Enhancing the utility of freshwater mussel (*Pilsbryoconcha* sp.) Shell waste as an abrasive substance in toothpaste

Santhy Wisuda Sidauruk[1*], *Tjipto* Leksono[1], *Rizky Febriansyah* Siregar[1], *Noor* Ira Sari[1], and *Stephanie* Oktavia[1]

[1]Dept. of Fish Product Technology, Faculty of Fisheries and Marine Science, Universitas Riau, Indonesia

Abstract. Untapped potential lies in the solid waste of freshwater mussel shells. Indeed, the shells possess a substantial amount of calcium. Valorization is essential for enhancing added value by utilizing calcium as an abrasive ingredient in toothpaste. This study aimed to investigate the characteristics of toothpaste containing abrasive components derived from freshwater mussel shells. The research procedure consisted of the production of CaO flour and the synthesis of hydroxyapatite through the calcination method. The test parameters consisted of evaluation of toothpaste preparations, analysis of calcium content, and antibacterial activity of *S. aureus* in 4 toothpaste formulations, namely T1 (CaO flour), T2 (commercial $CaCO_3$), T3 (hydroxyapatite), and T4 (commercial toothpaste). Except for treatment T1, the results demonstrated that toothpaste's pH, spreadability, and foam formation fulfilled toothpaste quality standards. Every treatment's toothpaste had a homogenous paste with good cleaning abilities. Treatment T2 had the greatest calcium level in toothpaste, measuring 2132.5 ppm. Subsequently, toothpaste T3 and T4 had antibacterial properties, however T1 and T2 did not possess any antibacterial action. The toothpaste formulation including hydroxyapatite as an abrasive, known as treatment T3, can be determined as the most effective.

1 Introduction

Mussels (*Pilsbryoconcha* sp.) are one type of freshwater shellfish from the mollusk phylum that is widely favored by the community. Currently, this mussel is used as a bioremediation agent in open waters and fish farming ponds [1-2]. In addition, freshwater mussels are also used as food by the surrounding community. The use of mussels as food is still limited to the use of meat only, while mussel shells have not been optimally utilized and have the potential to be solid waste. In fact, the proportion of mussel meat and offal is 48.10%; and the remaining largest proportion is mussel shells at 51.90% [3].

The large potential of solid waste from the utilization of mussels, so that a breakthrough is needed so that the utilization of mussel shells becomes more optimal. One way is through the valorization of mussel shells which aims to increase added value and generate potential

[*] Corresponding author: santhy.sidauruk@lecturer.unri.ac.id

profits. The results of the study showed that mussel shells are rich in minerals, especially high with a calcium content of 61.39% [4]. The high calcium content in mussel shells is the basis for valorizing them into products with high added value. So far, the valorization of mussel shells has been carried out in the food sector, namely the manufacture of mussel shell flour which is applied to mineral-rich cookies [4-5]. In addition, the high calcium in mussel shells has been utilized by being converted into CaO flour with a calcium content of 76.27% which functions as a calcium precursor in the synthesis of hydroxyapatite by the calcination method [3].

The calcium content in freshwater mussel shells can be used as an abrasive in toothpaste preparations. Abrasive materials in toothpaste function to clean and polish the tooth surface without damaging the enamel, and prevent stain accumulation [6]. Toothpaste that does not contain calcium or abrasive compounds will cause a brown layer on the tooth surface, but those that exceed abrasiveness will result in abrasion of the tooth surface and cause demineralization due to loss of tooth minerals such as calcium, phosphorus, and phosphate which can damage the enamel [7]. Based on this potential, freshwater mussel shell waste has the potential to be an abrasive in toothpaste preparations, so it is necessary to conduct research on increasing the utility value of freshwater mussel shell waste as a source of calcium. This study aims to determine the characteristics of toothpaste containing abrasive materials from freshwater mussel shells.

2 Materials and methods of research

2.1 Materials

The materials used in this study were mussel shells (*Pilsbryoconcha* sp.) obtained from the Paku River, Kampar Kiri District, Kampar Regency, Riau Province, diammonium hydrogen phosphate $NH_4H_2PO_4$ (Merck, Germany), commercial $CaCO_3$, commercial toothpaste (sensitive) and toothpaste formulation materials consisting of Na-CMC, glycerin, sodium benzoate, saccharin, sodium lauryl sulfate, titanium oxide, menthol, phosphoric acid, and distilled water.

2.2 Production of CaO flour

Initially, the mussel shells were washed using distilled water to remove dirt that was attached and then dried under the sun, then floured and sieved. The mussel shell flour was then calcined to obtain CaO flour. The mussel shell calcination was carried out twice, namely the first at a temperature of 1000°C for 12 hours, the second at the same temperature for 24 hours [8].

2.3 Synthesis of hydroxyapatite

Hydroxyapatite synthesis was carried out using a low-temperature hydrothermal method. Calcined mussel shell CaO flour of 45.91 grams was mixed with 55.63 grams of ammonium dihydrogen phosphate and 600 mL of distilled water. The mixture was put into a 1000 mL beaker and heated on a hot plate at 90°C while stirring using a stirrer for 6 hours. The synthesis results in the form of slurry were then dried in an oven at 120°C for 15 hours. The dried sample will be calcined at 1000°C with a temperature increase rate of 10°C/minute for 2 hours [9].

2.4 Production of toothpaste made from calcium abrasive from freshwater mussel shells

Toothpaste production was carried out using 4 treatments including T1 (45% CaO Flour from freshwater mussel shells), T2 (45% commercial $CaCO_3$), T3 (45% Hydroxyapatite from freshwater mussel shells), and T4 (commercial toothpaste). The formulation of toothpaste based on natural hydroxyapatite is presented in Table 1 [10]:

Table 1. Toothpaste formulation containing calcium abrasive

Material	Category	Composition			T4
		T1	T2	T3	
Commercial $CaCO_3$ (g)	Abrasive	-	45	-	Commercial toothpaste
CaO freshwater mussel shell (g)	Abrasive	45	-	-	
HAp freshwater mussel shell (g)	Abrasive	-	-	45	
Na-CMC (g)	Binding agent	1.5	1.5	1.5	
Glycerin (mL)	Humectant	25	25	25	
Sodium benzoate (g)	Preservative	0.1	0.1	0.1	
Saccharin	Sweetner	0.4	0.4	0.4	
Sodium Lauryl Sulfate (g)	Surfactant	1	1	1	
Titanium dioxide (g)	Colour additive	6	6	6	
Menthol (g)	Aroma	0.4	0.4	0.4	
Aquadest (mL)	Solvent	Add 100	Add 100	Add100	

Description: T1 (45% CaO flour from freshwater mussel shells), T2 (45% commercial $CaCO_3$), T3 (45% Hydroxyapatite from freshwater mussel shells), and T4 (commercial toothpaste).

Toothpaste production begins with weighing all the ingredients. Na-CMC is ground and warm distilled water is added for 15 minutes, after which it is stirred. Hydroxyapatite is ground according to the treatment, then titanium oxide, sodium benzoate, and saccharin are added and then stirred until homogeneous. The homogeneous Na-CMC solution is added with sodium lauryl sulfate and glycerin. After the paste is formed, add menthol. The toothpaste preparation was analyzed consisting of organoleptic tests [11], pH tests [12], homogeneity tests [13], spreadability tests [14], foam formation tests [13], calcium content tests [15], cleaning ability [16], and antibacterial tests [14].

2.4 Data analysis

Data on pH, spread power, foam height and organoleptic were analyzed statistically by analysis of variance (ANOVA) using SPSS Software. While data on homogeneity, clean ability, calcium content, and antibacterial activity were analyzed descriptively.

3 Results

3.1 pH

The pH value of toothpaste containing calcium abrasive from freshwater mussel shells is presented in Figure 1.

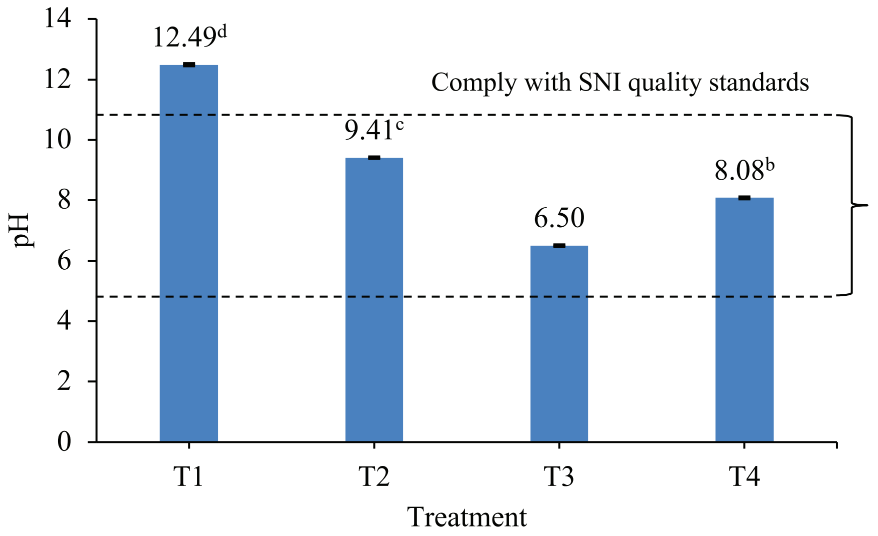


Fig.1. The pH value of toothpaste containing calcium abrasive from freshwater mussel shells

Figure 1 shows that the pH of toothpaste T2, T3, and T4 has met the SNI quality standards for toothpaste with pH values ranging from 6.50 to 9.41. However, the pH value of toothpaste T1 does not meet the SNI quality standards, which is 12.49. The pH quality requirements for toothpaste are 4.5-10.5. pH incompatibility can potentially cause irritation to the oral mucosa, as well as support bacterial growth, so maintaining the right pH in toothpaste is very important [17]. Toothpaste with a low pH can irritate and facilitate the growth of acidogenic bacteria that live in acidic environments such as *Streptococcus mutans* and *Lactobacillus* at pH 4.5-5.5. In addition, pH below 5.5 has the potential to cause demineralization and damage to tooth enamel, causing tooth caries [18].

Based on the analysis of variance, it shows that the difference in the addition of different abrasive materials has a very significant effect on the pH of toothpaste with F count (31832.12) > F table (7.85) at a confidence level of 99%, so the hypothesis (H0) is rejected. To see the different treatments, further tests were carried out. The results of further tests showed that the pH value of toothpaste for each treatment was different. T3 has the lowest pH value compared to other treatments, which is 6.50. The pH value of toothpaste in treatment T1 with CaO flour abrasive material from mussel shells has the highest pH value because mussel shells have a basic pH value, the basic pH value in mussel shells is thought to come from the lime (Ca) contained in mussel shells [23]. T1 has the lowest pH value compared to other treatments, which is 12.49. Treatment T3 with hydroxyapatite abrasive material has the lowest pH value because the hydroxyapatite used is given the addition of phosphoric acid.

3.2 Spread power

The spreadability of toothpaste containing calcium abrasive from freshwater mussel shells is presented in Table 2 and Figure 2.

Table 2. The spreadability of toothpaste containing calcium abrasive from freshwater mussel shells

Treatment	Spread Power (cm)
T1	2.27±0.07[a]
T2	3.04±0.15[b]
T3	3.08±0.19[c]
T4	4.34±0.14[d]

Table 2 shows that the diameter of the spread power of the toothpaste produced ranges from 2.27 - 4.34 cm. The diameter of the spread power of T1 toothpaste does not meet the criteria for spread power set at 2.61 - 5.32 cm. The diameter of the spread power of this toothpaste functions to measure the level of smooth distribution of the preparation, so that it provides comfort when used [17], in addition, the spread power also shows the ability of the gel to spread which is an important characteristic and affects the transfer of active ingredients [19]. Spread power that is too high indicates that the toothpaste is too thin so it is difficult to use [7].

Based on the analysis of variance, it shows that the difference in the addition of different abrasive materials has a very significant effect on the spreadability of toothpaste with F count (10866.537) > F table (7.85) at a confidence level of 99%, so the hypothesis (H0) is rejected. To see the different treatments, further tests were carried out. The results of further tests showed that the spreadability value of toothpaste for each treatment was different. T4 has the highest spreadability value compared to other treatments, namely 4.34 cm. T1 has the lowest spreadability value compared to other treatments, namely 2.27 cm.

3.3 Homogeneity

The homogeneity of toothpaste containing calcium abrasive from freshwater mussel shells is presented in Table 3.

Table 3. Homogeneity of toothpaste containing calcium abrasive from freshwater mussel shells

Treatment	Homogeneity
T1	 Homogeneous
T2	 Homogeneous

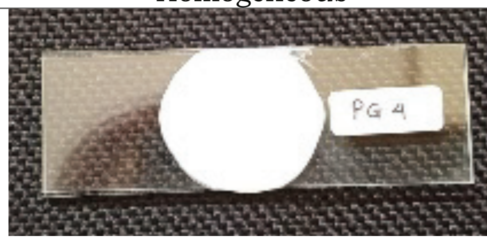
Treatment	Homogeneity
T3	 Homogeneous
T4	 Homogeneous

Table 3 shows that toothpaste with various different abrasive sources meets the criteria of a homogeneous paste, characterized by the absence of visible coarse particles or grains on the surface of the glass object [20]. This means that the mixing of ingredients in the toothpaste formulation is effective and evenly distributed [17]. All treatments T1, T2, T3, and T4 have met the ideal requirements for the homogeneity test of toothpaste. This causes the toothpaste to feel soft and can provide optimal therapeutic effects because the basic ingredients for making it have been mixed homogeneously [25].

3.4 Foam formation and cleaning ability

Foam formation and cleaning ability of toothpaste containing calcium abrasive from freshwater mussel shells are presented in Table 4.

Table 4. Foam formation and cleaning ability of toothpaste containing calcium abrasive from freshwater mussel shells

Treatment	Foam Height (mm)	Cleaning Ability
T1	$7.67{\pm}0.06^{b}$	Good
T2	$8.83{\pm}0.11^{c}$	Good
T3	$5.62{\pm}0.06^{a}$	Good
T4	$35.13{\pm}0.05^{d}$	Good

Table 4 shows that toothpaste with various different abrasive sources has a foam height ranging from 5.62–8.83 mm which has met the requirements for toothpaste with a minimum foam height of 15 mm [17]. Meanwhile, the foam height of commercial toothpaste is 35.13. This is because the use of sodium lauryl sulfate (SLS) is added more to commercial toothpaste. The amount of foam formed is influenced by the concentration of SLS which is an anionic surfactant with effective cleaning ability. In terms of cleaning ability, all toothpastes have good cleaning ability. This is because the abrasive materials used have the ability to clean and remove stains and plaque [21]. Abrasive materials are also used to clean and polish the tooth surface without damaging the enamel, and prevent the accumulation of stains on the teeth [24].

Based on the analysis of variance, it shows that the difference in the addition of different abrasive materials has a very significant effect on the height of toothpaste foam with F count (110530.907) > F table (7.85) at a confidence level of 99%, so the hypothesis (H0) is rejected. To see the different treatments, further tests were carried out. The results of further tests showed that the height of toothpaste foam for each treatment was different. T4 has the highest foam height value compared to other treatments, namely 35.13 mm. T3 has the lowest foam height value compared to other treatments, namely 5.62 mm.

3.5 Organoleptic

The organoleptic value of toothpaste made from calcium abrasive from freshwater mussel shells is presented in Figure 2.

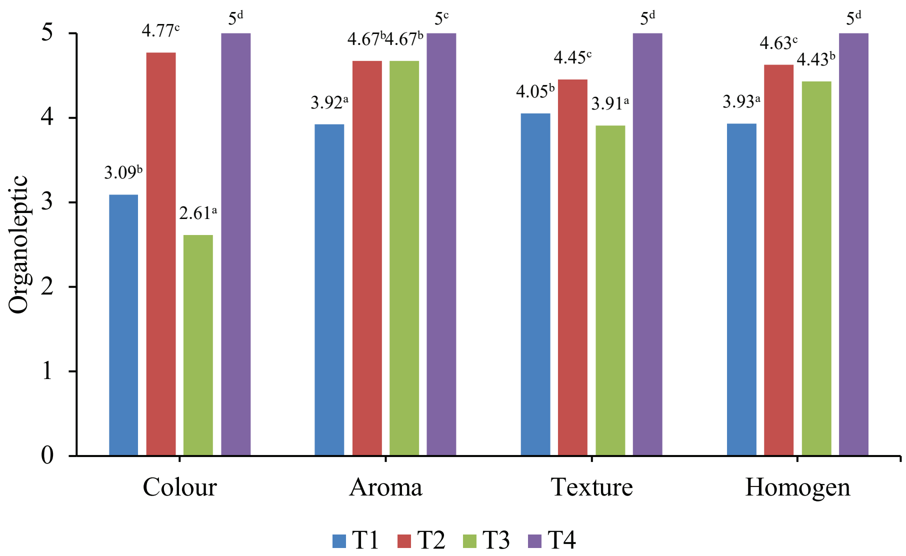


Fig.2. Organoleptic value of toothpaste containing calcium abrasive from freshwater mussel shells

Figure 2 shows that the average panelist rated the color of the toothpaste ranging from 2.61 to 5. The texture of the toothpaste produced, the panelist's assessment tends to decrease in the HAp abrasive material used because the texture of the toothpaste produced is getting rougher. The aroma and homogeneity of toothpaste with the addition of HAp have values between 3.92-5. This is because the mint aroma is still felt and homogeneous. Based on SNI 8861-2020, a toothpaste preparation is said to be good if it has a soft, homogeneous texture, no air bubbles, lumps, and separate particles [22].

Based on the analysis of variance, it shows that the difference in the addition of different abrasive materials has a very significant effect on the color of toothpaste with F count (1266.278) > F table (7.85) at a 99% confidence level, so the hypothesis (H0) is rejected. To see the different treatments, further testing was carried out. The results of the further testing showed that the color value of the toothpaste for each treatment was different. T4 has the highest color value compared to other treatments, namely 5. T3 has the lowest color value compared to other treatments, namely 2.61.

The difference in the addition of different abrasive materials has a very significant effect on the aroma of toothpaste with F count (103.175) > F table (7.85) at a 99% confidence level, so the hypothesis (H0) is rejected. To see the different treatments, further testing was carried out. The results of the further testing showed that the aroma value of the toothpaste for each treatment was different. T4 has the highest aroma value compared to other treatments, namely 5. T1 has the lowest aroma value compared to other treatments, namely 3.92.

The difference in the addition of different abrasive materials gives a very significant effect on the texture of toothpaste with F count (321.134) > F table (7.85) at a 99% confidence level, so the hypothesis (H0) is rejected. To see the different treatments, further testing was carried out. The results of the further testing showed that the texture value of the toothpaste for each treatment was different. T4 has the highest texture value compared to other treatments, which is 5. T3 has the lowest texture value compared to other treatments, which is 3.91. The difference in the addition of different abrasive materials gives a very significant effect on the homogeneity of toothpaste with F count (104.552) > F table (7.85) at a 99% confidence level, so the hypothesis (H0) is rejected. To see the different treatments, further testing was carried out. The results of the further testing showed that the homogeneity value of the toothpaste for each treatment was different. T4 has the highest homogeneity value compared to other treatments, which is 5. T1 has the lowest homogeneity value compared to other treatments, which is 3.93.

3.5 Calcium content and antibacterial activity of dental caries

The calcium content and antibacterial activity of dental caries-causing toothpastes containing calcium abrasive from freshwater mussel shell waste are presented in Table 5.

Table 5. Calcium content and antibacterial activity of toothpaste containing calcium abrasive from freshwater mussel shells on caries-causing toothpastes

Treatment	Calcium Content (ppm)	Antibacterial Activity of *Staphylococcus aureus* (mm)
T1	2132.5	0
T2	1028.1	0
T3	1022.5	7.93
T4	50.61	27.49

Table 5 shows that toothpaste with commercial $CaCO_3$ abrasive has a calcium content of 1028.1 ppm, while toothpaste with freshwater mussel shell HAp abrasive has a calcium content of around 1022.5 ppm and toothpaste with freshwater mussel shell CaO abrasive is 2132.5 ppm. Meanwhile, commercial toothpaste (positive control) containing HAp only has a calcium content of 50.61 ppm. Antibacterial activity against *S. aureus* bacteria with an inhibition zone diameter of around 7.93 in toothpaste with HAp abrasive, while commercial toothpaste has an inhibition zone diameter of 27.49 mm.

4 Conclusions

Toothpaste pH, spreadability, and foam formation have met the quality standards of toothpaste except for treatment T1. Toothpaste of all treatments has a good cleaning ability category and homogeneous paste preparation. The highest calcium content of toothpaste is treatment T2 (2132.5 ppm) and the lowest calcium content is in T4 (50.61 ppm), and the calcium content of T1 is 1028.1 ppm and T3 is 1022.5 ppm. Next, toothpaste T3 and T4 have antibacterial activity, while T1 and T2 do not have antibacterial activity. It can be concluded that treatment T3 is the best toothpaste formulation with hydroxyapatite as an abrasive.

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