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MINERALS COMPOSITION AND CHARACTERIZATION OF THE HATCHERY EGGSHELL WASTE TREATED WITH DIFFERENT PREPARATION METHODS

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ABSTRACT

The preparation process needs to be carried out in the treatment of hatchery waste. The objective of the study was to determine the mineral content and characterization of the eggshell waste after preparation. An experiment was arranged on a completely randomized design (CRD). Preparation treatment consisted of; T1: shell waste watered with distilled water; T2: shell waste soaked in distilled water for 12 h; T3: shell waste soaked in 0.5% NaOH for 720 min; and T4: shell waste boiled at 80°C for 15 min and soaked in 5% H_3PO_4 for 15 min. The data were analyzed using Analysis of Variance (ANOVA), and if any differences, a Duncan Multiple Range Test (DMRT) post hoc tests were carried out. Composition of proximate and mineral were detected by using X-Ray Fluorescence (XRF). Results indicated that proximate analysis of the dry matter content were T4: 98.98±0.01; T3: 98.58±0.04; T2: 98.75±0.08; and T1: 98.72±0.03. The calcium content of the treatment from the highest was T3: 24.22±0.31; T1: 22.80±0.57; T2: 22.77±0.71; and T4: 21.55±0.46. It may be inferred that the treatment technique had no major impact on the eggshells physical characteristics. However, boiling treatment at 80°C for 15 min and soaking in 5% H_3PO_4 reduced the eggshell waste's Mg, Si, and Ca content. The addition of 0.5% NaOH immersion did not degrade Mg, Ca, and Si of eggshell waste.

Keywords: Calcium; eggshell; proximate; silica; XRF

INTRODUCTION

The population of poultry in Gunungkidul Regency in 2020 has increased, especially broilers. The broiler population increased from 1,626,260 to 1,635,000 (BPS, 2020). Population increase is directly proportional to the population of day-old chicks as well as eggshell production. Eggshell was part of the waste that had not been handled properly. Mittal *et al.* (2016) explained that the largest contribution of waste is from eggshells of hatcheries.

The survey conducted in Gunungkidul Regency has two hatcheries with a production capacity of 100,000 hatching eggs/month. Hatchery waste was not only shells, infertile eggs, dead embryos, cracked eggs, failed chicks to hatch, or culling chicks (Dhaliwal *et al.,* 1997; Glatz *et al.,* 2011). Environmental pollution, odors, flies, and problems with eggshell waste disposal and zoonoses are negative impacts if the waste is not handled (Glatz *et al*., 2011; Orrico *et al*., 2020). According to Khairiyah (2016), zoonoses are diseases transmitted from animals to humans and vice versa. For this reason, before processing eggshell waste, it is necessary to sterilize it. Kismiati *et al*. (2013) explained that using 3-5% phosphoric acid (H_3PO_4) could reduce eggshell bacteria.

However, it has not been explained the effect of preparation on the mineral content of the eggshell. According to Setiyawan *et al.* (2021), hatchery eggshells contain 22.52% Ca, 0.56% Mg, and 0.19% P. The mineral content in the shell has the potential to be utilized. Minerals in the shell are usually bound to several other compounds or components (Wowor *et al*., 2015). According to Yonata *et al.*, (2017), the use of the solution can affect the mineral content in the shell. It was further explained that the use of HCl as a solution to soak the shells reduced the calcium content by 2%. Based on the description above, a review of the hatchery eggshell preparation material was carried out. This research aimed to determine the mineral content and characterization of the eggshell waste after preparation with different solvents.

MATERIALS AND METHODS

The research was conducted in the Research Division for Natural Product Technology laboratory, The National Research and Innovation Agency. Eggshell samples were obtained from the hatchery of PT Widodo Makmur Unggas in Semanu sub-district, Gunungkidul district. In addition to the eggshell material samples, chemicals that were also used for proximate analysis included aquades, 0.5% NaOH, 5% H_3PO_4, and cellulose for X-Ray Fluorescence (XRF) preparation. The equipment used was a digital scale with a capacity of 5 kg, a beaker glass, hydraulics for sample pressing (XRF preparation), and a set of proximate analyzers.

Research Methods

The study was conducted using a completely randomized design method (CRD) with preparation treatments (T). T1: shell waste was flushed with distilled water; T2: shell waste soaked in distilled water for 12 h; T3: shell waste soaked in NaOH 0.5% for 720 min; and T4: shell waste was boiled at 80°C for 15 min and soaked in 5% H_3PO_4

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for 30 min referring to the eggshell preparation method of Kismiati *et al*. (2013). After the sample treatment, milling was carried out with a size of 20 mesh before analysis. Then a proximate analysis was carried out (AOAC, 2005). XRF analysis was carried out to determine the elements contained in eggshells. Before testing the sample, preparation was carried out first. First, the milled and sifted samples were mixed with cellulose in a ratio of 7:1. Then, it was mixed until homogeneous and pressed using hydraulics. After that, readings were carried out using an XRF device with the brand Epsilon 4 made by Malvern Panalytical.

Fourier transform infrared (FTIR) analysis was carried out to determine the functional group of eggshells. IR spectra of treatments were then recorded by FTIR model Bruker Vertex 80 in the range of 500-4000 cm^{-1}. Eggshell profiles using SEM (Hitachi SU 3500) at the same magnification, namely 1K with Vacc 3 kV, and previously prepared by coating with gold (Au) using an ion device Sputter MC1000 (Hitachi Corp.) (Suryani et al., 2021). They set parameters for the Hitachi SU3500 SEM and eggshell sample preparation using modified (Karimy et al., 2020; Prasetyo et al., 2019) techniques.

Data Analysis

The data were analyzed using Analysis of Variance (ANOVA); when significant, a Duncan Multiple Range Test (DMRT) post hoc tests were carried out. Data analysis was performed using the SPSS application for Windows 16.0.

RESULTS AND DISCUSSION

Proximate Composition

Dry matter was the content of materials that had been removed from the water. Treatment T4 has the highest dry matter content than T3 (Table 1). There was a correlation between the water content and the content of organic or non-organic materials in a material (Soekarto, 2012). Further explained, dry matter such as carbohydrates, proteins, fats, and vitamins can bind water. The decrease in protein content using H_3PO_4 solution was possible to reduce the water content of the material so that the dry matter content increased.

Ash content was the value of inorganic materials in a material. The inorganic material content of T1 was lower (90.37±0.76%) than the other methods. This value indicates that T1 still contains organic material. It can be seen from the value of crude protein content (7.88±0.06%) of the method. For the T4 preparation method, the inorganic material content was not different from the T1 and T3 methods. According to Nurhidayah *et al.* (2019), inorganic materials were mineral components. The mineral components of eggshell waste used the T2, T3, and T4 preparation methods, namely 91.54±0.66%, 91.07±0.14%, and 92.01±0.75%.

Tabel 1. Proximate composition of hatchery eggshell waste with different preparation methods

Variable	T1	T2	T3	T4
DM (%)	$98.72±0.03^b$	$98.75±0.08^b$	$98.58±0.04^a$	$98.98±0.01^c$
Ash (%)	$90.37±0.76^a$	$91.54±0.66^{ab}$	$91.07±0.14^{ab}$	$92.01±0.75^b$
EE (%)	0.07±0.00	0.07±0.01	0.07±0.02	0.07±0.02
CF (%)	$0.51±0.01^a$	$0.59±0.02^b$	$0.58±0.02^b$	$0.79±0.01^c$
CP (%)	$7.88±0.06^c$	$6.72±0.32^b$	$6.92±0.27^b$	$4.18±0.27^a$

Remarks : *Mean values in same row with the different letters are significantly different at $p < 0.05$. T1: shell waste was flushed with distilled water; T2: shell waste soaked in distilled water for 12 h; T3: shell waste soaked in NaOH 0.5% for 12 h; and T4: shell waste was boiled at 80°C for 15 min and soaked in 5% H_3PO_4 for 30 min. DM : dry matter; EE : extract eter; CF : crude fiber; CP : crude protein.

The protein content of eggshell waste with different preparation methods contains a protein content of 4.18%-7.88%. The protein content of eggshells treated by T4 was lower protein content than others. It was closely related to boiling and soaking H_3PO_4. Kismiati *et al.* (2013) stated that soaking with H_3PO_4 causes the eggshell to be a hollower. It can be seen from the particle size of T4, which was smaller and uniform than the other treatments (Figure 4). Therefore, changes in the structure and content of eggshells may occur. In addition, the heating process will cause the protein to be denatured. The heating process starting from $80^{O}C$ reduces the protein content at a temperature of $70^{O}C$ (37.54% CP) to $80^{O}C$ (28.40% CP) (Novia *et al.*, 2011).

The T3 and T2 preparation methods also reduce the protein content of eggshell waste. NaOH was an alkaline solution that was used as a deproteination agent. The use of NaOH affects the deproteination reaction that causes changes in protein structure (Rohyami and Istiningrum, 2016). Hatchery eggshell waste contains a shell membrane containing protein. The eggshell membrane contains collagen (Cordeiro and Hincke, 2012). Protein is a compound that comes from several molecules (C, H, O, N), then it is hydrolyzed by water molecules (Paramita, 2013). Fiber content was divided into two types i.e., soluble and insoluble fiber. Insoluble fibers were cellulose, hemicellulose, and lignin (Mudgil, 2017). Eggshell waste contains more insoluble fiber. Therefore, T4 treatment increased the fiber content in shell waste. Furthermore, as described above, the treatment loosens the structure of the eggshell to release the bonds between the fibers (Kismiati *et al.*, 2013). Likewise, the process of soaking with water and NaOH changes the structure of eggshell waste.

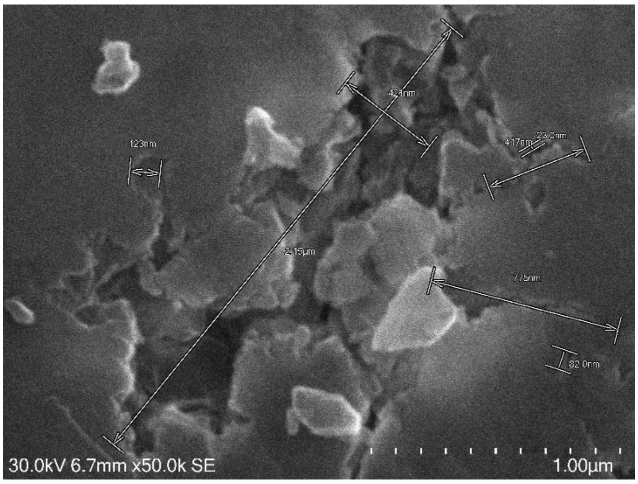


Figure 1. Fractional width of eggshell waste was boiled at 80°C for 15 min and soaked in 5% H_3PO_4 for 30 min.

Eggshell waste mineral content

Components of organic compounds have C, H, O, and N (Sriharti & Salim, 2010). These components are found in the structure of carbohydrates, proteins, fats, and fibers. The T3 and T4 methods were the lowest C, H, O, N components (Table 2). In this treatment, the protein content decreased, especially the use of the T4 method (Table 1). Protein contains the element N in it (Uauy *et al.*, 2015). Therefore, the low protein content indicates the low N content in the sample. The deproteination process by 0.5% NaOH and boiling at 80°C then soaked with 5% H_3PO_4 for 15 min reduced CHON levels.

The mineral content of the XRF detection results showed a significant difference between the four treatments. 80°C boiling treatment and 5% H_3PO_4 immersion (T4) showed that the Ca content was lower than the others. Compared with the use of 0.5% NaOH (T3), with a Ca content of 24.22±0.31%. The decrease in mineral content in T4 can release the bonds that bind these minerals to dissolve in water. Nurlaela *et al.* (2014) stated that 94-97% Ca in the shell is $CaCO_3$.

Tabel 2. XRF analysis of hatchery eggshell waste with different preparation methods.

Parameter	T1	T2	T3	T4
CHON (%)	$76.17±0.60^b$	$76.15±0.66^b$	$74.47±0.34^a$	$74.44±0.65^a$
Mg (%)	$0.20±0.01^c$	$0.18±0.01^b$	$0.22±0.00^d$	$0.14±0.01^a$
Si (ppm)	$89.15±9.75^a$	$88.00±0.70^a$	$144.75±31.35^b$	$106.55±17.45^a$
P (%)	$0.16±0.01^a$	$0.17±0.01^a$	$0.15±0.01^a$	$2.89±0.17^b$
Ca (%)	$22.80±0.57^b$	$22.77±0.71^b$	$24.22±0.31^c$	$21.55±0.46^a$

Remarks : *Mean values in same row with the different letters are significantly different at $p < 0.05$. – T1: shell waste was flushed with distilled water; T2: shell waste soaked in distilled water for 12 h; T3: shell waste soaked in NaOH 0.5% for 12 h; and T4: shell waste was boiled at 80°C for 15 min and soaked in 5% H_3PO_4 for 30 min. CHON : Carbon, Hydrogen, Oxygen, Nitrogen.

On the other hand, the decrease in calcium content increased the phosphorus (P) T4 content (Table 2). In addition to organic materials, eggshells contain inorganics such as calcium carbonate, magnesium carbonate, calcium phosphate, and magnesium phosphate (Laohavisuti *et al.*, 2021). T4 eggshells were treated using acid. Calcium was easily degraded using acids (Amjad and Zuhl, 2007). It causes the release of bonds between calcium and phosphorus. So that the calcium content decreased and phosphorus increased.

Si (Silica) content in eggshells using 0.5% NaOH preparation method was higher than other methods. NaOH was a deproteinizing agent that reduces collagen (protein) content marked by a decrease in the content of C, H, O, N (Table 2.). However, it did not break the bonds of Ca and Si. According to Shen and Chen (2003), Ca served as a constituent of the palisade tissue, in the presence of Si can strengthen the tissue. It was further explained that the higher the amount of calcium, the more compact the shell layer.

Hatchery eggshell waste contains magnesium (Mg). The Mg content of each treatment showed a significant difference among treatments. The T4 treatment tends to be lower than the other treatments. Acids very easily degrade magnesium. The use of inorganic compounds (HCO_3^-, HPO_4^{2-} and $H_2PO_4^-$) can react and cause deposits (Gonzalez *et al.*, 2018; Wang *et al.*, 2017).

FTIR Spectra of hatchery eggshell waste

FTIR was used to examine the structure and functional group of the samples. FTIR analysis was performed and compared for the hatchery eggshell waste with the different preparation methods, as presented in Figure 1. T1, T2, T3, and T4 samples presented similar spectra at around 1397 cm^{-1}, 1123 cm^{-1}, 872 cm^{-1}, and 711 cm^{-1} were associated with vibrations of carbonate (CO_3^{2-}) groups (R. Ahmad et al., 2012; Wembabazi et al., 2015). Band 1397 cm^{-1} represented the asymmetric C-O stretch, 872 cm^{-1} represented the out of plane bend, and 711 cm^{-1} represented the in plane bend (Wembabazi et al., 2015). The band observed at 1060 cm^{-1} corresponds to the vibration of the phosphate group (A. Ahmad et al., 2020).

The peaks observed from the spectrum at around 3267 cm^{-1} suggest the presence of an amide group (Cheung, 2006). The spectrum followed the results of the proximate analysis in Table 1. There was a tendency for spectrum results in T4 to be lower than other treatments.

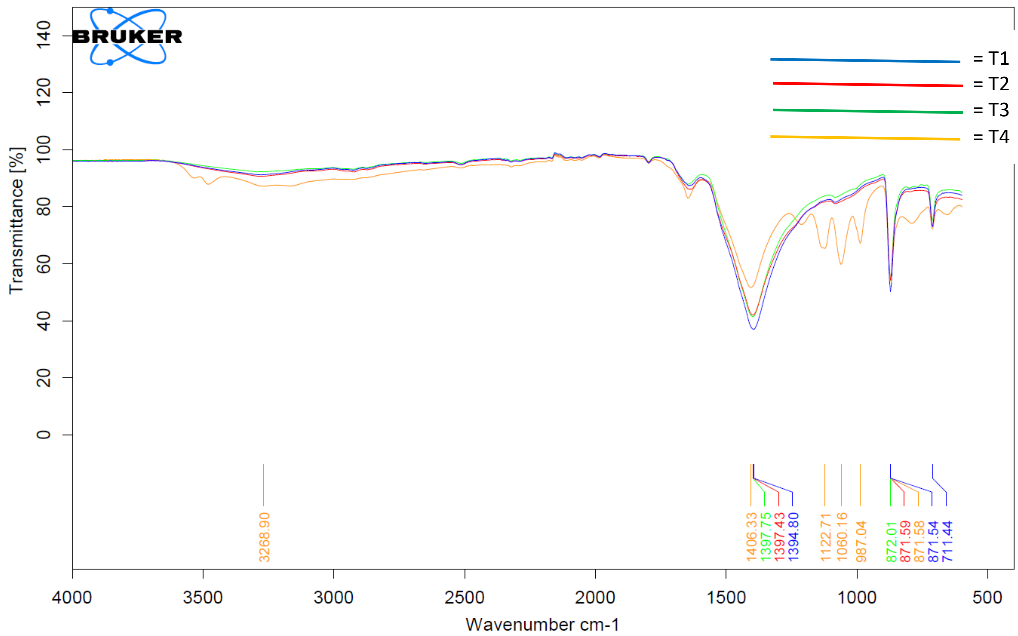
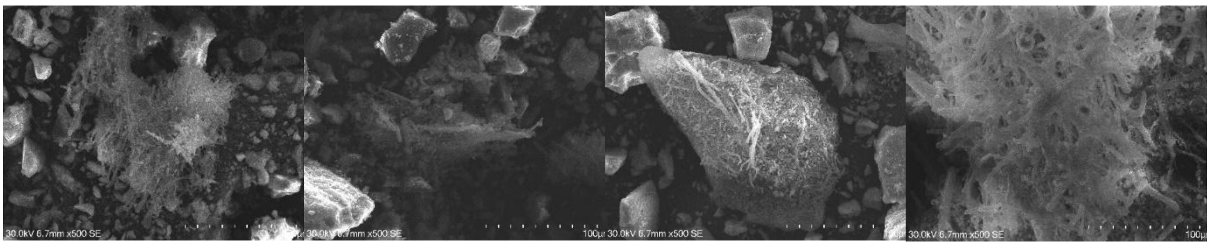


Figure 2. FTIR Spectra of hatchery eggshell waste with the different preparation methods

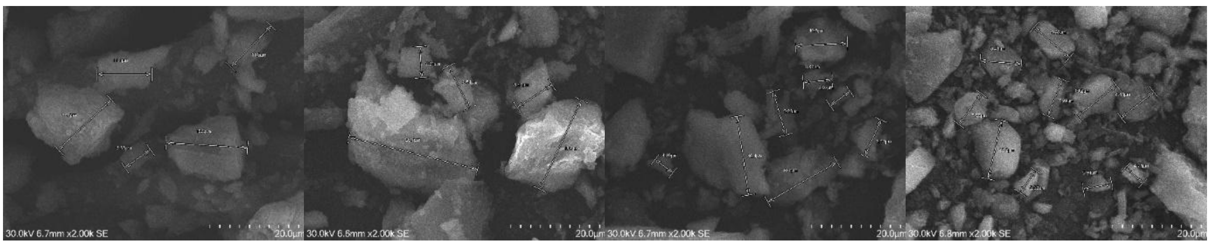
Profile SEM of eggshell

There was no significant variation in the profile of the SEM picture in the eggshell membrane samples seen by SEM with the tool configuration of magnification of 500 times, Vacc 30 kV, secondary electron (SE) detector, and high vacuum mode. The membrane fibres appear cleaner, and the membrane fibres form more noticeable in the T4 treatment.



T1 T2 T3 T4

Figure 3. Eggshell membrane of hatchery eggshell waste with the different preparation methods. T1: shell waste was flushed with distilled water; T2: shell waste soaked in distilled water for 12 h; T3: shell waste soaked in NaOH 0.5% for 12 h; and T4: shell waste was boiled at 80°C for 15 min and soaked in 5% H_3PO_4 for 30 min.



T1 T2 T3 T4

Figure 4. The particle of hatchery eggshell waste with the different preparation methods. T1: shell waste was flushed with distilled water; T2: shell waste soaked in distilled water for 12 h; T3: shell waste soaked in NaOH 0.5% for 12 h; and T4: shell waste was boiled at 80°C for 15 min and soaked in 5% H_3PO_4 for 30 min.

When comparing the particle sizes of the eggshells amongst treatments T1, T2, T3, and T4, there were only minor differences in particle size. It can be seen from the description of the SEM particle size profile between treatments that the particle size is about the same, i.e. T1: particle size between 6.33-16.5 µm, T2: particle size between 6.31-26.9 µm, T3: particle size between 9.23-16.2 µm, T4: particle size between 8.41-12.2 µm. The particle size in the T3 and T4 treatments was more uniform than in the T1 and T2 treatments, with higher size fluctuations.

CONCLUSION

It may be inferred that the treatment technique had no major impact on the egg shell's physical characteristics. However, boiling treatment at 80°C for 15 min and soaking in 5% H_3PO_4 reduced the eggshell waste's Mg, Si, and Ca content. The addition of 0.5% NaOH immersion did not degrade Mg, Ca, and Si of eggshell waste. On this basis, it may be inferred that the treatment technique had no major impact on the egg shell's physical characteristics.

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