



Physicocemical and Microbiological Characterization of Hydroxyapatite Based Toothpaste From Freshwater Mussel (*Pilsbryconcha exilis*) Shell

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Abstract: Utilizing freshwater mussel shell waste as a sustainable source of calcium-based biomaterials represents an eco-friendly approach to producing hydroxyapatite for oral care applications. Hydroxyapatite acts as an active component in toothpaste formulations, functioning as an abrasive and inhibiting bacterial growth responsible for dental plaque. This study aimed to evaluate the effect of adding hydroxyapatite to toothpaste on its physicochemical and microbiological characteristics and to determine the best formulation of freshwater mussel shell-based hydroxyapatite toothpaste. The research included hydroxyapatite synthesis, toothpaste production, and evaluation of physicochemical and microbiological properties. An experimental method using a completely randomized design was employed, consisting of four treatments: TH35 (35% hydroxyapatite), TH45 (45% hydroxyapatite), TH55 (55% hydroxyapatite), and TK (commercial toothpaste). The results showed that the addition of hydroxyapatite significantly affected the physicochemical characteristics (color, aroma, texture, homogeneity, spreadability, pH, and foam height) and microbiological activity (inhibition zone against *Staphylococcus aureus*) at a 95% confidence level ($p < 0.001$). The best formulation, based on pH, calcium content, and bacterial inhibition zone, was obtained with 55% hydroxyapatite, having a pH of 7.39, calcium content of 15.97%, and an inhibition zone of 22.24 mm against *Staphylococcus aureus*. These findings demonstrate that mussel shell-derived hydroxyapatite can serve as a sustainable and functional ingredient in toothpaste formulations with enhanced antibacterial and physicochemical performance.

Introduction

Freshwater mussels (*Pilsbryconcha exilis*) are a type of shellfish from the mollusc phylum commonly found in freshwater environments such as lakes, rivers, and aquaculture ponds (1, 2). These shellfish can tolerate a wide temperature range (11–29°C) and pH values between 4.8–9.8 (3). In Riau Province, this species is widely distributed and harvested, contributing to a total shellfish production of 64,355.54 tons in 2021(4). However, despite this substantial production, the effective utilization of shell waste remains limited, as most people perceive mussels only as a food source. Shells account for approximately 51.93% of total shellfish biomass, leading to considerable shell waste accumulation that poses environmental challenges if not sustainably managed (3, 5).

In recent years, shell-derived biomaterials have gained significant attention as part of sustainable material science initiatives aimed at converting biowaste into high-value products (6, 7). *Pilsbryconcha exilis* shells, in particular, have great potential as calcium precursors for synthesizing

hydroxyapatite (HAp) due to their high calcium content (61.39%) (8). Compared to marine shells such as oyster or snail shells, which typically exhibit Ca/P ratios between 1.6–1.8, *P. exilis* shows a favorable Ca/P ratio closer to the stoichiometric HAp value (1.67), suggesting suitability as a biomaterial source (1).

Hydroxyapatite is a calcium phosphate compound with the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ that exhibits excellent biocompatibility and bioactivity, making it ideal for applications in bone and dental tissue engineering (9, 10). Biomedical-grade HAp mimics the mineral composition of human hard tissues and has been reported to support remineralization and antibacterial functions when incorporated into oral care formulations (11, 12). Thus, developing HAp from freshwater mussel shells not only contributes to environmental sustainability through biowaste valorization but also offers a renewable source of functional biomaterials for dental applications.

Previous studies have shown that hydroxyapatite synthesized from *P. exilis* shells contains high levels of calcium (48.26%), phosphorus (1.47%), and potassium

(0.009%) (1). Its antibacterial performance has been demonstrated against *Escherichia coli* and *Pseudomonas aeruginosa*, with inhibition zones of 3.77 mm and 5.04 mm, respectively, at a concentration of 50 mg/mL (13, 14). Similarly, HAP derived from snail shells showed a 5 mm inhibition zone against *Staphylococcus aureus* (15). These findings suggest that the antibacterial activity of HAP could be influenced by its concentration and physicochemical properties such as particle size, surface area, and crystallinity.

However, limited studies have examined the correlation between the concentration of freshwater mussel shell-derived hydroxyapatite and its antibacterial or physicochemical performance in toothpaste formulations. Therefore, this study aims to evaluate the potential of *Pilsbryconcha exilis* shell-derived hydroxyapatite as an active ingredient in toothpaste by investigating its antibacterial activity and physicochemical properties at varying concentrations. It is hypothesized that higher concentrations of hydroxyapatite will enhance both antibacterial efficacy and physicochemical characteristics, thus improving its potential as a sustainable biomaterial for dental applications.

Experimental Section

Materials

The materials in this study were mussel shells (*Pilsbryconcha exilis*) obtained from Sungai Paku Village, Kampar Kiri District, Kampar Regency, ammonium dihydrogen phosphate ((NH₄)H₂PO₄) (HIMEDIA), commercial toothpaste (sensitive), and toothpaste formulation materials consisting of glycerin (ONEMED), Na CMC (Raja Kimia), SLS (KAO Japan), sodium benzoate (C₇H₅NaO₂) (Puroxs Grains), saccharin (Sosweet SP), menthol (Polar Bear Brand EX PRC), titanium dioxide, phosphoric acid, and distilled water (Aqua SciencΣ).

Hydroxyapatite Production

The mussel shells were crushed, ground using a grinder, and sieved through a 100-mesh sieve. The resulting shell powder was calcined at 1000 °C for 6 h with a heating rate of 10 °C/min to obtain CaO powder, which was then cooled in a desiccator for 1 hour.

Hydroxyapatite was synthesized by reacting calcium oxide and phosphate precursors. A 1 M CaO solution (as the calcium source) was reacted with a 0.6 M (NH₄)H₂PO₄ solution (as the phosphate source) while maintaining a Ca/P molar ratio of 1.67, corresponding to stoichiometric hydroxyapatite. The CaO solution was heated at 90 °C for 1 hour on a hot plate under continuous magnetic stirring, while the (NH₄)H₂PO₄ solution was added dropwise. The mixture was aged for 18 h, followed by centrifugation at 4500 rpm for 15 min to obtain the precipitate. The precipitate was dried in an oven at 65 °C for 72 h, ground using a mortar, and calcined again at 1000 °C for 2 h before being cooled in a desiccator for 1 hour.

To confirm that hydroxyapatite was successfully formed, characterization was conducted using X-ray diffraction (XRD) to identify the crystalline phase, Fourier-transform infrared spectroscopy (FTIR) to verify functional groups (PO₄³⁻, OH⁻), and scanning electron microscopy (SEM) to examine surface morphology and particle size.

Each synthesis was performed in triplicate (n = 3) to ensure reproducibility.

Table 1. Formulation and functional components of hydroxyapatite-based toothpaste treatments.

Material	Function	Treatments			
		T _{H35}	T _{H45}	T _{H55}	T _K
Hydroxyapatite (g)	Abrasive	35	45	55	Commercial toothpaste
Glycerin (mL)	Humectant	25	25	25	
Na CMC (g)	Paste base	1.5	1.5	1.5	
SLS (g)	Surfactant	1	1	1	
Natrium benzoate (g)	Preservative	0.1	0.1	0.1	
Saccharin (g)	Sweetener	0.4	0.4	0.4	
Menthol (g)	Fragrance	0.4	0.4	0.4	
Titanium dioxide (g)	Bleach	6	6	6	Commercial toothpaste
Distilled water add (mL)	Solvent	100	100	100	

Note: T_{H35} (35% hydroxyapatite from freshwater mussel shells), T_{H45} (45% hydroxyapatite from freshwater mussel shells), T_{H55} (55% hydroxyapatite from freshwater mussel shells), T_K (commercial toothpaste)

Toothpaste Production

Hydroxyapatite from *P. exilis* was incorporated into toothpaste formulations at concentrations of 35%, 45%, and 55% (w/w). These concentration ranges were chosen based on preliminary results indicating optimal texture and antibacterial performance between these values, while higher concentrations (>55%) led to unstable paste consistency and reduced spreadability.

Na CMC was dissolved in hot water and left to stand for 15 min (Mass 1). Glycerin and sodium benzoate were mixed separately (Mass 2). Mass 1 and Mass 2 were combined and ground to form Mass 3. Hydroxyapatite pre-mixed with 50% phosphoric acid was added gradually to Mass 3 and homogenized (Mass 4). SLS, saccharin, and menthol were added to Mass 4 (Mass 5), and finally titanium dioxide was incorporated to form a homogeneous toothpaste base.

The full composition and total percentage (100%) of ingredients are presented in **Table 1** to ensure reproducibility.

Physicochemical Characterization

Organoleptic Test

Thirty semi-trained panelists evaluated color, aroma, texture, and homogeneity using a 5-point hedonic scale. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test ($\alpha = 0.05$) to determine significant differences among formulations (16).

Homogeneity Test

The homogeneity of the toothpaste was assessed by applying 0.1 g of the sample onto a glass slide, followed by visual observation of its texture (17).

Spreading Power Test

A total of 0.5 g of toothpaste was placed on a glass plate, covered with another glass plate, and then subjected to a 200 g load for 1 minute. The diameter of the spread was subsequently measured (18).

pH Test

One gram of toothpaste was dissolved in 10 mL of distilled water. The pH electrode was immersed in the solution until a stable reading was obtained (19).

Foam Height Test

A 0.1 g sample of toothpaste was dissolved in 10 mL of water, shaken for 1 minute, and the resulting foam height was measured (20).

Cleaning Ability Test

Cleaning ability was evaluated using eggshells stained with food coloring. Each eggshell was brushed 5–10 times, and the removal of color was observed (Lakshmi et al., 2022) (21).

Calcium Content Test

Calcium concentration was determined using an Atomic Absorption Spectrophotometer (AAS) at 422.7 nm. A CaCl_2 standard curve (0–10 mg/L) was prepared for calibration, and results were expressed as % Ca content (22).

Microbiological Characterization

MHA medium (15–20 mL) was poured into sterile Petri dishes. After solidification, *Staphylococcus aureus* was inoculated using an inoculating loop, and wells were created with a sterile stainless-steel borer.

The wells were filled with the following:

1. Toothpaste formulations at 35%, 45%, and 55%,
2. Positive control: commercial fluoride-containing toothpaste,
3. Negative control: sterile distilled water.

The plates were incubated at 37 °C for 24 h, after which inhibition zones were measured.

Results and Discussion

Physicochemical Characteristics of Toothpaste

The physicochemical characteristics of toothpaste in this study included organoleptic assessment, homogeneity, spreadability, pH, foam height, cleaning ability, and calcium content of toothpaste (see **Figure 1**).

The analysis of variance showed that hydroxyapatite concentration significantly affected the color of the toothpaste ($F(3, 8) = 1187.11$, $p < 0.001$). The higher the hydroxyapatite concentration, the grayer the color produced, as hydroxyapatite imparts a natural grayish-white hue (23).

A significant difference was also found in aroma ($F(3, 8) = 8.38$, $p = 0.007$), although the differences among treatments were minor, likely because all formulations contained identical concentrations of menthol. The texture of toothpaste was also significantly affected ($F(3, 8) = 711.33$, $p < 0.001$), where higher hydroxyapatite concentrations resulted in a slightly rougher texture. This is attributed to the granular surface morphology of hydroxyapatite particles (24). Homogeneity differed significantly as well ($F(3, 8) = 51.30$, $p < 0.001$) as seen in **Figure 2**, with panelists preferring TH35 due to its smoother and more uniform consistency. All formulations met the SNI 12-3524-1995 standards for toothpaste homogeneity, which require that products be free from air bubbles, lumps, or separation (25).

Hydroxyapatite concentration significantly affected the spreadability of toothpaste ($F(3, 8) = 9321.90$, $p < 0.001$). All formulations were within the acceptable spreadability range (26.10–53.20 mm), indicating satisfactory ease of application and consumer usability (see **Table 2**).

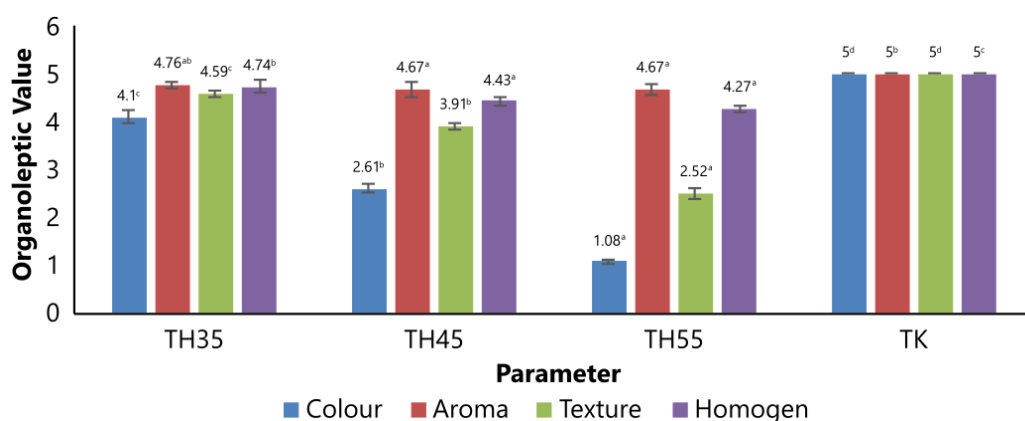


Figure 1. Organoleptic value of hydroxyapatite-based toothpaste from freshwater mussel shells. Note: TH35 = 35% hydroxyapatite, TH45 = 45% hydroxyapatite, TH55 = 55% hydroxyapatite, TK = commercial toothpaste. Different superscript letters indicate a significant statistical difference ($p < 0.05$).



Figure 2. Homogeneity of hydroxyapatite-based toothpaste from freshwater mussel shells. Note: TH35 = 35% hydroxyapatite, TH45 = 45% hydroxyapatite, TH55 = 55% hydroxyapatite, TK = commercial toothpaste.

Table 2. Spreadability, pH, foam height, and cleaning ability of hydroxyapatite-based toothpaste from freshwater mussel shells.

Treatment	Spread power (mm)	pH	Foam height (mm)	Cleaning ability
T _{H35}	35.48±0.08 ^c	4.47±0.16 ^a	12.51±0.19 ^c	Good
T _{H45}	30.78±0.19 ^b	6.50±0.02 ^b	5.62±0.06 ^b	Good
T _{H55}	26.07±0.11 ^a	7.39±0.03 ^c	4.92±0.11 ^a	Good
T _K	43.36±0.14 ^d	8.08±0.03 ^d	35.13±0.05 ^d	Good

Note: Different superscript letters indicate a significant statistical difference ($p < 0.05$). TH35 = 35% hydroxyapatite, TH45 = 45% hydroxyapatite, TH55 = 55% hydroxyapatite, TK = commercial toothpaste.

Table 3. Calcium content of hydroxyapatite-based toothpaste from freshwater mussel shells.

Treatment	Calcium content (%)
T _{H35}	7.58
T _{H45}	11.42
T _{H55}	15.97
T _K	1.05

Note: TH35 = 35% hydroxyapatite, TH45 = 45% hydroxyapatite, TH55 = 55% hydroxyapatite, TK = commercial toothpaste.

A significant effect was also observed for pH ($F(3, 8) = 1174.40$, $p < 0.001$). TH35 exhibited an acidic pH (4.47), slightly below the SNI standard (4.5–10.5), while TH45, TH55, and TK were within the acceptable range. This variation can be explained by the basicity of residual calcium oxide (CaO) present in hydroxyapatite. At higher hydroxyapatite concentrations, traces of unreacted CaO increase alkalinity, thereby elevating the pH of the toothpaste.

Foam height was significantly affected as well ($F(3, 8) = 43633.70$, $p < 0.001$). Although all formulations met market standards (≤ 15 mm), TK (commercial toothpaste) exhibited a much higher foam height (35.13 mm) due to higher surfactant levels such as SLS.

All toothpastes demonstrated good cleaning ability. The abrasive properties of hydroxyapatite facilitated effective

removal of surface stains from eggshells without damaging their structure, consistent with its recognized cleaning and polishing function

The calcium content of toothpaste increased proportionally with the concentration of hydroxyapatite (see **Table 3**). The highest calcium level was observed in TH55 (15.97%), while the lowest was in TK (1.05%). The high calcium value in TH55 reflects the substantial calcium content in freshwater mussel shell hydroxyapatite (48.26%) (26).

Microbiological Characterization of Toothpaste

Hydroxyapatite concentration had a significant effect on antibacterial activity ($F(3, 8) = 214.52$, $p < 0.001$). Increasing hydroxyapatite concentration enhanced inhibition zone diameter, with TH35 classified as moderate, TH45 as strong, and TH55 as very strong inhibition levels (see **Figure 3** and **Table 4**).

The antibacterial mechanism of hydroxyapatite involves multiple processes. Beyond Ca^{2+} and Na^+ ion diffusion, antibacterial activity is also influenced by surface charge interactions and ionic exchange between HAp and bacterial membranes (27). The negatively charged phosphate and hydroxyl groups on hydroxyapatite adsorb onto the positively charged bacterial cell surface, disturbing membrane integrity, permeability, and ionic balance. This mechanism has been supported by recent studies on nano-hydroxyapatite (28).

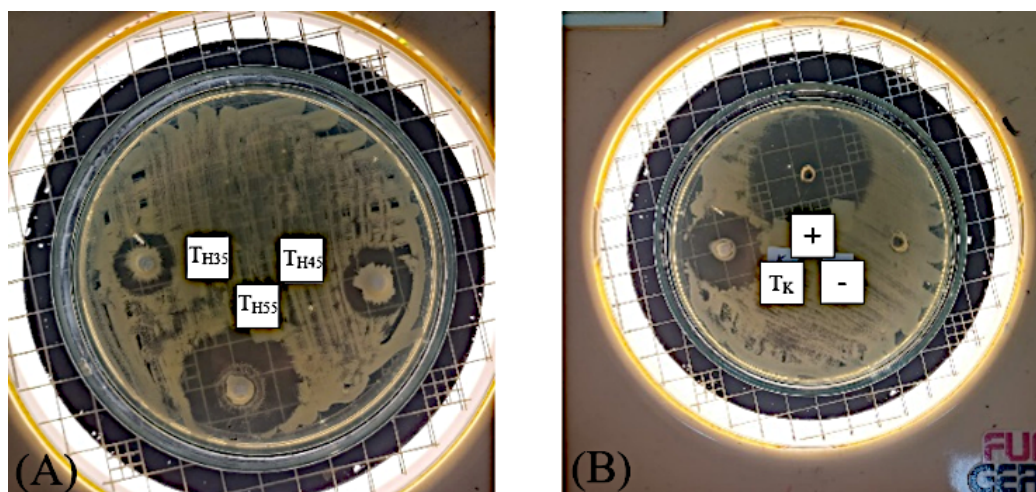


Figure 3. Clear zone of hydroxyapatite based toothpaste from freshwater mussel shells against *Staphylococcus aureus* bacteria (A), clear zone of commercial toothpaste, positive control, and negative control against *Staphylococcus aureus* bacteria (B).

Table 4. Inhibition zones of hydroxyapatite-based toothpaste from freshwater mussel shells against *Staphylococcus aureus*.

Treatment	Inhibition zone (mm)
T _{H35}	9.16±0.07 ^c
T _{H45}	11.69±0.04 ^d
T _{H55}	22.24±0.08 ^f
T _K	20.68±0.11 ^e

Note: TH35 = 35% hydroxyapatite, TH45 = 45% hydroxyapatite, TH55 = 55% hydroxyapatite, TK = commercial toothpaste.

However, the comparison between TH55 and the commercial toothpaste (TK) should be interpreted with caution. The composition of commercial toothpaste is proprietary and not fully disclosed. Thus, the superior inhibition of TH55 may not directly correspond to hydroxyapatite content alone but could also be influenced by differences in surfactant or fluoride levels between formulations.

Conclusion

Toothpaste formulations containing hydroxyapatite derived from freshwater mussel shells at concentrations of 35–55% significantly affected physicochemical parameters (color, texture, homogeneity, spreadability, pH, foam height) and microbiological properties (inhibition against *Staphylococcus aureus*). The observed increase in pH and calcium content with hydroxyapatite concentration is attributed to the alkaline nature of residual CaO, while antibacterial activity is governed by both ionic exchange and surface charge mechanisms. Among the tested formulations, TH55 (55% hydroxyapatite) demonstrated the best overall performance, combining optimal pH (7.39), highest calcium content (15.97%), and strong antibacterial activity (22.24 mm inhibition zone). This formulation exhibits potential not only for oral health enhancement but also for promoting enamel remineralization and bacterial control. The findings highlight the potential commercialization of freshwater mussel shell-derived hydroxyapatite as an eco-friendly, low-cost biomaterial for toothpaste production. Utilizing shell waste as a calcium source contributes to sustainable resource management, reduces environmental pollution, and supports circular economy practices in aquaculture regions. Therefore, this study provides both scientific and environmental value by integrating waste utilization with functional oral care product development.

Abbreviations

MHA = Mueller Hinton Agar; CaO = Calcium Carbonate; Na CMC = Natrium Carboxymethylcellulose; SLS = Sodium Lauryl Sulfate; SNI = Standar Nasional Indonesia.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

Ethics Statement

Not applicable.

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Additional Information


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