International Journal of Chemical and Biochemical Sciences (IJCBS), 25(19) (2024): 594-600

International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Chemical Characteristics of Hydroxyapatite (HAp) from Broiler Eggshell Waste at Different Sintering Temperature Treatments

M.I. Said[1*], F. N. Yuliati[1], A. Gani[2], and P. Taba[3]

[1]Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Indonesia
[2]Department of Periodontology, Faculty of Dentistry, Hasanuddin University, Indonesia
[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Indonesia

Abstract

World bodies have claimed the livestock industry as one of the contributors to the increase in pollution. This is a factor that needs attention. Waste from the livestock industry could be a trigger for this statement. The hatchery industry is one of the livestock industries contributing to pollution worldwide. One of them is eggshell waste produced from broiler egg-hatching activities. This study aimed to examine the properties of hydroxyapatite (HAp) from broiler eggshell waste from the hatchery industry. A total of 6 temperature variations were applied (550°C, 600°C, 650°C, 700°C, 750°C and 800°C). Applying different sintering temperatures produces different physicochemical properties of HAp, especially those using broiler eggshell waste as raw material. Applying a sintering temperature of 550ºC significantly increases the yield (Y) value and reduces mass loss (ML). Crystal formation and functional properties were not significantly different between different sintering temperatures. An application temperature of 550ºC is considered appropriate for HAp production efficiency. Utilizing broiler eggshell waste as a raw material for hydroxyapatite is essential in providing hydroxyapatite products that are easy to obtain, cheap, and environmentally friendly.

Keywords: Hydroxyapatite, Broiler, Eggshell Waste, Sintering, Temperature

**Corresponding Author*, e-mail: irfanunhas@gmail.com

Doi # https://doi.org/10.62877/68-IJCBS-24-25-19-68

1. Introduction

The agricultural sector, especially the livestock sector, is a source of waste. The need for food has increased along with the increase in human population. The increasing need for animal food, of course, supports the growing need for food. As a result, the production of livestock by-products is also increasing. Eggshells are a type of by-product often produced by the hatchery industry. The increase in demand for chicken meat is a driving factor in increasing production of eggshell by-products [1,2]. One of the livestock industries that produces eggshell waste is the egg hatchery industry. Eggshell waste is produced after the chicks are harvested. The eggshell waste still contains other organic materials such as protein and fat and inorganic materials such as minerals. The composition of the eggshell includes large amounts of mineral compounds, namely 94-97%. The dominant mineral composition is calcium (Ca) [3–5]. Eggshells are one of the livestock wastes that can produce hydroxyapatite (HAp) compounds. This compound has the molecular formula $Ca_{10}(PO_4)_6(OH)_2$). HAp compounds can be produced from eggshell raw materials after an impregnation process using phosphate precursors. Apart from that, eggshells can also function as a cheap, absorbent raw material. This is due to its structure in phosphate and hydroxyl groups [6]. The production of eggshell waste is from the hatchery industry and mostly from the processed food industry. The processed food industry uses eggs as the main raw material in production [7]. Most of the eggshell waste is disposed of in landfills. The membranous part of the eggshell is rich in protein. As a result, the waste is beautiful to animals (rats and pests), so the impact is hazardous [8]. Therefore, quite bad negative consequences may occur to the environment if they are not handled seriously and in a structured manner [9].

Broiler eggshell waste is a type of eggshell waste that has the highest production. This is because broilers are the largest chicken population consumed by both people in the world. As a result of an increase in population due to the fulfillment of needs, the production of broiler eggshell waste has also increased significantly. This waste has not been widely utilized because some of it has been mixed with eggs containing embryos, making it very easy to experience the decomposition process. The study's results reported that pollution due to eggshell waste from the food processing industry was ranked 15th out of other waste producers. [10, 11]. Eggshell waste contains calcium carbonate compounds, which are nano calcium sources. The content of nano calcium is a constituent material and source of HAp [12]. Different preparation processes will produce physicochemical properties that tend to be distinct [13]. This research is fundamental as an effort to increase the added value of broiler eggshell waste so that the impact of pollution from the chicken breeding industry can be reduced.

The use of eggshell waste as raw material from broilers is proof of the novelty of this research. So far, researchers have used eggshell waste as raw material from laying hens, while eggshell waste from broilers is still very rare. Previous search results show that publications that use broiler eggshell waste as raw material for HAp production have never been found. This study aims to evaluate the effect of different sintering temperatures on the physicochemical properties of HAp produced from broiler eggshell waste.

2. Materials and methods

2.1. Materials

The main ingredient used is broiler eggshell waste (BEW). The main ingredients are obtained from the hatchery industry unit at the company PT. Multi Breeder Adirama Indonesia (MBAI) (Japfa Group).

The supporting materials are distilled water, $(NH_4)_2HPO_4$ 0.3 M, Whatman filter paper No.42, and aluminum foil. The production and testing process equipment uses several types of equipment, namely SEM-EDS (*JEOL Type JCM 6000 plus*), FTIR (*IR Prestige-21*), furnace (*Furnace 6000*), oven (*BMT Incucell V-LSIS-B2V/IC55*), analytical balance (*OHAUS PA214C*), magnetic stirrer (*Stuart SD162*) and some supporting equipment (thermometer, desiccator, stopwatch, beaker glass, Erlenmeyer, measuring cup and volume pipette).

2.2. Methods

2.2.1. Preparation process of BEW materials

The material preparation process of broiler eggshell waste (BEW). A total of 200 g of BEW from the hatchery industry was prepared, washed with running water, and then weighed to determine the yields.

2.2.2. Production process of HAp-BEW

The HAp-BEW production process was carried out according to the method used by [14]. The method applied in the HAp production process is hydrothermal. In general, the HAp production process that uses eggshell waste as raw material involves several process methods. Several known process methods include the dry method and the hydrothermal method. Apart from that, several methods are also known, including the co-precipitation and sol-gel methods. Among these several methods, the choice is more towards the hydrothermal method. One of the reasons is that this method can produce HAp products that are more homogeneous and able to form crystals well [15]. The stages of the HAp-BEW production process were carried out in 4 stages, namely: preparation, calcination, precipitation, and sintering.

2.2.3. Research parameters

The treatment applied is a difference in 5 sintering temperature levels in the HAp synthesis process, namely: 1) 550°C; 2) 600°C; 3) 650°C; 4) 700°C; 5) 750°C; 6) 800°C. The data obtained were then processed and analyzed on the basis of several observed parameters. The research parameters include (1) Yield (Y), (2) Mass loss (ML), (3) Fourier Transform infrared (FTIR) analysis, and (4) microstructural evolution analysis.

2.2.4. Data analysis

Data analysis for each parameter consists of (1) Y value on HAp products, which are determined by weighing the broiler eggshell raw material (g) and the HAp products after the synthesis process (g). The equation used is Y(%) = a/bx100%, where a = weight of the synthesized HAp product (g); b = weight of raw material for broiler eggshells (g); (2) The ML parameter is calculated based on the equation ML=(100%-Y(%); (3) The FTIR analysis using an FTIR spectrometer. This analysis aims to evaluate the functional characteristics of HAp products from BEW. The analysis was carried out on the resulting spectra; (4) Microstructural evolution parameters were used to evaluate the characteristics of the HAp particles resulting from the different temperatures using a Scanning Electron Microscope (SEM) to determine the microstructural properties of the particles.

2.2.5. Statistical analysis

This research was based on a completely randomized design with a unidirectional pattern. A total of 5 treatments were applied with three repetitions each. The data set was analyzed using ANOVA. The data thus obtained were then processed and analyzed statistically to determine the effect of the treatment. This treatment showed a significant effect, which was analyzed using a significant difference test (Duncan's Multiple Range Test (DMRT) at the 5% level) [16].

3. Results and Discussion

3.1. Yields (Y)

The yield value of the BEW products is important to determine the effectiveness of the production process based on the application of different sintering temperatures. A high Y value indicates that the process has a high-efficiency value. This value is highly important in designing the production process. HAp-BEW Y values produced from BEW materials by the application of different sintering temperatures are presented in Figure 1. Based on Figure 1 it can be seen that the difference in the application of the sintering temperature in the HAp-BEW production process shows a very significant difference ($P<0.01$) in the Y value. The efficiency of the hydroxyapatite production process was highest in broiler eggshell waste samples resulting from the 550ºC combustion process, namely 57.11%. However, statistically, the yield values are relatively the same for the sintering process at 600°C and 650°C with production process efficiencies of 55.97% and 56.42%, respectively (superscript "a").

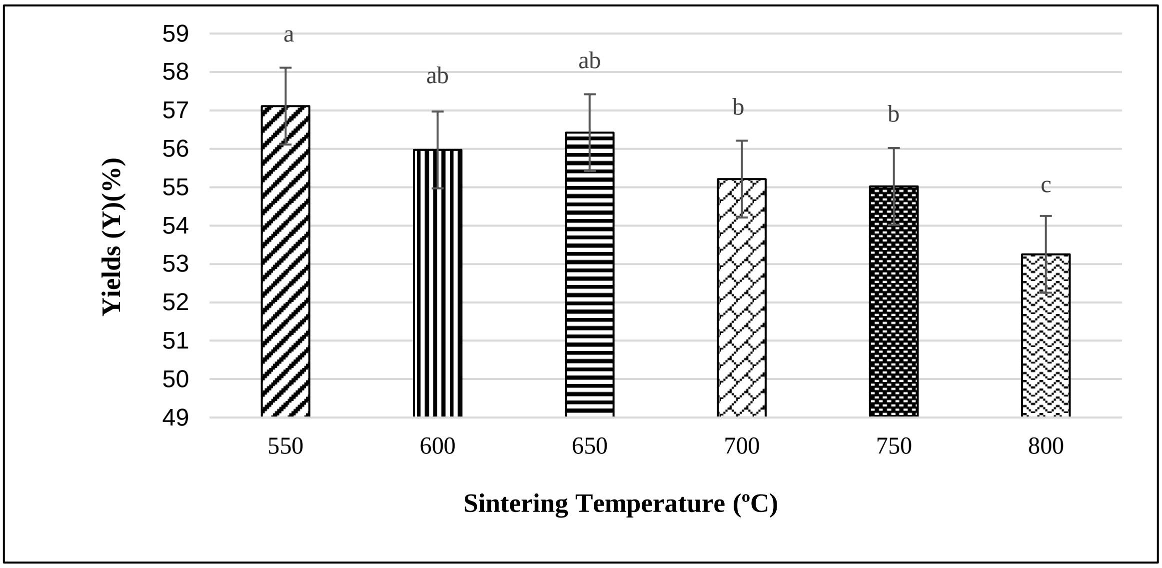

Figure 1: Graph of yields (Y)(%) of HAp-BEW product at different sintering temperatures. Note: [a-c]; Different superscripts in the graph show very significant effect differences (p<0.01)

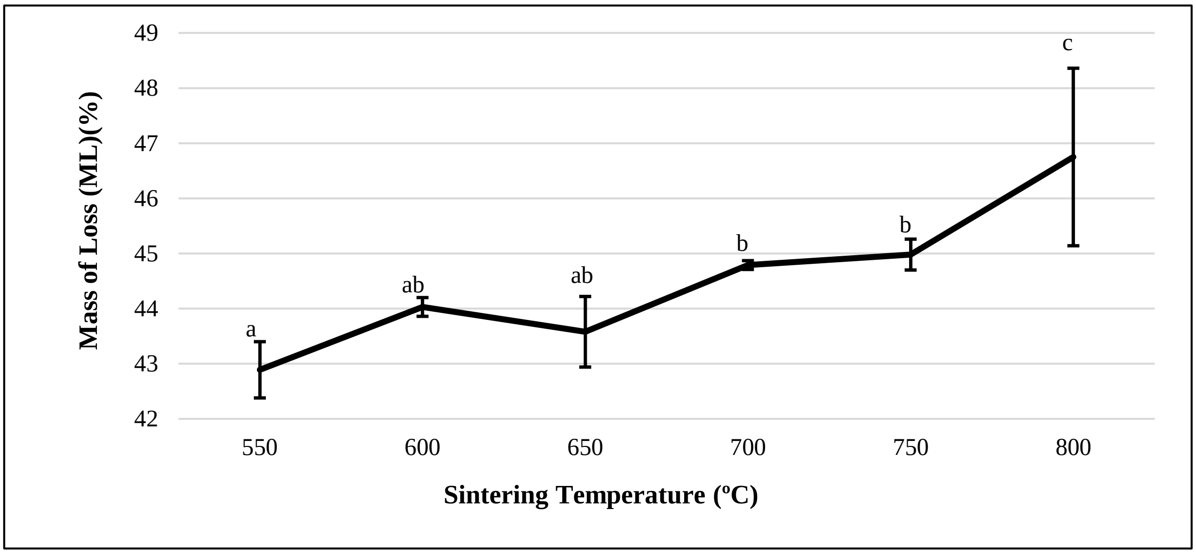

Figure 2: Graph of Mass Loss (ML)(%) of HAp-BEW product at different sintering temperatures. Note: [a-e]; Different superscripts in the graph show very significant effect differences (p<0.01)

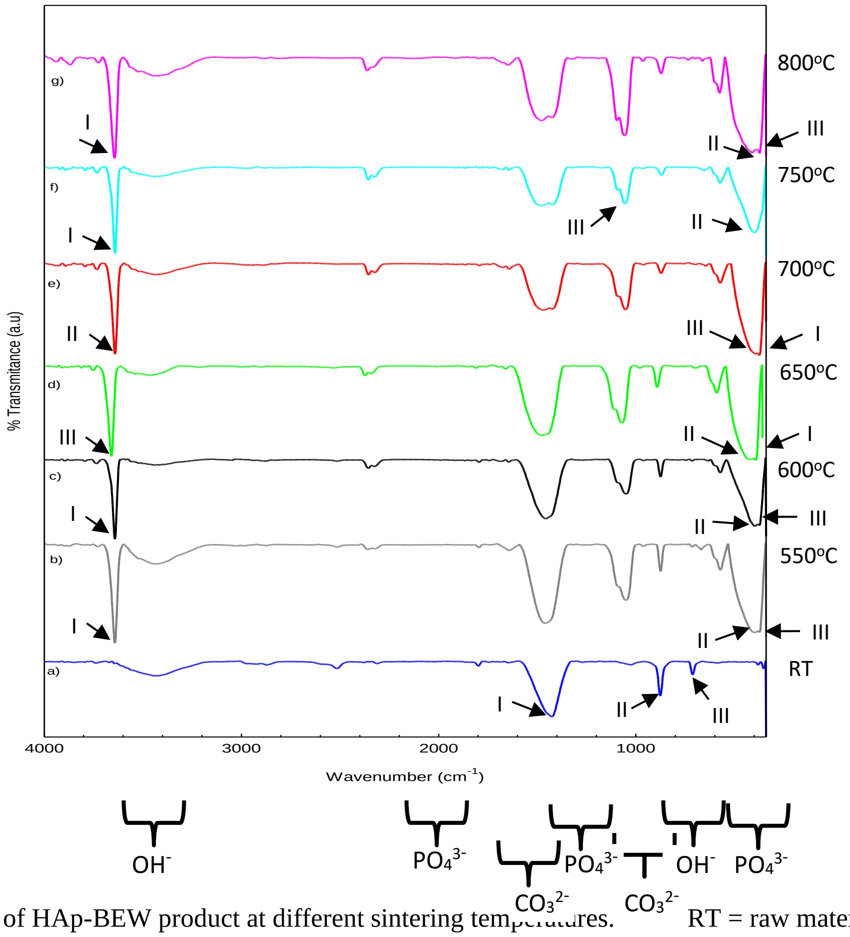

Figure 3: FTIR spectra of HAp-BEW product at different sintering temperatures. RT = raw material at room temperature as control; wavelength intensity (I=strongest intensity ; II= strong intensity; III=weak intensity); arrow = peak wavelength.

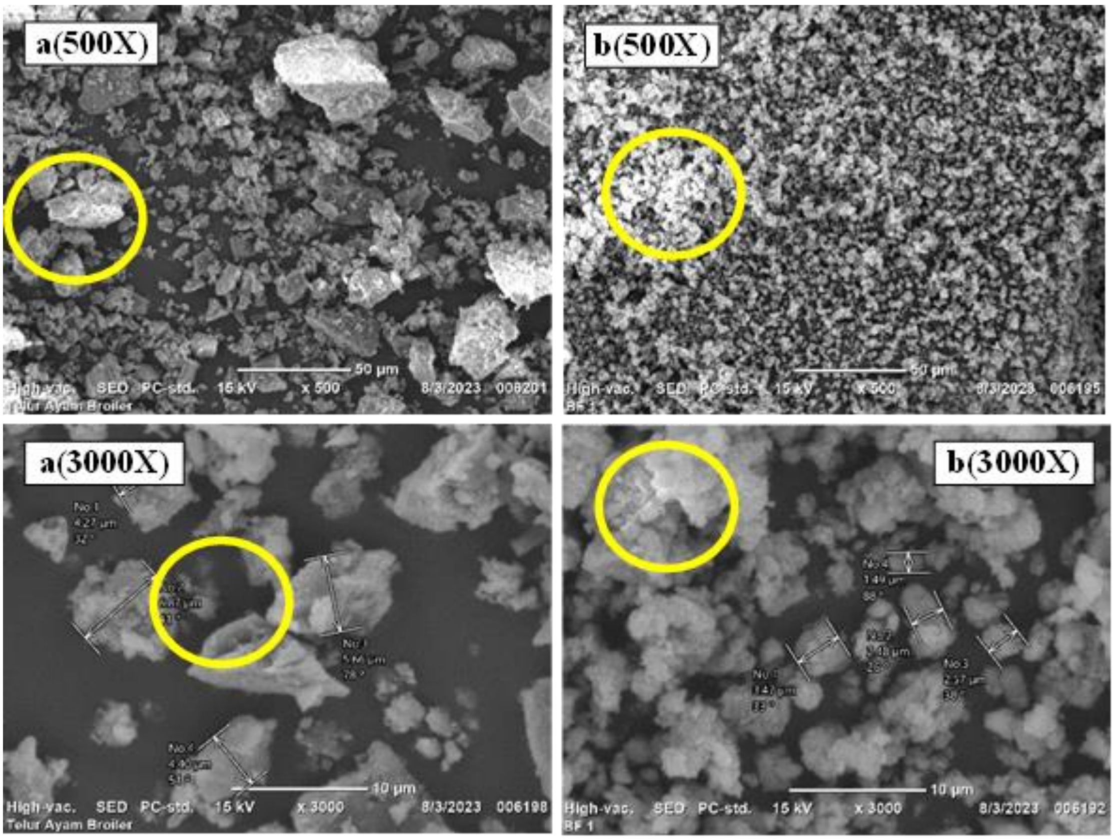

Figure 4: SEM images of raw material (a500X) and (a3000X) of BEW at room temperature and (b500X) and

(b3000X) HAp-BEW, each with a magnification of 500x and 3000x

The lowest efficiency of the production process is the sintering process at 800°C. The decrease in yield value at 800°C can occur due to an imbalance between Ca/P ions in HAp in the composition of BEW. As reported by [17], heating at high temperatures (up to 1000°C) can cause Na ions to enter the apatite structure, which contains OH^- ions, so that the composition of the material changes. Waste from eggshells consists of organic and inorganic materials. The weight loss rate of 40-45% indicates that there is removal of organic content and water during the sintering process. This is consistent with the results reported in [18]. Figure 2 shows that the yield of HAp-BEW decreased as the sintering temperature decreased. This is associated with the HAp dehydroxylation process and the slow elimination process, especially in carbonate ions, as has been reported by [19]. In connection with the sintering process carried out in this study, the results obtained can be related to the theory published by [20] that the decrease in weight after the sintering process is related to 1) the number of water particles that are lost from a material, 2) the presence of a mixture of several types of organic matter in egg shells such as protein and fat residues that have been completely burned, 3) the process of dissociation of carbonate or hydroxide ions in forming oxide molecules and 4) the presence of a dihydroxylation process in the HAp formation phase.

3.2. Mass loss

The value of mass loss (ML)(%) of samples of broiler eggshell waste that underwent sintering processes at different temperature variations is presented in Figure 2. The ML is related to the loss of mass due to the heating process, which occurs in the preparation, calcination, precipitation, and sintering stages. The ML in question is in the form of loss of organic molecules as well as some inorganic ones. Some organic molecules that are lost during the sintering process include proteins, fats, and carbohydrates. Apart from that, several inorganic molecules that also experience loss during the sintering process are the minerals calcium carbonate, phosphorus, magnesium, sodium, potassium, zinc, manganese, iron, and copper. The missing organic and inorganic molecules are related to the composition of broiler eggshells. As much as 97% of eggshells consist of calcium carbonate. Apart from that, eggshells also contain 95.1% organic salts. Of the organic material, 3.3% is protein, and the remaining 1.6% is water [21]. Based on Figure 3, it can be seen that the ML of broiler eggshell samples during the calcination and sintering process averaged 44.50%. Increasing the sintering temperature from 550°C to 800°C tends to increase ML. The results obtained are similar to the results of a study conducted by thermogravimetric analysis (TGA) of 45.30% [22] for eggshell samples from laying hens (layer) and 33.21 (bovine bone) [23]. Differences can occur due to differences in the type of sample used, the temperature applied, and the source of the eggshells. The differences in sources and breeds of chickens used in the samples will potentially result in different loss mass due to the different composition, shell thickness, and morphological properties. The decrease in Y values is directly proportional to ML in the HAp production process. The sintering process is a process used to form bonds between particles or powders after the compaction process. This process is carried out by providing a temperature below the melting point so that mass transfer occurs on the powder surface and bonds are formed between the powders.

3.3. Fourier Transform Infra-red (FTIR) analysis

Analysis using FTIR aims to identify functional groups in the sample. The results of the FTIR analysis on HAp-BEW are presented in Figure 3. Figure 3 shows that the spectra of the three most substantial peaks between the raw material (RT) and the sample that has undergone the sintering process are very different. The peak points are the PO_4^{3-}, CO_3^{-2}, and OH^- functional groups, which are the ions that makeup HAp. The detected ions have been able to prove that BEW samples contained PO_4^{3-}, CO_3^{-2}, and OH^- ions. This shows that BEW has potential as a material for HAp. The peak point for the formation of PO_4^{3-} ions occurred in samples with sintering temperatures of 650°C and 700°C. The wave number shown is the same, namely 374.19 cm^{-1}, but the intensity is different. These results are in line with research conducted by [24]; peaks in the phosphate functional group were identified at wavelengths of 640-560 cm^{-1} and 1100-1000 cm^{-1}. This result is attributed to the symmetric stretching of the phosphate group (PO_4^{-3}). The temperature intensity of 650ºC (6.289%T) is stronger than the temperature of 700ºC (8.797%T). The highest peak in the formation of CO_3^{2-} ions also occurred in the raw material (RT), namely 1425.4 cm^{-1} with an intensity of 45.071%T. The observation results have many similarities to the research reported by [25]. It can be seen that in the raw samples, none of them have peak points at wave numbers 4000 cm^{-1} to 3000 cm^{-1}, while samples that have been sintered at 550-800ºC have an average peak point at wave numbers 4000 cm^{-1} to 3000 cm^{-1}. The three strongest peak points are on average at wave numbers 1000 cm^{-1} to 300 cm^{-1}. Sintering temperatures 550°C, 600°C, 750°C, and 800°C have the strongest peak point (peak I) (at wave numbers 4000 cm^{-1} to 3000 cm^{-1}, while 650°C and 700°C peak I is at wave numbers 400 cm^{-1} to 300 cm^{-1}. The differences in absorption peaks between raw and sintered samples have proven that the sintering process has an influence on the process of forming HAp compound molecules. The results obtained are similar to the results of research reported by [26]. In carbonate molecules (CO_3^{2-}), strong peak intensity is found at wavelengths 1500-1490 cm^{-1} and 2000-1900 cm^{-1}), followed by hydroxyl groups with different peaks (2900 and 3400 cm^{-1})[27].

3.4. Microstructural evolution

Analysis using SEM is an in-depth analysis that examines the surface morphological structure of a material. Figure 4 shows a representative appearance of BEW(a) base material with HAp produced by applying a sintering temperature of 800ºC (b). The appearance of the microstructure in Figure 4(a) shows a denser structure. The solid structure is composed of organic matter molecules that are between the shell matrix. In Figure 4(b) it can be seen that there is a porous structure left due to the sintering process applied. The porous structure occurs due to the loss of organic substance molecules that fill the matrix between calcium molecules that make up the composition of the eggshell. In particular, it can be seen that in Figure 4(b), there is an agglomeration of the particles, whereas previously, in Figure 4(a), the particles were separated. This is the effect of the sintering process applied. The agglomeration process on HAp indicates that the sintering process has reached its final stage. This process is indicated by grain boundaries that begin to enlarge and pores that are closed/isolated by grains. This result is also in line with the results of research that has been reported [28]. Based on the morphology of the resulting HAp, particles have a round shape and are not sharp. This makes HAp products from BEW have potential as materials in the medical field and are not harmful to other tissues. Materials that have a sharp and sharp morphology have the potential to injure the underlying tissue [29]. Figure 4 also shows that the application of sintering to eggshells causes a reduction in particle size. This can be seen in Figure 4(a); the average particle size of the raw material is 4-5 microns, whereas when the sintering temperature is 800°C, the particle size decreases to an average of 1-3 microns. Increasing temperature causes agglomeration, which facilitates the formation of hydroxyapatite. Evaluation results from previous research show that different types of materials and sintering temperatures influence the physicochemical properties of HAp. The sintering temperature applied in the process is positively correlated with the structure of the raw materials used. Types of materials can have different crystal arrangements and molecular bonds so that they will produce different products [25, 30].

4. Conclusion

The research results show that the hydroxyapatite extraction process from BEW material using varying sintering temperatures has been successfully implemented. These results have answered public concerns regarding the increasing potential for broiler eggshell waste production. The research results show that the hydroxyapatite extraction process from broiler eggshell material using varying sintering temperatures has been successfully implemented. The properties of hydroxyapatite were evaluated quantitatively (yields and mass loss) and qualitatively (FTIR and SEM). The application of different sintering temperatures to broiler eggshells produces HAp with different physicochemical properties (crystal formation and functional properties). Applying a temperature of 550ºC significantly increases the Y value and reduces ML. Sintering temperature has a significant effect on the agglomeration process for the formation of HAp crystal molecules during the production process. Utilizing BEW is a solution in reducing the production of livestock waste and is a solution in providing environmentally friendly alternative raw materials.

Acknowledgments

The author expresses his appreciation and gratitude to the Ministry of Education and Culture, Research and Technology of the Republic of Indonesia, the Directorate General of Higher Education, Research and Technology, the Directorate of Research, Technology and Community Service, the Rector of Hasanuddin University and the University Head of Institute Research and Community Service, Hasanuddin University for research financing support through the "Skim Penelitian Fundamental-Reguler" program for Fiscal Year 2023 contract number 124/E5/PG.02.00.PL/2023 dated June 19, 2023 and 02381/UN4.22/PT.01.03/2023 dated June 20, 2023.

References

[1] M. Khalil, M.A. Berawi, R. Heryanto, A. Rizalie. (2019). Renew. Sustain. Energy Rev. 105:323.

[2] N.P.A. Widjanarko, A.P. Siregar. (2022). Nat. Environ. Pollut. Technol. 21:1445.

[3] E.R. Muñoz-Sanchez, C.D. Arrieta-Gonzalez, A. Quinto-Hernandez, E. Garcia-Hernandez, J. Porcayo-Calderon. (2023). Synthesis of hydroxyapatite from eggshell and its electrochemical characterization as a coating on titanium. Int. J Electrochem Sci. 18: 100204.

[4] R. Vinayagam, S. Kandati, G. Murugesan, L.C. Goveas, A. Baliga, S. Pai, T. Varadavenkatesan, K. Kaviyarasu, R. Selvaraj. (2023). Bioinspiration synthesis of hydroxyapatite nanoparticles using eggshells as a calcium source: Evaluation of Congo red dye adsorption potential. J. of Materials Research and Technology. 22: 69–80.

[5] S. Pai, M.S. Kini, R. Selvaraj. (2021). A review on adsorptive removal of dyes from wastewater by hydroxyapatite nanocomposites. Env. Sci. and Pollution Research. 28. 11835–49.

[6] B. Kizilkaya, A.A. Tekmay. (2014). Utilization to remove Pb (II) ions from aqueous environments using waste fish bones by ion exchange. J. Chem 1-12.

[7] R. Ravindran, A.K. Jaiswal. (2016). Exploitation of food industry waste for high-value products. Trends Biotechnol. 34: 58–69.

[8] I. Abdulrahman I, H.I. Tijani, B.A. Mohammed, H. Saidu, H. Yusuf, M.N. Jibrin, S. Mohammed. (2014). From garbage to biomaterials: an overview on eggshell based hydroxyapatite. J. Mater. 1–6.

[9] K. Wheeling. (2019). Pasific Standar. Are We Headed Toward the Worst-Case Climate Change Scenario?.

[10] M. Waheed, M. Yousaf, A. Shehzad, M. Inam-Ur-Raheem, M.K.I. Khan, M.R. Khan, N. Ahmad, Abdullah, R.M. Aadil. (2020). Channelling eggshell waste to valuable and utilizable products: A comprehensive review. Trends Food Sci Technol. 106: 78–90.

[11] E.O. Ajala, O.A. Eletta, M.A. Ajala, S.K. Oyeniyi. (2018). Characterization and evaluation of chicken eggshell for use as a bio-resource. J of Engineer Tech and Environ. 14: 26-40.

[12] S. Sultana, Md.S. Hossain, M. Mahmud, M.B. Mobarak, M.H. Kabir, N. Sharmin, S. Ahmed. (2021). UV-assisted synthesis of hydroxyapatite from eggshells at ambient temperature: cytotoxicity, drug delivery and bioactivity. RSC Adv. 11: 3686–94.

[13] A.D. Sobczak-kuupiec, M.R. Kijkowska (2012). Comparative study of hydroxyapatite prepared by the authors with selected commercially available ceramics. Dig. J. Nanomater. Biostruct. 7(1): 385-391.

[14] M.Z.A. Khiri, K.A. Matori, N. Zainuddin, C.A.C. Abdullah, Z.N. Alassan, N.F. Baharuddin, M.H.M. Zaid. (2016). The usability of ark clam shell (*Anadara granosa*) as calcium precursor to produce hydroxyapatite nanoparticle via wet chemical precipitate method in various sintering temperature. Springerplus. 5: 1206.

[15] S. Pokhrel. (2018). Hydroxyapatite: Preparation, Properties and Its Biomedical Applications, Advances in Chemical Eng. Sci. 08(04): 225-240.

[16] O. Heinisch, R.G.D. Steel, J.H. Torrie. (1960). Principles and Procedures of Statistics. McGraw-Hill Book Company, New York, Toronto, London, 481 S., 15 Abb.

[17] C. Rey, J.C. Trombe, G. Montel. (1973). Retention of molecular oxygen by the lattice of certain alkaline earth apatites (C R Acad Sci Ser C).

[18] R.V. Silva, J.A. Camilli, C.A. Bertran, N.H. Moreira. (2005). The use of hydroxyapatite and autogenous cancellous bone grafts to repair bone defects in rat. Int. J. Oral Maxillofac Surg. 34: 178–84.

[19] J.P. Davim, N. Marques. (2004). Dynamical experimental study of friction and wear behavior of bovine cancellous bone sliding against a metallic counterface in a water lubricated environment. J. Mater Process Technol. 152: 389–94.

[20] E. Landi, A. Tampieri, G. Celotti, R. Langenati, M. Sandri, S. Sprio. (2005). Nucleation of biomimetic apatite in synthetic body fluids: dense and porous scaffold development. Biomaterials. 26: 2835–45.

[21] G.D. Butcher, R. Miles. (2012). Concepts of eggshell quality. University of Florida. The Institute of Food and Agricultural Sciences (IFAS) extension. 1–2.

[22] S. Joschek, B. Nies, R. Krotz, A. Göpferich. (2000). Chemical and physicochemical characterization of porous hydroxyapatite ceramics made of natural bone. Biomaterials. 21: 1645–58.

[23] E. Bulus, D. Ismik, D.S. Mansuroglu, Y.M. Sahin, G. Tosun. (2017). Synthesis and characterization of hydroxyapatite powders from eggshell for functional biomedical application. Electric Electronics, Computer Science, Biomedical Engineerings' Meeting (EBBT), Istanbul, Turkey, 1-3.

[24]. M.I. Said, H. Heryanto, D. Tahir. (2024). Hydroxyapatite (HA) Synthesis from Leg Bone by-product of beef cattle: Structural and optical characteristics for various sintering temperatures. JOM. The Journal of The Minerals, Metals & Materials Society (TMS).

[25] Y. Han, S. Li, X. Wang, L. Jia, J. He. (2007). Preparation of hydroxyapatite rod-like crystals by protein precursor method. Mater Res Bull. 42(6): 1169–77.

[26] C.Y. Ooi, M. Hamdi, S. Ramesh. (2007). Properties of hydroxyapatite produced by annealing of bovine bone. Ceram Int. 33:1171–7.

[27] V.K.C, Kumar, J.T. Subha, K.G. Ahila, B. Ravindran, S.W. Chang, A.H. Mahmoud, O.B. Mohammed, M.A. Rathi. (2021). Spectral characterization of hydroxyapatite extracted from Black Sumatra and Fighting cock bone samples: A comparative analysis. Saudi J. Biol Sci. 28(1): 840-846.

[28] G.E.J. Poinern, R.K.Brundavanam, D. Fawcett. (2013). Nanometer scale hydroxyapatite ceramics for bone tissue engineering. Am J. Biomed. Eng. 3(6): 148–68.

[29] A. Szcześ, L. Hołysz, E. Chibowski. (2017). Synthesis of hydroxyapatite for biomedical applications. Adv Colloid Interface Sci. 249: 321–30.

[30] M.I. Said, E. Abustam, A. Gani, P. Taba, A. Atirah. (2018). Production and evaluation of hydroxyapatite (HAp) properties of broiler's composite bone (BCB) waste at different sintering temperatures. Online J.of Biological Sciences. 18(3): 290-297.