. (2015)**,who concluded that the ash content of chicken eggshell nanocalcium with a 1N NaOH solvent and 1-,2-, and 3-hour extraction gave the highest value of ash content in the 3-hour extraction time: 98.07%, 98.01%, and 98.03% for 1-,2-, and 3-hour extraction, respectively.

Calcium content

The calcium content of microcalcium and nanocalcium of all samples is shown in Table 2.Microcalcium'scalcium content (149 µm) of chicken eggshell was higher than that of duck eggshell. This was because $CaCO_3$ of duck eggshell (96.48%) was higher than that of duck eggshell (90.90%), resulting in the higher amount of extracted calcium. Moreover, the calcium content of nanocalcium was higher than that of microcalcium both from chicken and duck eggshells. This was due to demineralization, precipitation, and calcination processes during nanocalcium preparation, maximally opening eggshell spores and extracting more calcium extracted. The steps of the reactions are as follows:

Demineralization:

$$CaCO_{3(s)} + 2HCl_{(aq)} \longrightarrow CaCl_{2(aq)} + H_2O_{(l)} + CO_{2(g)} \quad (1)$$

Precipitation:

$$CaCl_{2(aq)} + 2NaOH_{(aq)} \longrightarrow Ca(OH)_{2(s)} + 2NaCl_{(aq)} \quad (2)$$

Calcination:

$$Ca(OH)_{2(s)} + \text{Heat} \longrightarrow CaO_{(s)} + H_2O_{(l)} \quad (3)$$

The observation supported by previous research **(Navarro et al., 2009; Mohamed et al., 2012; Mosaddegh et al., 2014; Zuhra et al., 2015)** concluded that $CaCO_3$ can be converted to CaO through thermal decomposition (calcination).

Moreover, Table 2 shows that the calcium content of nanocalcium from chicken and duck eggshells decreased with increasing extraction time. This may be due to $CaCO_3$ reformation, resulting from the reversible reaction of the calcium carbonate cycle. The steps are as follows:

$$CO_{2(g)} + H_2O_{(l)} \longrightarrow H_2CO_3 \quad (4)$$

$$CaCO_{3(s)} + H_2CO_{3(aq)} \longrightarrow Ca(HCO_3)_{2(aq)} \quad (5)$$

$$Ca(HCO_3)_2 \longrightarrow CaCO_{3(s)} + CO_{2\,(g)} + H_2O_{(l)} \quad (6)$$

X-Ray Diffraction

The XRD results are used to determine the crystalline structure of nanocalcium powder. The XRD is monitored at 2Θ = 5°–90°. The results of the XRD analysis of nanocalcium from chicken eggshells with extraction times of 1, 1.5, and 2 hours are presented in Figure 1. Figure 2 shows the result of the XRD analysis of nanocalcium from duck eggshells with extraction times of 1, 1.5, and 2 hours.

The main peak appeared at 2Θ = 37.44° of nanocalcium from chicken eggshells and 2Θ = 37.36° of nanocalcium from duck eggshells. Peaks of nanocalcium oxide (CaO) from chicken eggshells appeared at 2Θ = 32.28°, 37.44°, 53.94°, 64.21°, and 67.44°. Moreover, peaks of nanocalcium oxide (CaO) from duck eggshells appeared at 2Θ = 32.15°, 37.36°, 53.85°, 64.10°, and 67.32°. The peaks that appeared were all identified and corresponded to the database International Center for Diffraction Data (ICDD) of calcium oxide (CaO) (PDF No. 99-0070). This result was supported by **Taufiq_Yap et al. (2011)**,and **Habte et al. (2019)** stated that the peaks at 2Θ = 32.22°, 37.36°, 53.8°, 64.1°, and 67.3° were assigned to planes of pure CaO phase. Nanocalcium oxide powder of chicken and duck eggshells had a polycrystalline structure **(Habte et al., 2019;Khan et al., 2018)**.

The XRD patterns displayed diffractograms of CaO, $Ca(OH)_2$, and $CaCO_3$. The XRD patterns of nanocalcium from chicken eggshells have peaks of CaO (100%) at 1-hour extraction time; $Ca(OH)_2$ (77.4%), $CaCO_3$ (11.0%), and CaO (11.6%) at 1.5-hour extraction time; and $Ca(OH)_2$ (52.2%), $CaCO_3$ (9.0%), and CaO (38.8%) at 2-hour extraction time. Moreover, the XRD patterns of nanocalcium from duck eggshells have peaks of CaO (100%) at 1-hour extraction time; $Ca(OH)_2$ (48.1%), $CaCO_3$ (6.9%), CaO (45.1%) at 1.5-hour extraction time; and $Ca(OH)_2$ (87.3%), $CaCO_3$ (11.2%), and CaO (1.5%) at 2-hour extraction time.

The nanocalcium oxide (CaO) content from chicken and duck eggshells at 1 hour of extraction was formed 100%. This indicated that transformation of a chemical composition from $CaCO_3$ to CaO was completely achieved after the whole process took place. This observation was supported by **Dasgupta et al. (2004)**,who stated that $CaCO_3$ turned to CaO at 540°C. The appearance of peak $Ca(OH)_2$ of nanocalcium from chicken and duck eggshells at extraction time 1.5 and 2 hours due to the hydration reaction between hygroscopic CaO and water vapor. In the same condition, the appearance of peak $CaCO_3$ must be due to the incomplete decomposition of $CaCO_3$ to CaO.

Based on the XRD analysis, the crystallite size and density of nanocalcium oxide from chicken and duck eggshells were 41.54 nm and 3.36 g/cm^3 and 24.90 nm and 3.34 g/cm^3, respectively. Previous studies showed that the crystallite sizes of nanocalcium from chicken eggshells were 10.46 nm (**Sunardi, Krismawati, and Mahayana, 2020)** and50–198 nm **(Habte et al., 2019)**,and the crystallite size of nanocalcium from duck eggshells was 262 nm **(Prayitno, Prasetyo, and Sutirtoadi, 2019)**. These values were different from the values of nanocalcium crystallite sizes from this study. This was due to differences in raw material since the substances contained in eggshells depend on the breed, feed, and environment of the chicken.

The density values of nanocalcium oxide from chicken and duck eggshells were 3.361 g/cm^3 and 3.342 g/cm^3, respectively. These values were lower than density values of the chicken eggshells (2.16 g/cm^3) and duck eggshells (2.84 g/cm^3) calcinated at 900°C for 1 hour (**Tangboriboon et al., 2012).** The differences are caused by differencesin temperature and calcination times; the species and the feeding of the chickens and ducksalso influenced the nutrient content during the egg formation.

CONCLUSION

The XRD showed that CaO was formed from nanocalcium powder of chicken and duck eggshellsat 1 hour of extraction. The size of nanocalcium oxide crystals from chicken and duck eggshells were 41.54 nm and 24.90 nm.

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Acknowledgments:

The authors would like to thank Sriwijaya University for the funding provided for the implementation of this research.

Conflict of interest:

The authors declare no conflict of interest.

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Table 1 Physicochemical characteristics of nanocalcium powder from chicken and duck eggshells with different extraction times.

Treatment	Yield (%)	Colour			Moisture content (%)	Ash content (%)
		Lightness (%)	Chroma (%)	Hue (°)		
A_1B_1	11.62±0.28^a	93.67±3.71^a	7.07±0.06^a	26.30±0.20^a	0.33±0.01^a	96.80±0.44^a
A_1B_2	13.30±0.27^b	93.73±0.12^a	6.80±0.10^a	28.30±0.30^a	0.25±0.01^a	98.32±0.05^a
A_1B_3	13.59±0.20^b	93.67±0.35^a	6.83±0.12^a	28.00±0.17^a	0.26±0.01^a	98.28±0.05^a
A_2B_1	13.83±0.03^b	93.53±0.15^a	7.03±0.06^a	26.93±0.35^a	0.18±0.03^b	97.91±0.64^a
A_2B_2	15.27±0.32^c	93.37±1.01^a	6.73±0.12^a	28.87±0.46^a	0.18±0.03^b	98.69±0.10^a
A_2B_3	15.20±0.20^c	93.87±0.21^a	6.77±0.06^a	28.27±0.23^a	0.17±0.03^b	98.67±0.10^a

Note: Data are presented as mean ± standard deviation of triplicate determinations.
Means for each characteristic followed by the same superscript within the same row are not significantly different at p<0.05 by the LSD test.
A_1B_1 = chicken eggshell; 1-hour extraction time
A_1B_2 = chicken eggshell; 1.5-hour extraction time
A_1B_3 = chicken eggshell; 2-hour extraction time
A_2B_1 = duck eggshell; 1-hour extraction time
A_2B_2 = duck eggshell; 1.5-hour extraction time
A2B_3 = duck eggshell; 2-hour extraction time

Table 2 Calcium content of microcalcium and nanocalcium powders from chicken and duck eggshells.

Treatment	Calcium content (%)
Microcalcium powder (149 µm)	
Chicken	11.41±0.03
Duck	12.32±0.14
Nanocalcium powder	
A_1B_1	40.80±0.06
A_1B_2	24.78±0.04
A_1B_3	24.52±0.05
A_2B_1	49.39±0.08
A_2B_2	27.38±0.05
A_2B_3	25.42±0.04

Note: A_1B_1 = chicken eggshell; 1-hour extraction time
A_1B_2 = chicken eggshell; 1.5-hour extraction time
A_1B_3 = chicken eggshell; 2-hour extraction time
A_2B_1 = duck eggshell; 1-hour extraction time
A_2B_2 = duck eggshell; 1.5-hour extraction time
A_2B_3 = duck eggshell; 2-hour extraction time

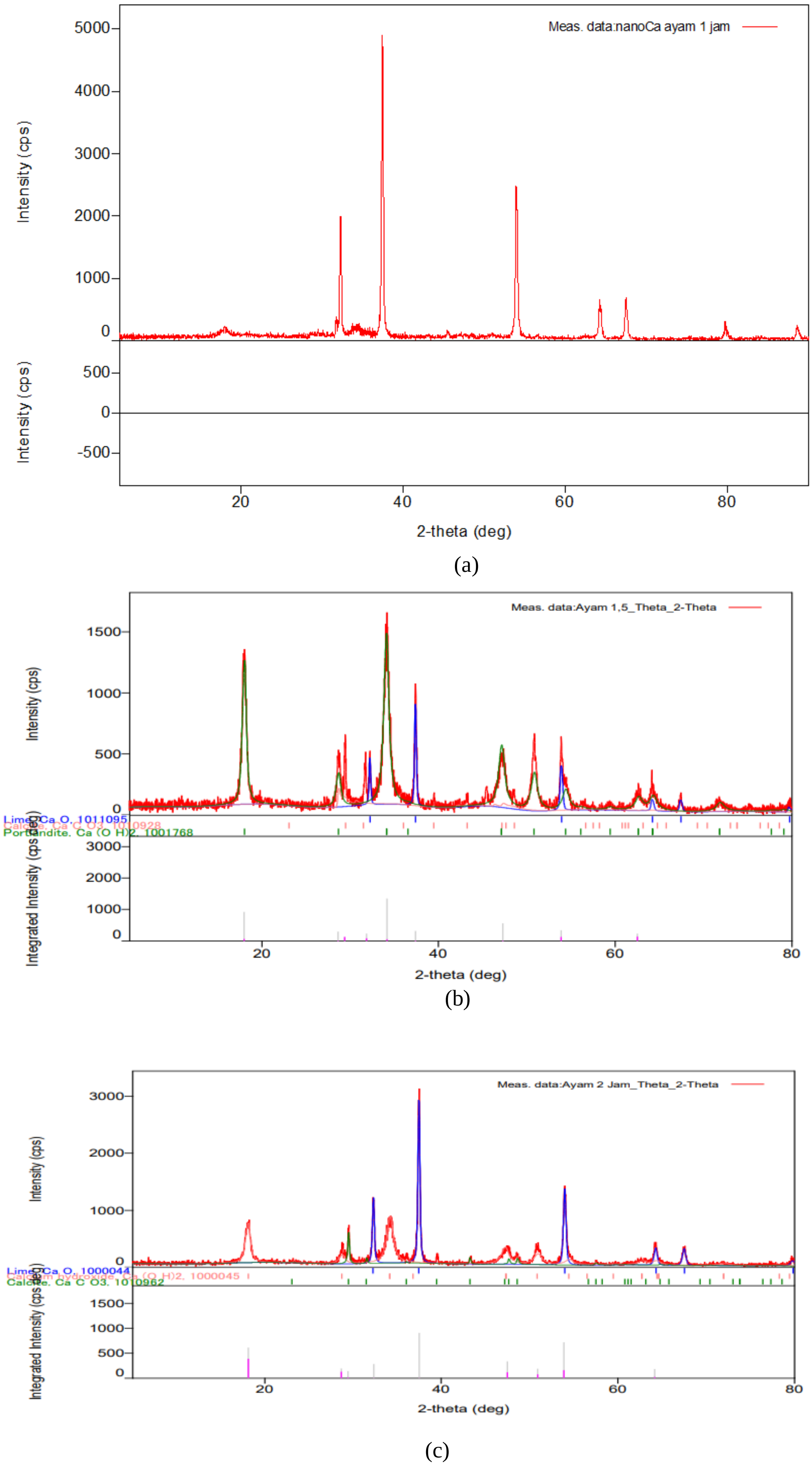
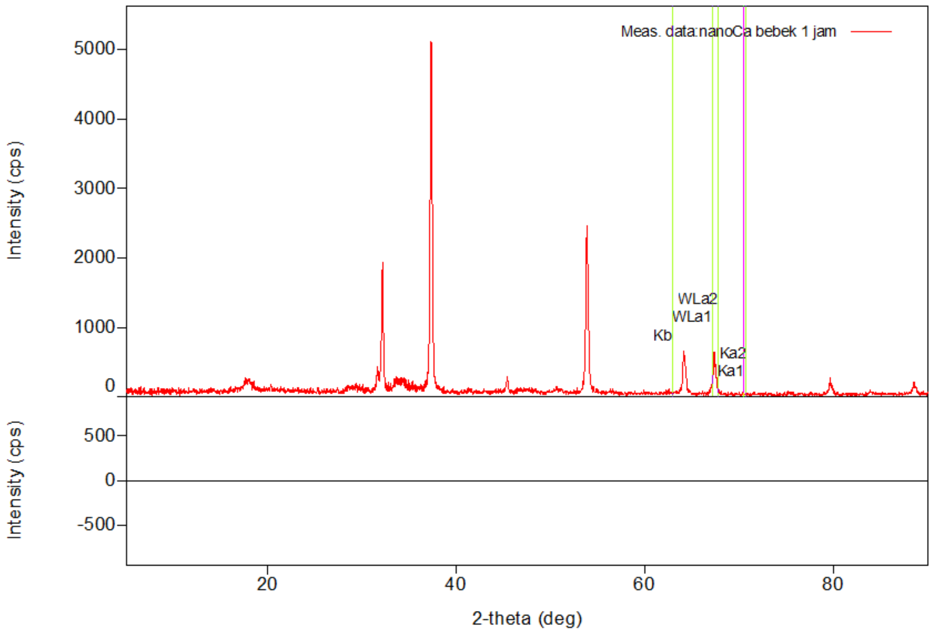


Figure 1 XRD of nanocalcium chicken eggshells from (a) 1-hour extraction, (b) 1.5-hour extraction, and (c) 2-hour extraction.



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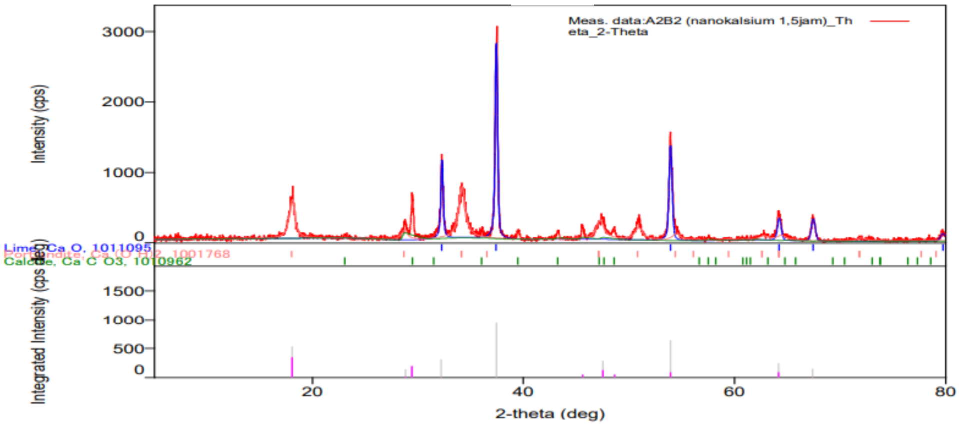

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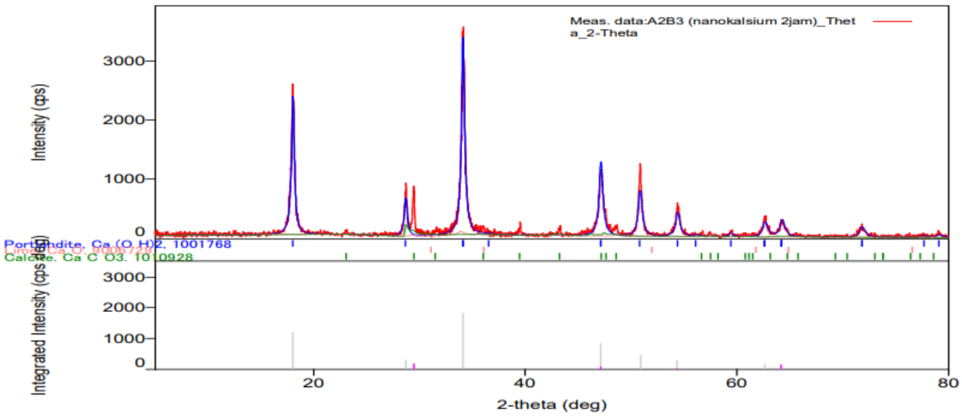

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Figure 2 XRD of nanocalcium duck eggshells from (a) 1-hour extraction, (b) 1.5-hour extraction, and (c) 2-hour extraction.

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Potravinarstvo Slovak Journal of Food Sciences
vol. XX, XXXX, p. XX-XX
https://doi.org/10.5219/XXX
Received: XXXXXXXXXXXX. Accepted: XXXXXXXXXXXX.
Available online: XXXXXXXXXXXX at www.potravinarstvo.com

ISSN 1337-0960 (online)

EFFECT OF EXTRACTION TIME ON THE PHYSICOCHEMICAL CHARACTERISTICS OF NANOCALCIUM POWDER FROM CHICKEN AND DUCK EGGSHELLS

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Nura Malahayati, Tri Wardhani Widowati, Nurul Saniah Alsoyuna

ABSTRACT

Calcium deficiency is associated with the risks of bone fracture and osteoporosis. This type of malnutrition has been a concern of governments and the World Health Organization for decades, and extensive efforts have been made to address it. There are several solutions to increase calcium intake. One is to take calcium in the form of nanocalcium. The objective of the present research was to determine the effect of extraction time on the physicochemical characteristics of nanocalcium powder extracted from chicken and duck eggshells through precipitation. This research was conducted by using a completely randomized factorial design with two factors, and each treatment analysis was repeated three times. The first factor was the type of eggshell (chicken and duck), and the second was the extraction time (1, 1.5, and 2 hours). The observed parameters were physical (yield and color) and chemical characteristics (moisture, ash, calcium content, and crystalline structure). The results showed that the type of eggshell had a significant effect ($p<0.05$) on yield, color (lightness), and moisture content and that extraction time had significant effect ($p<0.05$) on yield, color (chroma and hue), moisture, and ash content. The highest content of the crystalline structure of nanocalcium formation (100%) was nanocalcium powder from chicken and duck eggshells, with 1 hour of extraction time. The particle size of the crystalline structure of nanocalcium from chicken and duck eggshells were 41.54 nm and 24.90 nm, respectively.

Keywords: nanocalcium; chicken; duck; eggshells; extraction

INTRODUCTION

Calcium is the main mineral in the human body, with as much as 1.5%–2% of adult body weight or about 1 kg **(Ariyanti, 2012)**. Calcium plays an important role in stiffness, bone strength, and most metabolic processes, including blood clots, muscle contractions, hormonal systems, glycogen metabolism, cell proliferation, and differentiation **(WHO, 2006)**. People's calcium consumption varies according to age and sex.

Indonesian people's dietary intakes of calcium, 254 mg/day, are far below the requirement to build proper bone mass. It is widely accepted in the literature that a low calcium intake over the years contributes to the development of bone fracture and osteoporosis. The factors that lead to calcium deficiency among Indonesians are poor dietary habits, low intake of natural sources of calcium (e.g., dairy products), low intake of other calcium sources that must be consumed in large amounts to meet the recommended dietary intake for calcium, and low bioavailability (e.g., cereals, nuts, and green leafy vegetables). Another factor is consumer behavior, such as purchasing power, food preferences, and special conditions (e.g., teen years, young adult years, childbearing years, pregnancy, breastfeeding, later adult years, lactose intolerance, and a vegetarian diet).

There are several solutions to increase calcium intake. One of these is consuming calcium-fortified foods. However, calcium in these food products is more available in the form of microcalcium, so the absorption of calcium in the body (only about 50%) is not optimal **(Lekahena et al., 2014)**.

Current technological advances, particularly nanotechnology, can overcome the problem of eggshell waste by extracting nanocalcium powder with particle sizes of 10–100 nm in diameter. This process increases the economic value of abundant eggshell waste in line with the concept of zero waste products. It also reduces calcium deficiency by converting calcium from the eggshells into absorbable forms **(Suptijah, Jacoeb and Deviyanti, 2012)**.

Using nanotechnology in the fractionation process of the precipitation method converts calcium carbonate into calcium oxide so that it produces nanocalcium powder with good physical and chemical characteristics. This method controls the solubility of the material in the solution through pH and temperature changes by adding certain chemicals to convert soluble compounds into insoluble solids or by mixing acidic bases, producing solids and water **(Purwasasmita and Gultom, 2008)**. This method is very effective because it produces nanoparticles through a simple and low-cost process **(Gulsun, Gursoy and Oner, 2009)**.

Eggs are the main protein source in Indonesia. They are popular with the community because they are highly nutritious, relatively cheap compared to other animal protein sources, delicious, and easily digested in the body. In Indonesia, the production of chicken eggs in 2018 reached 1,644,460 tons, with an average consumption per capita as much as 302.71 grams per day **(Central Statistics Agency, 2016)**.

Increasing the value of egg production is in response to the increasing demand for eggs. Thus, the potential for eggshell waste in Indonesia is quite large but has not been used optimally. People generally dispose of eggshells without using them first even though 96% of their calcium content (94% calcium carbonate, 1% magnesium carbonate, and 1% calcium phosphate) can be additional material extracted for food minerals. The main composition of calcium carbonate in eggshells can cause environmental pollution because soil microbes can't degrade it easily **(Trilaksani, Salamah and Nabil, 2006)**.

Several studies confirmed that nanosizing increases the bioavailability of calcium **(Park et al., 2008; Seo et al., 2009; Hilty et al., 2011).** Nanocalcium powder can be used in various products and food fortification as a form of functional food that benefits people's health. Therefore, research was conducted on the effect of eggshell type (chicken and duck) and extraction time on the physicochemical characteristics of nanocalcium powder.

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Scientific Hypothesis

It is hypothesized that different types of eggshells and extraction time had significant effects on the physicochemical charactericties of eggshells' nanocalcium powder.

MATERIAL AND METHODOLOGY

Samples

Chicken and duck eggshells were obtained from the cake home industry in Palembang City, South Sumatera Province, Indonesia.

Chemicals

The main chemicals used in this study were lanthanum, chloric acid, potassium hydroxide, and demineralized water.

Instruments

The instruments used in this study were an atomic absorption spectrophotometer (AAS, Shimadzu AA-7000, Japan), a chroma meter (CR-410 Konica Minolta, Japan), and X-ray diffraction (XRD, Rigaku Benchtop XRD Miniflex 60, Japan).

Laboratory Method

Eggshell powder preparation (Rahmawati and Nisa, 2015)

Chicken and duck eggshells (500 g) were washed with water until clean. The cleaned eggshells were boiled at 100^{o}C for 10 minutes to kill pathogenic microbes. They were drained and then dried in an oven for 2 hours at 60^{o}C. Then, the eggshells were placed at room temperature. They were ground to powder and sieved with a 100 mesh sieve. The eggshell powder was included in the oriented polystyrene) plastic and stored at 4°C for analysis.

Eggshell nanocalcium powder preparation (Khoerunnisa, 2011)

Eggshell powder was immersed in 1N HCl solvent (1:5) for 48 hours and then extracted at 90°C for 1, 1.5, and 2 hours. The extracts were then filtered with filter paper to obtain filtrates and sediments. The filtrate was precipitated by adding 3N NaOH. It was stirred and left until the precipitate was formed. The precipitate was then neutralized by using aquabidest to pH neutral water. The solution was separated from the sediment by pouring it slowly so that the precipitate was not wasted. The sediment was dried in an oven for 3 hours at 105°C. It was ashed in a muffle furnace at 600°C for 5 hours and refined with a mortar. The nanocalcium powder was packed in airtight plastic bags and stored at 4°C until it was used.

Yield determination

The nanocalcium yield was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{weight of nanocalcium powder}}{\text{weight of eggshell powder}} \text{ x } 100\%$$

Color determination of nanocalcium

Nanocalcium powder's color was measured using Munsell (1977). The values were L (lighteness), C (chroma), and H (hue) scales.

Moisture content (AOAC, 2005) of nanocalcium

A sample of approximately 1 g (W_1) was placed on a dish and dried at 105°C for 18 hours. After drying, a dish containing samples was transferred to a desiccator for 15 minutes. Then, the dish was reweighted until its weight was constant (W_2). Nanocalcium's moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{(W1 - W2)}{W2} \text{ x } 100\%$$

W_1 = weight (g) of sample before drying
W_2 = weight (g) of sample after drying

Moisture content (AOAC, 2005) of nanocalcium

A sample of about 1 g sample (W_1) was placed on a porcelain crucible. Then, it was put in a muffle furnace at 550°C until the samples turned whitish gray. The ash content of the nanocalcium was calculated using the following formula:

$$\text{Ash content (\%)} = \frac{\text{weight of ash}}{\text{weight of the sample}} \text{ x } 100\%$$

Calcium determination (AOAC, 2005) of nanocalcium

The calcium content of samples was determined using an AAS (Shimadzu AA-7000, Japan) according to the AOAC (Association of Official Analytical Chemists) method (AOAC, 2005). The ground sample (5 g) was placed in an ashing vessel, charred in a muffle furnace, and then ashed at 500°C overnight. The completely ashed sample was dissolved in 10 mL of concentrated hydrochloric acid. The solution was boiled and evaporated nearly to dryness. The residue was redissolved in 20 mL of 2N hydrochloric acid and boiled gently. The solution was cooled and diluted to 100 mL with distilled deionized water. Its absorbance was then measured using the AAS at 422.7 nm. The measurements were calibrated using a commercial standard solution (Merck KGaA, Germany). To eliminate phosphorus interference in the measurements, lanthanum was added to the test ash and standard solutions so that the final solution contained 1% lanthanum.

Crystalline structure of nanocalcium powder

XRD was used to measure the crystal structure of samples.

Statistical Analysis

The research was conducted using a completely randomized factorial design with two factors, and each treatment analysis was repeated three times. The first factor (A) was the type of eggshell (A_1 = chicken and A_2 = duck), and the second factor (B) was the extraction time (B_1 = 1 hour, B_2 = 1.5 hour, and B3 = 2 hours). All analyses were performed in triplicates. The data were subjected to the analysis of variance followed by Fisher's least significant difference (LSD) test to compare treatment means. Differences were considered at a significant level of 95% ($p < 0.05$) by using SPSS v.19 software.

RESULTS AND DISCUSSION

Physical Characteristics of Nanocalcium Powder

Yield

The yield of nanocalcium powder ranged from 11.62% to 15.27% for all samples. The lowest average yield was found in the A_1B_1 treatment (chicken egg shells, 1 hour extraction time), while the highest yield value was found in the A_2B_2 treatment (duck eggshell, extraction time of 1.5 hours). The results showed that the type of eggshell and the extraction time had a significant effect on the yield of nanocalcium powder ($p < 0.05$). Table 1 shows the value of the average yield of nanocalcium powder.

The yield of duck nanocalcium powder was significantly higher ($p < 0.05$) than that of chicken nanocalcium powder due to the main components forming the eggshell, calcium carbonate ($CaCO_3$). Calcium carbonate is a major component of an eggshell **(Swetha et al., 2018).** Duck eggshells have a higher $CaCO_3$ content (96.48%) **(Sari, 2013)** than chicken eggshells (90.90%) (**Warsy et al., 2016**). However, the result of research conducted by **Ajayan et. al. (2020)**

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showed that the average percentage of calcium carbonate in eight varieties of chickens (89.05%) was higher than that in six varieties of ducks (84.63). **Adeyeye (2009)** stated that hen eggshells contain more calcium than duck eggshells. This is because the differences in calcium carbonate content between shells are due to differences in chicken or duck varieties. Also, calcium carbonate content depends on the ration of the dietary calcium of poultry feed **(Lestari, Riyanti, and Wanniatie, 2015)**.

During the extraction process, a higher $CaCO_3$ content and a longer extraction time caused a higher solvent penetration into the eggshell powder, resulting in more compounds to diffuse out of the shell and giving a higher yield. However, a yield of 2-hour extraction time was not significantly different from that of 1.5 hours. This is because the calcium carbonate cycle has a reversible reaction so that it can allow the return of products to reactants, where carbonic acid can react again with calcium carbonate to form calcium bicarbonate **(Dwi et al., 2015)**. **Risnojatiningsih (2012)** stated that the formation of $Ca(HCO_3)_2$ occurs when the formed $CaCO_3$ continues to react with water containing CO_2 gas. The result also agreed with the findings of **Khoerunnisa (2011)**, showing that the yield of local mussel shell nanocalcium with 1N HCl extraction significantly increased from 1-hour extraction (5.02%) to 1.5 hour (8.53%) and decreased insignificantly from 1.5 hours (8.53%) to 2 hours of extraction (7.89%).

Color

The color of nanocalcium powder from chicken and duck eggshells was white. This indicates that calcium oxide (CaO) has formed. This is in accordance with the statement of **Sing et al. (2011)** and **Tangboriboon et al. (2012)** that the eggshells' change in color to white during calcinations indicates that a complete chemical transformation from calcium carbonate to calcium oxide has been achieved.

The lightness value of nanocalcium powder ranged from 92.00% to 94.03%. The lowest average lightness value was found in A_1B_1 (chicken eggshell, extraction time of 1 hour), while the highest lightness value was found in treatment A_2B_2 (duck eggshell, extraction time of 1.5 hours). Chroma value of nanocalcium powder ranged from 6.73% to 7.07%. The lowest average chroma value was found in A_2B_2 (duck eggshell, extraction time of 1.5 hours), while the highest chroma value was found in A_1B_1 (chicken eggshell, 1 hour extraction time). The hue value of nanocalcium powder ranged from 26.30^o to 28.87^o. The lowest hue value was found in the A_1B_1 treatment (race chicken eggshell, extraction time of 1 hour), while the highest hue value was found in the A_2B_2 treatment (duck eggshell, extraction time of 1.5 hours). Table 1 shows the average lightness, chroma, and hue values of nanocalcium powder.

The value of lightness is the degree of brightness of a product. Chicken and duck eggshells produced high lightness values of nanocalcium powder. This was because the preparation of nanocalcium powder through HCl caused pigment deposition in each eggshell so it degraded easily and the color became brighter. Duck eggshells contain biliverdin pigments, hence their greenish blue color. On the other hand, chicken eggshells contain porphyrin pigments, hence their brownish shells **(Mushawwir and Latipudin, 2013; Yonata et al., 2017)**. The immersion process with HCl and extraction can cause the greenish color of the duck eggshells to degrade easily, resulting in a whiter color, while the brownish color of the chicken eggshell produces a darker color than the duck egg nanocalcium powder. The difference in the components of the constituent minerals in the eggshell also affects the lightness value of the nanocalcium powder. The main component of the constituent minerals of nanocalcium powder is calcium, which generally has a white color. Therefore, the lightness value of nanocalcium powder was also high.

Chroma is a parameter used to determine the color intensity of a product. The chroma value is inversely proportional to the lightness value because if a product has a dark color, the intensity of the resulting color will be stronger. If the lightness value is high, then the chroma value produced from a product will be lower. The longer the extraction, the more $CaCO_3$ hydrolyzed by HCl will produce a whiter color, resulting in a decreased chroma value.

Hue is a value to determine the dominant wavelength of the color in a product. The hue of nanocalcium powder was red (R), with a range of 18°–54°. The longer the extraction, the more the hue of nanocalcium powder turned yellowish red. This was because the longer the extraction, the more calcium was extracted from the eggshell. The constituent elements of calcium from the flame color test are brick red **(Permata et al., 2018)**. Therefore, the more calcium in eggshell powder, the more the hue of the nanocalcium powder turned yellowish red.

Chemical Characteristics of Nanocalcium Powder

Moisture content

The moisture content of nanocalcium powder ranged from 0.16% to 0.33% for all samples. These values were very small due to drying in the preparation of nanocalcium powder. The moisture content of nanocalcium of all samples is shown in Table 1. That of nanocalcium powder of chicken eggshells was significantly higher than that of duck eggshells ($p < 0.05$). This must be because the initial moisture content of duck eggshells (1.43±0.04%) was significantly lower than that of chicken eggshells (1.99±0.01%), decreasing the water content in the calcination stage of the nanocalcium preparation.

The extraction time significantly affected the moisture content of nanocalcium powder ($p < 0.05$). The longer the extraction time, the lower the moisture content of nanocalcium powder. This was due to the evaporation of water in the filtrate so that the water content of the nanoparticle powder became free water. The decrease in water content was also due to the protein content in eggshell powder being hydrolyzed when it is mixed with chloric acid

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and the heating process resulting in decreased water binding. This observation was in agreement with the result found by **Trilaksani et al. (2006)** and **Agustini et al. (2011)**, who stated that the low water content in tuna bone and clam shell powder was due to protein hydrolysis during heating in powder preparation.

Ash content

The ash content of nanocalcium powder ranged from 96.80% to 98.69% for all samples. Table 1 shows the ash content of all samples' nanocalcium. The extraction time significantly affected the ash content of nanocalcium powder ($p < 0.05$). The ash content increased as the extraction time increased. This may because the longer the extraction time, the longer the contact of nanoparticle powder and solvent (chloric acid). This, in turn, increased the chance of hydrolysis reaction. This result was supported by the findings by **Widyastuti et al. (2015)**, who concluded that the ash content of chicken eggshell nanocalcium with a 1N NaOH solvent and 1-, 2-, and 3-hour extraction gave the highest value of ash content in the 3-hour extraction time: 98.07%, 98.01%, and 98.03% for 1-, 2-, and 3-hour extraction, respectively.

Calcium content

The calcium content of microcalcium and nanocalcium of all samples is shown in Table 2. Microcalcium's calcium content (149 µm) of chicken eggshell was higher than that of duck eggshell. This was because $CaCO_3$ of duck eggshell (96.48%) was higher than that of duck eggshell (90.90%), resulting in the higher amount of extracted calcium. Moreover, the calcium content of nanocalcium was higher than that of microcalcium both from chicken and duck eggshells. This was due to demineralization, precipitation, and calcination processes during nanocalcium preparation, maximally opening eggshell spores and extracting more calcium extracted. The steps of the reactions are as follows:

Demineralization:

$$CaCO_{3(s)} + 2HCl_{(aq)} \longrightarrow CaCl_{2\,(aq)} + H_2O_{(l)} + CO_{2(g)} \quad (1)$$

Precipitation:

$$CaCl_{2(aq)} + 2NaOH_{(aq)} \longrightarrow Ca(OH)_{2(s)} + 2NaCl_{(aq)} \quad (2)$$

Calcination:

$$Ca(OH)_{2(s)} + \text{Heat} \longrightarrow CaO_{(s)} + H_2O_{(l)} \quad (3)$$

The observation supported by previous research **(Navarro et al., 2009; Mohamed et al., 2012; Mosaddegh et al., 2014; Zuhra et al., 2015)** concluded that $CaCO_3$ can be converted to CaO through thermal decomposition (calcination).

Moreover, Table 2 shows that the calcium content of nanocalcium from chicken and duck eggshells decreased with increasing extraction time. This may be due to $CaCO_3$ reformation, resulting from the reversible reaction of the calcium carbonate cycle. The steps are as follows:

$$CO_{2(g)} + H_2O_{(l)} \longrightarrow H_2CO_3 \quad (4)$$

$$CaCO_{3(s)} + H_2CO_{3(aq)} \longrightarrow Ca(HCO_3)_{2(aq)} \quad (5)$$

$$Ca(HCO_3)_2 \longrightarrow CaCO_{3(s)} + CO_{2\,(g)} + H_2O_{(l)} \quad (6)$$

X-Ray Diffraction

The XRD results are used to determine the crystalline structure of nanocalcium powder. The XRD is monitored at $2\Theta = 5^{\circ}–90^{\circ}$. The results of the XRD analysis of nanocalcium from chicken eggshells with extraction times of 1, 1.5, and 2 hours are presented in Figure 1. Figure 2 shows the result of the XRD analysis of nanocalcium from duck eggshells with extraction times of 1, 1.5, and 2 hours.

The main peak appeared at $2\Theta = 37.44°$ of nanocalcium from chicken eggshells and $2\Theta = 37.36°$ of nanocalcium from duck eggshells. Peaks of nanocalcium oxide (CaO) from chicken eggshells appeared at $2\Theta = 32.28°$, 37.44°, 53.94°, 64.21°, and 67.44°. Moreover, peaks of nanocalcium oxide (CaO) from duck eggshells appeared at $2\Theta = 32.15°$, 37.36°, 53.85°, 64.10°, and 67.32°. The peaks that appeared were all identified and corresponded to the database International Center for Diffraction Data (ICDD) of calcium oxide (CaO) (PDF No. 99-0070). This result was supported by **Taufiq_Yap et al. (2011)**, and **Habte et al. (2019)** stated that the peaks at $2\Theta = 32.22°$, 37.36°, 53.8°, 64.1°, and 67.3° were assigned to planes of pure CaO phase. Nanocalcium oxide powder of chicken and duck eggshells had a polycrystalline structure **(Habte et al., 2019; Khan et al., 2018)**.

The XRD patterns displayed diffractograms of CaO, $Ca(OH)_2$, and $CaCO_3$. The XRD patterns of nanocalcium from chicken eggshells have peaks of CaO (100%) at 1-hour extraction time; $Ca(OH)_2$ (77.4%), $CaCO_3$ (11.0%), and CaO (11.6%) at 1.5-hour extraction time; and $Ca(OH)_2$ (52.2%), $CaCO_3$ (9.0%), and CaO (38.8%) at 2-hour extraction time. Moreover, the XRD patterns of nanocalcium from duck eggshells have peaks of CaO (100%) at 1-hour extraction time; $Ca(OH)_2$ (48.1%), $CaCO_3$ (6.9%), CaO (45.1%) at 1.5-hour extraction time; and $Ca(OH)_2$ (87.3%), $CaCO_3$ (11.2%), and CaO (1.5%) at 2-hour extraction time.

The nanocalcium oxide (CaO) content from chicken and duck eggshells at 1 hour of extraction was formed 100%. This indicated that transformation of a chemical composition from $CaCO_3$ to CaO was completely achieved after the whole process took place. This observation was supported by **Dasgupta et al. (2004)**, who stated that $CaCO_3$ turned to CaO at 540°C. The appearance of peak $Ca(OH)_2$ of nanocalcium from chicken and duck eggshells at extraction time 1.5 and 2 hours due to the hydration reaction between hygroscopic CaO and water vapor. In the same condition, the appearance of peak $CaCO_3$ must be due to the incomplete decomposition of $CaCO_3$ to CaO.

Based on the XRD analysis, the crystallite size and density of nanocalcium oxide from chicken and duck eggshells were 41.54 nm and 3.36 g/cm^3 and 24.90 nm and 3.34 g/cm^3, respectively. Previous studies showed that the crystallite sizes of nanocalcium from chicken eggshells were 10.46 nm (**Sunardi, Krismawati, and Mahayana, 2020)** and 50–198 nm **(Habte et al., 2019)**, and the crystallite size of nanocalcium from duck eggshells was 262 nm **(Prayitno,**

Prasetyo, and Sutirtoadi, 2019). These values were different from the values of nanocalcium crystallite sizes from this study. This was due to differences in raw material since the substances contained in eggshells depend on the breed, feed, and environment of the chicken.

The density values of nanocalcium oxide from chicken and duck eggshells were 3.361 g/cm^3 and 3.342 g/cm^3, respectively. These values were lower than density values of the chicken eggshells (2.16 g/cm^3) and duck eggshells (2.84 g/cm^3) calcinated at 900°C for 1 hour (**Tangboriboon et al., 2012).** The differences are caused by differences in temperature and calcination times; the species and the feeding of the chickens and ducks also influenced the nutrient content during the egg formation.

CONCLUSION

The XRD showed that CaO was formed from nanocalcium powder of chicken and duck eggshells at 1 hour of extraction. The size of nanocalcium oxide crystals from chicken and duck eggshells were 41.54 nm and 24.90 nm.

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Acknowledgments:

The authors would like to thank Sriwijaya University for the funding provided for the implementation of this research.

Conflict of interest:

The authors declare no conflict of interest.

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Table 1 Physicochemical characteristics of nanocalcium powder from chicken and duck eggshells with different extraction times.

Treatment	Yield (%)	Colour			Moisture content (%)	Ash content (%)
		Lightness (%)	Chroma (%)	Hue (°)		
A_1B_1	11.62±0.28[a]	93.67±3.71[a]	7.07±0.06[a]	26.30±0.20[a]	0.33±0.01[a]	96.80±0.44[a]
A_1B_2	13.30±0.27[b]	93.73±0.12[a]	6.80±0.10[a]	28.30±0.30[a]	0.25±0.01[a]	98.32±0.05[a]
A_1B_3	13.59±0.20[b]	93.67±0.35[a]	6.83±0.12[a]	28.00±0.17[a]	0.26±0.01[a]	98.28±0.05[a]
A_2B_1	13.83±0.03[b]	93.53±0.15[a]	7.03±0.06[a]	26.93±0.35[a]	0.18±0.03[b]	97.91±0.64[a]
A_2B_2	15.27±0.32[c]	93.37±1.01[a]	6.73±0.12[a]	28.87±0.46[a]	0.18±0.03[b]	98.69±0.10[a]
A_2B_3	15.20±0.20[c]	93.87±0.21[a]	6.77±0.06[a]	28.27±0.23[a]	0.17±0.03[b]	98.67±0.10[a]

Note: Data are presented as mean ± standard deviation of triplicate determinations.
Means for each characteristic followed by the same superscript within the same row are not significantly different at $p < 0.05$ by the LSD test.
A_1B_1 = chicken eggshell; 1-hour extraction time
A_1B_2 = chicken eggshell; 1.5-hour extraction time
A_1B_3 = chicken eggshell; 2-hour extraction time
A_2B_1 = duck eggshell; 1-hour extraction time
A_2B_2 = duck eggshell; 1.5-hour extraction time
$A2B_3$ = duck eggshell; 2-hour extraction time

Table 2 Calcium content of microcalcium and nanocalcium powders from chicken and duck eggshells.

Treatment	Calcium content (%)
Microcalcium powder (149 µm)	
Chicken	11.41±0.03
Duck	12.32±0.14
Nanocalcium powder	
A_1B_1	40.80±0.06
A_1B_2	24.78±0.04
A_1B_3	24.52±0.05
A_2B_1	49.39±0.08
A_2B_2	27.38±0.05
A_2B_3	25.42±0.04

Note: A_1B_1 = chicken eggshell; 1-hour extraction time
A_1B_2 = chicken eggshell; 1.5-hour extraction time
A_1B_3 = chicken eggshell; 2-hour extraction time
A_2B_1 = duck eggshell; 1-hour extraction time
A_2B_2 = duck eggshell; 1.5-hour extraction time
A_2B_3 = duck eggshell; 2-hour extraction time

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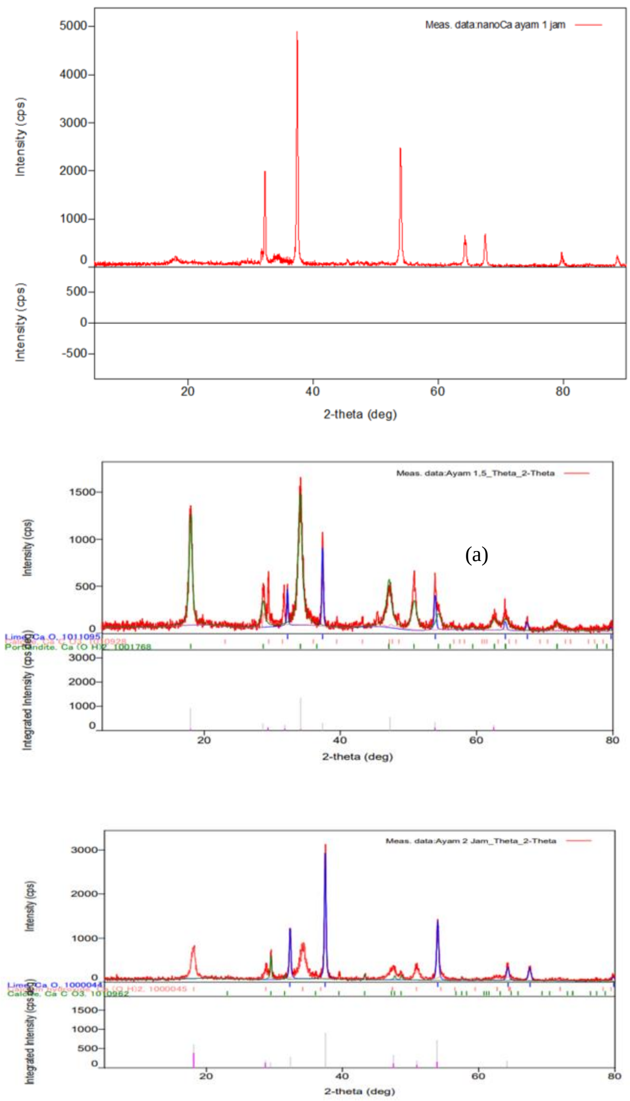

(a)

(c)

Figure 1 XRD of nanocalcium chicken eggshells from (a) 1-hour extraction, (b) 1.5-hour extraction, and (c) 2-hour extraction.

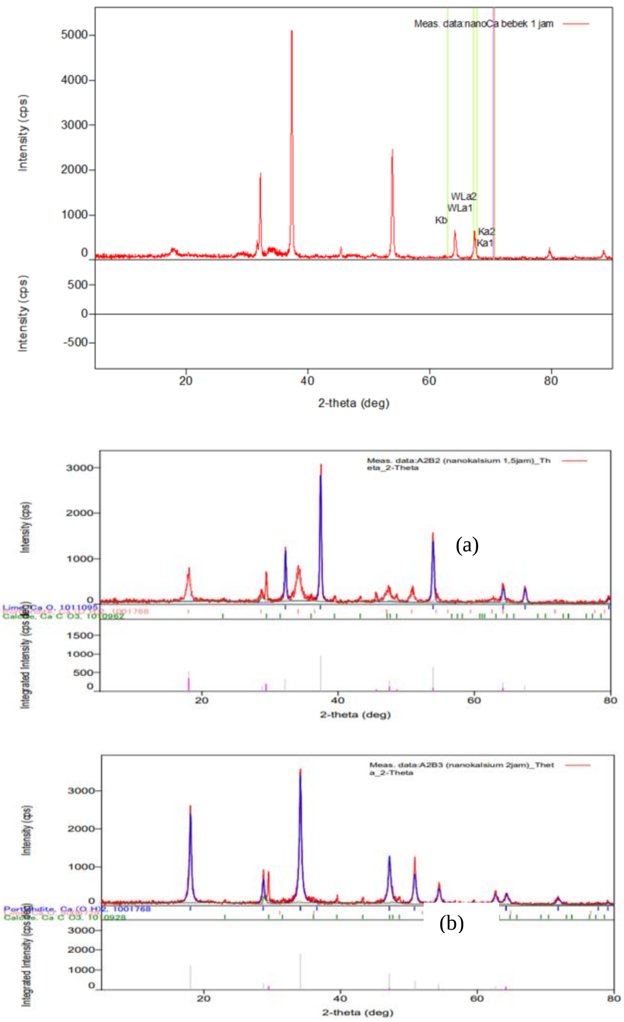

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Figure 2 XRD of nanocalcium duck eggshells from (a) 1-hour extraction, (b) 1.5-hour extraction, and (c) 2-hour extraction.

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vol. XX, XXXX, p. XX-XX
https://doi.org/10.5219/XXX
Received: XXXXXXXXXXXX. Accepted: XXXXXXXXXXXX.
Available online: XXXXXXXXXXXX at www.potravinarstvo.com

ISSN 1337-0960 (online)

EFFECT OF EXTRACTION TIME ON THE PHYSICOCHEMICAL CHARACTERISTICS OF NANOCALCIUM POWDER FROM CHICKEN AND DUCK EGGSHELLS

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ABSTRACT
Calcium deficiency is associated with the risks of bone fracture and osteoporosis. This type of malnutrition has been a concern of governments and the World Health Organization for decades, and extensive efforts have been made to address it. There are several solutions to increase calcium intake. One is to take calcium in the form of nanocalcium. The objective of the present research was to determine the effect of extraction time on the physicochemical characteristics of nanocalcium powder extracted from chicken and duck eggshells through precipitation. This research was conducted by using a completely randomized factorial design with two factors, and each treatment analysis was repeated three times. The first factor was the type of eggshell (chicken and duck), and the second was the extraction time (1,1.5, and 2 hours). The observed parameters were physical (yield and color) and chemical characteristics (moisture, ash, calcium content, and crystalline structure). The results showed that the type of eggshell had a significant effect ($p<0.05$) on yield, color (lightness), and moisture content and that extraction time had a significant effect ($p<0.05$) on yield, color (chroma and hue), moisture, and ash content. The highest content of the crystalline structure of nanocalcium formation (100%) was nanocalcium powder from chicken and duck eggshells, with 1 hour of extraction time. The particle size of the crystalline structure of nanocalcium from chicken and duck eggshells were 41.54 nm and 24.90 nm, respectively.

Keywords: nanocalcium; chicken; duck; eggshell; extraction

INTRODUCTION
Calcium is the main mineral in the human body, with as much as 1.5%–2% of adult body weight or about 1 kg **(Ariyanti, 2012)**. Calcium plays an important role in stiffness, bone strength, and most metabolic processes, including blood clots, muscle contractions, hormonal systems, glycogen metabolism, cell proliferation, and differentiation **(WHO, 2006)**. People's calcium consumption varies according to age and sex.

Indonesian people's dietary intakes of calcium, 254 mg/day, are far below the requirement to build proper bone mass. It is widely accepted in the literature that a low calcium intake over the years contributes to the development of bone and osteoporosis. Factors that lead to calcium deficiency among Indonesians are poor dietary habits, low intake of natural sources of calcium (e.g., dairy products), low intake of other calcium sources that must be consumed in large amounts to meet the recommended dietary intake for calcium, and low bioavailability (e.g., cereals, nuts, and green leafy vegetables). Another factor is consumer behavior, such as purchasing power, food preferences, and special conditions (e.g., teen years, young adult years, childbearing years, pregnancy, breastfeeding, later adult years, lactose intolerance, and a vegetarian diet).

There are several solutions to increase calcium intake. One of these is consuming calcium-fortified foods. However, calcium in these food products is more available in the form of microcalcium, so the absorption of calcium in the body (only about 50%) is not optimal **(Lekahena et al., 2014)**.

Current technological advances, particularly nanotechnology, can overcome the problem of eggshell waste by extracting nanocalcium powder with particle sizes of 10–100 nm in diameter. This process increases the economic value of abundant eggshell waste in line with the concept of zero waste products. It also reduces calcium deficiency by converting calcium from the eggshells into absorbable forms **(Suptijah, Jacoeb and Deviyanti, 2012)**.

Using nanotechnology in the fractionation process of the precipitation method converts calcium carbonate into calcium oxide so that it produces nanocalcium powder with good physical and chemical characteristics. This method controls the solubility of the material in the solution through pH and temperature changes by adding certain chemicals to convert soluble compounds into insoluble solids or by mixing acidic bases, producing solids and water **(Purwasasmita and Gultom, 2008)**. This method is very effective because it produces nanoparticles through a simple and low-cost process **(Gulsun, Gursoy and Oner, 2009)**.

Eggs are the main protein source in Indonesia. They are popular with the community because they are highly nutritious, relatively cheap compared to other animal protein sources, delicious, and easily digested in the body. In Indonesia, the production of chicken eggs in 2018 reached 1,644,460 tons, with an average consumption per capita of as much as 302.71 grams per day **(Central Statistics Agency, 2016)**.

Increasing the value of egg production is in response to the increasing demand for eggs. Thus, the potential for eggshell waste in Indonesia is quite large but has not been used optimally. People generally dispose of eggshells without using them first even though 96% of their calcium content (94% calcium carbonate, 1% magnesium carbonate, and 1% calcium phosphate) can be additional material extracted for food minerals. The main composition of calcium carbonate in eggshells can cause environmental pollution because soil microbes can't degrade it easily **(Trilaksani, Salamah and Nabil, 2006)**.

Several studies confirmed that nanosizing increases the bioavailability of calcium **(Park et al., 2008; Seo et al., 2009; Hilty et al., 2011).** Nanocalcium powder can be used in various products and food fortification as a form of functional food that benefits people's health. Therefore, research was conducted on the effect of eggshell type (chicken and duck) and extraction time on the physicochemical characteristics of nanocalcium powder.

Scientific Hypothesis

It is hypothesized that different types of eggshells and extraction time had significant effects on the physicochemical characteristics of eggshells' nanocalcium powder.

MATERIAL AND METHODOLOGY

Samples

Chicken and duck eggshells were obtained from the cake home industry in Palembang city, South Sumatera Province, Indonesia.

Chemicals

The main chemicals used in this study were lanthanum, chloric acid, potassium hydroxide, and demineralized water.

Instruments

The instruments used in this study were an atomic absorption spectrophotometer (AAS, Shimadzu AA-7000, Japan), a chromameter (CR-410 Konica Minolta, Japan), and X-ray diffraction (XRD, RigakuBenchtop XRDMiniflex 60, Japan).

Description of the Experiment

The sampel in this research was eggshell nanocalcium powder that prepared from eggshell powder.

Preparation of eggshell powder (Rahmawati and Nisa, 2015)

Chicken and duck eggshells (500 g) were washed with water until clean. The cleaned eggshells were boiled at 100°C for 10 minutes to kill pathogenic microbes. They were drained and then dried in an oven for 2 hours at 60°C. Then, the eggshells were placed at room temperature. They were ground to powder and sieved with a 100 mesh sieve. The eggshell powder was included in the oriented polystyrene) plastic and stored at 4°C for analysis.

Preparation of eggshell nanocalcium powder (Khoerunnisa, 2011)

Eggshell powder was immersed in 1N HCl solvent (1:5) for 48 hours and then extracted at 90°C for 1, 1.5, and 2 hours. The extracts were then filtered with filter paper to obtain filtrates and sediments. The filtrate was precipitated by adding 3N NaOH. It was stirred and left until the precipitate was formed. The precipitate was then neutralized by using aquabidest to pH neutral. The solution was separated from the sediment by pouring it slowly so that the precipitate was not wasted. The sediment was dried in an oven for 3 hours at 105°C. It was ashed in a muffle furnace at 600°C for 5 hours and refined with a mortar (Figure 1). The nanocalcium powder was packed in airtight plastic bags and stored at 4°C until it was used.

Number of sample analyze

The research was conducted using a completely randomized factorial design with two factors, and each treatment analysis was repeated three times. The first factor (A) was the type of eggshell (A_1 = chicken and A_2 = duck), and the second factor (B) was the extraction time (B_1 = 1hour, B_2 = 1.5 hour, and B3 = 2 hours).

Number of experiment replication

Experiment was performed in triplicates

Analyses of physicochemical characteristics of eggshell nanocalcium powder

Yield determination

The nanocalcium yield was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{weight of nanocalcium powder}}{\text{weight of eggshell powder}} \text{ x } 100\%$$

Color determination of nanocalcium

Nanocalcium powder's color was measured using Munsell (1977). The values were L (lightness), C (chroma), and H (hue) scales.

Moisture content (AOAC, 2005) of nanocalcium

A sample of approximately 1 g (W_1) was placed on a dish and dried at 105°C for 18 hours. After drying, a dish containing samples was transferred to a desiccator for 15 minutes. Then, the dish was reweighted until its weight was constant (W_2). Nanocalcium's moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{(W_1 - W_2)}{W_2} \times 100\%$$

W_1 = weight (g) of a sample before drying
W_2 = weight (g) of a sample after drying

Ash content (AOAC, 2005) of nanocalcium

A sample of about 1 g sample (W_1) was placed on a porcelain crucible. Then, it was put in a muffle furnace at 550°C until the samples turned whiteish gray. The ash content of the nanocalcium was calculated using the following formula:

$$\text{Ash content (\%)} = \frac{\text{weight of ash}}{\text{weight of the sample}} \times 100\%$$

Calcium determination (AOAC, 2005) of nanocalcium

The calcium content of samples was determined using an AAS (Shimadzu AA-7000, Japan) according to the AOAC (Association of Official Analytical Chemists) method (AOAC, 2005). The ground sample (5 g) was placed in an ashing vessel, charred in a muffle furnace, and then ashed at 500°C overnight. The completely ashed sample was dissolved in 10 mL of concentrated hydrochloric acid. The solution was boiled and evaporated nearly to dryness. The residue was redissolved in 20 mL of 2N hydrochloric acid and boiled gently. The solution was cooled and diluted to 100 mL with distilled deionized water. Its absorbance was then measured using the AAS at 422.7 nm. The measurements were calibrated using a commercial standard solution (Merck KGaA, Germany). To eliminate phosphorus interference in the measurements, lanthanum was added to the test ash and standard solutions so that the final solution contained 1% lanthanum.

The crystalline structure of nanocalcium powder

XRD was used to measure the crystal structure of samples.

Statistical Analysis

All analyses were performed in triplicates. The data were subjected to the analysis of variance followed by Fisher's least significant difference (LSD) test to compare treatment means. Differences were considered at a significant level of 95% (p<0.05) by using SPSS v.19 software.

RESULTS AND DISCUSSION

Physical Characteristics of Nanocalcium Powder

Yield

The yield of nanocalcium powder ranged from 11.62% to 15.27% for all samples. The lowest average yield was found in the A_1B_1 treatment (chicken eggshells, 1 hour extraction time), while the highest yield value was found in the A_2B_2 treatment (duck eggshell, extraction time of 1.5 hours). The results showed that the type of eggshell and the extraction time had a significant effect on the yield of nanocalcium powder (p<0.05). Table 1 shows the value of the average yield of nanocalcium powder.

The yield of duck nanocalcium powder was significantly higher (p<0.05) than that of chicken nanocalcium powder due to the main component forming the eggshell, calcium carbonate ($CaCO_3$). Calcium carbonate is a major component of an eggshell **(Shwetha et al., 2018).** Duck eggshells have a higher $CaCO_3$ content (96.48%) **(Sari, 2013)** than chicken eggshells (90.90%) (**Warsy et al., 2016**). However, the result of research conducted by **Ajayan et. al. (2020)** showed that the average percentage of calcium carbonate in eight varieties of chickens (89.05%) was higher than that in six varieties of ducks (84.63). **Adeyeye (2009)** stated that hen eggshells contain more calcium than duck eggshells. This is because the differences in calcium carbonate content between shells are due to differences in chicken or duck varieties. Also, calcium carbonate content depends on the ratio of the dietary calcium of poultry feed **(Lestari, Riyanti, and Wanniatie, 2015)**.

During the extraction process, higher $CaCO_3$ content and a longer extraction time caused a higher solvent penetration into the eggshell powder, resulting in more compounds diffusing out of the shell and giving a higher yield. However, a yield of 2-hour extraction time was not significantly different from that of 1.5 hours. This is because the calcium carbonate cycle has a reversible reaction so that it can allow the return of products to reactants, where carbonic acid can react again with calcium carbonate to form calcium bicarbonate **(Dewi et al., 2012)**. **Risnojatiningsih (2009)** stated that the formation of $Ca(HCO_3)_2$ occurs when the formed $CaCO_3$ continues to react with water containing CO_2 gas. The result also agreed with the findings of **Khoerunnisa (2011)**, showing that the yield of local mussel shell nanocalcium with 1N HCl extraction significantly increased from 1-hour extraction (5.02%) to 1.5 hours (8.53%) and decreased insignificantly from 1.5 hours (8.53%) to 2 hours of extraction (7.89%).

Color

The color of nanocalcium powder from chicken and duck eggshells was white. This indicates that calcium oxide (CaO) has formed. This is following the statement of **Sing et al. (2011)** and **Tangboriboon et al. (2012)** that the eggshells' change in color to white during calcinations indicates that a complete chemical transformation from calcium carbonate to calcium oxide has been achieved.

The lightness value of nanocalcium powder ranged from 92.00% to 94.03%. The lowest average lightness value was found in A_1B_1 (chicken eggshell, extraction time of 1 hour), while the highest lightness value was found in treatment A_2B_2 (duck eggshell, extraction time of 1.5 hours). Chroma value of nanocalcium powder ranged from 6.73% to 7.07%. The lowest average chroma value was found in A_2B_2 (duck eggshell, extraction time of 1.5 hours), while the highest chroma value was found in A_1B_1 (chicken eggshell, 1 hour extraction time). The hue value of nanocalcium powder ranged from 26.30° to 28.87°. The lowest hue value was found in the A_1B_1 treatment (race chicken eggshell, extraction time of 1 hour), while the highest hue value was found in the A_2B_2 treatment (duck eggshell, extraction time of 1.5 hours). Table 1 shows the average lightness, chroma, and hue values of nanocalcium powder.

The value of lightness is the degree of brightness of a product. Chicken and duck eggshells produced high lightness values of nanocalcium powder. This was because the preparation of nanocalcium powder through HCl caused pigment deposition in each eggshell so it degraded easily and the color became brighter. Duck eggshells contain biliverdin pigments,hence their greenish-blue color. On the other hand, chicken eggshells contain porphyrin pigments, hence their brownish shells **(Mushawwir and Latipudin, 2013; Yonata et al., 2017)**.

The immersion process with HCl and extraction can cause the greenish color of the duck eggshells to degrade easily, resulting in a whiter color, while the brownish color of the chicken eggshell produces a darker color than the duck egg nanocalcium powder. The difference in the components of the constituent minerals in the eggshell also affects the lightness value of the nanocalcium powder. The main component of the constituent minerals of nanocalcium powder is calcium, which generally has a white color. Therefore, the lightness value of nanocalcium powder was also high.

Chroma is a parameter used to determine the color intensity of a product. The chroma value is inversely proportional to the lightness value because if a product has a dark color, the intensity of the resulting color will be stronger.If the lightness value is high, then the chroma value produced from a product will be lower. The longer the extraction, the more $CaCO_3$ hydrolyzed by HCl will produce a whiter color, resulting in a decreased chroma value.

Hue is a value to determine the dominant wavelength of the color in a product. The hue of nanocalcium powder was red (R), with a range of 18°–54°. The longer the extraction, the more the hue of nanocalcium powder turned yellowish red. This was because the longer the extraction, the more calcium was extracted from the eggshell. The constituent elements of calcium from the flame color test are brick red **(Permata et al., 2018)**. Therefore, the more calcium in eggshell powder, the more the hue of the nanocalcium powder turned yellowish red.

Chemical Characteristics of Nanocalcium Powder

Moisture content

The moisture content of nanocalcium powder ranged from 0.16% to 0.33% for all samples. These values were very small due to drying in the preparation of nanocalcium powder. The moisture content of nanocalcium of all samples is shown in Table 1. That of nanocalcium powder of chicken eggshells was significantly higher than that of duck eggshells (p<0.05). This must be because the initial moisture content of duck eggshells (1.43±0.04%) was significantly lower than that of chicken eggshells (1.99±0.01%), decreasing the water content in the calcination stage of the nanocalcium preparation.

The extraction time significantly affected the moisture content of nanocalcium powder (p<0.05). The longer the extraction time, the lower the moisture content of nanocalcium powder. This was due to the evaporation of water in the filtrate so that the water content of the nanoparticle powder became free water. The decrease in water content was also due to the protein content in eggshell powder being hydrolyzed when it is mixed with chloric acid and the heating process resulting in decreased water binding. This observation was in agreement with the result found by **Trilaksani et al. (2006)** and **Agustini et al. (2011)**, who stated that the low water content in tuna bone and clamshell powder was due to protein hydrolysis during heating in powder preparation.

Ash content

The ash content of nanocalcium powder ranged from 96.80% to 98.69% for all samples. Table 1 shows the ash content of all samples' nanocalcium. The extraction time significantly affected the ash content of nanocalcium powder (p<0.05). The ash content increased as the extraction time increased. This may be because the longer the extraction time,the longer the contact of nanoparticle powder and solvent (chloric acid). This, in turn, increased the chance of hydrolysis reaction. This result was supported by the findings by **Widyastuti et al. (2015)**, who concluded that the ash content of chicken eggshell nanocalcium with a 1N NaOH solvent and 1-,2-, and 3-hour extraction gave the highest value of ash content in the 3-hour extraction time: 98.07%, 98.01%, and 98.03% for 1-,2-, and 3-hour extraction, respectively.

Calcium content

The calcium content of microcalcium and nanocalcium of all samples is shown in Table 2. Microcalcium content (149 μm) of chicken eggshells was higher than that of duck eggshell. This was because $CaCO_3$ of duck eggshell (96.48%) was higher than that of duck eggshell (90.90%), resulting in a higher amount of extracted calcium. Moreover, the calcium content of nanocalcium was higher than that of microcalcium both from chicken and duck eggshells. This was due to demineralization, precipitation, and calcination processes during nanocalcium preparation, maximally opening eggshell spores and extracting more calcium extracted. The steps of the reactions are as follows:

Demineralization:

$$\mathrm{CaCO_{3(s)} + 2HCl_{(aq)} \rightarrow CaCl_{2(aq)} + H_2O_{(l)} + CO_{2(g)}} \quad (1)$$

Precipitation:

$$\mathrm{CaCl_{2(aq)} + 2NaOH_{(aq)} \rightarrow Ca(OH)_{2(s)} + 2NaCl_{(aq)}} \quad (2)$$

Calcination:

$$\mathrm{Ca(OH)_{2(s)} + Heat \rightarrow CaO_{(s)} + H_2O_{(l)}} \quad (3)$$

The observation supported by previous research **(Navarro et al., 2009; Mohamed et al., 2012; Mosaddegh et al., 2014; Zuhra et al., 2015)** concluded that $CaCO_3$ can be converted to CaO through thermal decomposition (calcination).

Moreover, Table 2 shows that the calcium content of nanocalcium from chicken and duck eggshells decreased with increasing extraction time. This may be due to $CaCO_3$ reformation, resulting from the reversible reaction of the calcium carbonate cycle. The steps are as follows:

$$CO_{2(g)} + H_2O_{(l)} \rightarrow H_2CO_3 \quad (4)$$
$$CaCO_{3(s)} + H_2CO_{3(aq)} \rightarrow Ca(HCO_3)_{2(aq)} \quad (5)$$
$$Ca(HCO_3)_2 \rightarrow CaCO_{3(s)} + H_2O_{(l)} \quad (6)$$

X-Ray Diffraction

The XRD results are used to determine the crystalline structure of nanocalcium powder. The XRD is monitored at 2ϴ = 5°–90°. The results of the XRD analysis of nanocalcium from chicken eggshells with extraction times of 1, 1.5, and 2 hours are presented in Figure 2. Figure 3 shows the result of the XRD analysis of nanocalcium from duck eggshells with extraction times of 1, 1.5, and 2 hours.

The main peak appeared at 2ϴ = 37.44° of nanocalcium from chicken eggshells and 2ϴ = 37.36° of nanocalcium from duck eggshells. Peaks of nanocalcium oxide (CaO) from chicken eggshells appeared at 2ϴ = 32.28°, 37.44°, 53.94°, 64.21°, and 67.44°. Moreover, peaks of nanocalcium oxide (CaO) from duck eggshells appeared at 2ϴ = 32.15°, 37.36°, 53.85°, 64.10°, and 67.32°. The peaks that appeared were all identified and corresponded to the database International Center for Diffraction Data (ICDD) of calcium oxide (CaO) (PDF No. 99-0070). This result was supported by **Taufiq_Yap et al. (2011)**, and **Habte et al. (2019)** stated that the peaks at 2ϴ = 32.22°, 37.36°, 53.8°, 64.1°, and 67.3° were assigned to planes of pure CaO phase. Nanocalcium oxide powder of chicken and duck eggshells had a polycrystalline structure **(Habte et al., 2019; Khan et al., 2018)**.

The XRD patterns displayed diffractograms of CaO, $Ca(OH)_2$, and $CaCO_3$. The XRD patterns of nanocalcium from chicken eggshells have peaks of CaO (100%) at 1-hour extraction time; $Ca(OH)_2$ (77.4%), $CaCO_3$ (11.0%), and CaO (11.6%) at 1.5-hour extraction time; and $Ca(OH)_2$ (52.2%), $CaCO_3$ (9.0%), and CaO (38.8%) at 2-hour extraction time. Moreover, the XRD patterns of nanocalcium from duck eggshells have peaks of CaO (100%) at 1-hour extraction time; $Ca(OH)_2$ (48.1%), $CaCO_3$ (6.9%), CaO (45.1%) at 1.5-hour extraction time; and $Ca(OH)_2$ (87.3%), $CaCO_3$ (11.2%), and CaO (1.5%) at 2-hour extraction time.

The nanocalcium oxide (CaO) content from chicken and duck eggshells at 1 hour of extraction was formed 100%. This indicated that transformation of chemical composition from $CaCO_3$ to CaO was completely achieved after the whole process took place. This observation was supported by **Dasgupta et al. (2004)**, who stated that $CaCO_3$ turned to CaO at 540°C. The appearance of peak $Ca(OH)_2$ of nanocalcium from chicken and duck eggshells at extraction time 1.5 and 2 hours due to the hydration reaction between hygroscopic CaO and water vapor. In the same condition, the appearance of peak $CaCO_3$ must be due to the incomplete decomposition of $CaCO_3$ to CaO.

Based on the XRD analysis, the crystallite size and density of nanocalcium oxide from chicken and duck eggshells were 41.54 nm and 3.36 g/cm^3 and 24.90 nm and 3.34 g/cm^3, respectively. Previous studies showed that the crystallite sizes of nanocalcium from chicken eggshells were 10.46 nm (**Sunardi, Krismawati, and Mahayana, 2020)** and50–198 nm **(Habte et al., 2019)**, and the crystallite size of nanocalcium from duck eggshells was 262 nm **(Prayitno, Prasetyo, and Sutirtoadi, 2020).**

These values were different from the values of nanocalcium crystallite sizes from this study. This was due to differences in raw material since the substances contained in eggshells depend on the breed, feed, and environment of the chicken.

The density values of nanocalcium oxide from chicken and duck eggshells were 3.361 g/cm^3 and 3.342 g/cm^3, respectively. These values were lower than density values of the chicken eggshells (2.16 g/cm^3) and duck eggshells (2.84 g/cm^3) calcinated at 900°C for 1 hour (**Tangboriboon et al., 2012).** The differences are caused by differences in temperature and calcination times; the species and the feeding of the chickens and ducks also influenced the nutrient content during the egg formation.

CONCLUSION

The results of this study indicated that extraction time influenced yield, moisture content, ash content, and color in terms of chroma and hue of nanocalcium powder. Moreover, the type of eggshells influenced yield, moisture content, and lightness of nanocalcium powder. The XRD showed that CaO was formed from nanocalcium powder of chicken and duck eggshells at 1 hour of extraction. This showed that transformation of chemical composition from $CaCO_3$ to CaO was completely achieved after the calcination and precipitation processes process took place. The size of nanocalcium oxide crystals from chicken and duck eggshells were 41.54 nm and 24.90 nm.

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Acknowledgments:
The authors would like to thank Sriwijaya University for the funding provided for the implementation of this research.

Conflict of interest:
The authors declare no conflict of interest.

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Table 1 Physicochemical characteristics of nanocalcium powder from chicken and duck eggshells with different extraction times.

Treatment	Yield (%)	Colour			Moisture content (%)	Ash content (%)
		Lightness (%)	Chroma (%)	Hue (°)		
A_1B_1	11.62 ±0.28^a	93.67 ±3.71^a	7.07 ±0.06^a	26.30 ±0.20^a	0.33 ±0.01^a	96.80 ±0.44^a
A_1B_2	13.30 ±0.27^b	93.73 ±0.12^a	6.80 ±0.10^a	28.30 ±0.30^a	0.25 ±0.01^a	98.32 ±0.05^a
A_1B_3	13.59 ±0.20^b	93.67 ±0.35^a	6.83 ±0.12^a	28.00 ±0.17^a	0.26 ±0.01^a	98.28 ±0.05^a
A_2B_1	13.83 ±0.03^b	93.53 ±0.15^a	7.03 ±0.06^a	26.93 ±0.35^a	0.18 ±0.03^b	97.91 ±0.64^a
A_2B_2	15.27 ±0.32^c	93.37 ±1.01^a	6.73 ±0.12^a	28.87 ±0.46^a	0.18 ±0.03^b	98.69 ±0.10^a
A_2B_3	15.20 ±0.20^c	93.87 ±0.21^a	6.77 ±0.06^a	28.27 ±0.23^a	0.17 ±0.03^b	98.67 ±0.10^a

Note: Data are presented as mean ± standard deviation of triplicate determinations.
Means for each characteristic followed by the same superscript within the same row are not significantly different at $p<0.05$ by the LSD test.
A_1B_1 = chicken eggshell; 1-hour extraction time
A_1B_2 = chicken eggshell; 1.5-hour extraction time
A_1B_3 = chicken eggshell; 2-hour extraction time
A_2B_1 = duck eggshell; 1-hour extraction time
A_2B_2 = duck eggshell; 1.5-hour extraction time
$A2B_3$ = duck eggshell; 2-hour extraction time

Table 2 Calcium content of microcalcium and nanocalcium powders from chicken and duck eggshells.

Treatment	Calcium content (%)
Microcalcium powder (149 µm)	
Chicken	11.41 ±0.03
Duck	12.32 ±0.14
Nanocalcium powder	
A_1B_1	40.80 ±0.06
A_1B_2	24.78 ±0.04
A_1B_3	24.52 ±0.05
A_2B_1	49.39 ±0.08
A_2B_2	27.38 ±0.05
A_2B_3	25.42 ±0.04

Note: A_1B_1 = chicken eggshell; 1-hour extraction time
A_1B_2 = chicken eggshell; 1.5-hour extraction time
A_1B_3 = chicken eggshell; 2-hour extraction time
A_2B_1 = duck eggshell; 1-hour extraction time
A_2B_2 = duck eggshell; 1.5-hour extraction time
A_2B_3 = duck eggshell; 2-hour extraction time

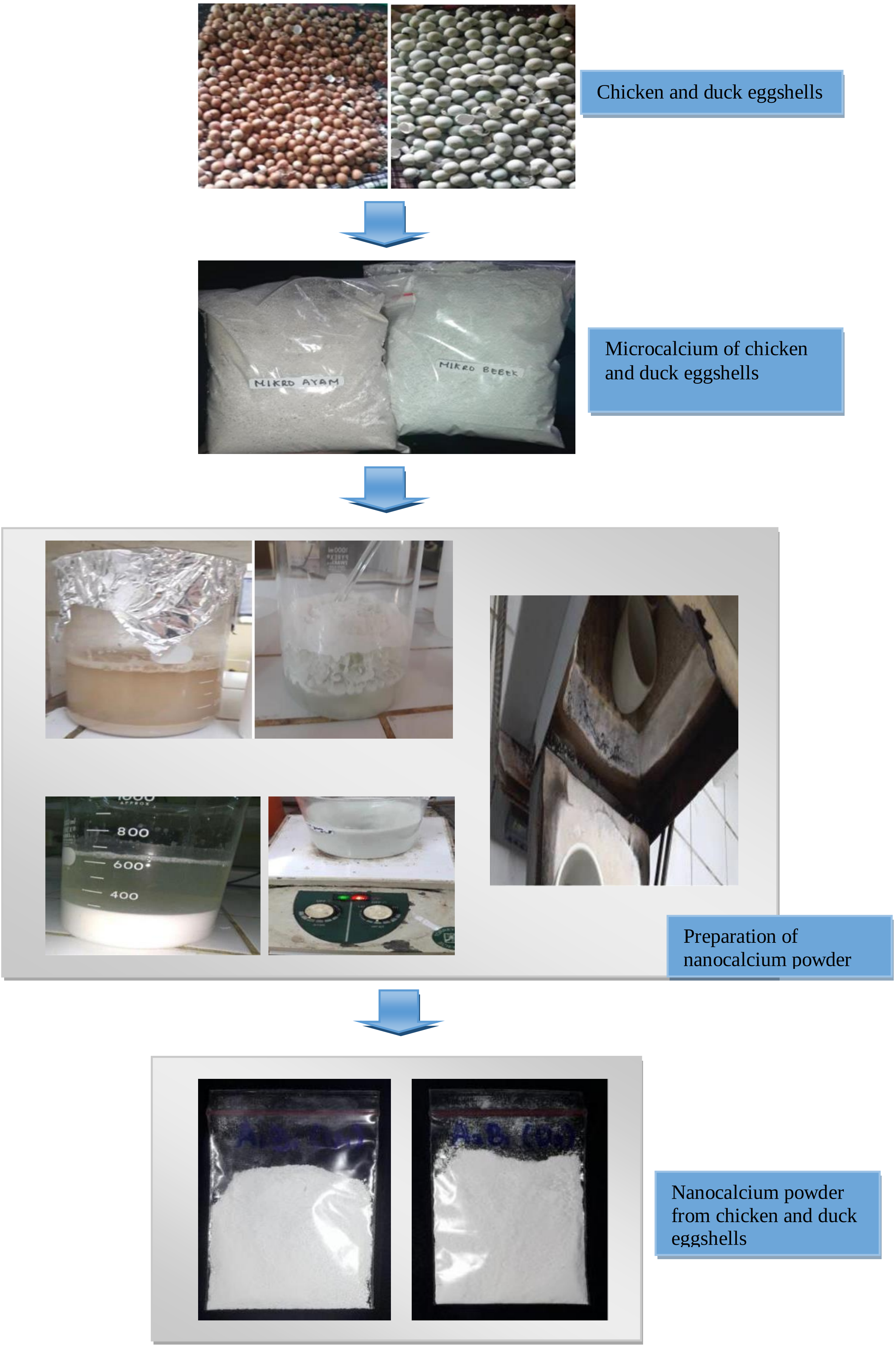
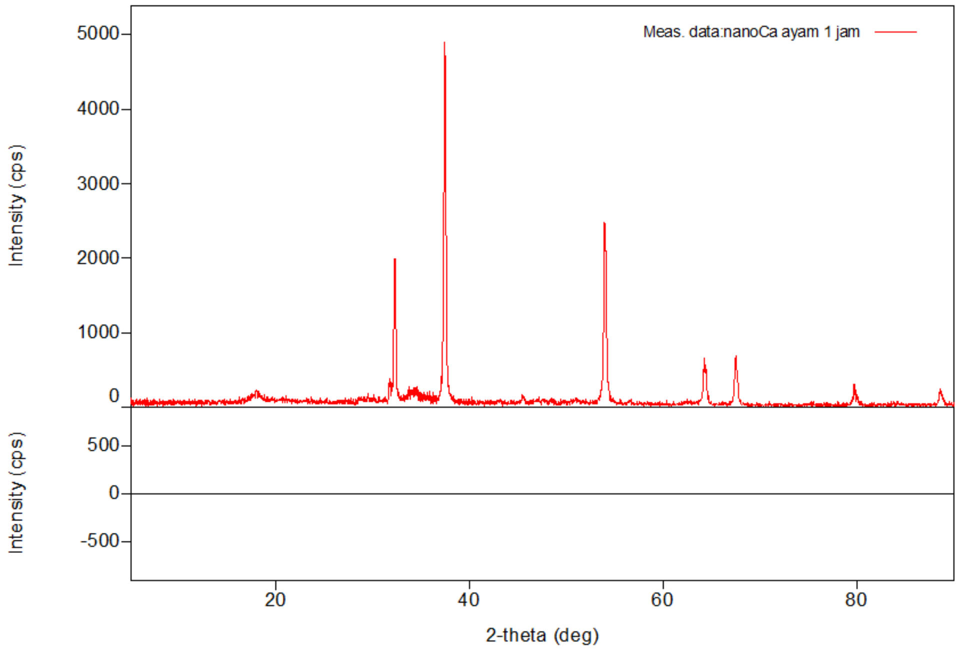


Figure 1 Processing of Nanocalcium from Egg and Duck



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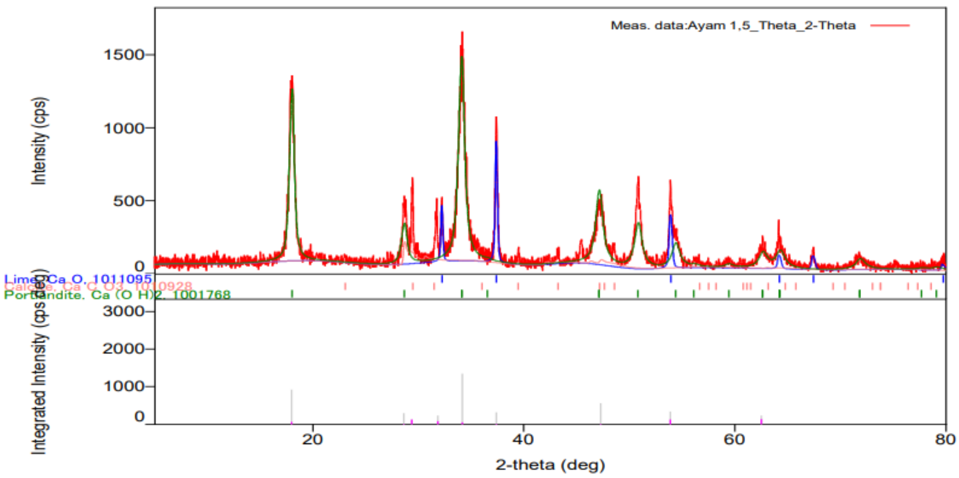

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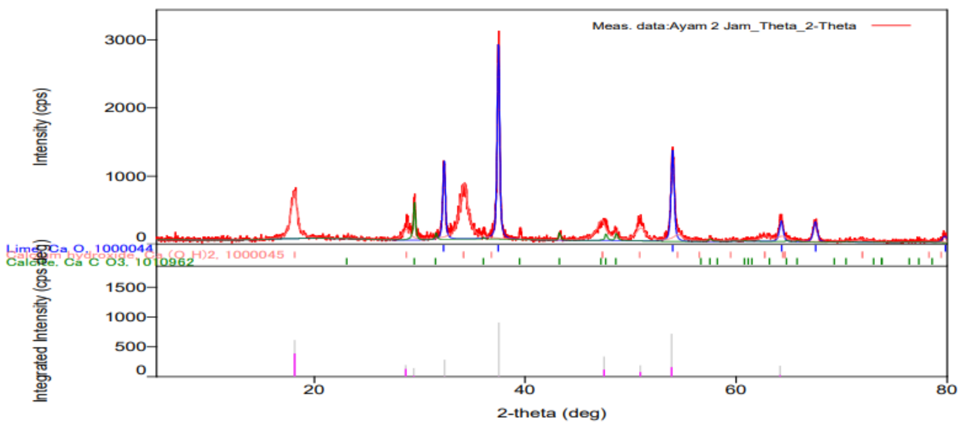

(c)

Figure 2 XRD of nanocalcium chicken eggshells from (a) 1-hour extraction, (b) 1.5-hour extraction, and (c) 2-hour extraction.

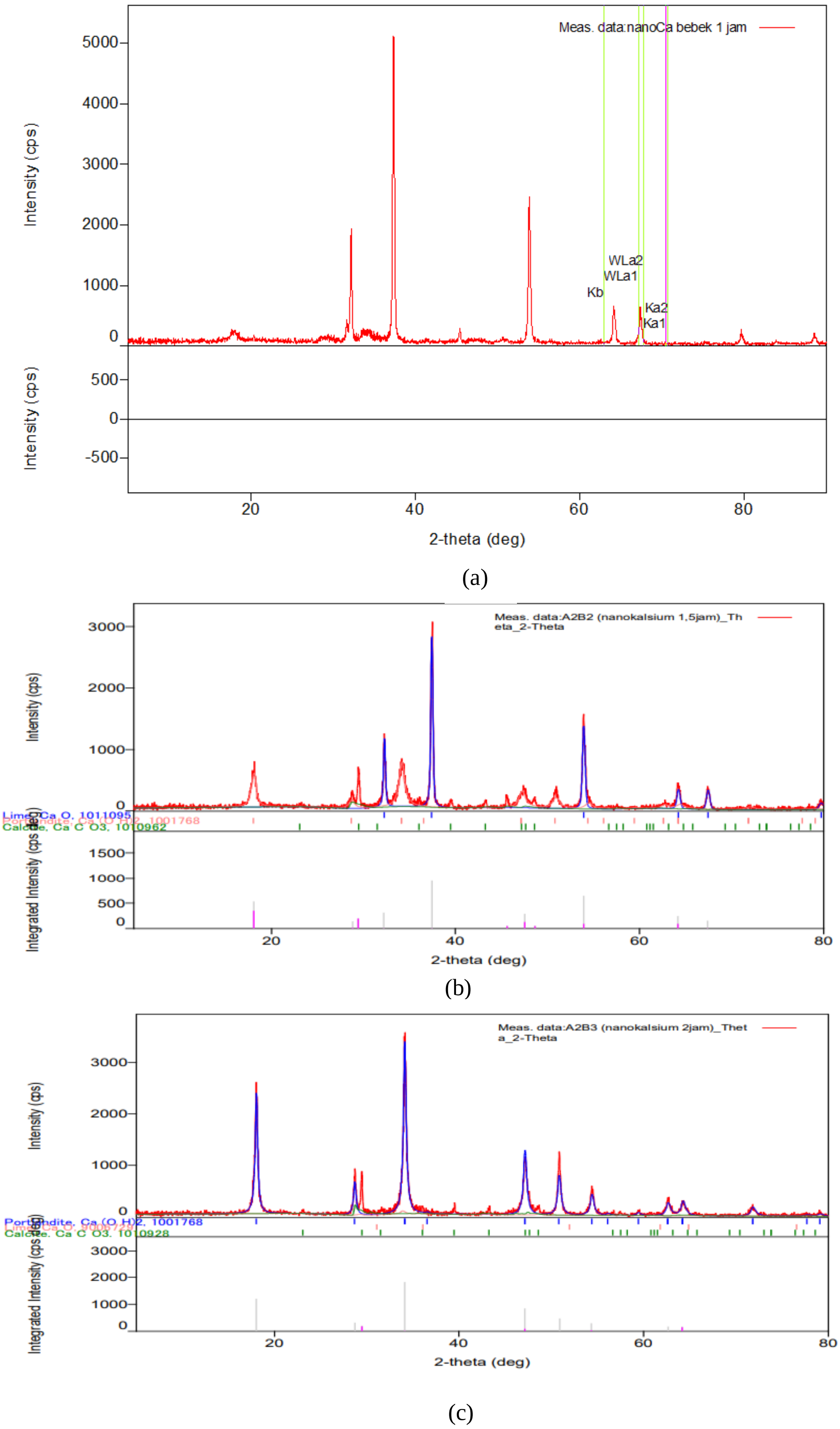

Figure 3 XRD of nanocalcium duck eggshells from (a) 1-hour extraction, (b) 1.5-hour extraction, and (c) 2-hour extraction.

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potravinárstvo® Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. XX, XXXX, p. XX-XX
https://doi.org/10.5219/XXX
Received: XXXXXXXXXXXX. Accepted: XXXXXXXXXXXX.
Available online: XXXXXXXXXXXX at www.potravinarstvo.com

ISSN 1337-0960 (online)

EFFECT OF EXTRACTION TIME ON THE PHYSICOCHEMICAL CHARACTERISTICS OF NANOCALSCIUM POWDER FROM CHICKEN AND DUCK EGGSHELLS

Nura Malahayati, Tri Wardhani Widowati, Nurul Saniah Alsoyuna

ABSTRACT

Calcium ~~D~~deficiency ~~of calcium~~ is associated with the risks of bone fracture and osteoporosis. This type of malnutrition has been a ~~focus of the~~concern of governments and the World Health Organization for decades, and extensive efforts ~~are~~have been made to address it. There are several solutions to increase calcium intake.~~,~~ ~~o~~One ~~of these~~ is to take~~ing~~ calcium in the form of nanocalcium. The objective of the present research was to determine the effect of extraction time on the physicochemical characteristics of nanocalcium powder extracted from chicken and duck eggshells ~~conducted by~~through precipitation~~ method~~. This research was conducted by using ~~Factorial~~ a ~~C~~completely ~~R~~randomized factorial ~~D~~design with two factors, and each treatment analysis was repeated three times. The first factor was the type of eggshell (chicken and duck), and the second ~~factor~~ was the extraction time (1, 1.5, and 2 hours). The ~~O~~observed parameters were physical ~~characteristics~~ (yield and color) and chemical characteristics (moisture, ash, calcium content, and crystalline structure). The results ~~of the research~~ showed that the type of eggshell had a significant effect (*p*<0.05) on yield, color (lightness), and moisture content~~.~~ ~~The treatment of~~and that extraction time had significant effect (*p*<0.05) on yield, color (chroma and hue), moisture, and ash content. ~~Based on t~~The highest content of the crystalline structure of nanocalcium formation (100%) was nanocalcium powder from chicken and duck eggshells, with 1 hour~~s~~ of extraction time. The particle size of the crystalline structure of nanocalcium from chicken and duck eggshells were 41.54 nm and 24.90 nm, respectively.

Keywords: nanocalcium; chicken; duck; eggshells; extraction

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INTRODUCTION

Calcium is the main mineral ~~contained~~ in the human body, with as much as 1.5% ~~to~~ –2% of adult body weight or about 1 kg **(Ariyanti, 2012)**. Calcium plays an important role in stiffness, bone strength, and most metabolic processes, including blood clots, muscle contractions, hormonal systems, glycogen metabolism, cell proliferation, and differentiation **(WHO, 2006)**. People's ~~C~~calcium consumption ~~in the human body~~ varies according to age and sex.

Indonesian people's ~~D~~dietary intakes of calcium– ~~by Indonesian people~~, 254 mg/day, are far below– ~~that needed~~the requirement to build proper bone mass. It is widely accepted in the literature that a low calcium intake over the years contributes to the development of– bone fracture and osteoporosis. The ~~problems~~factors that lead to calcium deficiency ~~in getting enough calcium of the people in~~ among Indonesians are poor ~~(low and inadequate)~~ dietary habits, ~~not many foods are~~low intake of naturall~~y a good~~ sources of calcium (e.g., ~~milk and~~ dairy products), ~~and~~ low intake of ~~an~~other calcium sources ~~(non-dairy foods) have to~~that must be consumed in large amounts to meet the ~~R~~recommended ~~D~~dietary ~~I~~intake ~~(RDI)~~ for calcium, and low bioavailability (e.g., cereals, nuts, and green leafy vegetables). Another ~~problem~~factor is consumer behavior, such as ~~low~~ purchasing power, ~~boring,~~food preferences, and special conditions (e.g., ~~the~~ teen years, ~~the~~ young adult years, ~~the~~ childbearing years, pregnancy~~t~~, breast-feeding, ~~the~~ later adult years, lactose intolerance, and a vegetarian diet).

There are several solutions to increase calcium intake.~~,~~ ~~o~~One of these is consuming calcium-fortified foods ~~as a benefit product~~. However, calcium in these food products is more available in the form of microcalcium, so the~~at~~ absorption of calcium in the body (only about 50%) is not optimal~~, only about 50%~~ **(Lekahena et al., 2014)**.

Current technological advances, ~~in the form of~~ particularly nanotechnology, ~~are able to~~can overcome the problem of eggshell waste by extracting ~~calcium into~~ nanocalcium powder with particle sizes of 10 ~~nm to~~ –100 nm in diameter. This process~~is an effort to~~ increases the economic value of abundant eggshell waste in line with the concept of zero waste products. ~~and~~It also reduces calcium deficiency by converting calcium from the eggshells into absorbable forms ~~because it is not optimal absorption of calcium in the body so that calcium in the eggshell must be converted into absorbable forms~~ **(Suptijah, Jaceob and Deviyanti, 2012)**.

~~The use of~~Using nanotechnology in the fractionation process ~~by~~of the precipitation method ~~is able to~~ converts calcium carbonate into calcium oxide so that it produces nanocalcium powder with good physical and chemical characteristics. This method controls the solubility of the material in the solution through ~~changes in~~ pH and temperature changes by adding ~~a number of~~ certain chemicals to convert soluble compounds into insoluble solids or by mixing acidic bases, producing~~which produces~~ solids and water **(Purwasasmita and Gultom, 2008)**. This method is very effective because it ~~can~~ produces nanoparticles ~~with~~through a simple and low-cost process **(Gulsun, Gursoy and Oner, 2009)**.

Eggs ~~is~~are the main protein source in Indonesia~~n~~. They are ~~food consumption that is~~ popular with the community because ~~it has a~~they are highly nutritious~~ent contents~~, ~~the price is~~ relatively cheap compared to other animal protein sources, ~~has a~~ delicious, ~~taste~~ and ~~is~~ easily digested in the body. In Indonesia, the production of chicken eggs in 2018 ~~was~~reached 1,644,460 tons, with an average consumption per capita as much as 302.71 grams per day **(Central Statistics Agency, 2016)**.

Increasing the value of egg production is in ~~line with~~response to the increasing ~~need~~demand for eggs. ~~so that the~~Thus, the potential ~~of~~for eggshell waste in Indonesia is quite large but has not been used optimally. People generally dispose of ~~the~~ eggshells ~~waste~~ without using ~~it~~them first~~,~~ even though 96% of their calcium content (94% calcium carbonate, 1% magnesium carbonate, and 1% calcium phosphate) ~~in the eggshell has the potential as~~can be ~~an~~ additional material extracted for food minerals. The main composition of calcium carbonate in eggshells can cause environmental pollution because ~~it is difficult to be degraded by~~ soil microbes can't degrade it easily **(Trilaksani, Salamah and Nabil, 2006)**.

~~It was approved by s~~Several studies confirmed that nanosizing– increase~~s~~d the bioavailability of calcium **(Park et al., 2008; Seo et al., 2009; Hilty et al., 2011)**. Nanocalcium powder can be used in various products and food fortification as a form of functional food that ~~is~~ benefits~~cial~~ ~~for~~people's health. Therefore, research was conducted on the ~~the~~ effect of eggshell type (chicken and duck) and extraction time on the physicochemical characteristics of ~~the~~ nanocalcium powder ~~produced~~.

Scientific Hypothesis

It is ~~suspected~~hypothesized that ~~the effect of~~ different types of eggshells and extraction time had ~~a~~ significant effects on the physicochemical characterictics of eggshells' nanocalcium powder.

MATERIAL AND METHODOLOGY

Samples

Chicken and duck eggshells were obtained from the cake home industry in Palembang City, South Sumatera Province,~~.~~ Indonesia.

Chemicals

The main chemicals used in this study were lanthanum, chloric acid, potassium hydroxide, and demineralized water.

Instruments

The ~~I~~instruments used in this study were an ~~A~~atomic ~~A~~absorption ~~S~~spectrophotomet~~ry~~er (AAS~~)~~, ~~(~~Shimadzu AA-7000, J~~e~~apan), a ~~C~~chroma meter (CR-410 Koni~~k~~ca Minolta,

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Japan~~epang~~), and X-~~R~~ray ~~D~~diffraction (XRD~~,)~~ ~~(~~Rigaku ~~The~~ Benchtop X-RD Miniflex 60, J~~e~~apan).

Laboratory Method

Eggshell powder preparation (Rahmawati and Nisa, 2015)

Chicken and duck eggshells (500 g) were washed with water until clean. The ~~C~~cleaned eggshells were boiled at 100°C for 10 minutes to kill pathogenic microbes.~~,~~ ~~then the~~They ~~eggshells~~ were drained~~.~~ ~~The eggshells were~~and then dried in an oven for 2 hours at 60°C.~~,~~ T~~t~~hen, the eggshells were placed at room temperature. ~~Eggshells~~They were ground to powder~~,~~ and~~then were~~ sieved with a 100 mesh sieve. The ~~E~~eggshell powder was included in the ~~OPP (O~~oriented ~~P~~polystyrene) plastic and stored at 4°C for analysis.

Eggshell nanocalcium powder preparation (Khoerunnisa, 2011)

Eggshell powder was immersed in 1N HCl solvent (1:~~-~~5) for 48 hours~~,~~ and then~~,~~ extracted ~~with~~at ~~a temperature of~~ 90°C for 1, 1.5, and 2 hours. The extracts were then filtered with filter paper to obtain filtrates and sediments. The filtrate was precipitated by adding 3N NaOH. ~~and~~It was stirred~~, then leaved it~~and left until the precipitate was formed. The precipitate was then neutralized by using aquabidest to pH neutral water. The solution was separated from the sediment by pouring it slowly so that the precipitate was not wasted. The sediment was dried in an oven for 3 hours at ~~a temperature of~~ 105°C.~~,~~ ~~then i~~It was ashed in a ~~M~~muffle furnace at 600°C for 5 hours~~, then were~~ and refined with a mortar. The ~~N~~nanocalcium powder ~~were~~was packed in airtight plastic bags and stored at 4°C until it was used.

Yield determination

The ~~yield of the~~ nanocalcium yield was calculated using~~by~~ the following formula:

$$\text{Yield (\%)} = \frac{\text{weight of nanocalcium powder}}{\text{weight of eggshell powder}} \text{ x } 100\%$$

Color determination of nanocalcium

~~The color of n~~Nanocalcium powder's color was measured using Munsell (1977).~~-~~ The values were L (~~L~~lighteness), C (~~C~~chroma), and H (~~H~~hue) scales.

Moisture content (AOAC, 2005) of nanocalcium

~~-~~A sample ~~A~~of approximately 1 g ~~sample~~ (W_1) was placed on a dish and dried at 105°C for 18 hours. After drying, a dish containing samples was transferred to a desiccator for 15 minutes. Then, the dish ~~and sample was~~was reweighted until its weight ~~is~~was constant (W_2). Nanocalcium's~~The~~ moisture content ~~of the nanocalcium~~ was calculated ~~by~~using the following formula:

$$\text{Moisture content (\%)} = \frac{(W1-W2)}{W2} \text{ x } 100\%$$

W_1 = weight (g) of sample before drying
W_2 = weight (g) of sample after drying

Moisture content (AOAC, 2005) of nanocalcium

A sample of ~~— Approximately~~about 1 g sample (W_1) was placed on a porcelain crucible. Then, it~~sample~~ was ~~placed~~put in a muffle furnace at ~~temperature~~ 550°C until the samples turned whitish gr~~e~~ay. The ash content of the nanocalcium was calculated ~~by~~using the following formula:

$$\text{Ash content (\%)} = \frac{\text{weight of ash}}{\text{weight of the sample}} \text{ x } 100\%$$

Calcium determination (AOAC, 2005) of nanocalcium

The ~~— C~~calcium content of samples was determined using an ~~atomic absorption spectrophotometer (~~AAS~~)~~ (Shimadzu AA-7000, J~~e~~apan) according to the AOAC (Association of Official Analytical Chemists) method (AOAC, 2005). The ground sample (5 g) was placed in an ashing vessel, charred in a muffle furnace, and then ashed at 500°C overnight. The completely ashed sample was dissolved in 10 mL of concentrated hydrochloric acid. The solution was boiled and evaporated nearly to dryness. The residue was redissolved in 20 mL of 2N hydrochloric acid and boiled gently. The solution was cooled and diluted to 100 mL with distilled deionized water. Its absorbance was then measured using the AAS at 422.7 nm. ~~Calibration of t~~The measurements were calibrated~~was performed~~ using a commercial standard solution (Merck KGaA~~- 64271 Darmstadt~~, Germany). To el~~e~~iminate phosphor~~o~~us interference in the ~~determination~~measurements, lanthanum was added to the test ash ~~solution~~ and standard solutions so that the final solution cont~~e~~ained 1% lanthanum.

Crystalline structure of nanocalcium powder

~~X-Ray Diffraction (~~XRD~~)~~ ~~is~~was used to measure the crystal structure of samples.

Statistical Analysis

The research was conducted ~~by~~ using ~~Factorial~~ a ~~C~~completely ~~R~~randomized factorial ~~D~~design with two factors, and each treatment analysis was repeated three times. The first factor (A) was the type of eggshell (A_1 = chicken and A_2 = duck), and the second factor (B) was the extraction time (B_1 = 1 hour, B_2 = 1.5 hour, and B3 = 2 hours). All analyses were performed in triplicates. The ~~D~~data were subjected to the analysis of variance ~~(ANOVA)~~ followed by Fisher's least significant difference ~~test~~ (LSD) test to compare treatment means.~~;~~ ~~d~~Differences were considered at a significant level of 95% ($p < 0.05$) by using SPSS v.19 software.

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RESULTS AND DISCUSSION

Physical ~~c~~Characteristics of ~~n~~Nanocalcium ~~p~~Powder

Yield

~~-~~The yield of nanocalcium powder ranged from 11.62% to 15.27% for all samples. The lowest average yield was found in the A_1B_1 treatment (chicken egg shells, 1 hour extraction time), while the highest yield value was found in the A_2B_2 treatment (duck eggshell, extraction time of 1.5 hours). The results showed that ~~There was a significant effect of~~the type of eggshell and the extraction time had a significant effect on the yield of nanocalcium powder (*p* < 0.05). Table 1 shows ~~T~~the value of the average yield of nanocalcium powder ~~was shown in~~ . ~~**Table 1.**~~

-The yield of duck nanocalcium powder was significantly higher (*p* < 0.05) than that of chicken nanocalcium powder due to the main components forming the eggshell, calcium carbonate ($CaCO_3$). Calcium carbonate is a major component ~~in~~of an eggshell **(Swetha et al., 2018).** Duck eggshells have~~s~~ a higher $CaCO_3$ content (96.48%) **(Sari, 2013)** than ~~that of~~ chicken eggshells (90.90%) (**Warsy et al., 2016**). However, the result of research conducted by **Ajayan et. al. (2020)** showed that the average percentage of calcium carbonate in eight varieties of chickens (89.05%) was higher than that in six varieties of ducks (84.63). **Adeyeye (2009)** ~~in a comparative study~~ stated that hen eggshells contain~~s~~ more calcium than ~~that of~~ duck eggshells. This is because the differences in calcium carbonate content ~~in~~between shells ~~is~~are due to differences in chicken or duck varieties. ~~Another reason~~Also, calcium carbonate content ~~is~~ depends on the ration of the dietary calcium of poultry feed **(Lestari, ~~et al.~~Riyanti, and Wanniatie, 2015)**.

~~-~~During the extraction process, ~~the~~a higher $CaCO_3$ content and ~~the~~a longer ~~the~~ extraction time caused a higher solvent penetration ~~of solvent~~ into the eggshell powder, resulting in more compounds to diffuse out of the shell and ~~gave~~giving a higher yield. However, ~~the~~a yield of 2-hour~~s~~ extraction time was not significantly different from that of 1.5 hours. This is because the calcium carbonate cycle has a reversible reaction so that it can allow the return of products to reactants, where carbonic acid can react again with calcium carbonate to form calcium bicarbonate (**Dwi et al., 2015**). **Risnojatiningsih (2012)** stated that the formation of $Ca(HCO_3)_2$ occurs when the formed $CaCO_3$ continues to react with water containing CO_2 gas. The result ~~was~~ also ~~in~~ agreed~~ment~~ with the findings of ~~the research conducted by~~ **Khoerunnisa (2011)**, showing~~ed~~ that the yield of local mussel shell nanocalcium with 1N HCl extraction ~~was~~ significantly increased from 1-hour extraction (5.02%) to 1.5 hour (8.53%)~~,~~ and ~~non significantly~~ decreased insignificantly ~~-~~from 1.5 hours (8.53%) to 2 hours of extraction (7.89%).

Color

~~-~~The color of nanocalcium powder from chicken and duck eggshells ~~were~~was white. This indicates~~d~~ that calcium oxide (CaO) has formed. This ~~was~~is in accordance with the statement of **Sing et al. (2011)** and **Tangboriboon et al. (2012)** that the eggshells' change~~ing~~ in color ~~of the eggshells~~ to white during calcinations indicates~~d~~ that a complete chemical transformation from calcium carbonate to calcium oxide has been achieved.

The ~~L~~lightness value of nanocalcium powder ranged from 92.00% to 94.03%. The lowest average lightness value was found in A_1B_1 (chicken eggshell, extraction time of 1 hour), while the highest lightness value was found in treatment A_2B_2 (duck eggshell, extraction time of 1.5 hours). Chroma value of nanocalcium powder ranged from 6.73% to 7.07%. The lowest average chroma value was found in A_2B_2 (duck eggshell, extraction time of 1.5 hours), while the highest chroma value was found in A_1B_1 (chicken eggshell, 1 hour extraction time). The ~~H~~hue value of nanocalcium powder ranged from 26.30° to 28.87°. The lowest hue value was found in the A_1B_1 treatment (race chicken eggshell, extraction time of 1 hour), while the highest hue value was found in the ~~treatment~~ A_2B_2 treatment (duck eggshell, extraction time of 1.5 hours). Table 1 shows ~~T~~the average lightness, chroma, and hue values of nanocalcium powder ~~were shown in **Table 1**~~.

The value of lightness is the degree of brightness of a product. Chicken and duck eggshells produced high lightness values of nanocalcium powder. This was ~~due to~~because the preparation ~~process~~ of nanocalcium powder ~~by using~~through HCl caused ~~the~~ pigment deposition ~~process~~ in each eggshell so ~~that~~ it ~~was easily~~ degraded easily and the color beca~~o~~me~~s~~ brighter. Duck eggshells contain biliverdin pigments, ~~so~~hence ~~that~~ their ~~shell has a~~ greenish blue color. ~~, while~~On the other hand, chicken eggshells contain porphyrin pigments, ~~so that~~hence their brownish~~-colored~~ shells **(Mushawwir and Latipudin, 2013; Yonata et al., 2017)**. The immersion process with HCl and extraction can cause the greenish color ~~of the shell~~ of the duck eggshells to ~~be more easily~~ degrade easily~~d~~, resulting in a whiter color, while the brownish color of the chicken eggshell produces a darker color than the ~~powder of~~ duck egg nanocalcium powder. The difference in the components of the constituent minerals in the eggshell also affects the lightness value of the nanocalcium powder. The main component of the constituent minerals of nanocalcium powder is calcium, which generally has a white color.~~,~~ ~~t~~Therefore, the lightness value of nanocalcium powder was also high.

Chroma is a parameter used to determine the color intensity of ~~the~~a product. The chroma value is inversely proportional to the lightness value because if a product has a dark color, the intensity of the resulting color will be stronger.~~,~~ ~~i~~If the lightness value is high, then the chroma value produced from a product will be lower. The longer the extraction, the more $CaCO_3$ hydrolyzed by HCl will produce~~d~~ a whiter color, resulting in a decreased chroma~~ing of the~~ value ~~of chroma~~.

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-Hue is a value to determine the dominant wavelength of the color in a product. The hue of nanocalcium powder was red (R), with a range of 18°–—54°. The longer the extraction, the more the hue of nanocalcium powder ~~was increasingly toward~~ turned ~~yellow red or~~ yellowish red. This was ~~due to~~because the longer the extraction, the more calcium was extracted from the eggshell. The constituent elements of calcium ~~with~~from the flame color test are brick red (**Permata et al., 2018**). Therefore, the more calcium in eggshell powder, the more the hue of the nanocalcium powder ~~had a color that increasingly leads to yellow red or~~turned yellowish red.

Chemical ~~c~~Characteristics of ~~n~~Nanocalcium ~~p~~Powder

Moisture content

The m~~—M~~oisture content of nanocalcium powder ranged from 0.16% to 0.33% for all samples. These values were very small due to drying in the preparation ~~method~~ of nanocalcium powder. The m~~M~~oisture content of nanocalcium of all samples is shown in Table 1. ~~Moisture content~~That of nanocalcium powder of chicken eggshells was significantly higher than that of duck eggshells (p < 0.05). This must be ~~due to~~because the initial moisture content of duck eggshells (1.43±0.04%) was significantly lower than that of chicken eggshells (1.99±0.01%), ~~resulting in a~~ decreasinge ~~in a~~the water content ~~during~~in the calcination~~s process~~ stage ~~during~~of the nanocalcium preparation.

The ~~E~~extraction time ~~gave~~ significantly affected~~effect on~~ the moisture content of nanocalcium powder (p < 0.05). The longer the extraction time, the lower the moisture content of nanocalcium powder. This was due to the evaporation of water in the filtrate so that the water content of the nanoparticle powder ~~came out to~~ beca~~o~~me free water. The decrease in water content was also due to the protein content in eggshell powder being hydrolyzed when it is mixed with chloric acid and the heating process resulting in decreased~~ing of~~ water binding. This observation was in agreement with the result found by **Trilaksani et al. (2006)** and **Agustini et al. (2011)**, who stated that the low water content in tuna bone and clam shell powder was due to protein hydrolysis during heating in powder preparation.

Ash content

The a~~—A~~sh content of nanocalcium powder ranged from 96.80% to 98.69% for all samples. Table 1 shows the ~~A~~ash content ~~of nanocalcium~~ of all samples' nanocalcium ~~is shown in **Table 1**~~. The ~~E~~extraction time ~~gave~~ significantly affected~~effect on~~ the ash content of nanocalcium powder (p < 0.05). The ~~A~~ash content ~~was~~ increased as the extraction time increased. This may because ~~due to~~ the longer the extraction time, ~~that could be~~the longer the contact of nanoparticle powder and solvent (chloric acid). This, in turn, increased the chance of hydrolysis reaction ~~occurred~~. This result was supported by the findings by **Widyastuti et al. (2015)**, who concluded that the ash content of chicken eggshell nanocalcium with a 1N NaOH solvent and 1-, 2-, and 3-hour extraction gave the highest value of ash content in the 3-hour extraction time~~;~~: 98.07%, 98.01%, and 98.03% for 1-, 2-, and 3-hour extraction, respectively.

Calcium content

The ~~C~~calcium content of microcalcium and nanocalcium of all samples is shown in Table 2. Microcalcium's ~~C~~calcium content ~~of microcalcium~~ (149 µm) of chicken eggshell was higher than that of duck eggshell. This was ~~due to~~because $CaCO_3$ of duck eggshell (96.48%) was higher than that of duck eggshell (90,90%), resulting in the higher amount of extracted calcium. Moreover, the calcium content of nanocalcium was higher than that of microcalcium both from chicken and duck eggshells. This was due to demineralization, precipitation, and calcination processes ~~happened~~ during nanocalcium preparation, ~~resulting in~~ maximally opening ~~of~~ eggshell spores and ~~in turn~~ extracting more calcium extracted. The steps of the reactions are as follows~~ed~~:

Demineralization:

$$CaCO_{3(s)} + 2HCl_{(aq)} \longrightarrow CaCl_{2\,(aq)} + H_2O_{(l)} + CO_{2(g)} \quad (1)$$

Precipitation:

$$CaCl_{2(aq)} + 2NaOH_{(aq)} \longrightarrow Ca(OH)_{2(s)} + 2NaCl_{(aq)} \quad (2)$$

Calcination:

$$Ca(OH)_{2(s)} + \text{Heat} \longrightarrow CaO_{(s)} + H_2O_{(l)} \quad (3)$$

The observation ~~was~~ supported by previous research (**Navarro et. al., 2009; Mohamed et. al., 2012; Mosaddegh et al., 2014; Zuhra et al., 2015**) concluded that $CaCO_3$ can be converted to CaO through thermal decomposition ~~method~~ (calcination).

Moreover, Table 2 shows~~ed~~ that the calcium content of nanocalcium from chicken and duck eggshells decreased with increasing extraction time. This may be due to $CaCO_3$ reformation, resulting from the reversible reaction of the calcium carbonate cycle. The steps are as follows~~ed~~:

$$CO_{2(g)} + H_2O_{(l)} \longrightarrow H_2CO_3 \quad (4)$$

$$CaCO_{3(s)} + H_2CO_{3(aq)} \longrightarrow Ca(HCO_3)_{2(aq)} \quad (5)$$

$$Ca(HCO_3)_2 \longrightarrow CaCO_{3(s)} + CO_{2\,(g)} + H_2O_{(l)} \quad (6)$$

X-Ray Diffraction ~~(XRD)~~

The XRD results ~~of XRD~~ are used to determine the crystalline structure of nanocalcium powder. The XRD is monitored at 2Θ = 5°–90°. The results of the XRD analysis of nanocalcium from chicken eggshells with extraction times of 1, 1.5, and 2 hours are presented in Figure 1. ~~Moreover,~~ Figure 2 shows~~ed~~ the result of the XRD analysis of nanocalcium from duck eggshells with extraction times of 1, 1.5, and 2 hours.

The main peak appeared at 2Θ = 37.44° of nanocalcium from chicken eggshells~~,~~ and 2Θ = 37.36° of nanocalcium from duck eggshells. Peaks of nanocalcium oxide (CaO) from chicken eggshells appeared at 2Θ = 32.28°, 37.44°,

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53.94°, 64.21°, and 67.44°. Moreover, peaks of nanocalcium oxide (CaO) from duck eggshells appeared at 2Θ = 32.15°, 37.36°, 53.85°, 64.10°, and 67.32°. The peaks that appeared were all identified and corresponded to the data-base International Center for Diffraction Data (ICDD) of calcium oxide (CaO) (PDF No. 99-0070). This result was supported by **Taufiq_Yap et al. (2011)**, and **Habte et al. (2019)** stated that the peaks ~~appeared~~ at 2Θ = 32.22°, 37.36°, 53.8°, 64.1°, and 67.3° were assigned to planes of pure CaO phase ~~respectively~~. Nanocalcium oxide powder of chicken and duck eggshells had a polycrystalline structure **(Habte et al., 2019; Khan et al., 2018)**.

The XRD patterns displayed diffractograms of CaO, $Ca(OH)_2$, and $CaCO_3$. The XRD patterns of nanocalcium from chicken eggshells have peaks ~~formed~~ of CaO (100%) ~~of~~at 1-hour extraction time; $Ca(OH)_2$ (77.4%), $CaCO_3$ (11.0%), and CaO (11.6%) ~~of~~at 1.5-hours extraction time; and $Ca(OH)_2$ (52.2%), $CaCO_3$ (9.0%), and CaO (38.8%) ~~of~~at 2-hours extraction time. Moreover, the XRD patterns of nanocalcium from duck eggshells have peaks ~~formed~~of CaO (100%) at~~of~~ 1-hour extraction time; $Ca(OH)_2$ (48.1%), $CaCO_3$ (6.9%), CaO (45.1%) ~~of~~at 1.5-hours extraction time; and $Ca(OH)_2$ (87.3%), $CaCO_3$ (11.2%), and CaO (1.5%) ~~of~~at 2-hours extraction time.

The nanocalcium oxide (CaO) content from chicken and duck eggshells ~~of~~at 1 hour of extraction ~~time~~ was formed 100%. This indicated that transformation of a chemical composition from $CaCO_3$ to CaO was completely achieved after the whole process ~~take~~took place. This observation was supported by **Dasgupta et al. (2004)**-, who stated that $CaCO_3$ turned to CaO at ~~temperature~~ 540°C. The appearance of peak $Ca(OH)_2$ of nanocalcium from chicken and duck eggshells at extraction time 1.5 and 2 hours due to the hydration reaction between hygroscopic CaO and water vapor. In the same condition, the appearance of peak $CaCO_3$ must be due to the incomplete decomposition of $CaCO_3$ to CaO.

Based on the XRD analysis, the crystallite size and density of nanocalcium oxide from chicken and duck eggshells were 41.54 nm and 3.36 g/cm^3~~,~~ and 24.90 nm and 3.34 g/cm^3, respectively. Previous studies showed that the crystallite sizes of nanocalcium from chicken eggshells were~~as~~ 10.46 nm (**Sunardi, Krismawati, and Mahayana, 2020**), and~~,~~ 50-198 nm **(Habte et al., 2019)**, and the crystallite size of nanocalcium from duck eggshells was 262 nm **(Prayitno, Prasetyo, and Sutirtoadi, 2019)**. These values were different from the values of nanocalcium crystallite sizes from this study. This was due to differences in raw material since the substances contained in eggshells depend on the breed, feed, and environment of the chicken.

The density values of nanocalcium oxide from ~~eggshell~~ chicken and duck eggshells were 3.361 g/cm^3 and 3.342 g/cm^3, respectively. These values were lower than density values of the chicken eggshells (2.16 g/cm^3) and duck eggshells (2.84 g/cm^3) calcinated at 900°C for 1 hour (**Tangboriboon et al., 2012).** The differences are caused by differences in temperature and ~~time of~~ calcinations times;~~process,~~ the species and the feeding of the chickens and ducks ~~that~~also influenced the ~~position of~~ nutrients content during the eggs formation.

CONCLUSION

The XRD showed that CaO was formed from nanocalcium powder of chicken and duck eggshells ~~with~~at 1 hours of extraction ~~time~~. The size of nanocalcium ox~~y~~ide crystals from chicken and duck eggshells were 41.54 nm and 24.90 nm.

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Acknowledgments:

The authors would like to thank Sriwijaya University for the funding provided for the implementation of this research.

Conflict of interest:

The authors declare no conflict of interest.

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Table 1 Physicochemical characteristics of nanocalcium powder from chicken and duck eggshells with different extraction times.

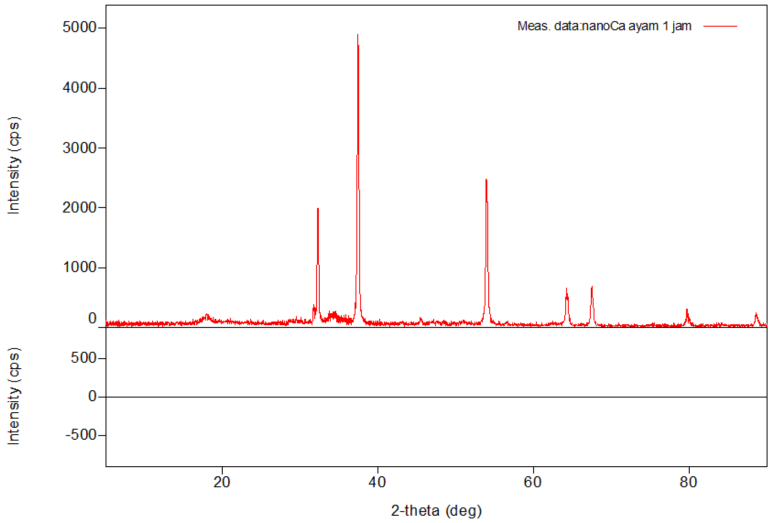
Treatment	Yield (%)	Colour			Moisture content (%)	Ash content (%)
		Lightness (%)	Chroma (%)	Hue (°)		
A_1B_1	$11.62±0.28^a$	$93.67±3.71^a$	$7.07±0.06^a$	$26.30±0.20^a$	$0.33±0.01^a$	$96.80±0.44^a$
A_1B_2	$13.30±0.27^b$	$93.73±0.12^a$	$6.80±0.10^a$	$28.30±0.30^a$	$0.25±0.01^a$	$98.32±0.05^a$
A_1B_3	$13.59±0.20^b$	$93.67±0.35^a$	$6.83±0.12^a$	$28.00±0.17^a$	$0.26±0.01^a$	$98.28±0.05^a$
A_2B_1	$13.83±0.03^b$	$93.53±0.15^a$	$7.03±0.06^a$	$26.93±0.35^a$	$0.18±0.03^b$	$97.91±0.64^a$
A_2B_2	$15.27±0.32^c$	$93.37±1.01^a$	$6.73±0.12^a$	$28.87±0.46^a$	$0.18±0.03^b$	$98.69±0.10^a$
A_2B_3	$15.20±0.20^c$	$93.87±0.21^a$	$6.77±0.06^a$	$28.27±0.23^a$	$0.17±0.03^b$	$98.67±0.10^a$

Note: Data are presented as mean ± standard deviation of triplicate determinations.
Means for each characteristic followed by the same superscript within the same row are not significantly different at $p < 0.05$ by the LSD test.
A_1B_1 = chicken eggshell; 1-hour extraction time
A_1B_2 = chicken eggshell; 1.5-hour extraction time
A_1B_3 = chicken eggshell; 2-hour~~s~~ extraction time
A_2B_1 = duck eggshell; 1-hour extraction time
A_2B_2 = duck eggshell; 1.5-hour extraction time
$A2B_3$ = duck eggshell; 2-hour extraction time

Table 2 Calcium content of microcalcium and nanocalcium powders from chicken and duck eggshells.

Treatment	Calcium content (%)
Microcalc~~s~~ium powder (149 µm)	
Chicken	11.41±0.03
Duck	12.32±0.14
Nanocalcium powder	
A_1B_1	40.80±0.06
A_1B_2	24.78±0.04
A_1B_3	24.52±0.05
A_2B_1	49.39±0.08
A_2B_2	27.38±0.05
A_2B_3	25.42±0.04

Note: A_1B_1 = chicken eggshell; 1-hour extraction time
A_1B_2 = chicken eggshell; 1.5-hour extraction time
A_1B_3 = chicken eggshell; 2-hour~~s~~ extraction time
A_2B_1 = duck eggshell; 1-hour extraction time
A_2B_2 = duck eggshell; 1.5-hour extraction time
A_2B_3 = duck eggshell; 2-hour~~s~~ extraction time



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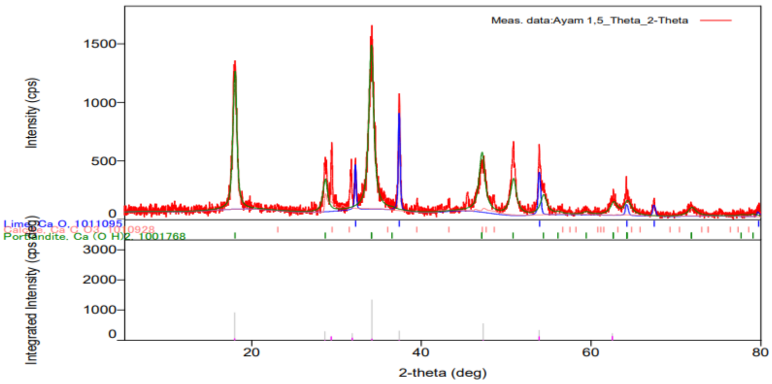

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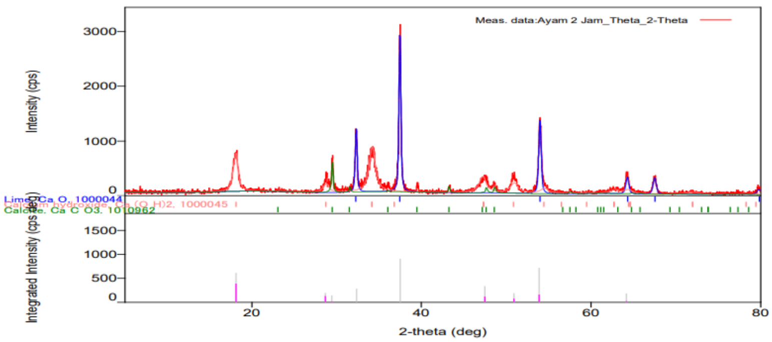

(c)

Figure 1 XRD of nanocalcium chicken eggshells from (a) 1-hour extraction, (b) 1.5-hour extraction, and (c) 2-hour~~s~~ extraction.~~ time~~

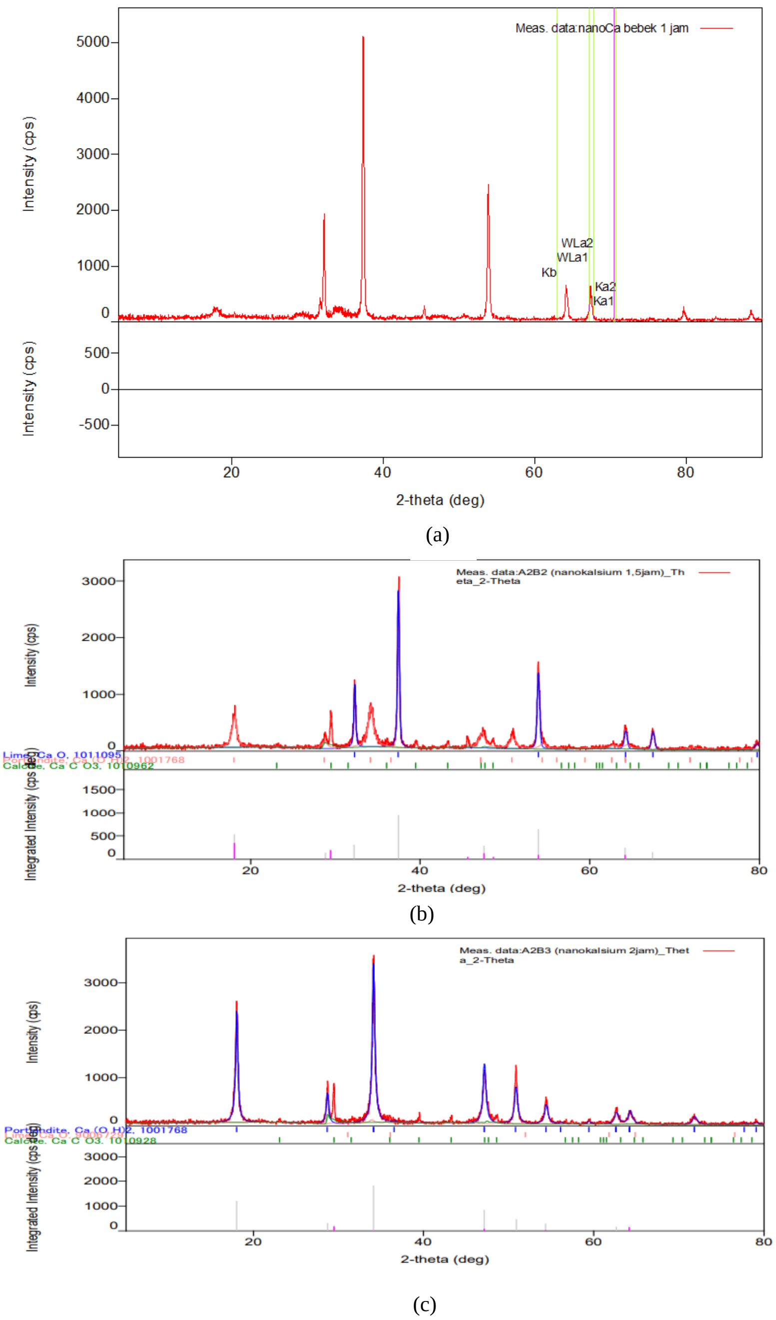

Figure 2 XRD of nanocalcium duck eggshells from (a) 1-hour extraction, (b) 1.5-hour extraction, and (c) 2-hour~~s~~ extraction ~~time~~.

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Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. XX, XXXX, p. XX-XX
https://doi.org/10.5219/XXX
Received: XXXXXXXXXXXX. Accepted: XXXXXXXXXXXX.
Available online: XXXXXXXXXXXX at www.potravinarstvo.com

ISSN 1337-0960 (online)

EFFECT OF EXTRACTION TIME ON PHYSICOCHEMICAL CHARACTERISTICS OF NANOCALSIUM POWDER FROM CHICKEN AND DUCK EGGSHELLS

Nura Malahayati, Tri Wardhani Widowati, Nurul Saniah Alsoyuna

ABSTRACT
Deficiency of calcium is associated with risk of bone fracture and osteoporosis. This malnutrition has been a focus of the governments and WHO for decades and extensive efforts are made to address it. There are several solutions to increase calcium intake, one of these is taking calcium in the form of nanocalcium. The objective of the research was to determine effect of extraction time on the physicochemical characteristics of nanocalcium powder from chicken and duck eggshells conducted by precipitation method. This research was conducted by using Factorial Completely Randomized Design with two factors and each treatment analysis was repeated three times. The first factor was type of eggshell (chicken and duck), and the second factor was extraction time (1,1.5 and 2 hours). Observed parameters were physical characteristics (yield and color) and chemical characteristics (moisture, ash, calcium content, and crystalline structure). The result of the research showed that type of eggshell had significant effect ($p<0.05$) on yield, color (lightness), and moisture content. The treatment of extraction time had significant effect ($p<0.05$) on yield, color (chroma and hue), moisture, and ash content. Based on the highest content of crystalline structure of nanocalcium formation (100%) was nanocalcium powder from chicken and duck eggshells with 1 hours of extraction time. The particle size of crystalline structure of nanocalcium from chicken and duck eggshells were 41.54 nm and 24.90 nm.

Keywords: nanocalcium; chicken; duck; eggshells; extraction

INTRODUCTION
Calcium is the main mineral contained in the human body as much as 1.5% to 2% of adult body weight or about 1 kg **(Ariyanti, 2012)**. Calcium plays an important role in stiffness, bone strength and most metabolic processes, including blood clots, muscle contractions, hormonal systems, glycogen metabolism, cell proliferation and differentiation **(WHO, 2006)**. Calcium consumption in the human body varies according to age and sex.

Dietary intakes of calcium by Indonesian people, 254 mg/day, are far below that needed to build proper bone mass. It is widely accepted in the literature that a low calcium intake over years contributes to the development of bone fracture and osteoporosis. The problems in getting enough calcium of the people in Indonesia are poor (low and inadequate) dietary habits, not many foods are naturally a good source of calcium (milk and dairy products), and another calcium sources (non-dairy foods) have to be consumed in large amounts to meet Recommended Dietary Intake (RDI) for calcium and low bioavailability (cereals, nuts, and green leafy vegetables). Another problem is consumer behavior such as low purchasing power, boring, preference, and special conditions (the teen years, the young adult years, the childbearing years, pregnant, breast-feeding, the later adult years, lactose intolerance, and vegetarian).

There are several solutions to increase calcium intake, one of these is calcium-fortified foods as a benefit product. However, calcium in food products is more available in the form of microcalcium so that absorption of calcium in the body is not optimal, only about 50% **(Lekahena et al., 2014)**.

Current technological advances in the form of nanotechnology are able to overcome the problem of eggshell waste by extracting calcium into nanocalcium powder with particle sizes of 10 nm to 100 nm in diameter. This is an effort to increase the economic value of abundant eggshell waste in line with the concept of zero waste products and reduce calcium deficiency because it is not optimal absorption of calcium in the body so that calcium in the eggshell must be converted into absorbable forms **(Suptijah, Jacoeb and Deviyanti, 2012)**.

The use of nanotechnology in the fractionation process by precipitation method is able to convert calcium carbonate to calcium oxide so that it produces nanocalcium powder with good physical and chemical characteristics. This method controls the solubility of the material in the solution through changes in pH and temperature by adding a number of certain chemicals to convert soluble compounds to insoluble solids or mixing acidic base which produces solids and water **(Purwasasmita and Gultom, 2008)**. This method is very effective because it can produce nanoparticles with a simple and low-cost process **(Gulsun, Gursoy and Oner, 2009)**.

Eggs is the main protein source in Indonesian food consumption that is popular with the community because it has a high nutrient contents, the price is relatively cheap compared to other animal protein sources, has a delicious taste and is easily digested in the body. In Indonesia, the production of chicken egg in 2018 was 1,644,460 tons with an average consumption per capita as much as 302.71 grams per day **(Central Statistics Agency, 2016)**.

Increasing the value of egg production is in line with the increasing need for eggs so that the potential of eggshell waste in Indonesia is quite large but has not been used optimally. People generally dispose of the eggshell waste without using it first, even though 96% of the calcium content (94% calcium carbonate, 1% magnesium carbonate, 1% calcium phosphate) in the eggshell has the potential as an additional material extracted for food minerals. The main composition of calcium carbonate in eggshells can cause environmental pollution because it is difficult to be degraded by soil microbes **(Trilaksani, Salamah and Nabil, 2006)**.

It was approved by several studies that nanosizing increased the bioavailability of calcium **(Park et al., 2008; Seo et al., 2009; Hilty et al., 2011).** Nanocalcium powder can be used in various products and food fortification as a form of functional food that is beneficial for health. Therefore, research on the the effect of eggshell type (chicken and duck) and extraction time on the physicochemical characteristics of the nanocalcium powder produced.

Scientific Hypothesis

It is suspected that the effect of different types of eggshells and extraction time had a significant effect on physicochemical charactericties of egshells nanocalcium powder.

MATERIAL AND METHODOLOGY

Samples

Chicken and duck eggshells were obtained from cake home industry in Palembang City, South Sumatera Province. Indonesia.

Chemicals

The main chemicals used in this study were lanthanum, chloric acid, potassium hydroxide, demineralized water.

Instruments

Instruments used in this study were *Atomic Absorption Spectrophotometry* (AAS) (Shimadzu AA-7000, Jepan), *Cromameter (CR*-410 Konika Minolta, Jepang), *X-Ray Diffraction* (XRD) (Rigaku *The Benchtop* X-RD *Miniflex* 60, Jepan).

Laboratory Method

Eggshell powder preparation (Rahmawati and Nisa, 2015)

Chicken and duck eggshells (500 g) were washed with water until clean. Cleaned eggshells were boiled at 100ºC for 10 minutes to kill pathogenic microbes, then the eggshells were drained. The eggshells were dried in an oven for 2 hours at 60ºC, then the eggshells were placed at room temperature. Eggshells were ground to powder, then were sieved with a 100 mesh sieve. Eggshell powder was included in the OPP (Oriented Polystyrene) plastic and stored at 4ºC for analysis.

Eggshell nanocalcium powder preparation (Khoerunnisa, 2011)

Eggshell powder was immersed in 1N HCl solvent (1: 5) for 48 hours, then, extracted with a temperature of 90ºC for 1, 1.5 and 2 hours. The extracts were then filtered with filter paper to obtain filtrate and sediment. The filtrate was precipitated by adding 3N NaOH and stirred, then leaved it until the precipitate was formed. The precipitate was then neutralized by using aquabidest to pH neutral. The solution was separated from the sediment by pouring it slowly so that the precipitate was not wasted. The sediment was dried in an oven for 3 hours at a temperature of 105ºC, then it was ashed in Muffle furnace at 600ºC for 5 hours, then were refined with mortar. Nanocalcium powder were packed in airtight plastic bags and stored at 4°C until used.

Yield determination

The yield of the nanocalcium was calculated by following formula:

$$\text{Yield (\%)} = \frac{weight\ of\ nanocalcium\ powder}{weight\ of\ eggshell\ powder} \text{ x } 100\%$$

Color determination of nanocalcium

The color of nanocalcium powder was measured using Munsell (1977). The values were L (Lighteness), C (Chroma) and H (Hue) scales.

Moisture content (AOAC, 2005) of nanocalcium

Approximately 1 g sample (W_1) was placed on a dish and dried at 105°C for 18 hours. After drying, a dish containing sample was transferred to desicator for 15 minutes. Then, dish and sample was reweighted until its weight is constant (W_2). The moisture content of the nanocalcium was calculated by following formula:

$$\text{Moisture content (\%)} = \frac{(W1-W2)}{W2} \text{ x } 100\%$$

W_1 = weight (g) of sample before drying
W_2 = weight (g) of sample after drying

Moisture content (AOAC, 2005) of nanocalcium

Approximately 1 g sample (W_1) was placed on a porcelain crucible. Then, sample placed in muffle furnace at temperature 550°C until the samples turned whitish grey. The ash content of the nanocalcium was calculated by following formula:

$$\text{Ash content (\%)} = \frac{\text{weight of ash}}{\text{weight of the sample}} \times 100\%$$

Calcium determination (AOAC, 2005) of nanocalcium

Calcium content of samples was determined using an atomic absorption spectrophotometer (AAS) (Shimadzu AA-7000, Jepan) according to AOAC method (AOAC, 2005). The ground sample (5 g) was placed in an ashing vessel, charred in a muffle furnace and then ashed at 500°C overnight. The completely ashed sample was dissolved in 10 mL of concentrated hydrochloric acid. The solution was boiled and evaporated nearly to dryness. The residue was redissolved in 20 mL of 2N hydrochloric acid and boiled gently. The solution was cooled and diluted to 100 mL with distilled deionized water. Its absorbance was then measured using the AAS at 422.7 nm. Calibration of the measurements was performed using commercial standard solution (Merck KGaA 64271 Darmstadt, Germany). To eleminate phosphorous interference in the determination, lanthanum was added to the test ash solution and standard solution so that the final solution conteined 1% lanthanum.

Crystalline structure of nanocalcium powder

X-Ray Diffraction (XRD) is used to mesure the crystal structure of samples.

Statistical Analysis

The research was conducted by using Factorial Completely Randomized Design with two factors and each treatment analysis was repeated three times. The first factor (A) was type of eggshell (A_1 = chicken and A_2 = duck), and the second factor (B) was extraction time (B_1 = 1hour, B_2 = 1.5 hour, and B3 = 2 hours). All analyses were performed in triplicates. Data were subjected to analysis of variance (ANOVA) followed by Fisher's least significant difference test (LSD) to compare treatment means; differences were considered at significant level of 95% ($p<0.05$) by using SPSS v.19 software.

RESULTS AND DISCUSSION

Physical characteristics of nanocalcium powder

Yield

The yield of nanocalcium powder ranged from 11.62% to 15.27% for all samples. The lowest average yield was found in A_1B_1 treatment (chicken egg shells, 1 hour extraction time), while the highest yield value was found in A_2B_2 treatment (duck eggshell, extraction time of 1.5 hours). There was a significant effect of type of eggshell and extraction time on the yield of nanocalcium powder ($p<0.05$). The value of the average yield of nanocalcium powder was shown in **Table 1.**

The yield of duck nanocalcium powder was significantly higher ($p<0.05$) than that of chicken nanocalcium powder due to the main components forming the eggshell, calcium carbonate ($CaCO_3$). Calcium carbonate is a major component in an eggshell **(Swetha et al., 2018).** Duck eggshell has a higher $CaCO_3$ content (96.48%) **(Sari, 2013)** than that of chicken eggshell (90.90%) (**Warsy et al., 2016)**. However, the result of research conducted by **Ajayan et. al. (2020)** showed that average percentage of calcium carbonate in eight varieties of chickens (89.05%) was higher than that in six varieties of duck (84.63). **Adeyeye (2009)** in a comparative study stated that hen eggshell contains more calcium than that of duck eggshells. This is because difference in calcium carbonate content in shell is due to difference in chicken or duck varieties. Another reason, calcium carbonate content is depends on ration of dietary calcium of poultry feed **(Lestari et al., 2015).**

During extraction process, the higher $CaCO_3$ content and the longer the extraction time caused a higher penetration of solvent into the eggshell powder resulting in more compounds to diffuse out of the shell and gave a higher yield. However, the yield of 2 hours extraction time was not significantly different from that of 1.5 hours. This is because the calcium carbonate cycle has a reversible reaction so that it can allow the return of products to reactants, where carbonic acid can react again with calcium carbonate to form calcium bicarbonate **(Dwi et al., 2015)**. **Risnojatiningsih (2012)** stated that the formation of $Ca(HCO_3)_2$ occurs when the formed $CaCO_3$ continues to react with water containing CO_2 gas. The result was also in agreement with the findings of the research conducted by **Khoerunnisa (2011)** showed that the yield of local mussel shell nanocalcium with 1N HCl extraction was significantly increased from 1 hour extraction (5.02%) to 1.5 hour (8.53%), and non significantly decreased from 1.5 hour (8.53%) to 2 hours extraction (7.89%).

Color

The color of nanocalcium powder from chicken and duck eggshell were white. This indicated that calcium oxide (CaO) has formed. This was in accordance with the statement of **Sing et al. (2011)** and **Tangboriboon et al. (2012)** that changing in color of the eggshells to white during calcinations indicated that a complete chemical transformation from calcium carbonate to calcium oxide has been achieved.

Lightness value of nanocalcium powder ranged from 92.00% to 94.03%. The lowest average lightness value was found in A_1B_1 (chicken eggshell, extraction time of 1 hour), while the highest lightness value was found in treatment A_2B_2 (duck eggshell, extraction time of 1.5 hours). Chroma value of nanocalcium powder ranged from 6.73% to 7.07%. The lowest average chroma value was found in A_2B_2 (duck eggshell, extraction time of 1.5 hours), while the highest chroma value was found in A_1B_1 (chicken eggshell, 1 hour extraction time). Hue value of nanocalcium powder ranged from 26.30^o to 28.87^o. The lowest hue value was found in A_1B_1 treatment (race chicken eggshell, extraction time of 1 hour), while the highest hue value was found in treatment A_2B_2 (duck eggshell, extraction time of 1.5 hours). The average lightness, chroma and hue value of nanocalcium powder were shown in **Table 1.**

The value of lightness is the degree of brightness of a product. Chicken and duck eggshell produced high lightness values of nanocalcium powder. This was due to preparation process of nanocalcium powder by using HCl caused the pigment deposition process in each eggshell so that it was easily degraded the color becomes brighter. Duck eggshells contain biliverdin pigments so that the shell has a greenish blue color, while chicken eggshells contain porphyrin pigments so that brownish-colored shells **(Mushawwir and Latipudin, 2013; Yonata et al., 2017)**. The immersion process with HCl and extraction can cause the greenish color of the shell of the duck egg to be more easily degraded resulting in a whiter color while the brownish color of the chicken eggshell produces a darker color than the powder of duck egg nanocalcium. The difference in the components of the constituent minerals in the eggshell also affects the lightness value of the nanocalcium powder. The main component of the constituent minerals of nanocalcium powder is calcium which generally has a white color, therefore the lightness value of nanocalcium powder was also high.

Chroma is a parameter used to determine the color intensity of the product. The chroma value is inversely proportional to the lightness value because if a product has dark color, the intensity of the resulting color will be stronger, if the lightness value is high then the chroma value produced from a product will be lower. The longer the extraction, the more $CaCO_3$ hydrolyzed by HCl produced a whiter color resulting in decreasing of the value of chroma.

Hue is a value to determine the dominant wavelength of the color in a product. The hue of nanocalcium powder was red (R) with a range of 18° - 54°. The longer the extraction, the hue of nanocalcium powder was increasingly toward yellow red or yellowish red. This was due to the longer the extraction the more calcium was extracted from the eggshell. The constituent elements of calcium with the flame color test are brick red **(Permata et al, 2018)**. Therefore, the more calcium in eggshell powder, the hue of the nanocalcium powder had a color that increasingly leads to yellow red or yellowish red.

Chemical characteristics of nanocalcium powder

Moisture content

Moisture content of nanocalcium powder ranged from 0.16% to 0.33 for all samples. These values were very small due to drying in preparion method of nanocalcium powder. Moisture content of nanocalcium of all samples is shown in **Table 1**. Moisture content of nanocalcium powder of chicken eggshells was significantly higher than that of duck eggshells ($p<0.05$). This must be due to initial moisture content of duck eggshell (1.43±0.04%) was significantly lower than that of chicken eggshell (1.99±0.01%) resulting in a decrease in a water content during calcinations process during nanocalcium preparation.

Extraction time gave significant effect on moisture content of nanocalcium powder ($p<0.05$). The longer the extraction time the lower the moisture content of nanocalcium powder. This was due to the evaporation of water in the filtrate so that the water content of the nanoparticle powder came out to become free water. The decrease in water content was also due to the protein content in eggshell powder being hydrolyzed when it is mixed with chloric acid and the heating process resulting in decreasing of water binding. This observation was in agreement with the result found by **Trilaksani et al. (2006)** and **Agustini et al. (2011)**, who stated that the low water content in tuna bone and clam shell powder due to protein hydrolysis during heating in powder preparation.

Ash content

Ash content of nanocalcium powder ranged from 96.80% to 98.69% for all samples. Ash content of nanocalcium of all samples is shown in **Table 1**. Extraction time gave significant effect on ash content of nanocalcium powder ($p<0.05$). Ash content was increased as extraction time increased. This may be due to the longer the extraction time that could be longer the contact of nanoparticle powder and solvent (chloric acid) in turn increased the chance of hydrolysis reaction occurred. This result was supported by the findings by **Widyastuti et al. (2015),** who concluded that the ash content of chicken eggshell nanocalcium with 1N NaOH solvent and 1,2, and 3 hour extraction gave the highest value of ash content in the 3 hour extraction time; 98.07%, 98.01%, and 98.03% for 1,2, and 3 hour extraction.

Calcium content

Calcium content of microcalcium and nanocalcium of all samples is shown in **Table 2**. Calcium content of microcalcium (149 µm) of chicken eggshell was higher than that of duck eggshell. This was due to $CaCO_3$ of duck eggshell (96.48%) was higher than that of duck eggshell (90,90%) resulting in the higher amount of extracted calcium. Moreover, the calcium content of nanocalcium was higher than that of microcalcium both from chicken and duck eggshells. This was due to demineralization, precipitation, and calcination processes happened during nanocalcium preparation resulting in maximally opening of eggshell spores in turn more calcium extracted. The steps of reactions as followed:

Demineralization:

$$CaCO_{3(s)} + 2HCl_{(aq)} \longrightarrow CaCl_{2\,(aq)} + H_2O_{(l)} + CO_{2(g)} \quad (1)$$

Precipitation:

$$CaCl_{2(aq)} + 2NaOH_{(aq)} \longrightarrow Ca(OH)_{2(s)} + 2NaCl_{(aq)} \quad (2)$$

Calcination:

$$Ca(OH)_{2(s)} + \text{Heat} \longrightarrow CaO_{(s)} + H_2O_{(l)} \quad (3)$$

The observation was supported by previous research **(Navarro et. al., 2009; Mohamed et. al., 2012; Mosaddegh et al., 2014; Zuhra et al., 2015)** concluded that $CaCO_3$ can converted to CaO through thermal decomposition method (calcination).

Moreover, Table 2 showed that calcium content of nanocalcium from chicken and duck eggshells decreased with increasing extraction time. This may be due to $CaCO_3$ reformation resulting from reversible reaction of calcium carbonat cycle. The steps as followed:

$$CO_{2(g)} + H_2O_{(l)} \longrightarrow H_2CO_3 \quad (4)$$

$$CaCO_{3(s)} + H_2CO_{3(aq)} \longrightarrow Ca(HCO_3)_{2(aq)} \quad (5)$$

$$Ca(HCO_3)_2 \longrightarrow CaCO_{3(s)} + CO_{2\,(g)} + H_2O_{(l)} \quad (6)$$

X-Ray Diffraction (XRD)

The results of XRD are used to determine the crystalline structure of nanocalcium powder. The XRD is monitored at 2ϴ = 5-90°. The result of XRD analysis of nanocalcium from chicken eggshell with extraction time of 1, 1.5, and 2 hours are presented in **Figure 1**. Moreover, **Figure 2** showed the result of XRD analysis of nanocalcium from duck eggshell with extraction time of 1, 1.5, and 2 hours.

The main peak appeared at 2ϴ = 37.44° of nanocalcium from chicken eggshell, and 2ϴ = 37.36° of nanocalcium from duck eggshell. Peaks of nanocalcium oxide (CaO) from chicken eggshell appeared at 2ϴ = 32.28°, 37.44°, 53.94°, 64.21° and 67.44°. Moreover, peaks of nanocalcium oxide (CaO) from duck eggshell appeared at 2ϴ = 32.15°, 37.36°, 53.85°, 64.10° and 67.32°. The peaks that appear were all identified and corresponded to the data base International Center for Diffraction Data (ICDD) of calcium oxide (CaO) (PDF No. 99-0070). This result was supported by **Taufiq_Yap et al. (2011)** and **Habte et al. (2019)** stated that the peaks appeared at 2ϴ = 32.22°, 37.36°, 53.8°, 64.1° and 67.3° were assigned to planes of pure CaO phase respectively. Nanocalcium oxide powder of chicken and duck eggshells had a polycrystalline structure **(Habte et al., 2019; Khan et al., 2018)**.

The XRD patterns displayed diffractograms of CaO, $Ca(OH)_2$, and $CaCO_3$. XRD patterns of nanocalcium from chicken eggshell have peaks formed CaO (100%) of 1 hour extraction time; $Ca(OH)_2$ (77.4%), $CaCO_3$ (11.0%), CaO (11.6%) of 1.5 hours extraction time; and $Ca(OH)_2$ (52.2%), $CaCO_3$ (9.0%), CaO (38.8%) of 2 hours extraction time. Moreover, XRD patterns of nanocalcium from duck eggshell have peaks formed CaO (100%) of 1 hour extraction time; $Ca(OH)_2$ (48.1%), $CaCO_3$ (6.9%), CaO (45.1%) of 1.5 hours extraction time; and $Ca(OH)_2$ (87.3%), $CaCO_3$ (11.2%), CaO (1.5%) of 2 hours extraction time.

The nanocalcium oxide (CaO) content from chicken and duck eggshells of 1 hour extraction time was formed 100%. This indicated that transformation of a chemical composition from $CaCO_3$ to CaO was completely achieved after the whole process take place. This observation was supported by **Dasgupta et al. (2004)** who stated that $CaCO_3$ turned to CaO at temperature 540°C. The appearance of peak $Ca(OH)_2$ of nanocalcium from chicken and duck eggshells at extraction time 1.5 and 2 hours due to the hydration reaction between hygroscopic CaO and water vapor. In the same condition, appearance of peak $CaCO_3$ must be due to incomplete decomposition of $CaCO_3$ to CaO.

Based on XRD analysis, crystallite size and density of nanocalcium oxide from chicken and duck eggshells were 41.54 nm and 3.36 g/cm^3, and 24.90 nm and 3.34 g/cm^3, respectively. Previous studies showed that crystallite size of nanocalcium from chicken eggshell was 10.46 nm (**Sunardi, Krismawati, Mahayana, 2020),** 50-198 nm **(Habte et al., 2019),** and crystallite size of nanocalcium from duck eggshell was 262 nm **(Prayitno, Prasetyo, Sutirtoadi, 2019)**. These values were different from the values of nanocalcium crystallite size from this study. This was due to differences in raw material since the substances contained in eggshells depend on the breed, feed, and environment of the chicken.

The density values of nanocalcium oxide from eggshell chicken and duck were 3.361 g/cm^3 and 3.342 g/cm^3, respectively. These values were lower than density values of the chicken eggshells (2.16 g/cm^3) and duck eggshells (2.84 g/cm^3) calcinated at 900°C for 1 hour (**Tangboriboon et al., 2012).** The differences are caused by difference temperature and time of calcinations process, the species and the feeding of chicken and duck that influence the position of nutrients during eggs formation.

CONCLUSION

The XRD showed that CaO was formed from nanocalcium powder of chicken and duck eggshell with 1 hours extraction time. The size of nanocalcium oxyde crystal from chicken and duck eggshells were 41.54 nm and 24.90 nm.

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Acknowledgments:
The authors would like to thank Sriwijaya University for the funding provided for implementation of this research.

Conflict of interest:
The authors declare no conflict of interest.

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Table 1 Physicochemical characteristics of nanocalcium powder from chicken and duck eggshells with different extraction time

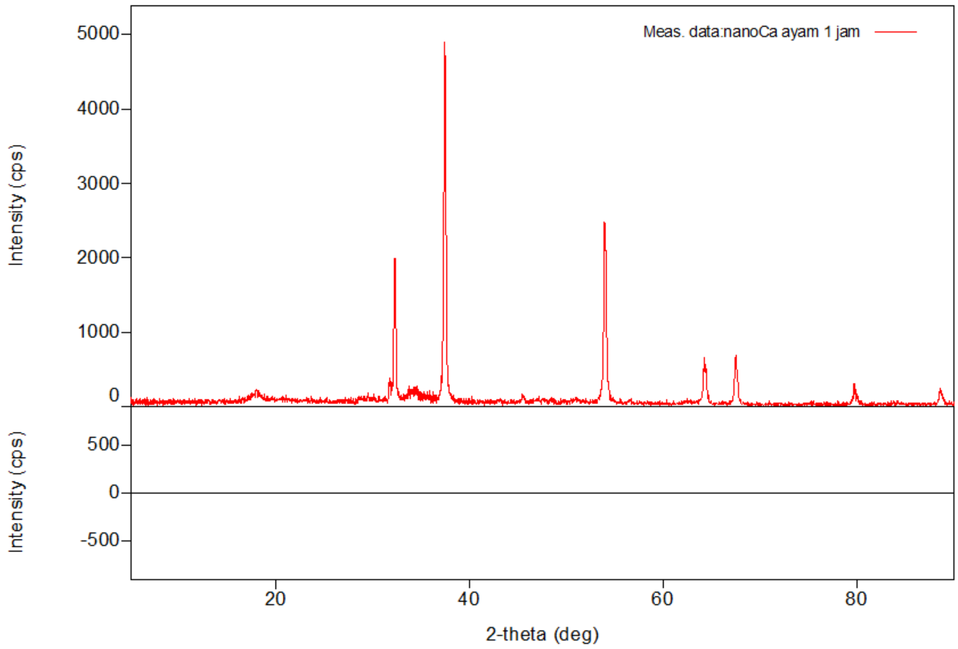
Treatment	Yield (%)	Colour			Moisture content (%)	Ash content (%)
		Lightness (%)	Chroma (%)	Hue (°)		
A_1B_1	11.62 ± 0.28^a	93.67 ± 3.71^a	7.07 ± 0.06^a	26.30 ± 0.20^a	0.33 ± 0.01^a	96.80 ± 0.44^a
A_1B_2	13.30 ± 0.27^b	93.73 ± 0.12^a	6.80 ± 0.10^a	28.30 ± 0.30^a	0.25 ± 0.01^a	98.32 ± 0.05^a
A_1B_3	13.59 ± 0.20^b	93.67 ± 0.35^a	6.83 ± 0.12^a	28.00 ± 0.17^a	0.26 ± 0.01^a	98.28 ± 0.05^a
A_2B_1	13.83 ± 0.03^b	93.53 ± 0.15^a	7.03 ± 0.06^a	26.93 ± 0.35^a	0.18 ± 0.03^b	97.91 ± 0.64^a
A_2B_2	15.27 ± 0.32^c	93.37 ± 1.01^a	6.73 ± 0.12^a	28.87 ± 0.46^a	0.18 ± 0.03^b	98.69 ± 0.10^a
A_2B_3	15.20 ± 0.20^c	93.87 ± 0.21^a	6.77 ± 0.06^a	28.27 ± 0.23^a	0.17 ± 0.03^b	98.67 ± 0.10^a

Note: Data are presented as mean ± standard deviation of triplicate determinations.
Means for each characteristic followed by the same superscript within the same row are not significantly different at $p<0.05$ by LSD test.
A_1B_1 = chicken eggshell; 1 hour extraction time
A_1B_2 = chicken eggshell; 1.5 hour extraction time
A_1B_3 = chicken eggshell; 2 hours extraction time
A_2B_1 = duck eggshell; 1 hour extraction time
A_2B_2 = duck eggshell; 1.5 hour extraction time
$A2B_3$ = duck eggshell; 2 hour extraction time

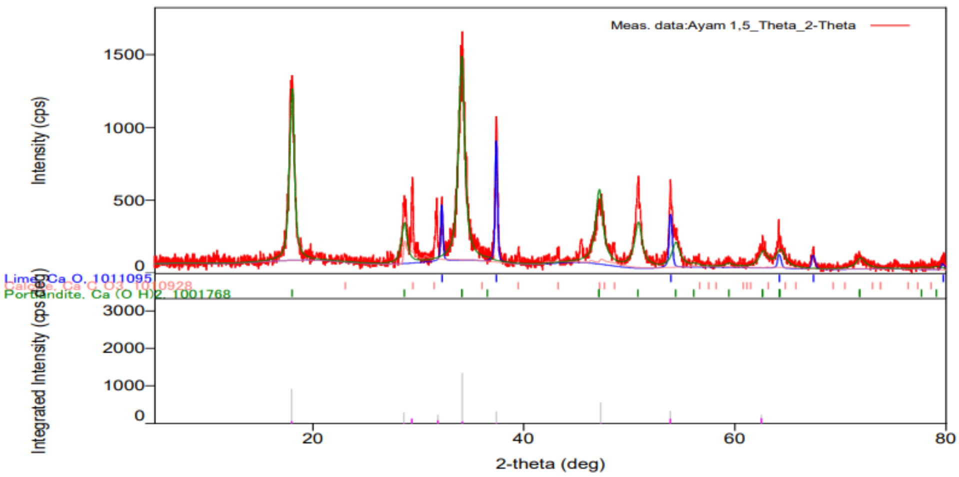
Table 2 Calcium content of microcalcium and nanocalcium powders from chicken and duck eggshells

Treatment	Calcium content (%)
Microcalsium powder (149 µm)	
Chicken	11.41±0.03
Duck	12.32±0.14
Nanocalcium powder	
A_1B_1	40.80±0.06
A_1B_2	24.78±0.04
A_1B_3	24.52±0.05
A_2B_1	49.39±0.08
A_2B_2	27.38±0.05
A_2B_3	25.42±0.04

Note: A_1B_1 = chicken eggshell; 1 hour extraction time
A_1B_2 = chicken eggshell; 1.5 hour extraction time
A_1B_3 = chicken eggshell; 2 hours extraction time
A_2B_1 = duck eggshell; 1 hour extraction time
A_2B_2 = duck eggshell; 1.5 hour extraction time
A_2B_3 = duck eggshell; 2 hours extraction time



(a)



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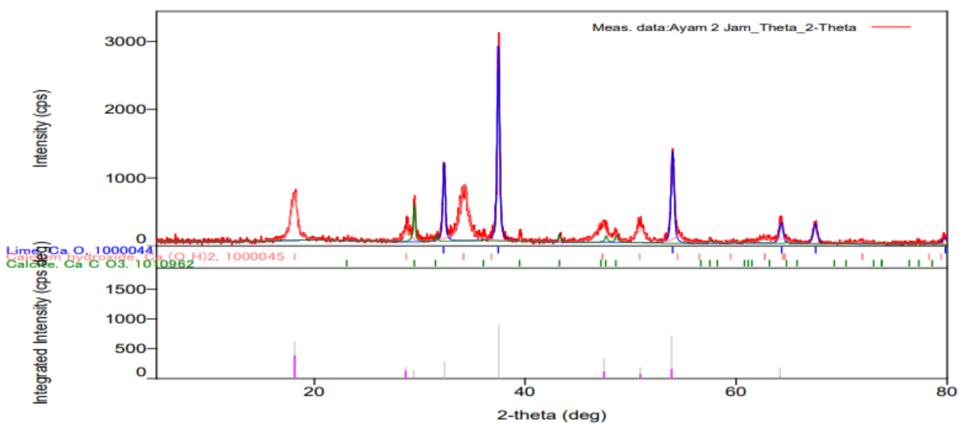
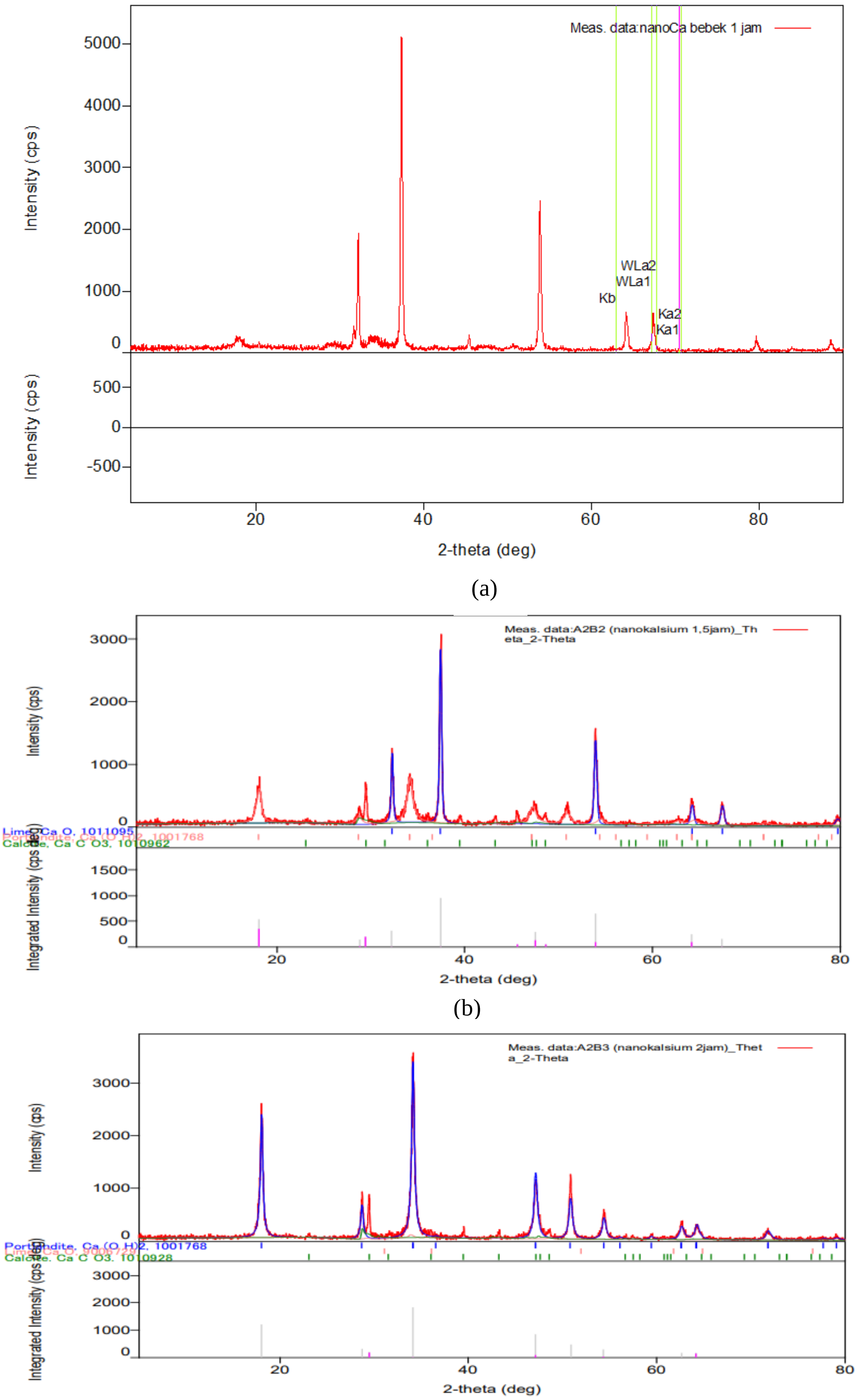


(c)

Figure 1 XRD of nanocalcium chicken eggshells from (a) 1 hour extraction, (b) 1.5 hour extraction, (c) 2 hours extraction time



(a)

(b)

(c)

Figure 2 XRD of nanocalcium duck eggshells from (a) 1 hour extraction, (b) 1.5 hour extraction, (c) 2 hours extraction time