Remineralization Potential of Philippine Cupped Oyster Shells (*Magallana bilineata)* Powder Formulated as Toothpaste: An In-Vitro Study

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Abstract

Dental caries is a prevalent oral health condition linked to enamel demineralization, highlighting the need for effective remineralizing agents. This study evaluated the remineralization potential of a 10% toothpaste formulated from hydroxyapatite derived from *Magallana bilineata* oyster shells using an in vitro bovine enamel model. The shells were processed through calcination and wet precipitation, with hydroxyapatite formation confirmed via FTIR analysis. Enamel samples were assigned to a negative control (toothpaste base), positive control (Brand X commercial toothpaste), and experimental group and were treated under simulated oral conditions for 14 days. Baseline results showed uniform enamel characteristics across all groups. After demineralization, all samples exhibited increased surface roughness and reduced calcium, phosphorus, and Ca/P ratio values. Following treatment, the experimental and positive control groups showed reductions in surface scores and increases in mineral content, while the negative control showed minimal changes. The experimental group demonstrated improvement in surface morphology and mineral recovery, with some variation among samples. One-way ANOVA revealed significant differences among groups in surface morphology, calcium content, and Ca/P ratio, while phosphorus showed no significant difference. Post hoc analysis identified difference was observed between the negative control and treatment groups, while no significant differences between the negative control and treatment groups, while no significant difference was observed between the experimental and positive control groups in key parameters. Physicochemical and organoleptic evaluation indicated acceptable formulation quality, with a pleasant menthol odor, light gray color, smooth texture, pH of 6, specific gravity of 1.65, and spreadability of 5.03 cm. Overall, the 10% *Magallana bilineata* toothpaste demonstrated remineralization potential under the study conditions.

Keywords: Dental caries, Remineralization, Hydroxyapatite, *Magallana bilineata*, Oyster shell toothpaste

1. Introduction

Teeth are highly mineralized structures composed primarily of enamel, dentin, cementum, and pulp. Enamel, the outermost protective layer, is the hardest tissue in the human body and serves as the primary barrier against mechanical, thermal, and chemical damage. Despite its strength, enamel is vulnerable to acid-induced demineralization, which can lead to dental caries (Cleveland Clinic, 2023; Roberts et al., 2022). Dental caries remains a chronic and multifactorial disease caused by acid-producing bacteria, particularly *Streptococcus mutans*, that metabolize dietary sugar and progressively destroy tooth structure (Rathee & Sapre, 2023). If untreated, enamel demineralization progresses from white spot lesions to cavitation, dentin involvement, pulp damage, and eventual tooth loss (Seladi-Schulman, 2020).

Dental caries continues to be a major global public health concern, affecting billions of individuals worldwide. Recent reports estimate that over 2.24 billion people suffer from untreated caries in permanent teeth, with particularly high prevalence rates in Southeast Asia and developing countries (Machado, 2025; Li et al., 2025). In the Philippines, dental caries remain highly prevalent, with national surveys reporting that approximately 72% of Filipinos experience tooth decay (Philippine Dental Association, 2023). Factors such as limited access to dental care, inadequate oral health awareness, high consumption of sugary foods, and socioeconomic disparities contribute significantly to the burden of disease. In La Union alone, more than 15,000 children aged 1-4 years were affected b y dental caries in 2023 (Marzan, 2024).

To address enamel demineralization, dentistry has utilized various remineralizing agents, including fluoride, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), tricalcium phosphate (TCP), and bioactive glass. Fluoride remains the gold standard for caries prevention because of its ability to enhance enamel resistance by forming fluorapatite and promoting mineral redeposition (Yeh et al., 2025). However, excessive fluoride exposure may result in dental and skeletal fluorosis, particularly among children (Wong et al., 2024). Moreover, fluoride primarily strengthens partially demineralized enamel rather than fully restoring the original enamel structure.

These limitations have driven the search for biomimetic and sustainable alternatives capable of replicating natural tooth mineralization. Marine-derived biomaterials such as fish bone, coral, mussel shells, and oyster shells have gained increasing attention because of their high calcium content and ability to be converted into hydroxyapatite, the primary mineral component of enamel and bone (Firdaus Hussin et al., 2022; Muntean et al., 2024). Previous studies demonstrated that marine-derived hydroxyapatite can effectively promote enamel remineralization and improve mineral density in vitro (Dewi et al., Revankar et al., 2021).

Among these materials, Philippine cupped oyster shells (*Magallana bilineata*) represent a promising and sustainable source of hydroxyapatite because they contain approximately 95% calcium carbonate (Ulagesan et al, 2022). Through calcination and chemical conversion, oyster shells can be transformed into hydroxyapatite with properties comparable to synthetic dental biomaterials (Fernández-Penas et al., 2023). Studies have also shown that oyster shell-derived hydroxyapatite exhibits antimicrobial activity against cariogenic microorganisms and demonstrates remineralization potential comparable to fluoride-based products (Nisa et al., 2022; Pushpalatha et al., 2023).

In the Philippines, oyster farming generates substantial amounts of shell waste, particularly in coastal areas such as La Union, creating an opportunity to convert an abundant byproduct into an inexpensive and eco-friendly dental biomaterial. This approach not only supports sustainable waste management and circular-economy practices but also provides a potentially safer and more accessible alternative to conventional remineralizing agents. Thus, the present study aims to investigate the remineralization potential of hydroxyapatite derived from Philippine cupped oyster shells (*Magallana bilineata*) formulated as toothpaste for dental applications.

2. Objectives

This Study investigated the remineralization Potential of toothpaste formulated with Philippine cupped oyster shell (*Magallana bilineata*) powder. Specifically, it aimed to determine enamel remineralization across the experimental stages by analyzing surface morphology, elemental composition, and mineral content. The study also evaluated the differences in remineralization efficacy between the experimental toothpaste and the control group. In addition, the physicochemical and organoleptic properties of the formulated toothpaste were assessed to determine its suitability for dental application.

3. Materials and Methods

This section presents the materials and methods used in determining the remineralization potential of Philippine cupped oyster (*Magallana bilineata*) shell powder formulated as toothpaste on dental enamel. It includes the research design, duration, and locale of the study; test specimens; data-gathering tools; and the methodology employed in evaluating the enamel remineralization potential of the formulated toothpaste.

Research Design

This study employed an experimental research design to evaluate the remineralization potential of toothpaste formulated with 10% Philippine cupped oyster (*Magallana bilineata*) shell-derived hydroxyapatite (HAp). The design enabled the assessment of the cause-and-effect relationship between the formulated toothpaste and enamel remineralization. The experimental toothpaste was applied to artificially demineralized bovine enamel specimens, and its remineralizing efficacy was evaluated through analyses of enamel surface morphology, mineral content, and elemental composition.

Collection and Preparation of the Marine Sample Bioactive Compound

Philippine cupped oyster (*Magallana bilineata*) shells were collected from Barangay Dulao, Aringay, La Union, and morphologically identified by the Bureau of Fisheries and Aquatic Resources – National Fisheries Development Center, Pangasinan to ensure species authenticity and uniformity. The shells were cleaned, dried, pulverized, and calcined to produce calcium oxide, which was synthesized into hydroxyapatite (HAp) using the wet precipitation method. The synthesized HAp was subsequently characterized through Fourier Transform Infrared (FT-IR) spectroscopy to confirm hydroxyapatite formation.

Formulation of the Toothpaste Base

The toothpaste formulation was adapted from the Textbook of Cosmetic Formulation by Sharma et al. (2018). A toothpaste base was prepared using the wet gum method, incorporating glycerin, distilled water, gum tragacanth, sodium saccharin, calcium carbonate, sodium lauryl sulfate, peppermint oil, and sodium benzoate. Philippine cupped oyster shell-derived hydroxyapatite (*Magallana bilineata*) was then incorporated at a 10% concentration to produce the experimental toothpaste formulation.

In-Vitro Testing

The study utilized bovine permanent incisors obtained post-mortem from cattle approximately 30 months old. The teeth were carefully cleaned, sectioned, and artificially demineralized to mimic early enamel carious lesions. The specimens were then divided into three (3) groups, with three (3) bovine enamel samples assigned to each group: Group I: (Positive Control), treated with Brand X commercially available toothpaste; Group II (Negative Control), treated with toothpaste base only; and Group III (Experimental Group), treated with toothpaste containing 10% Philippine cupped oyster shell-derived hydroxyapatite (*Magallana bilineata*). Each specimen was brushed twice daily for 14 days and stored in artificial saliva between treatments under controlled laboratory conditions to assess the remineralization potential of the formulated toothpaste.

Determination of Remineralization Potential

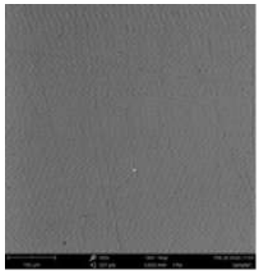
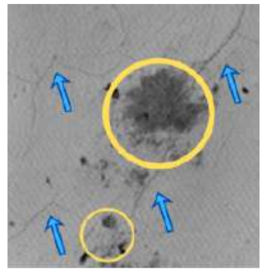
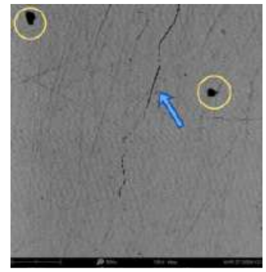
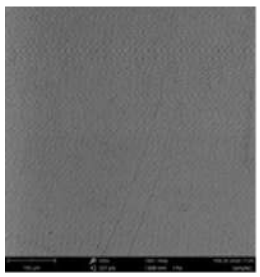
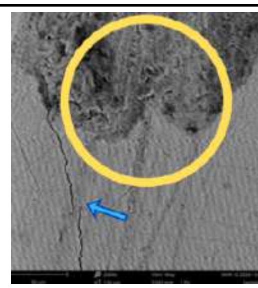
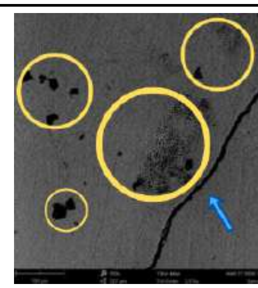
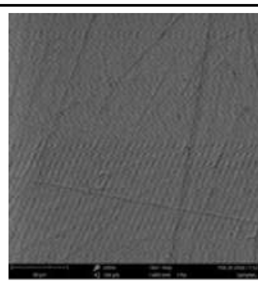
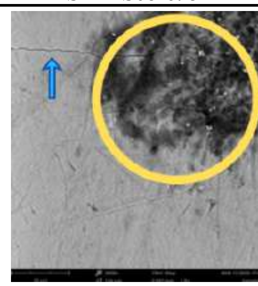
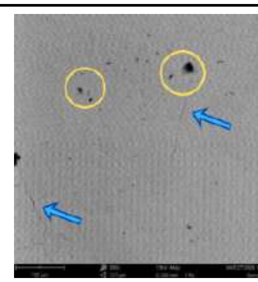
The remineralization potential of the enamel samples was assessed through surface morphological and elemental analyses using Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX). These analyses were conducted before demineralization, after demineralization, and following treatment. SEM was used to evaluate changes in enamel surface morphology, including surface smoothness, particle deposition, and pore closure, while EDX quantified calcium (Ca) and phosphorus (P) content and determined the calcium-to-phosphorus (Ca/P) ratio as an indicator of remineralization efficacy.

Evaluation Tests

The formulated toothpaste containing Philippine cupped oyster (*Magallana bilineata*) shell powder was evaluated for its organoleptic and physicochemical properties, including color, odor, texture, pH, specific gravity, and spreadability. Seven evaluators assessed the formulation using a guided questionnaire to document sensory perceptions and user responses. The pH was determined using pH strips, specific gravity was measured using a pycnometer, and spreadability was evaluated through the glass-slide method. The results provided insights into the overall acceptability, usability, and quality of the formulated toothpaste.

4. Results

Table 1 SEM Micrographs of Enamel Surface Changes Across Different Experimental Stages

	BD	AD	AT
POS	 SEM Score: 1	 SEM Score: 4	 SEM Score: 3
NEG	 SEM Score: 1	 SEM Score: 5	 SEM Score: 4
TX	 SEM Score: 1	 SEM Score: 5	 SEM Score: 3

Note: POS - Positive Control Group; NEG - Negative Control Group; TX- 10% *Magallana bilineata* Toothpaste; BD - Before Demineralization; AD - After Demineralization; AT - After Treatment

Table 2 Descriptive Statistics of Surface Morphology (SEM Scores) Across Different Experimental Stages

Surface Morphology Table				
Group	**Tooth**	**BD**	**AD**	**AT**
	1	1	3	2
POS	2	1	4	3
	3	1	4	3
	Overall	1.000	3.667 (SD 0.577)	2.33 (SD 0.577)
	4	1	4	4
NEG	5	1	5	4
	6	1	4	3
	Overall	1.000	4.333 (SD 0.577)	3.67 (SD 0.577)
	7	1	3	2
TX	8	1	4	3
	9	1	4	1
	Overall	1.000	4.000 (SD 1.000)	2 (SD 1.000)

Note: POS - Positive Control Group; NEG - Negative Control Group; TX- 10% *Magallana bilineata* Toothpaste; BD - Before Demineralization; AD - After Demineralization; AT - After Treatment; SD - Standard Deviation

Table 3 Descriptive Statistics of Elemental Calcium Content Across Different Experimental Stages

Elemental Calcium Table				
Group	**Tooth**	**BD**	**AD**	**AT**
	1	27.99	22.39	33.58
POS	2	30.37	23.91	44.09
	3	41.04	28.15	46.72
	Overall	33.131 (SD 6.950)	24.817 (SD 2.985)	41.463 (SD 6.953)
	4	29.92	23.36	24.03
NEG	5	38.96	27.47	29.99
	6	33.11	26.52	29.91
	Overall	33.997 (SD 4.585)	2.950 (SD 1.871)	27.977 (SD 3.422)
	7	36.15	25.93	40.51
TX	8	34.24	25.22	36.61
	9	27.77	22.77	33.69
	Overall	32.720 (SD 4.391)	24.640 (SD 1.658)	36.937 (SD 3.421)

Note: POS - Positive Control Group; NEG - Negative Control Group; TX- 10% *Magallana bilineata* Toothpaste; BD - Before Demineralization; AD - After Demineralization; AT - After Treatment; SD - Standard Deviation

Table 4 Descriptive Statistics of Elemental Phosphorus Content Across Different Experimental Stages

Elemental Phosphorus Table				
Group	**Tooth**	**BD**	**AD**	**AT**
	1	14.64	12.11	15.62
POS	2	15.65	12.92	17.69
	3	17.61	13.82	19.61
	Overall	15.967 (SD 1.510)	12.950 (SD 0.855)	17.640 (SD 1.995)
	4	14.59	12.98	13.66
NEG	5	17.71	14.55	15.44
	6	16.21	14.50	15.32
	Overall	16.170 (SD 1.560)	14.010 (0.892)	14.807 (SD 0.995)
	7	16.87	13.40	17.00
TX	8	16.24	13.20	16.97
	9	13.82	11.97	15.22
	Overall	15.643 (SD 1.610)	12.857 (SD 0.774)	16.397 (SD 1.019)

Note: POS - Positive Control Group; NEG - Negative Control Group; TX- 10% *Magallana bilineata* Toothpaste; BD - Before Demineralization; AD - After Demineralization; AT - After Treatment; SD - Standard Deviation

Table 5 Descriptive Statistics of Mineral Content (Ca/P Ratio) Across Different Experimental Stages

Mineral Content Table				
Group	**Tooth**	**BD**	**AD**	**AT**
POS	1	1.92	1.86	2.23
	2	1.95	1.90	2.42
	3	2.34	1.85	2.34
	Overall	2.070 (SD 0.234)	1.923 (SD 0.110)	2.330 (SD 0.095)
NEG	4	2.09	1.95	1.89
	5	2.21	1.95	2.19
	6	2.05	1.93	2.17
	Overall	2.117 (SD 0.823)	1.880 (SD 0.026)	1.897 (SD 0.031)
TX	7	2.15	1.86	2.23
	8	2.12	1.86	2.19
	9	2.03	2.05	2.17
	Overall	2.100 (SD 0.062)	1.943 (SD 0.012)	2.220 (SD 0.070)

Note: POS - Positive Control Group; NEG - Negative Control Group; TX- 10% *Magallana bilineata* Toothpaste; BD - Before Demineralization; AD - After Demineralization; AT - After Treatment; SD - Standard Deviation

Table 6 Test of Normality for Enamel Surface Morphology, Elemental Composition, and Mineral Content

Parameters		**TP**	**Shapiro-Wilk**			**Remarks**
			W	**df**	**p-value**	
Surface Morphology		BD	—	9	—	Normally Distributed By Default
		AD	0.978	9	0.951	Normally Distributed
		AT	0.978	9	0.951	Normally Distributed
Elemental Composition	Calcium	BD	0.923	9	0.419	Normally Distributed
		AD	0.936	9	0.545	Normally Distributed
		AT	0.931	9	0.495	Normally Distributed
	Phosphorus	BD	0.909	9	0.312	Normally Distributed
		AD	0.851	9	0.077	Normally Distributed
		AT	0.924	9	0.427	Normally Distributed
Mineral Content		BD	0.924	9	0.422	Normally Distributed
		AD	0.859	9	0.094	Normally Distributed
		AT	0.968	9	0.878	Normally Distributed

Note: p-value > 0.05 = Normally Distributed

Table 7 One-Way ANOVA Results for Significant Differences in Surface Morphology

ANOVA Table							
Source	**SS**	**df**	**MS**	**F**	**F crit**	**p-value**	**Remarks**
Treatment	4.667	2	2.333	4.2	3.463	0.072	Significant
Error	3.333	6	0.556				
Total	8	8					

Table 8 Tukey Post Hoc Comparisons of the Surface Morphology Among Treatment Groups

Post hoc Analysis		
Group	**p-value**	**Remarks**
POS vs NEG	0.151	Not Significant
NEG vs TX	0.075	Significant
TX vs POS	0.851	Not Significant

Note: POS - Positive Control Group; NEG - Negative Control Group; TX- 10% *Magallana bilineata* Toothpaste

Table 9 One-Way ANOVA Results for Significant Differences in Calcium Content

ANOVA Table							
Source	**SS**	**df**	**MS**	**F**	**F crit**	**p-value**	**Remarks**
Treatment	282.708	2	141.354	5.909	3.463	0.038	Significant
Error	351.008	18	19.500				
Total	1172.320	26					

Table 10 Tukey Post Hoc Comparisons of the Calcium Content Among Treatment Groups

Post hoc Analysis		
Group	**p-value**	**Remarks**
POS vs NEG	0.034	Significant
NEG vs TX	0.142	Not Significant
TX vs POS	0.530	Not Significant

Note: POS - Positive Control Group; NEG - Negative Control Group; TX- 10% *Magallana bilineata* Toothpaste

Table 11 One-Way ANOVA Results for Significant Differences in Phosphorus Content

ANOVA Table							
Source	**SS**	**df**	**MS**	**F**	**F crit**	**p-value**	**Remarks**
Treatment	12.123	2	6.062	3.030	3.463	0.123	Not Significant
Error	12.003	6	2.001				
Total	24.127	8					

Table 12 One-Way ANOVA Results for Significant Differences in Mineral Content

ANOVA Table							
Source	**SS**	**df**	**MS**	**F**	**F crit**	**p-value**	**Remarks**
Treatment	0.304	2	0.152	30.578	3.463	0.0007	Significant
Error	0.030	6	0.005				
Total	0.334	8					

Table 13 Tukey Post Hoc Comparisons of the Mineral Content Among Treatment Groups

Post hoc Analysis P		
Group	**p-value**	**Remarks**
POS vs NEG	<.001	Significant
NEG vs TX	0.003	Significant
TX vs POS	0.216	Not Significant

Note: POS - Positive Control Group; NEG - Negative Control Group; TX- 10% *Magallana bilineata* Toothpaste

Table 14 Summary of Physicochemical and Organoleptic Properties of the 10% *Magallana bilineata* Toothpaste

Properties		**Evaluation**
Organoleptic	Odor	Pleasant menthol odor
	Color	Whitish color to light gray
	Texture	Smooth and homogenous consistency
Physicochemical	pH	6 – slightly acidic
	Specific Gravity	1.65
	Spreadability	5.03 cm

5. Discussion

Table 1 presents the SEM micrographs of enamel surface changes across the different experimental stages, namely before demineralization (BD), after demineralization (AD), and after treatment (AT). Prior to demineralization, all groups exhibited smooth and intact enamel surfaces with minimal irregularities, corresponding to an SEM score of 1, which is characteristic of sound enamel morphology. Minor surface features, such as natural ridges, were observed; however, these are considered inherent characteristics of healthy enamel and are not indicative of structural damage or mineral loss (Ishak et al., 2020). The absence of porosity, fissures, and surface breakdown further confirms the integrity of the enamel prior to experimental intervention.

Following demineralization, evident structural deterioration was observed across the samples, characterized by increased surface porosity, widened interprismatic spaces, and honeycomb-like surface patterns associated with mineral dissolution. These alterations corresponded to SEM scores ranging from 3 to 5, indicating moderate to severe enamel demineralization. Similar findings were described by AbdElbar et al. (2022), who associated porosity and irregular enamel morphology with progressive mineral loss and early carious lesion formation.

After treatment, the positive control (POS) and treatment group (TX) demonstrated visible improvement in enamel surface morphology, as evidenced by reduced porosity and fewer structural irregularities, suggesting partial remineralization. The observed surfaces exhibited moderate porosity with less pronounced disruption, indicative of incomplete but evident enamel repair, consistent with the findings of Rangappa et al. (2022). In contrast, the negative control (NEG) continued to exhibit marked surface irregularities and persistent structural defects. These findings suggest that the 10% *Magallana bilineata* toothpaste promoted enamel surface recovery comparable to the positive control. This observation is supported by O'Hagan-Wong et al. (2022), who reported that hydroxyapatite particles can adhere to demineralized enamel surfaces, occupy microscopic defects, and facilitate the repair of early enamel lesions through biomimetic remineralization.

Table 2 presents the descriptive statistics of enamel surface morphology scores across the experimental stages. At baseline, all groups demonstrated a uniform mean SEM score of 1.000 with no variability, indicating consistent enamel integrity prior to treatment. Following demineralization, mean SEM scores increased across all groups, reflecting enamel deterioration caused by acid-induced mineral loss and hydroxyapatite dissolution (Yan et al., 2022). The negative control exhibited the highest mean score of 4.333 (SD = 0.577), indicating greater structural damage, whereas the TX group demonstrated a mean score of 4.000 (SD = 1.000), suggesting greater variability in demineralization response among samples.

After treatment, both the positive control and TX groups demonstrated reduced mean SEM scores, indicating improvement in enamel morphology and partial remineralization. The TX group decreased from 4.000 to 2.000, while the positive control decreased from 3.667 to 2.330. In contrast, the negative control exhibited only minimal improvement. These findings indicate that the hydroxyapatite toothpaste facilitated enamel surface repair through mineral redeposition and partial reconstruction of hydroxyapatite crystals (Anjum, 2023). Comparable findings were reported by Juntavee et al. (2021), who observed reduced enamel porosity and improved surface smoothness following hydroxyapatite treatment.

The improvement observed in the positive control may be attributed to fluoride-mediated remineralization. Fluoride promotes the formation of fluorapatite and calcium fluoride reservoirs, enhancing calcium and phosphate retention and increasing enamel resistance to acid dissolution (Simmer et al., 2020; Koontongkaew et al., 2024). Despite these improvements, none of the treated groups returned completely to baseline morphology. This may be attributed to the gradual nature of remineralization, limitations of in vitro conditions, and possible irreversible structural damage following extensive demineralization (Khairy et al., 2025; Malcangi et al., 2023).

The improvement observed in the TX group may be explained by the biomimetic properties of hydroxyapatite derived from *Magallana bilineata* shells. Hydroxyapatite can supply calcium and phosphate ions necessary for enamel repair while integrating into microscopic surface defects, thereby reducing porosity and improving enamel morphology (O'Hagan-Wong et al., 2022). Nano-hydroxyapatite has likewise been shown to penetrate enamel irregularities and enhance the repair of early enamel lesions (Juntavee et al., 2021).

Table 3 presents the descriptive statistics of elemental calcium content across the experimental stages. At baseline, calcium levels were relatively comparable among groups, indicating homogeneity in initial mineral composition. Following demineralization, calcium levels decreased across all groups due to acid-induced dissolution of hydroxyapatite crystals, resulting in mineral loss and enamel weakening (Kolumban et al., 2021). The relatively uniform reduction across groups suggests that the demineralization protocol was consistently applied.

After treatment, both the positive control and TX groups demonstrated marked increases in calcium content, whereas the negative control exhibited only limited recovery. The TX group increased from 24.640 to 36.937, while the positive control increased to 41.463, indicating substantial mineral redeposition within the enamel structure. These findings suggest that both treatments promoted enamel remineralization through calcium reuptake and hydroxyapatite reconstruction.

The calcium recovery observed in the TX group may be attributed to the calcium-rich hydroxyapatite derived from *Magallana bilineata* shells, which supplied calcium and phosphate ions necessary for enamel repair. Hydroxyapatite functions as a biomimetic material capable of integrating into the enamel matrix and supporting crystal reformation. Similar findings were reported by Rodemer et al. (2022), who noted that remineralizing agents enhance calcium retention and contribute to structural restoration of demineralized enamel. The greater calcium recovery observed in the positive control may be associated with fluoride action, which promotes the formation of calcium-rich surface deposits and enhances mineral retention at the enamel surface (Rodemer et al., 2022). Overall, the TX group demonstrated substantial and consistent calcium recovery comparable to the positive control and markedly greater than the negative control.

Table 4 presents the descriptive statistics of elemental phosphorus content across the experimental stages. Similar to calcium, phosphorus levels decreased after demineralization, indicating mineral loss from the enamel structure. Following treatment, phosphorus levels increased in both the positive control and TX groups, whereas the negative control demonstrated only minimal recovery. However, the changes in phosphorus content were less pronounced than those observed for calcium.

The relatively smaller variation in phosphorus levels may be attributed to the structural stability of phosphorus within the hydroxyapatite lattice, making it less responsive to short-term remineralization changes. Despite this, the TX group demonstrated phosphorus recovery beyond demineralized values, suggesting that mineral redeposition occurred following treatment. Kim et al. (2025) similarly reported that phosphorus exhibits less dynamic fluctuation during enamel remineralization due to its stable integration within enamel crystals.

Table 5 presents the descriptive statistics of mineral content based on the calcium-to-phosphorus (Ca/P) ratio across the experimental stages. Baseline Ca/P ratios were closely aligned among groups, indicating comparable initial mineral composition. Following demineralization, all groups exhibited reduced Ca/P ratios, reflecting mineral depletion and enamel weakening associated with hydroxyapatite dissolution.

After treatment, the positive control and TX groups demonstrated notable increases in Ca/P ratio, indicating mineral restoration and remineralization. The TX group increased from 1.943 to 2.220, while the positive control reached 2.330. In contrast, the negative control remained near its demineralized value, indicating limited mineral recovery. These findings suggest that the experimental toothpaste effectively promoted calcium and phosphate redeposition within the enamel structure. Similar increases in Ca/P ratio following hydroxyapatite treatment were reported by Al Ankily et al. (2025), who associated higher Ca/P values with enhanced enamel remineralization and structural recovery.

The increase in Ca/P ratio may be attributed to the redeposition of calcium and phosphate ions onto the enamel surface and their incorporation into the hydroxyapatite matrix (Anjum, 2023). The comparable Ca/P values observed between the TX and positive control groups further suggest that the remineralization potential of the *Magallana bilineata* hydroxyapatite toothpaste was similar to that of the fluoride-based treatment under the conditions of the study.

Table 6 presents the results of the Shapiro–Wilk test for enamel surface morphology, elemental composition, and mineral content across the experimental stages. All parameters yielded p-values greater than 0.05, indicating normal distribution of the data and satisfying the assumptions required for parametric statistical analysis. These findings support the use of One-Way ANOVA for determining differences among treatment groups. Mishra et al. (2021) emphasized the importance of normality testing prior to parametric analysis to ensure statistical validity, while Farooq et al. (2021) similarly applied normality testing in dental studies before conducting ANOVA.

Table 7 presents the One-Way ANOVA results for enamel surface morphology. The computed F-value of 4.2 exceeded the F-critical value of 3.463, while the p-value of 0.072 was lower than the significance level of $\alpha = 0.10$, indicating a statistically significant difference among treatment groups. These findings suggest that the treatments significantly influenced enamel surface morphology following remineralization.

Table 8 presents the Tukey post hoc comparisons for enamel surface morphology. A significant difference was observed between the negative control and TX group ($p = 0.075$), indicating that the 10% *Magallana bilineata* toothpaste significantly improved enamel surface morphology compared to untreated enamel. No significant difference was observed between the TX and positive control groups ($p = 0.851$), suggesting comparable remineralization effects between the experimental toothpaste and the fluoride-based treatment.

The observed improvement in the TX group supports the biomimetic action of hydroxyapatite in promoting enamel surface repair through calcium and phosphate redeposition and reduction of surface porosity. Hydroxyapatite can occupy microscopic surface defects and integrate into the enamel matrix, whereas fluoride enhances enamel resistance through the formation of acid-resistant mineral phases (O'Hagan-Wong et al., 2022; Simmer et al., 2020). These findings indicate that the 10% *Magallana bilineata* toothpaste effectively improved enamel surface morphology relative to the negative control.

Table 9 presents the One-Way ANOVA results for calcium content. The computed F-value of 5.909 exceeded the F-critical value of 3.463, while the p-value of 0.038 indicated a statistically significant difference among treatment groups. These findings confirm that the treatments significantly influenced calcium recovery following enamel demineralization.

Table 10 presents the Tukey post hoc comparisons for calcium content. A significant difference was observed between the positive and negative control groups ($p = 0.034$), demonstrating the effectiveness of fluoride in enhancing calcium redeposition. No significant difference was observed between the TX and positive control groups, indicating that the hydroxyapatite toothpaste produced calcium recovery comparable to the fluoride-based treatment. Similar findings were reported by O'Hagan-Wong et al. (2022), who demonstrated that hydroxyapatite formulations effectively supply calcium ions necessary for enamel remineralization.

The significant calcium recovery associated with fluoride may be attributed to the formation of calcium fluoride and fluorapatite, which enhance mineral retention and enamel resistance to acid dissolution (Simmer et al., 2020; Rodemer et al., 2022). Meanwhile, hydroxyapatite promotes remineralization through biomimetic integration into the enamel surface and direct mineral supplementation (Juntavee et al., 2021). These findings indicate that the experimental toothpaste effectively supported calcium restoration comparable to the standard treatment.

Table 11 presents the One-Way ANOVA results for phosphorus content. The computed F-value of 3.030 did not exceed the F-critical value of 3.463, while the p-value of 0.123 exceeded the significance level of $\alpha = 0.10$, indicating no statistically significant difference among treatment groups. These findings suggest that phosphorus remained relatively stable throughout the remineralization process and was less responsive to short-term mineral changes.

This observation may be explained by the structural stability of phosphorus within the hydroxyapatite lattice. Compared to calcium, phosphorus demonstrates less dynamic fluctuation during remineralization, resulting in smaller measurable changes that may not reach statistical significance (Rebeca et al., 2025).

Table 12 presents the One-Way ANOVA results for mineral content based on the Ca/P ratio. The computed F-value of 30.578 greatly exceeded the F-critical value of 3.463, while the p-value of 0.0007 indicated a highly significant difference among treatment groups. These findings demonstrate that the treatments significantly influenced enamel mineral restoration.

Table 13 presents the Tukey post hoc comparisons for mineral content. Significant differences were observed between the positive and negative control groups ($p < 0.001$) and between the negative control and TX group ($p = 0.003$), indicating that both fluoride and the experimental hydroxyapatite toothpaste significantly improved mineral recovery compared to untreated enamel. No significant difference was observed between the TX and positive control groups, suggesting that the remineralization capability of the 10% *Magallana bilineata* toothpaste was comparable to the fluoride-based treatment.

The remineralization observed in the positive control may be attributed to fluoride-induced formation of fluorapatite and calcium-rich mineral reservoirs that enhance enamel resistance and mineral retention (Simmer et al., 2020; Rodemer et al., 2022). In contrast, the experimental toothpaste promoted remineralization through the biomimetic properties of hydroxyapatite, which resembles the natural mineral structure of enamel and supplies calcium and phosphate ions necessary for enamel repair (O'Hagan-Wong et al., 2022; Juntavee et al., 2021). These findings demonstrate that treatments containing active remineralizing agents significantly enhanced enamel mineral recovery compared to untreated enamel.

Table 14 presents the physicochemical and organoleptic properties of the formulated 10% *Magallana bilineata* toothpaste. Organoleptic evaluation revealed a pleasant menthol odor, whitish to light gray color, and smooth homogeneous consistency, indicating acceptable sensory characteristics and user acceptability. The absence of unpleasant odor suggests that calcination effectively removed organic residues from the oyster shell-derived hydroxyapatite.

Physicochemical analysis demonstrated a pH of 6, which falls within the acceptable range for oral care products and supports compatibility with the oral environment. The toothpaste also exhibited a specific gravity of 1.65, reflecting the presence of mineral-rich components, and a mean spreadability of 5.03 cm, indicating suitable consistency and ease of application. Overall, these findings demonstrate that the formulated toothpaste possessed acceptable formulation stability and physicochemical characteristics consistent with established dentifrice standards.

6. Conclusion

The findings of this study demonstrate that the 10% *Magallana bilineata* oyster shell-derived hydroxyapatite toothpaste promotes enamel remineralization, as evidenced by improved surface morphology, increased calcium levels, and enhanced Ca/P ratios following treatment. Descriptive and microscopic analyses showed reduced surface porosity and partial restoration of enamel structure, while elemental analysis confirmed calcium redeposition and an increase in the Ca/P ratio, indicating mineral recovery after demineralization.

Statistical analysis further supports this remineralization potential. Significant differences between the experimental and negative control group in surface morphology and mineral content highlight the formulation's ability to facilitate enamel recovery. Moreover, the absence of a statistically significant difference between the experimental and positive control groups in selected parameters indicates that the formulation produces similar effects to the positive control under the conditions of the study. Phosphorus levels did not show significant variation, suggesting limited responsiveness of this parameter during the remineralization process and indicating that calcium deposition may play a more dominant role.

Furthermore, the formulated toothpaste exhibited physicochemical and organoleptic properties consistent with established criteria reported in related literature for dentifrice formulation, supporting its formulation stability and suitability for use.

Overall, the results support the potential 10% *Magallana bilineata* oyster shell-derived hydroxyapatite as a remineralizing agent for enamel restoration.

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To God be the glory.

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9. Appendices

APPENDIX A
Timetable

Parts	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
INTRODUCTION Background of the Study											
Conceptual Framework											
Operational Paradigm											
Statement of the Problem, Statement of Hypothesis											
Scope and Limitation											
METHODOLOGY Research Design and Method, Population and Locale of the study											
Data Gathering Tools, Data Gathering Procedures, Ethical Considerations, Treatment of Data											
Filing of LC-REC Approval Letter											
Perform Data Gathering											
RESULTS AND DISCUSSION											
CONCLUSIONS AND RECOMMENDATIONS											
Thesis Approval											
Bound Manuscript											
Copyright/Patent Filing											

APPENDIX B
Materials, Equipment, Chemicals, and Reagents

Materials	Equipment	Chemicals and Reagents
Mortar and Pestle	Drying Oven	Phosphoric acid
Sieve No. 60	Hammer mill	Sodium hydroxide
Sieve No. 120	Analytical balance	Glycerin
Beaker	Incinerator	Gum tragacanth
pH strip	Hotplate w/ stirrer	Sodium saccharin
Funnel	Perkin Elmer FT-IR	Calcium carbonate
Spatula	Spectrometer Frontier	Sodium lauryl sulfate
Stirring Rod	Thermo Scientific Phenom	Peppermint oil
	XL Benchtop SEM with EDX	Sodium benzoate
		0.1% thymol solution
		Epoxy Resin
		2.2 mmol/L Calcium chloride
		2.2 mmol/L Diammonium hydrogen phosphate
		0.15 mol/L Sodium chloride
		0.1 mol/L Acetic acid
		Artificial Saliva

APPENDIX C
Identification Certificate of Marine Species Material

Republic of the Philippines
Department of Agriculture
Bureau of Fisheries and Aquatic Resources
Fisheries Building Complex, BPI Compound, Brgy. Vasra
Visayas Ave. Quezon City
| do@bfar.da.gov.ph | records@bfar.da.gov.ph |
+63(02) 8539-5685

PHYSICAL CERTIFICATION


Figure 1

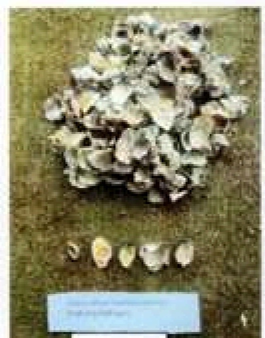
Figure 2


Figure 3

Figure 4

Based on the samples collected and analyzed physically, majority of the samples are Magallana bilineata that are commonly named slipper-shaped oyster or talabang tsinelas. Among the 5 kg at 916 pcs. shell samples submitted and identified are the following:

a. 593 pcs of samples are Magallana bilineata (see figure 1)

b. 189 pcs of samples are Saccostrea malabonensis (see figure 2)

c. 107 pcs of samples are Saccostrea palmipes (see figure 3)

d. 27 pcs of samples are Saccostrea cucullata (see figure 4)

Republic of the Philippines
Department of Agriculture
Bureau of Fisheries and Aquatic Resources
Fisheries Building Complex, BPI Compound, Brgy. Vasra
Visayas Ave. Quezon City
| do@bfar.da.gov.ph | records@bfar.da.gov.ph |
+63(02) 8539-5685

According to the morphological characteristic of the sample, the organism is identified as follows:

Kingdom: **Animalia**

Phylum: **Mollusca**

Subphylum: **Vertebrata**

Class: **Bivalvia**

Order: **Ostreida**

Family: **Ostreidae**

Genus: ***Magallana***

Species: ***bilineata***

Republic of the Philippines
Department of Agriculture
Bureau of Fisheries and Aquatic Resources
Fisheries Building Complex, BPI Compound, Brgy. Vasra
Visayas Ave. Quezon City
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+63(02) 8539-5685

Local/ common name: *talaba* or *talabang tsinelas* ("slipper oyster")

Shell Characteristics

Size: This oyster is notably large, reaching up to 18-21 cm in shell length.
Shape: The shell shape varies depending on what the oyster attaches to, but it can be generally oval or pear-shaped.
Coloration: The shell's exterior is typically pale yellow or purplish, with thin, flaky layers.
Valves: The lower attached valve is deeper and cup-shaped compared to the smaller, arched upper valve.

Internal Features

Adductor Muscle Scar: A key identifier is the distinctive dark or black scar on the inside of both shell valves where the adductor muscle attaches.

Identified by:

Francisco Reyes, Jr.
Head, Shellfish Unit
BFAR-National Fisheries Development Center Dagupan City, Pangasinan

APPENDIX D
Certification of Exempt from REC Review

LORMA COLLEGES

LC-REC Form #039
CERTIFICATE OF EXEMPTION FROM REVIEW

CERTIFICATION OF EXEMPTION FROM REVIEW

REC Reference #: 2026-074

To: Daryll Jade M. Abriam, Jasmine Andrei D. Flores, Hannah Jane A Nang, Cheryl Joyce M. Ulanday

From: LORMA Colleges - Research Ethics Committee

Date: January 20, 2026

This is to certify that the Research Proposal entitled, "REMINERALIZATION POTENTIAL OF PHILIPPINE CUPPED OYSTER SHELLS *(MAGALLANA BILINEATA)* POWDER FORMULATED AS TOOTHPASTE: AN IN-VITRO STUDY" submitted by Daryll Jade M. Abriam, Jasmine Andrei D. Flores, Hannah Jane A Nang and Cheryl Joyce M. Ulanday of College of Pharmacy has been reviewed by the Research Ethics Committee of LORMA Colleges and found that all ethical considerations are in place to conduct the research in the stated locale of the study. Hence, this research proposal is exempted from review.

JEROME P. VERA, LPT
Chairman, LC-REC

APPENDIX E
Collection, Identification, Preparation, Preparation, and Extraction of Marine Sample Bioactive Compound Documentation

Collection of Philippine Cupped Oyster (*Magallana bilineata*) Shells	 
Authentication of Philippine Cupped Oyster (*Magallana bilineata*) Shells	 
Preparation and Drying of Philippine Cupped Oyster (*Magallana bilineata*) Shells	 
Pulverization of Philippine Cupped Oyster (*Magallana bilineata*) Shells	 

Calcination of Philippine Cupped Oyster (*Magallana bilineata*) Shells	

APPENDIX F
Results and Interpretation of FT-IR Confirmatory Testing

Republic of the Philippines
Department of Science and Technology
INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE
ADVANCED DEVICE AND MATERIALS TESTING LABORATORY
DOST Cpd., General Santos Ave., Bicutan, Taguig City
Tel. Nos. (Direct Line): (02) 8837-0461, 8837-0503, 8837-0674, 8837-0650
http://www.itdi.gov.ph, http://www.admatel.com

REPORT OF ANALYSIS

Reference No.	:	**ADMATEL 2511-0555**
Customer	:	**LORMA COLLEGES** **Daryll Jade Abriam**
Address/E-mail	:	jasmineandrei.flores@lorma.edu
Sample Label	:	Hydroxyapatite powder
Analysis Requested	:	Fourier Transform Infrared (FTIR) Spectroscopy-No Identification
Reference Method	:	In-house Method AL-TP-400 Fourier Transform Infrared Spectroscopy
Date Received	:	November 04, 2025
Date Tested	:	November 05, 2025

I. Test Description

1. The equipment and parameters used for the test are as follows:

Testing Equipment	:	Perkin Elmer FT-IR Spectrometer Frontier
Technique	:	Attenuated Total Reflectance (ATR)
Range	:	4000 – 600 cm^{-1}
No. of scans	:	20

2. Baseline correction was applied to the spectrum to improve its quality without distorting the band intensities in the final spectrum.

II. Summary

The *Hydroxyapatite powder* sample as shown in **Figure 1** was tested as-received. The FT-IR spectrum and spectral library search are presented in **Figures 2** and **3**, respectively.

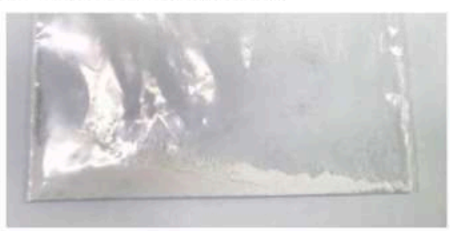

Figure 1. Photograph of the as-received *Hydroxyapatite powder* sample

Page 1 of 4

Republic of the Philippines
Department of Science and Technology
INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE
ADVANCED DEVICE AND MATERIALS TESTING LABORATORY
DOST Cpd., General Santos Ave., Bicutan, Taguig City
Tel. Nos. (Direct Line): (02) 8837-0461, 8837-0503, 8837-0674, 8837-0650
http://www.itdi.gov.ph, http://www.admatel.com

VALIDITY OF THE REPORT: The test results are those obtained at the time of the test and pertain only to the sample/s received by ADMATEL.

Prepared by:

CHARISSE M. MENDOZA
Analyst
Date: November 06, 2025

Reviewed by:

ANGELENE J. ALCAIN
Laboratory Head
Date: November 06, 2025

Issued under the authority of:

for:
DR. MARIANITO T. MARGARITO
Chief, MSD-ITDI
Date: November 06, 2025

Form: AL-21-F25
Issue: February 8, 2023
Revision: 05

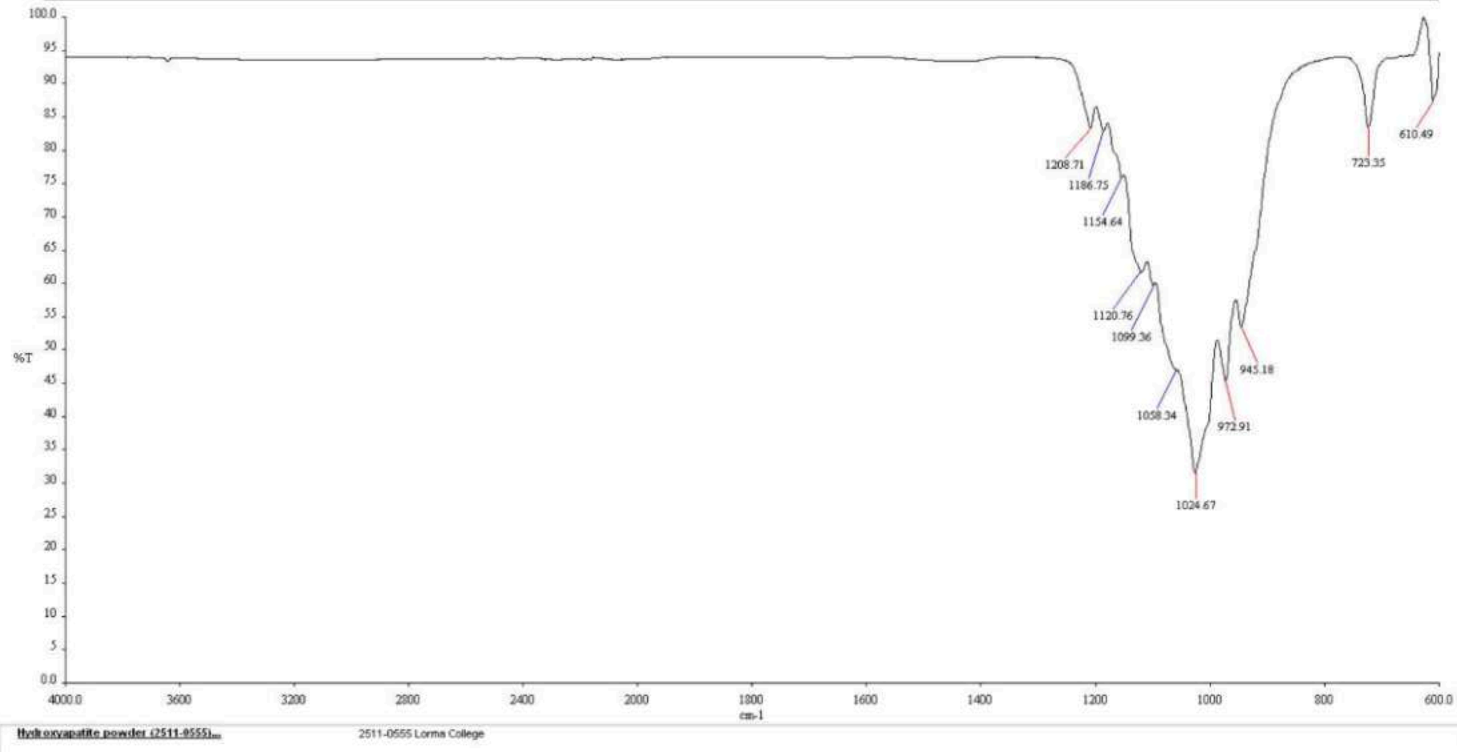

Figure 2. FT-IR spectrum of *Hydroxyapatite powder*

Republic of the Philippines
Department of Science and Technology
INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE
ADVANCED DEVICE AND MATERIALS TESTING LABORATORY
DOST Cpd., General Santos Ave., Bicutan, Taguig City
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http://www.itdi.gov.ph, http://www.admatel.com

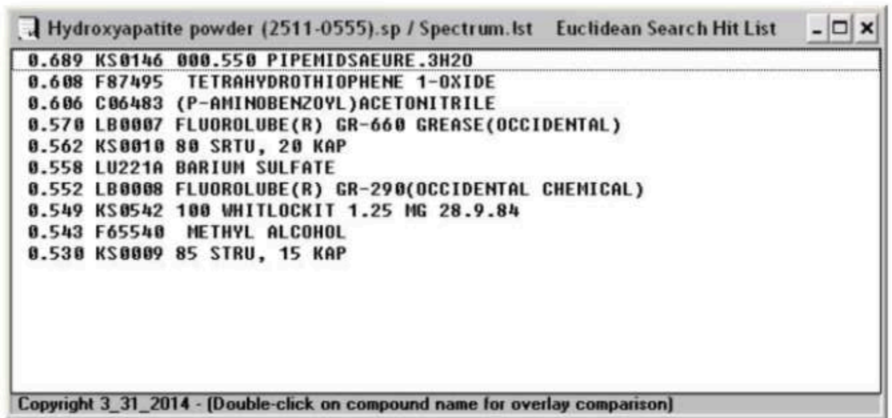


Figure 3. Spectral Library of *Hydroxyapatite powder*

Remarks:
**The above results and indicated values in the library search may serve as a guide for further verification and identification. These is for screening purposes only and cannot adequately serve as sole reference.*
**The results obtained herein are qualitative and only pertaining to the samples tested as received. This report should not be served as supporting document for any official function such as commercial and legal purposes.*

SUMMARY OF THE ASSIGNMENT PEAKS OF FTIR SPECTRUM RESULT

Peaks (cm^{-1})	Stretching	Functional Group
3600	O-H stretching	Hydroxyl group
1208.71	Asymmetric stretching	Phosphate group
1186.75	Asymmetric stretching	Phosphate group
1154.64	Asymmetric stretching	Phosphate group
1120.76	P-O stretching	Phosphate group
1099.36	P-O stretching	Phosphate group
1058.34	P-O stretching	Phosphate group
1024.67	P-O vibration	Phosphate group
972.91	O-P-O bending	Phosphate group
945.18	O-P-O bending	Phosphate group
723.35	P-O bending	Phosphate group
610.49	P-O bending	Phosphate group

INTERPRETATION:

The FTIR spectrum of the *Magallana bilineata oyster shells* (Mbs-HAp) powder showed several characteristic peaks that confirm the formation of hydroxyapatite, as seen in the result. A broad band around 3600 cm^{-1} was observed, which can be attributed to O-H stretching vibrations. This indicates the presence of hydroxyl groups, an essential component of the hydroxyapatite structure.

In the mid-infrared region, peaks at 1208.71, 1186.75, and 1154.64 cm^{-1} were observed and are associated with the asymmetric stretching of phosphate groups. Additional peaks at 1120.76, 1099.36, 1058.34, and 1024.67 cm^{-1} correspond to P-O stretching vibrations. Meanwhile, the peaks at 972.91 and 945.18 cm^{-1} are related to O-P-O bending, while those at 723.35 and 610.49 cm^{-1} represent P-O bending modes. These peaks are consistent with the known FTIR profile of hydroxyapatite, particularly the phosphate stretching and bending regions, as supported by study made by Hossain & Ahmed (2023).

The presence of both hydroxyl and phosphate groups confirms the formation of hydroxyapatite, which is chemically similar to the mineral component of enamel. This similarity is important because it allows the material to act as a source of calcium and phosphate ions, which are needed for enamel remineralization.

In addition, the absence of unexpected peaks suggests that the synthesized material is relatively pure. This observation is supported by recent studies showing that well-defined hydroxyl and phosphate peaks are indicative of high-purity hydroxyapatite suitable for biomedical use (Ciobanu et al., 2024).

APPENDIX G
Toothpaste Base Formulation

Formulation:

Calcium carbonate	28 g
Sodium lauryl sulfate	0.5 g
Glycerin	11 g
Gum tragacanth	0.75 g
Water	9.7 g
Saccharin sodium	0.05 g
Peppermint oil, q.s	
Sodium benzoate, q.s	
To make	50 g

APPENDIX H
Computation for the 10% Concentration of *Magallana bilineata* Toothpaste Formulation

Formulation of 10% w/w *Magallana bilineata* Toothpaste

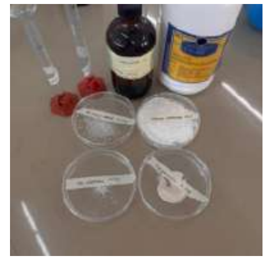
Magallana bilineata shell-derived HAp	5 g
Toothpaste base, q.s	45 g
To make	50 g

Computation:

Preparation of 10% w/w *Magallana bilineata* Toothpaste
$\frac{10\ g}{100\ g} \times \frac{X}{50\ g}$ $X = \frac{10\ g \times 50\ g}{100}$ $X = 5\ g$ 5 g of HAp powder was used to formulate 10% w/w *Magallana bilineata* toothpaste

APPENDIX I

Formulation of 10% Concentration of *Magallana bilineata* Toothpaste Documentation

Preparing and Weighing the Ingredients	 
Mixing the Ingredients as per Instruction	     

APPENDIX J
Certificate of Analysis for SEM-EDX

De La Salle University
i-NANO
RESEARCH FACILITY

CERTIFICATION OF ANALYSIS

This is to certify that the following analyses were performed by **i-Nano Research Facility, DLSU-Manila** on February 28, 2026 using **Phenom** (brand) **XL** (model) Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) equipment for the sample(s) provided by the client:

Analyses Information

Name of Client:	Jasmine Andrei D. Flores
Client's Affiliation/ School/ Company:	Lorma Colleges Inc
Analyses ID:	20260226
Date of Analysis:	February 28, 2026
Type of tests:	SEM and EDX

Equipment Configuration

Vacuum level:	High (1Pa)
Detector:	BSD full
Accelerating Voltage:	10kV for SEM analysis, while 15kV for EDX analysis
Signal Intensity:	Image mode for SEM analysis, while Map mode for EDX analysis
Working distance:	Please refer to each SEM micrograph. The value of the working distance is beside the vacuum level.

The laboratory analyses were performed following iNano's Research Facility standard operating procedures. The results are accurate and reliable to the best of our knowledge. The analyses were conducted by Dr. James Salveo Olarve. The certification is issued on February 28, 2026.

Certified by:

Dr. James Salveo Olarve
Laboratory Coordinator, i-Nano Research Facility
Assistant Professorial Lecturer, Department of Physics, DLSU-Manila

CERTIFICATION OF ANALYSIS

This is to certify that the following analyses were performed by **i-Nano Research Facility, DLSU-Manila** on March 12, 2026 using **Phenom** (brand) **XL** (model) Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) equipment for the sample(s) provided by the client:

Analyses Information

Name of Client:	Jasmine Andrei D. Flores
Client's Affiliation/ School/ Company:	Lorma Colleges
Analyses ID:	20260311
Date of Analysis:	March 12, 2026
Type of tests:	SEM and EDX

Equipment Configuration

Vacuum level:	High (1Pa)
Detector:	BSD full
Accelerating Voltage:	10kV for SEM analysis, while 15kV for EDX analysis
Signal Intensity:	Image mode for SEM analysis, while Map mode for EDX analysis
Working distance:	Please refer to each SEM micrograph. The value of the working distance is beside the vacuum level.

The laboratory analyses were performed following iNano's Research Facility standard operating procedures. The results are accurate and reliable to the best of our knowledge. The analyses were conducted by Dr. James Salveo Olarve. The certification is issued on March 12, 2026.

Certified by:

Dr. James Salveo Olarve
Laboratory Coordinator, i-Nano Research Facility
Assistant Professorial Lecturer, Department of Physics, DLSU-Manila

CERTIFICATION OF ANALYSIS

This is to certify that the following analyses were performed by **i-Nano Research Facility, DLSU-Manila** on March 27, 2026 using **Phenom** (brand) **XL** (model) Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) equipment for the sample(s) provided by the client:

Analyses Information

Name of Client:	Jasmine Andrei D. Flores
Client's Affiliation/ School/ Company:	Lorma Colleges
Analyses ID:	20260350
Date of Analysis:	March 27, 2026
Type of tests:	SEM and EDX

Equipment Configuration

Vacuum level:	High (1Pa)
Detector:	BSD full
Accelerating Voltage:	10kV for SEM analysis, while 15kV for EDX analysis
Signal Intensity:	Image mode for SEM analysis, while Map mode for EDX analysis
Working distance:	Please refer to each SEM micrograph. The value of the working distance is beside the vacuum level.

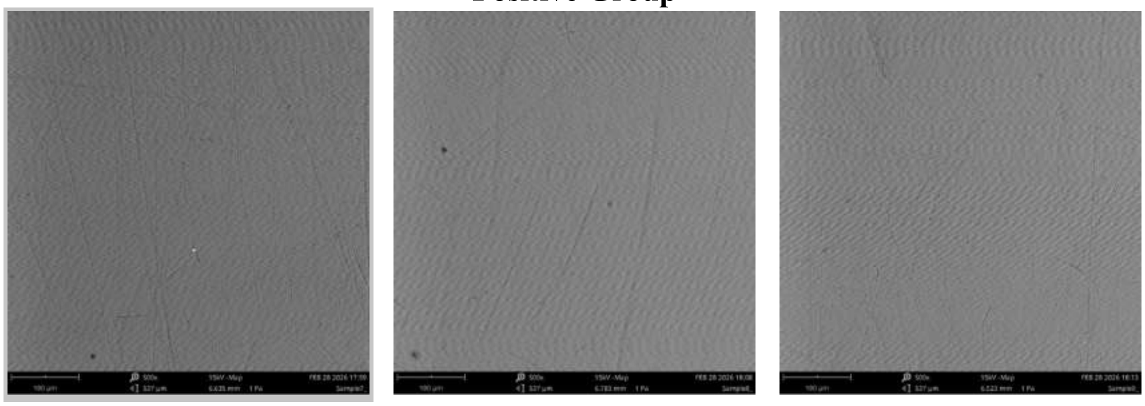
The laboratory analyses were performed following iNano's Research Facility standard operating procedures. The results are accurate and reliable to the best of our knowledge. The analyses were conducted by Dr. James Salveo Olarve. The certification is issued on March 27, 2026.

Certified by:

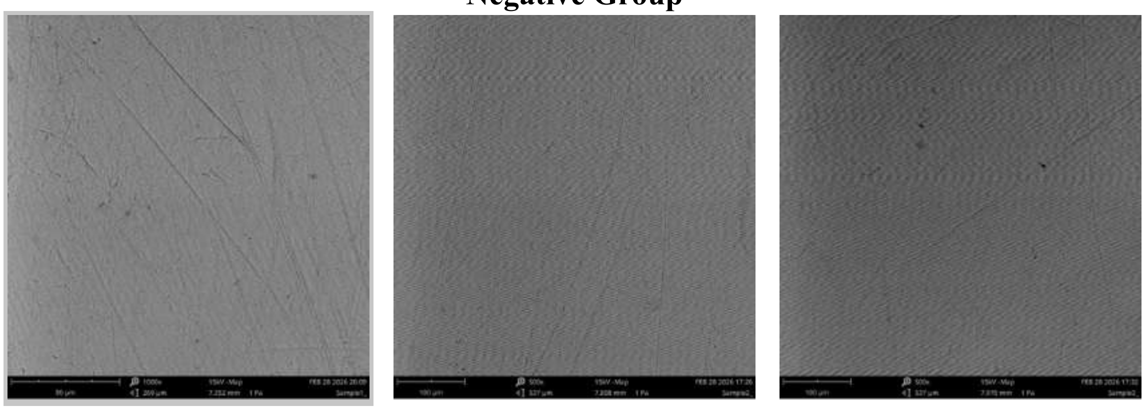
Dr. James Salveo Olarve
Laboratory Coordinator, i-Nano Research Facility
Assistant Professorial Lecturer, Department of Physics, DLSU-Manila

APPENDIX K
Surface Morphology Raw Data

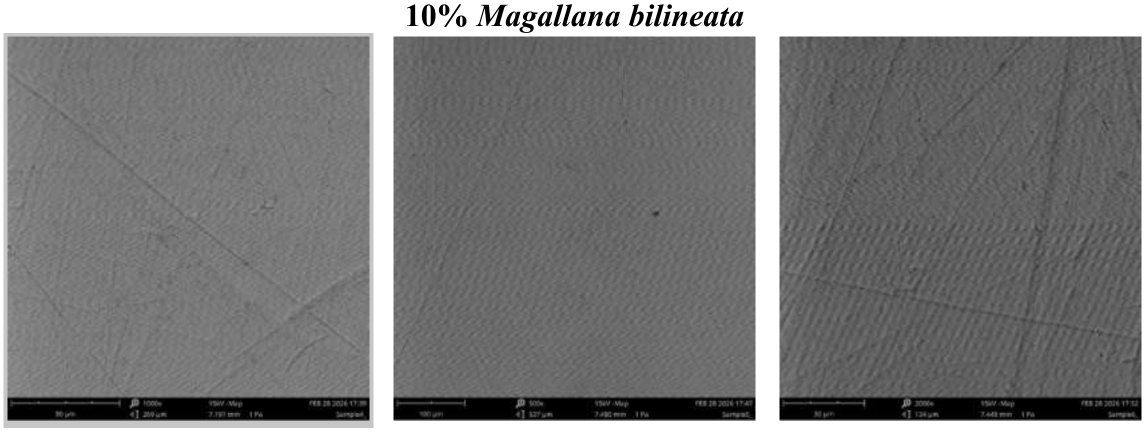
Before Demineralization Micrographs
Positive Group



Negative Group

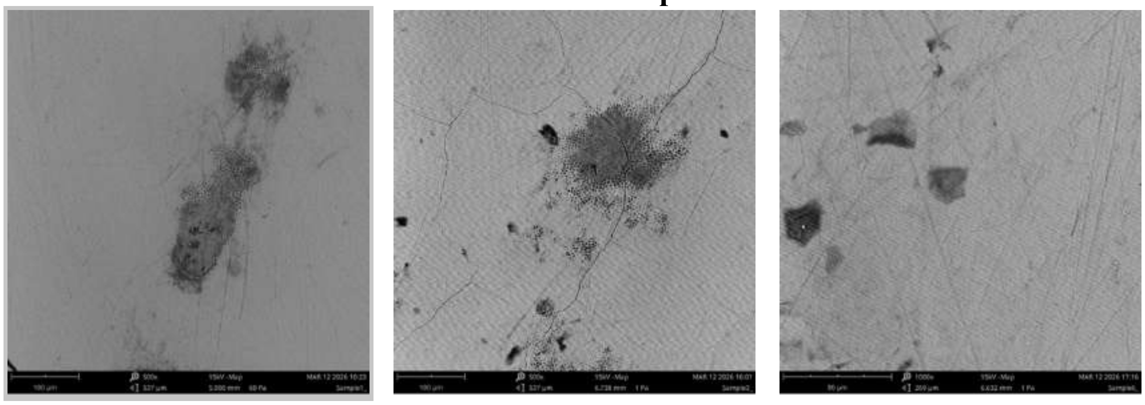


10% *Magallana bilineata*

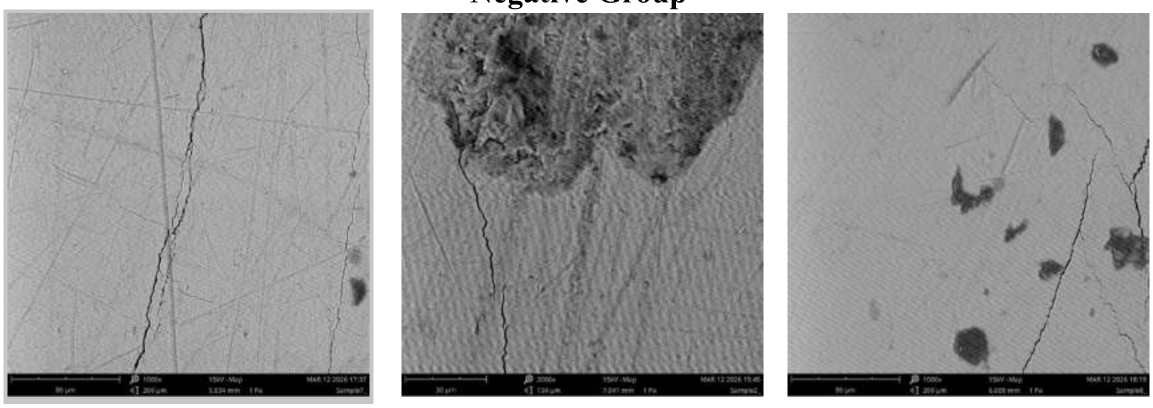


After Demineralization Micrographs

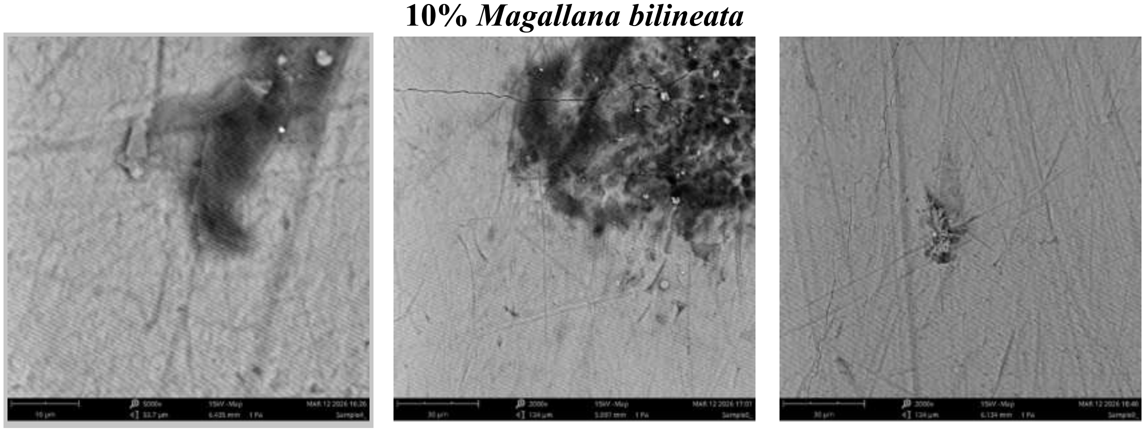
Positive Group



Negative Group

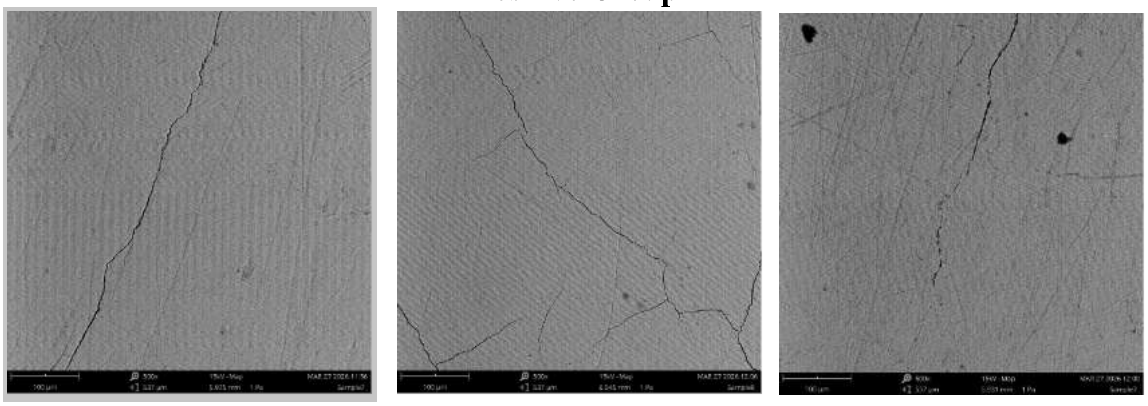


10% *Magallana bilineata*

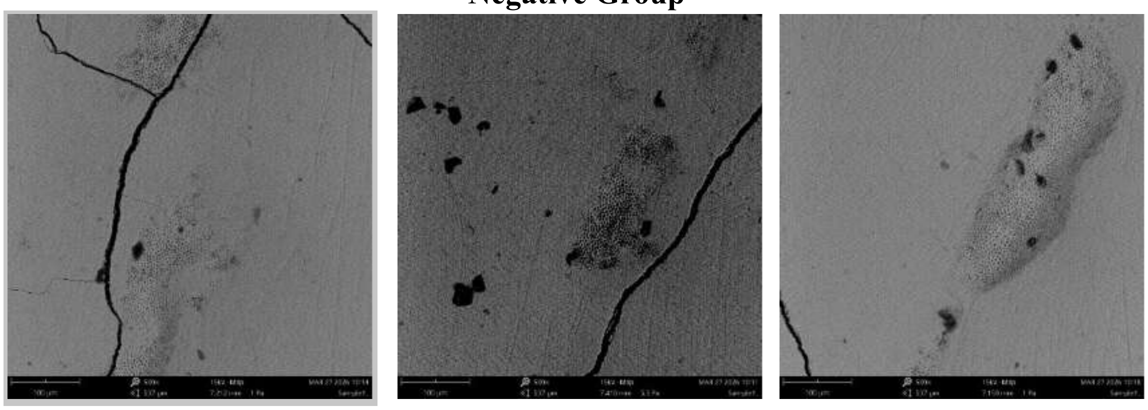


After Treatment Micrographs

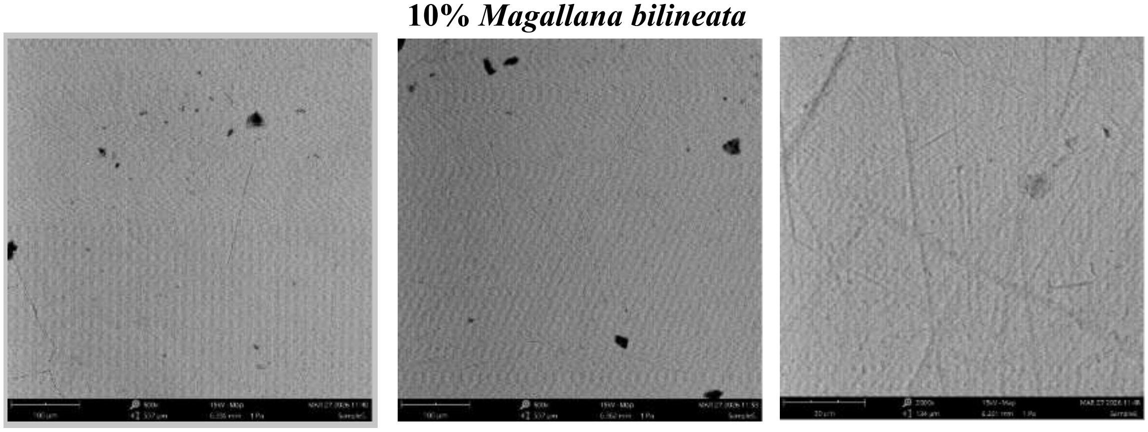
Positive Group



Negative Group



10% *Magallana bilineata*



	Tooth	Baseline	After Demineralization	After Treatment
Positive Control Group	1	1	3	2
	2	1	4	2
	3	1	4	3
Negative Control Group	4	1	4	4
	5	1	5	4
	6	1	4	3
10% *Magallana bilineata*	7	1	4	2
	8	1	5	3
	9	1	3	1

APPENDIX L
Elemental Composition Raw Data

Group	Tooth	BASELINE		AFTER DEMINERALIZATION		AFTER TREATMENT	
		Elemental Composition		Elemental Composition		Elemental Composition	
		Ca	P	Ca	P	Ca	P
Positive Control Group	1	27.99	14.64	22.39	12.11	33.58	15.62
	2	30.37	15.65	23.91	12.92	44.09	17.69
	3	41.04	17.61	28.15	13.82	46.72	19.61
Negative Control Group	1	29.92	14.59	23.36	12.98	24.03	13.66
	2	38.96	17.71	27.47	14.55	29.99	15.44
	3	33.11	16.21	26.52	14.50	29.91	15.32
10% *Magallana bilineata*	1	36.15	16.87	25.93	13.40	40.51	17.00
	2	34.24	16.24	25.22	13.20	36.61	16.97
	3	27.77	13.82	22.77	11.97	33.69	15.22

APPENDIX M
Physicochemical Properties Documentation

Specific Gravity Determination Using Pycnometer

$$\text{Specific gravity} = \frac{w2 - w1}{w3 - w1}$$

Where:
w1 = weight of empty pycnometer
w2 = weight of pycnometer filled with toothpaste
w3 = weight of pycnometer filled with water

$$\text{Specific gravity} = \frac{59.488g - 19.420g}{43.764g - 19.420g} = \frac{40.068\ g}{24.344\ g}$$

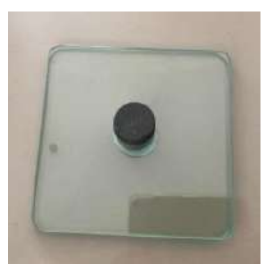
$$= 1.65$$

Spreadability Using Glass Slide Method

Glass Slide Method in 3 trials

1st Trial: 5.3 cm
2nd Trial: 5.1 cm
3rd Trial: 4.7 cm

Mean: 5.03 cm



pH Determination Using pH strip

The range goes from 0 – 14, pH of 7 and below indicates acidity, while pH of 7 and above indicate alkalinity. Our toothpaste has a pH of 6 which indicates slight acidity.

APPENDIX N
Questionnaire Tool for Evaluation Organoleptic Properties

Lorma Colleges
Center for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

Name (Optional): **Date:**
Course & Year/Position:

Evaluator's Questionnaire for Oyster Shell Toothpaste

Good day!

We are currently conducting a study entitled **"Remineralization Potential of Philippine Cupped Oyster Shells (*Magallana bilineata*) Powder Formulated as Toothpaste: An In-Vitro Study"**, in partial fulfillment of the requirements in the subject PHARMACY RESEARCH METHODS WITH STATISTICS.

As part of our research, we humbly seek your participation in evaluating the sensory characteristics of our formulated toothpaste. Your valuable insights regarding its color, odor, and texture will greatly assist us in determining the product's quality, acceptability, and potential for oral application. Rest assured that your responses will be treated with utmost confidentiality and will be used solely for academic purposes.

We sincerely appreciate your time, effort, and cooperation in supporting our study. Your participation plays a vital role in helping us achieve meaningful and reliable results.

Respectfully,
The Researchers

Instructions: *Please assess the product honestly by checking (✓) the description that best matches your observations.*

Color
What is the observed appearance of the toothpaste?
- ☐ Darker or uneven color
- ☐ Whitish to light gray
- ☐ Whiter or brighter than expected

Odor
How would you describe the odor intensity of the toothpaste?
- ☐ Weak or unpleasant odor
- ☐ Pleasant menthol scent (mild and refreshing)
- ☐ Strong or overly intense menthol aroma

Texture/Consistency
How would you describe the texture during application?
- ☐ Gritty or uneven consistency
- ☐ Smooth and homogenous consistency
- ☐ Overly viscous consistency

Kindly write down any further remarks, suggestions, or observations regarding the color, odor, texture, taste, foaming ability, or overall quality of the product.

APPENDIX O
Evaluation of Organoleptic Properties Documentation

Faculty

Lorma Colleges
Center for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

Name (Optional): Date:
Course & Year/Position:

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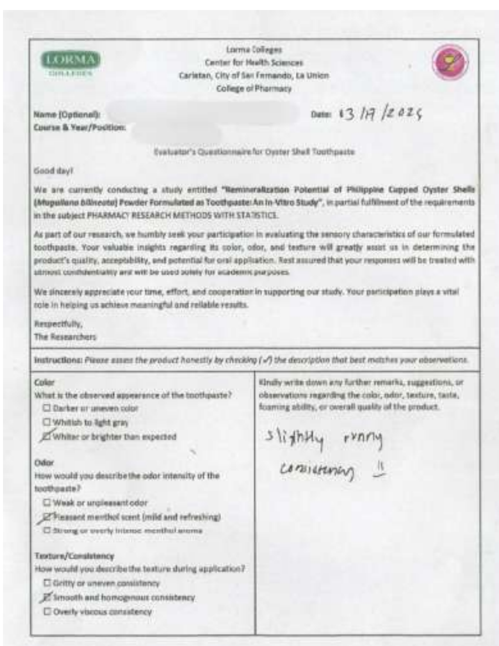
make your product more stiff.

BS Pharmacy Students



Lorma Colleges
Center for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

Name (Optional): Date: 13/17/2025
Course & Year/Position:

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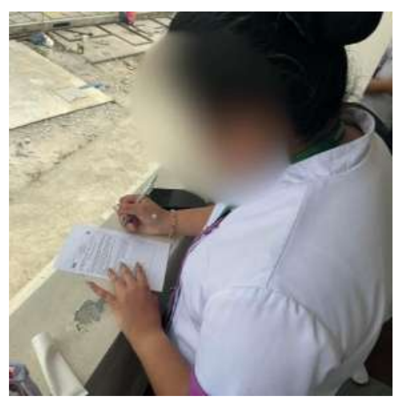
slightly runny consistency !!



Lorma Colleges
Center for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

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Course & Year/Position:
Date: 03/17/26

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pwede po ba different odor and color

Lorma Colleges
Center for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

Name (Optional):
Course & Year/Position:
Date: 3-17-26

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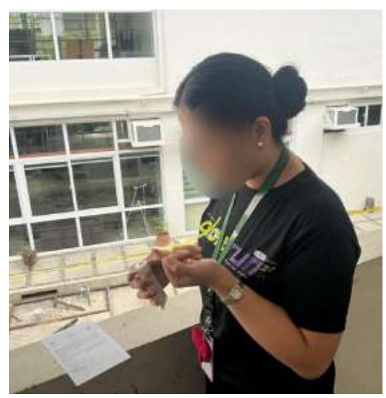
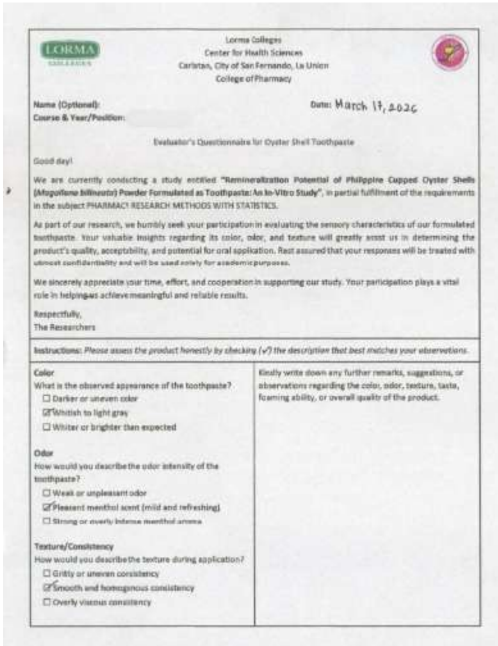


Other Department



Lorma Colleges
Center for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

Name (Optional):
Course & Year/Position:
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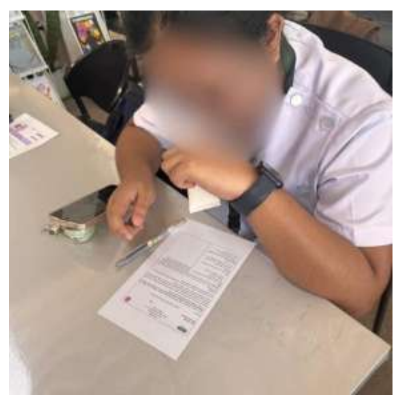
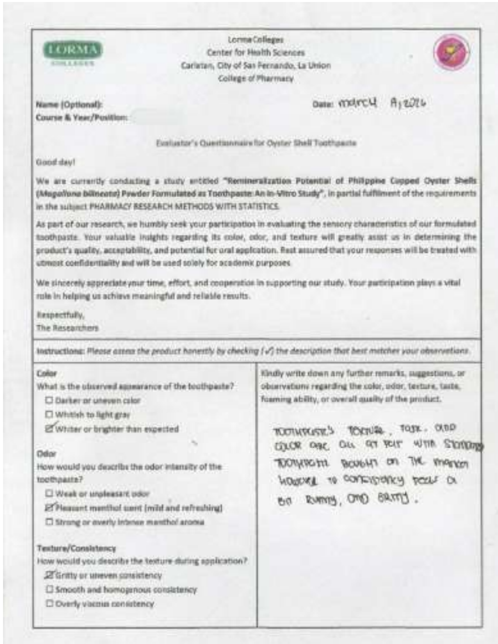
the odor was pleasant but I want it more stronger.





Lorma Colleges
Center for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

Name (Optional):
Course & Year/Position:

Date: march 9, 2026

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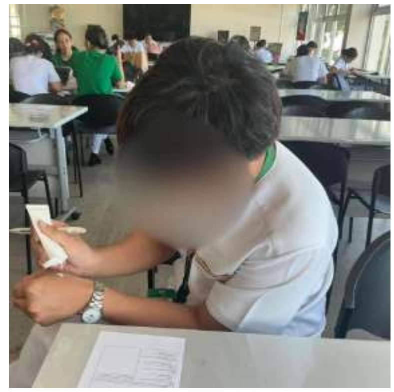
TOOTHPASTE'S TEXTURE, TASTE, AND COLOR ARE ALL AT PAR WITH STANDARD TOOTHPASTE BOUGHT ON THE MARKET HOWEVER ITS CONSISTENCY TENDS TO BE RUNNY, AND GRITTY.



APPENDIX P
Certificate from Statistician

CERTIFICATE OF DATA STATISTICAL ANALYSIS AND TREATMENT

CERTIFICATION

This certifies that the instrument used in the thesis entitled.

Remineralization Potential of Philippine Cupped Oyster Shells (Magallana bilineata) Powder Formulated as Toothpaste: An In-Vitro Study

By

Daryll Jade M. Abriam, Jasmine Andrei D. Flores, Hannah Jane A. Nang, & Cheryl Joyce M. Ulanday

This document certifies that the coursework for a Research Study, undertaken as part of the Bachelor of Science in Pharmacy at Lorma Colleges, City of San Fernando, La Union, has been subjected to thorough scrutiny, meticulous examination, and comprehensive statistical analysis in accordance with the study's requirements.

This certification is issued on the **1st of April 2026**, upon the request of the researchers for whatever legal purpose it may serve.

IWYNE MENDOZA ABENIS
Statistician
DepEd Cavite Province

10. Author(s)

Jasmine Andrei D. Flores, is a Pharmacy student at LORMA Colleges, who has demonstrated both academic excellence and strong leadership throughout her college journey. She served as a Year Representative of the College of Pharmacy-Student Body Organization during the academic years 2023-2024 and 2025-2026, and currently holds the position of Vice President of the ROTARACT Club of LORMA Colleges for the academic years 2024-2026. Her dedication to academics and student leadership has earned her consistent honorable distinctions.

Ellen Mae P. Abiqui, RPh, MSPharm, CPT, is the Dean of the College of Pharmacy at LORMA Colleges and also the Higher Education Academic Director. Her passion for learning and dedication to her field are clear in the path she had taken, becoming a Pharmacist, earning her Master of Science in Pharmacy, and achieving her CPT-certification at LORMA Colleges.. Each milestone reflects not just her academic excellence but also her commitment to growing and excelling in her profession.

Daryll Jade M. Abriam, is a Pharmacy Student at LORMA Colleges. He is determined to develop and enhance skills in pharmaceutical sciences aiming to contribute to quality healthcare. With a focus on patient care and promoting drug use, he aspires to become a medical professional and play a role in advancing an accessible healthcare system.

Karlo D. Legaspi, RPh, is a dedicated pharmacist with extensive experience in hospital pharmacy, clinical pharmacy, pharmaceutical manufacturing, and public health service. His career reflects a strong commitment to patient care, professional growth, and excellence in pharmacy practice. Currently serving as Pharmacist II at Department of Health - Ilocos Center for Health Development, he continues to contribute to the healthcare sector through his expertise and dedication to service.

Hannah Jane A. Nang, is a Pharmacy Student at LORMA Colleges. She is dedicated to expanding her knowledge and developing her skills in the field of healthcare. With a strong commitment to continuous learning and professional growth, she aspires to become a competent and compassionate healthcare professional who can provide quality patient-centered care and contribute positively to the medical community.

Ivy Rose C. Orozco, RPh, CIP, is the Research Coordinator of the College of Pharmacy at LORMA Colleges. She earned her bachelor’s degree in the University of Santo Tomas, and later pursued her Certified Immunization Pharmacist (CIP) credential through the Philippine Pharmacists Association. This reflects her drive to learning and dedication to advancing patient care.

Cheryl Joyce M. Ulanday, is a Pharmacy Student at LORMA Colleges. She is driven by a passion for healthcare and a strong desire to make a difference in the lives of others. She continuously works toward academic excellence while developing the skills, discipline, and compassion essential to become a future healthcare professional. With a vision to become a trusted healthcare provider, she is committed to promote patient well-being and embody the values of integrity and service.