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Synthesis of biocompatible hydroxyapatite from quail eggshell, oyster shell, and periwinkle snail shell

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ABSTRACT

This study focuses on the synthesis of hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) from calcium carbonate ($CaCO_3$)-rich quail eggshells, oyster shells, and periwinkle snail shells (Filopaludina bengalensis) through the use of the wet precipitation method. The methodology involved calcining the shell waste to convert $CaCO_3$ to calcium oxide (CaO), undergoing hydration, and reacting with phosphoric acid (H_3PO_4) to synthesize HA. The results indicated that periwinkle snail shells had the highest percent yield of HA at 92.12%, followed closely by quail eggshells at 92.01%, and oyster shells at 73.65%. For producing CaO, oyster shells provided the highest percent yield of CaO at 103.72%, followed by quail eggshells at 98.6% and periwinkle snail shells at 92.09%. The synthesized HA exhibited high biocompatibility, which is crucial for its potential applications in medical fields such as bone replacement and regeneration. The X-ray diffraction (XRD) analysis confirmed the successful synthesis of high-quality HA, with characteristic peaks indicative of excellent crystallinity and purity and near identicality to the standard XRD pattern of HA of ICDD 9-432 and the XRD pattern of successfully synthesized HA in other studies, indicating high biocompatibility. The research highlights the potential of recycling food waste, specifically shell waste, into valuable biomaterials. This not only addresses environmental concerns but also supports sustainable practices in the food industry. Moreover, the study contributes to advancements in biomaterials for medical applications, emphasizing the viability of utilizing organic waste for high-value products. By transforming food waste into useful medical materials, this research offers promising solutions for waste management and resource utilization, particularly within Thailand's ecological and industrial framework.

Keywords: Hydroxyapatite synthesis, Seashells, Calcium carbonate, X-ray diffraction, Calcium oxide

INTRODUCTION

It is estimated that a third of human-produced foods are wasted globally, which accounts for around 1.3 billion tons of food wasted annually [1]. The average human wastes 65 kg of food on average per year, with dairy and eggs contributing to 7% of total food waste weight, leading to the wasting of essential nutrients such as calcium, choline, riboflavin, zinc, and vitamin B12 [2].

In Thailand, eggs are a prevalent food source, with more than 15,000 million eggs being consumed per year. The estimated waste generated from the eggshells is around 90,000 tons per year [3]. Similarly, oyster shells generate a staggering 18.86 million tons of waste globally [4]. Shell waste produced from the rapidly increasing production and consumption of eggs and oysters is posing serious environmental threats in countless countries around the world, which require immediate action. It is suggested that these shell wastes be efficiently recycled and transformed into practical materials for sustainable advancement [5]. One solution is to utilize these shells, particularly eggshells, as an alternative source of biocompatible material to synthesize substitutive substances and materials in the medical field, since these shells are rich in valuable minerals such as calcium [6]. Eggshells and oyster shells contain high contents of calcium carbonate ($CaCO_3$), with eggshells at 93-97% and oyster shells at approximately 96% [7-9].

Quail eggs were selected for this study due to their substantial consumption and production in Thailand. In 2016, a particular supermarket franchise in Thailand reported a monthly consumption of 350,000 quail eggs, while the production of quail eggs for international export was approximately 125,000,000 eggs per year [10]. Quail shells consist of 96% or more $CaCO_3$ [11], which is considered a sufficient amount for hydroxyapatite synthesis [12]. Similarly, oyster shells were chosen due to their substantial $CaCO_3$ content and their high production and consumption rates in Thailand [9]. Oysters are a popular food widely consumed in Thailand, with the marine shellfish culture

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statistics survey by the Department of Fisheries, Ministry of Agriculture and Cooperatives reporting a production of 17,903.26 Tons of oysters with a total commercial value of over 749 million baht in the 2019 Department of Fisheries [13]. Additionally, periwinkle snail shells (Filopaludina bengalensis) contain 96% $CaCO_3$ [14]. In summary, these three materials (quail eggs, oyster shells, and periwinkle snail shells) were selected due to their high demand and popularity of consumption in Thailand, as well as the presence of $CaCO_3$ in their shells, making them suitable for hydroxyapatite synthesis. Furthermore, producing hydroxyapatite from these shell wastes contributes to reducing household and industrial waste from food shells, as they are currently not efficiently utilized to their maximum capacity.

$CaCO_3$ is the least expensive variation and the most common type of calcium. For medical uses and applications, $CaCO_3$ is generally used as a supplement or fundamental material for creating other commercial or medical consumables [15]. With more sophisticated materials and procedures, the compound $CaCO_3$ found in eggshells and snail shells is also popularly used as a calcium precursor in the methodology of the synthesis and production of HA, which is a proficient material for various medical and dental applications such as bone replacement, bone regeneration, and therapy [6].

Hydroxyapatite (HA) is an inorganic material that contains calcium (Ca), phosphate (PO_4^{3-}), and hydroxide (OH^-) in its standard apatite lattice structure with the chemical formula structure of $Ca_{10}(PO_4)_6(OH)_2$ [16]. It is a key component of human bone, which comprises approximately 60% HA, 30% proteins, and 10% water, respectively [17]. HA is a biocompatible ceramic with no toxicity, and numerous studies have demonstrated its similarities in composition, bioactivity, biocompatibility, osteoconductive, and chemical stability to the inorganic components of human bones and teeth [18, 19]. These close analogies to organic bone components have led to the development and production of synthetic HA via various methods. HA has the unique property of biologically interacting with organic materials, including chemically bonding with living tissues [16]. Due to its biocompatibility and osteoconductive nature, HA is widely used in orthopedics, dentistry, maxillofacial surgery, and orthopedic surgery. It is typically employed as a bone tissue replacement, for bone defect restoration, and as a coating substance for metallic implants [20]. Given its numerous applications and variations, synthetic hydroxyapatite can be manufactured utilizing different techniques and methodologies. A variety of methods may be employed for the synthesis of synthetic hydroxyapatite, such as high-temperature processes (combustion and pyrolysis), wet methods (chemical precipitation, hydrolysis, sol-gel, hydrothermal, emulsion, and sonochemical), and dry methods (solid-state and mechanochemical) [21].

Saeri et al. [22] proposed the precipitation method due to its simplicity, with water being the sole by-product. This method involves the process of sintering, which can have a substantial influence on the morphology and size of the synthesized HA. Saeri et al. [22] also used field emission electron microscopy (FESEM), XRD, and Raman spectroscopy methods to verify and examine as well as show the morphology and size of particles that the samples produced after each step.

Azis et al. [23] conducted a similar study on the synthesis of hydroxyapatite from duck eggshells by using a similar method, which was the precipitation method. In their study, the duck eggshells were converted into precipitate calcium carbonate (PCC) by undergoing calcination, hydration, and carbonation. Then, it was added with $(NH_4)_2HPO_4$ in various Ca/P molar ratios (1.67, 1.77, 1.87) and stirring speeds (200, 250, 300 rpm) in a basic environment of pH 10-11. The most proficient conditions are the molar ratio of 1.77 and a stirring speed of 200 rpm. Then, the characteristics of the synthesized hydroxyapatite were analyzed by X-ray Diffraction (XRD).

Kareem & Eyiler (2024) [24] characterize the wet precipitation method as low cost, low operating temperature, enabling control of the morphology and the mean size of the powder, as well as not requiring an organic solvent, which is why the method was selected and used.

Regarding the feasibility, Ibrahim et al. [25] state that HA does not only offer economic benefits but also contributes to global waste management processes. They also found that HA has economic gain to be had via using HA in biodiesel production, which would reduce the cost in various departments of production.

This study focuses on the synthesis of HA utilizing three distinct organic sources: quail eggshells, oyster shells, and the periwinkle snail shells taxonomically classified as Filopaludina bengalensis. The research methodology employs a wet precipitation technique for HA production due to its balance of simplicity, cost-effectiveness, environmental friendliness, the production of high-quality HA, and profound scalability for potential industrial-scale production, encompassing a series of processes from initial shell preparation to final product obtainment [26]. The primary objective of this study is to synthesize and conduct a comparative analysis of HA derived from these three shell types, evaluating their respective compositions and properties in relation to human osseous tissue. By ascertaining which shell source yields HA most analogous to human bone, this research endeavor contributes to potential advancements in the medical industry, with particular emphasis on the field of biomaterials. This study investigates the synthesis of HA from the three understudied materials, proposing potential uses for these materials which contain abundant availability and high cost-effectiveness while maintaining the

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essential properties of high crystallinity and biocompatibility, as alternatives compared to the conventional methods. Moreover, this study explores the viability of repurposing food waste byproducts in Thailand, specifically concentrating on shells from consumed aquatic species. This approach not only addresses waste management concerns but also aligns with sustainable practices in the Thai food industry. Through comprehensive analysis and comparison, this research aims to enhance the current understanding of biomaterial synthesis while concurrently promoting environmental sustainability. The findings may potentially facilitate innovative applications in medical science and offer valuable insights into the efficient utilization of organic waste materials within the context of Thailand's unique ecological and industrial landscape.

This study offers a novel approach and valuable contribution to the field of hydroxyapatite synthesis by utilizing three specific types of shells (quail eggshells, oyster shells, and periwinkle snail shells) studied together. The combination of these shells has not been extensively explored in research before, providing a new perspective on hydroxyapatite synthesis and valorizing shell waste into usable biomaterials for potential biomedical applications with low-cost and accessible materials. Moreover, this research contributes to the growing knowledge of quail eggshells, oyster shells, and periwinkle snail shells, highlighting their potential usability in hydroxyapatite synthesis.

MATERIALS AND METHODS

Materials

Quail eggshell, oyster shell, and periwinkle snail shell waste were procured from a supermarket in Bangkok, Khlong Toei market, as shown in Figure 1. Other materials also include distilled water, laboratory-grade H_3PO_4 from RCI Labscan Limited, laboratory-grade ethanol (95%), weighing machine, VELP AREC T. heating magnetic stirrer, SH-2 magnetic stirrer, Whatman filter paper No. 1, AS29 Oilless vacuum pump, and calcination furnace.

(a) (b) (c)

Figure 1 (a) Quail eggshell, (b) oyster shell, (c) periwinkle snail shell.

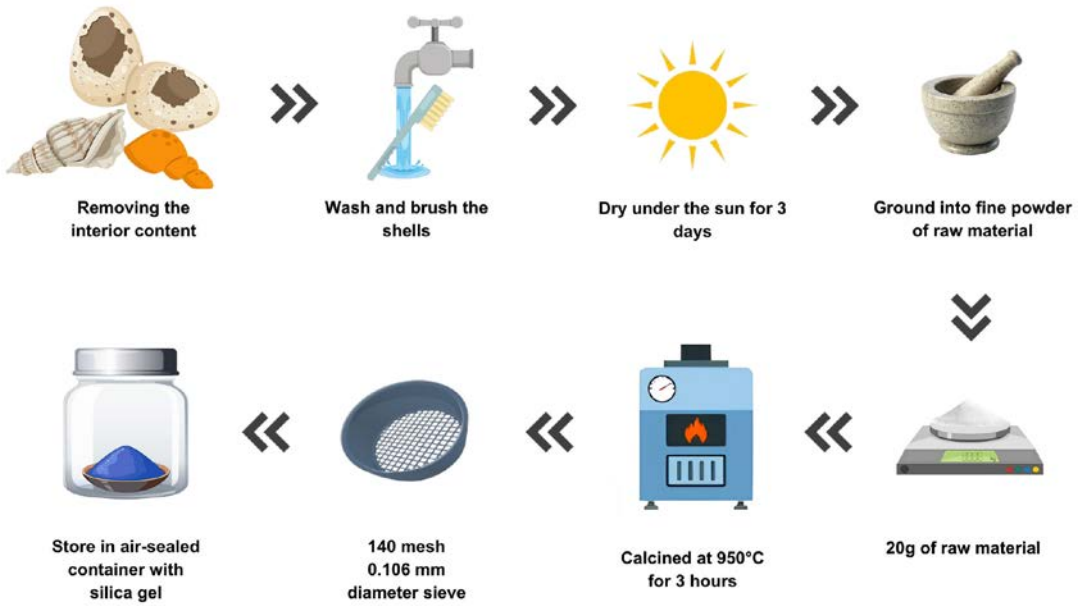

Figure 2 Diagram of preparation of CaO Precursor.

CaO precursor preparation

Prior to all the steps that require operating on the laboratory bench or table, the surfaces of the operating areas were sterilized with ethanol. Figure 2. shows the process of preparing CaO precursor, initiating with the contents of quail eggshells, periwinkle snail shells, and oyster shells being extracted. The shells were then thoroughly washed, brushed, and cleansed of dirt and membrane. These three shell materials were subsequently sun-dried for 3 days. After drying, the shells were ground into 20 g of fine powder of raw material using a mortar and were calcined in a furnace at 950°C for 3 hours. This sintering process transformed the raw material powder with $CaCO_3$ from the shell waste into CaO, serving as a calcium precursor, as represented by Eq. (1):

$$CaCO_{3(s)} + heat \rightarrow CaO_{(s)} + CO_{2(g)} \quad (1)$$

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The samples were then sieved through a 140-mesh screen with an aperture of 0.106 mm. Finally, they were weighed and stored in a container with silica gel to absorb moisture and prevent phase alteration of the substance.

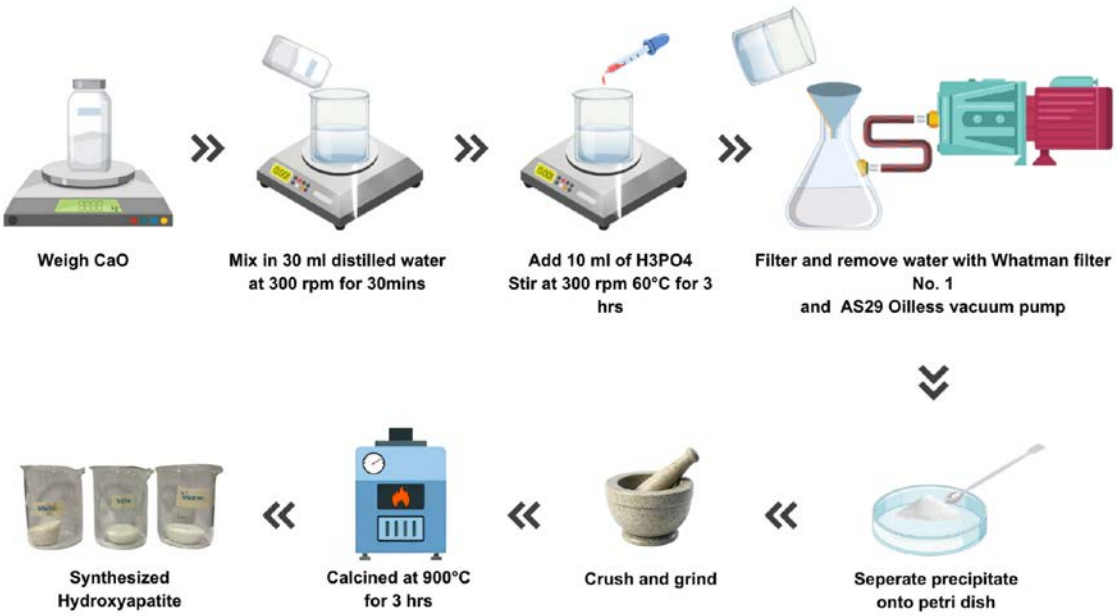

Figure 3 Illustration of hydroxyapatite synthesis process.

Synthesis of hydroxyapatite

Figure 3 demonstrates the process of Then, the CaO precursor, approximately 10 g, was weighed in the laboratory using an analytical balance. Each sample was then mixed with 30 mL of distilled water and stirred using a magnetic stirrer for 30 minutes at 300 rpm. The CaO reacted with distilled water to form calcium hydroxide, $Ca(OH)_2$, according to Eq. (2):

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

The precipitation method was conducted by adding 10 mL of H_3PO_4 to each solution of $Ca(OH)_2$. Each mixture was then stirred at 300 rpm and 60°C for 3 hours. Its pH was measured with litmus paper. The reaction can be represented by Eq. (3):

$$10Ca(OH)_{2(aq)} + 6H_3PO_{4(l)} \rightarrow Ca_{10}(PO_4)_6(OH)_{2(s)} + 18H_2O_{(l)} \quad (3)$$

After cooling, the solution was filtered using airtight connection to a flask. The flask was connected to an AS29 oil-free vacuum pump to remove the water content, leaving hydroxyapatite as the residue or the precipitate. The HA precipitate was carefully removed from the filter paper onto a petri dish, and it was crushed and grinded into fine powder before being sintered in a furnace at the temperature of 900°C for 3 hours.

Characterization of hydroxyapatite

The crystal structure of hydroxyapatite powder was characterized by X-ray diffraction, as 1 g of HA samples from the three types of shell were analyzed by the EMPYREAN PANalytical X-ray diffractometer - SC with the XRD patterns recorded in the 2θ range 5° - 90°.

RESULTS AND DISCUSSION

Calculate the amount yielded according to calculations made

Before commencing the laboratory experiment, chemical equilibrium equations were developed to determine the theoretical quantities of reactants ($CaCO_3$), intermediates (CaO and $Ca(OH)_2$), and the expected yield of the product (HA). In this study, the process began by preparing 20 g of crushed, fine powder of the raw material of three different shell types: quail eggshells, oyster shells, and periwinkle snail shells. However, the raw material of the three shell types contains approximately 96% calcium carbonate ($CaCO_3$) [9, 11, 13]. Thus, 20 g of raw material is equivalent to 19.2 g of $CaCO_3$, which represents the actual starting material for the whole process of the synthesis of HA. In each step of the chemical reactions, the theoretical yield of the producing essential compounds (CaO, $Ca(OH)_2$, and $Ca_{10}(PO_4)_6(OH)_2$) will be calculated and elucidated.

Decomposition of calcium carbonate ($CaCO_3$)

The process began with the calcination of eggshell, oyster shell, and periwinkle snail shell to eliminate organic compounds from the shell waste and to convert calcium carbonate ($CaCO_3$) to calcium oxide (CaO). The molar mass of $CaCO_3$ is 100 g/mol, while the molar mass of CaO is 56 g/mol. According to Eq.

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(4), the moles of a compound are defined by the mass of the compound divided by the molar mass of the compound. Since the process in this study starts with 19.2 g of $CaCO_3$, to calculate the moles of $CaCO_3$, divide 19.2 g of $CaCO_3$ by 100 g/mol of $CaCO_3$, which equals 0.192 mol of $CaCO_3$.

$$n\ (moles) = \frac{m\ (mass)}{M\ (molar\ mass)} \quad (4)$$

According to Eq. (1), the reaction shows a 1:1 stoichiometric ratio between $CaCO_3$ and CaO; therefore, 0.192 mol of $CaCO_3$ produces 0.192 mol CaO. According to Eq. (5), the mass of the substance can be calculated by multiplying the moles of the compound by the molar mass of the compound. To calculate the mass of CaO produced, multiply 0.192 mol CaO by 56 g/mol of CaO, which equals 10.75 g of CaO. In short, 19.2 g of $CaCO_3$ will theoretically yield 10.75 g of CaO from the decomposition of $CaCO_3$ during the calcination process.

$$m\ (mass) = n(moles) \times M(molar\ mass) \quad (5)$$

In this stage, the percent yield of CaO from the sintering process at the temperature of 950°C for 3 hours is shown in Table 1. The major changes in this process include the color transformation and mass reduction. For the former occurrence, there are color changes of the quail eggshell from brown to white, oyster shells from gray to white, and periwinkle shells from brown to cream white, in which all of the color alterations represent the organic decomposition in each sample. For the latter occurrence, it is apparent that the results of the production of CaO from the calcination process is relatively efficient, as the percent yield of CaO for quail eggshell, oyster shell, and periwinkle shell were 98.6, 103.72 and 92.09 percent which is calculated from the following Eq. (6).

$$Percent\ yield = \frac{Experimental\ mass}{Theoretical\ mass} \times 100 \quad (6)$$

The slight mass reduction can be due to the release of carbon and organic compounds during the sintering process, which causes the compounds to shrink [27]. The CaO production from oyster shells has the highest percent yield as a result of the oyster's prominent crystalline structures and the natural shell's content of $CaCO_3$ [28, 29]. However, the mass of CaO and thus the experimental yield of CaO from oyster shells are slightly higher than the theoretical yield. The excessive amount of such can be due to the rare impurities presented in the oyster shells, which were not removed during the calcination. As a result, the resulting mass of the sintered CaO also contains the mass of other compounds, which suggests that higher temperatures and periods of calcination, such as 1000°C for 10 h or 1200°C for 1 h, are required [30]. Due to the limitation of the capability of the calcinating machine, which reaches the highest temperature of only 950°C, the impurities remain, which causes the excessive mass and experimental yield of CaO from the oyster shells. Another explanation is that there were experimental errors, such as the inaccuracy of the weighing instrument, which led to plausibly false data and abnormal results. Nonetheless, these CaO compounds will be the fundamental materials for producing hydroxyapatite compounds via the precipitation method.

Hydration of calcium oxide (CaO)

In this reaction, the formation of calcium hydroxide ($Ca(OH)_2$) occurs from the dissolution of calcium oxide (CaO) in 30 ml distilled water (H_2O) and stirred at 300 rpm for 30 minutes. CaO was the limiting reactant, while distilled water is the excess reactant.

According to Eq. (2), the reaction shows a 1:1 stoichiometric ratio between CaO and $Ca(OH)_2$, therefore, 0.192 mol CaO produces 0.192 mol $Ca(OH)_2$. The molar mass of $Ca(OH)_2$ is 74 g/mol. Using Eq. (5), the mass of $Ca(OH)_2$ produced is calculated by multiplying 0.192 mol of $Ca(OH)_2$ by 74 g/mol of $Ca(OH)_2$, which equals 14.21 g of $Ca(OH)_2$. This indicates that the mixture of 10.75 g of CaO, previously produced from the decomposition of 19.2 g of $CaCO_3$, and 30 ml distilled water will subsequently yield 14.21 g of $Ca(OH)_2$.

Synthesis of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$)

In the final stage of the synthesis of hydroxyapatite, the hydroxyapatite compound is the product of the precipitation method between $Ca(OH)_2$ and phosphoric acid, a phosphorus precursor selected because of its cost-effectiveness with the production of water as the sole by-product of the reaction, with a mixing duration of 3 hours.

According to Eq. (3), the stoichiometry indicates that 10 mol of $Ca(OH)_2$ react with 6 mol of H_3PO_4 to produce 1 mol of HA and 18 mol of H_2O. Stoichiometrically, if 10 mol of $Ca(OH)_2$ requires 6 mol of H_3PO_4, then 0.115 mol of H_3PO_4 should be used, as 0.192 mol of $Ca(OH)_2$ is cross multiplied with 6 mol of H_3PO_4 and divided by 10 mol of $Ca(OH)_2$. Therefore, 11.27 g of H_3PO_4 (calculated from 0.115 mol H_3PO_4 × 98 g/mol H_3PO_4) or 6.98 ml of H_3PO_4 (the density of the H_3PO_4 used in this study is 1.685 g/ml) should be used. However, as $Ca(OH)_2$ is designated as the limiting reactant while H_3PO_4 is designated as the excess reactant, the amount of H_3PO_4 used in this study is 10 ml, which does not affect the result or the property of the synthesized HA as the pH level is being regulated and the solution remained basic, as confirmed by using litmus paper, despite the addition of the excess H_3PO_4.

Onto the calculation of HA theoretical yield. Firstly, moles of HA must be determined. According to Eq. (3), the reaction shows a 10:1 stoichiometric ratio between $Ca(OH)_2$ and HA; therefore, 0.192 mol of $Ca(OH)_2$ produces 0.0192 mol of HA, calculated from cross-multiplying 0.192 mol $Ca(OH)_2$ with 1 mol HA and being divided by 10 mol $Ca(OH)_2$. As the molar

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mass of HA ($Ca_{10}(PO_4)_6(OH)_2$) is 1004 g/mol, using Eq. (5), the mass of HA produced can be determined by multiplying 0.0192 mol HA by 1004 g/mol HA, which equals 19.28 g of HA. Ultimately, this indicates that the mixture of previously produced 14.21 g $Ca(OH)_2$ and 10 ml H_3PO_4 will theoretically yield 19.28 g of HA, which originated from the initial substance of 20 g of raw material of the shell waste or 19.2 g of $CaCO_3$.

Table 1 demonstrates the quantified relationship between the final product of the HA solid compound, the initial starting compound of $CaCO_3$, and the intermediate compound of CaO for better effectiveness of comparison of different masses of products, as well as the percent yield of the resulting HA compounds.

Comparing the experimental yield of HA to the theoretical yield of HA, it is perceptible that there are losses of compound mass in the process of hydration and synthesis of HA. For quail eggshell and periwinkle shell, the percent yield of HA is slightly lower than the percent yield of CaO, signifying that there is slight weight loss during the transformation from CaO to Ca(OH)2 or the transformation from Ca(OH)2 to HA, in which the slight reduction will be explained later on. More importantly, for oyster shells, the percent yield of HA is markedly lower than its percent yield of CaO, signifying a lot more weight loss compared to the other two groups. This is due to the difference in the crystallinity in the shell types and the calcium-deficient structure in oyster shell composition [31]. To elucidate, the crystal structure of calcium carbonate ($CaCO_3$) in the three shell types has two main crystalline forms: calcite and aragonite. The shells of periwinkle snail quail eggs contain only 10% or less aragonite and 90%, and their main crystal structure is 90% or almost pure calcite in periwinkle snail shells and quail eggshells, respectively [11, 32]. Meanwhile, the composition of the crystal structure of $CaCO_3$ in the oyster shells is different, as it contains 72.3% calcite and 27.7% aragonite [33]. Aragonite is less stable and more soluble compared to calcite, in which its higher solubility and less ordered structure, when mixed with distilled water during the hydration and H_3PO_4 during the synthesis, can contribute to a calcium-deficient structure. As the composition of the oyster shells contains more aragonite, the larger portion of the crystalline structure in the oyster shell group with higher solubility and less stability can immensely affect and reduce the final mass and experimental yield of the synthesized HA product from the oyster shells, compared to the periwinkle snail shells and egg shells [34, 35].

Moreover, the weight loss in all shell types can be caused by the loss of HA compounds during the process of separating, grinding, and purifying HA precipitates after they were filtered. Nevertheless, the efficiency of production of HA compounds for all types of shell is greater than 50%, which marks the valuable success of the hydroxyapatite synthesis. [36, 37].

Table 1 Mass and percentage yield of CaO and HA from different $CaCO_3$ sources.

Sample Type	Mass (g)			Percent Yield	
	$CaCO_3$	CaO	HA	CaO	HA
Theoretical Value	19.2	10.75	19.28	100	100
Quail eggshell	19.2	10.6	17.74	98.6	92.01
Oyster snail shell	19.2	11.15	14.2	103.72	73.65
Periwinkle shell	19.2	9.9	17.76	92.09	92.12

Overall, the entire process of the production of synthesized hydroxyapatite is derived from the initial compound of $CaCO_3$. For all types of shells, the initial quantity was 20 g of raw material or 19.2 g of $CaCO_3$, and the theoretical yield of synthesized HA compound was 19.28 g. The experimental yield of HA compounds was 17.74 g for quail eggshell, 14.20 g for oyster shell, and 17.76 g for periwinkle shell, as represented in Table 1. Regarding percent yield of HA, calculated from Eq. (6), the periwinkle shell had the highest percentage yield of 92.12%, followed by the quail eggshell with a slightly lower percent yield of 92.01%; meanwhile, the oyster shell had a considerably lower percent yield of 73.65% compared to the other two sample types. Quail eggshell and oyster snail shell have marginally lower percent yield compared to the experimental yield, while CaO has drastically lower percent yield. The lower experimental yield of all samples was affected by the experimental errors during different processes of separation and purification of the solutions, where $CaCO_3$, CaO, and HA compounds may be lost throughout various procedures, mainly during the separation of HA precipitate from the filter paper, as accumulation of leftover HA compound on the filter paper can account for the considerable amount of weight loss and yield difference.

In addition, the reasons for the dramatic difference in percent yield in CaO are possibly due to similar reasons as to the weight loss and HA production efficiency from various CaO sample sources [27, 36].

Thermal analysis

Figure 4 reveals the exothermic nature of the reactions involving oyster shells, periwinkle snail shells, and quail eggshells during the synthesis process. These images provide valuable insights into the heat

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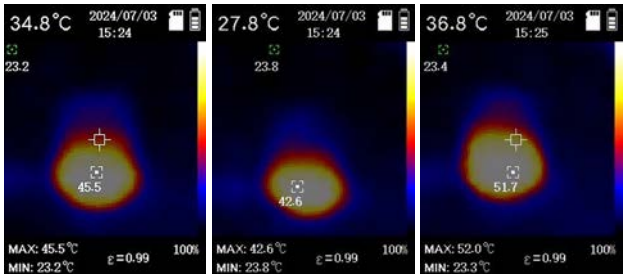
release patterns and maximum temperatures reached for each material. Oyster shells exhibited the lowest peak temperature (42.6°C), suggesting a more moderate heat release during the reaction. Quail eggshells showed an intermediate thermal profile, reaching a maximum of 45.5°C. Periwinkle snails demonstrated the highest exothermic activity, with temperatures peaking at 51.7°C.

The varying thermal profiles can be attributed to differences in the chemical composition and structural characteristics of each shell type. Oyster shells, primarily composed of calcium carbonate in the form of calcite, tend to have a more stable crystal structure, which may contribute to their lower heat release [37]. In contrast, quail eggshells, while also rich in calcium carbonate, typically contain a higher proportion of organic matter and a more porous structure, potentially leading to more rapid decomposition and higher heat generation during the reaction [38].

The higher exothermic activity observed in periwinkle snail shells could result in faster reaction rates and potentially affect the crystallinity and particle size of the resulting hydroxyapatite. This increased thermal energy might promote better crystallization and could influence the final product's properties [39].



(a) (b) (c)

Figure 4 Thermal images showing the exothermic reaction during hydroxyapatite synthesis from different shell sources: (a) quail eggshell, (b) oyster shell, and (c) periwinkle shell.

XRD characterization

Figure 5 illustrates the XRD patterns of the calcium oxide (CaO) samples, which were gathered from calcined quail eggshells, oyster shells, and periwinkle snail shells. Typical characteristic peaks of CaO can be observed at 2θ values of approximately 37.4° and 53.8°, and all three samples exhibit pronounced peaks at these positions. The calcination process was conducted at 950°C for 3 hours and successfully converted $CaCO_3$ to CaO across all shell types. Subtle variations in peak intensities among the three sources suggest slight differences in crystallinity or purity.

The characterization of the CaO samples closely aligns with the findings and results reported by Meshkatalsadat (2023) [40], who observed diffraction peaks (2θ) at approximately 32°, 37°, 53°, 64°, and 67°. This correlation further confirms the successful conversion of $CaCO_3$ to CaO during the calcination process.

In a similar study, Rujitanapanich et al. (2014) [41] reported XRD patterns for CaO obtained from oyster shells after being calcined at 1200°C for 2 hours. Their analysis revealed prominent CaO peaks at 37.4° and 53.8°, which closely correspond to the findings of the present study. This consistency across different research efforts highlights the reliability of the calcination method employed in this investigation for producing high-quality CaO precursors from various shell sources.

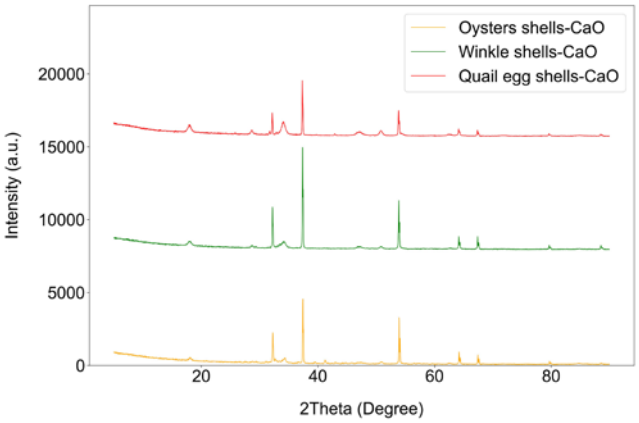


Figure 5 XRD patterns of CaO precursors.

Figure 6 illustrates the XRD patterns of HA synthesized from three distinct sources: quail eggshells, oyster shells, and periwinkle snail shells. Prominent HA peaks can be seen at 2θ values of approximately 25.9°, 31.8°, 32.2°, 32.9°, 34.1°, 39.8°, 46.7°, and 49.5°. The intensity peak of the XRD diffraction pattern of the synthesized HA from the three shell types is compared and closely resembles the standard XRD pattern of HA based on ICDD 9-432, which authenticates the high crystallinity and phase purity of the synthesized HA. The well-defined nature and high intensity of these peaks are indicative of the synthesized HA's excellent crystallinity. Moreover, the absence of significant extraneous peaks suggests a high degree of phase purity in the synthesized HA samples. These results demonstrate the successful synthesis of high-quality HA from the three different shell sources, with diffraction patterns that closely align with established standards for pure HA. The confirmation of the successful and high quality of the synthesized HA also suggests excellent biocompatibility and bioactivity, which are crucial for successful integration with human bone tissue. The HA obtained from the three shell sources closely mimics the mineral composition of natural bone, verifying that they contain the properties of osteoconductive and possible cell attachment, which are essential for bone regeneration applications. Moreover, the high crystallinity and purity of the synthesized HA ensure that it maintains structural integrity for supporting new bone growth, making it highly suitable and useful for usage in orthopedic and dental applications, in which osseointegration is important. This enhances the

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material's clinical performance in bone grafts and implants, highlighting its suitability for medical applications where bone regeneration is required [42].

Regarding the difference in the degree of crystallinity, the difference could be attributed to many different factors in the process that we used in the synthesis of HA. The difference in raw material is one possible explanation that could influence the crystallinity due to the variations in the mineral composition's organic content as well as impurities found in the raw material. Another possibility could be attributed to the conditions under which the HA was synthesized as well as post-synthesis treatment with factors such as temperature, pH, and concentration of reactants possibly affecting the crystallinity.

In a similar study, Shahabi et al. (2014) [43] reported that the standard XRD pattern based on ICDD 9-432 closely matches the XRD pattern of their HA samples, which also closely match the XRD pattern of the synthesized HA samples for further confirmation of the successful synthesis.

Venkatesan and Kim (2010) [44] investigated HA synthesis from fish bones. Their XRD results also revealed typical HA diffraction patterns similar to those observed in the present study, further corroborating the successful synthesis of HA from marine biowaste materials.

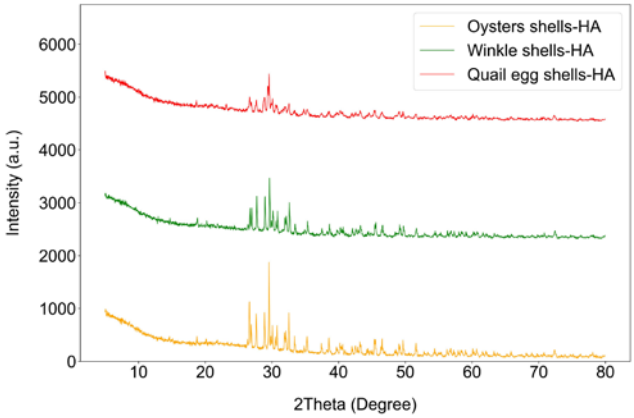

Figure 6 XRD patterns of synthesized hydroxyapatite (HA).

CONCLUSIONS

This study successfully produces synthesized HA from quail eggshells, oyster shells, and periwinkle snail shells using a wet precipitation technique, with periwinkle shell containing the highest percentage yield of producing HA compounds of 92.12%, followed by quail eggshell at 92.01%, respectively.

The oyster shell has a relatively lower percentage yield of 73.65% due to weight loss during the procedure of separation and purification of the HA compound as well as a crystallinity difference, particularly the greater prevalence of aragonite in the oyster shells. The XRD results for both CaO precursors and synthesized HA aligned with previous studies, which verifies the reliability and credibility of the study's methodology. As the CaO precursors showed characteristic peaks at 2θ values of approximately 37.4° and 53.8°, they confirm the successful transformation of calcium carbonate to calcium oxide. Moreover, the synthesized HA compound revealed peaks at 2θ values of approximately 25.9°, 31.8°, 32.2°, 32.9°, 34.1°, 39.8°, 46.7°, and 49.5°, which indicate the high properties of crystallinity and purity of the HA compound.

The successful synthesis of high-quality HA from these shell sources demonstrates high crystallinity, phase purity, excellent biocompatibility, bioactivity, and osteoconductive property of the synthesized HA, which are essential for integration with human body tissues, foundation of bone growth, and bone regeneration applications. This suggests its suitability for orthopedic, dental, and medical applications due to its clinical effectiveness in bone grafts and implants.

Additionally, the environmentally friendly approach of repurposing waste shells aligns with the growing emphasis on sustainability in healthcare, potentially influencing the next generation of biomaterial development. The findings of this study open up the possibilities and pathways for further research into tailored HA composites, promoting innovations that improve patient outcomes while reducing environmental impact, thus bridging the gap between sustainable practices and advanced medical technologies.

The findings contribute to the potential development of recycling food waste in Thailand while addressing the waste management issues and aligning with sustainable practices in the food industry.

Future research could focus on optimizing large-scale production, exploring additional applications, and investigating the economic feasibility for industrial implementation. In the medical field, further studies could address the development of advanced, sustainable biomaterials or products such as bone scaffolds and coatings for medical implants to maximize the cost-effectiveness and environmental benefits of HA synthesized from shell waste.

Several key areas can be explored in future analysis to enhance the characterization of hydroxyapatite (HA) synthesized from quail eggs, oyster shells, and periwinkle shells. We could evaluate the mechanical properties, including compressive strength and fracture toughness, to ensure suitability for load-bearing applications. Biocompatibility could be assessed through in vitro cell viability assays and in vivo studies. Surface characterization could be conducted using SEM and AFM to analyze morphology and porosity. Chemical composition and purity could be verified with FTIR and ICP-OES, focusing on the Ca/P ratio and impurities. Thermal stability could be examined with TGA and DSC, while long-term stability and degradation could be tested in simulated body fluids. Additionally, process

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optimization and cost analysis could be performed to facilitate large-scale production. Comparative studies with commercial HA and exploration of alternative synthesis methods could also be undertaken to benchmark performance and refine material properties.

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