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Effectiveness of Green Mussel Shell Waste and Lime Peel as Toothpaste

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ABSTRACT The problem is that waste produced from green mussel shells can result in environmental pollution. Green mussel shells can be used as raw material for making toothpaste. Toothpaste is a care product that has the function of caring for healthy teeth to prevent cavities that use a combination of green mussel shells and lime peel as the main ingredients. This study aims to determine the effectiveness of toothpaste derived from green mussel shells and lime peel. This research was carried out in four stages, namely making green mussel shell flour, making lime peel extract, making toothpaste formulations and testing which included organoleptic, carbohydrate, pH, antibacterial, calcium, viscosity, centrifugation and stability testing. The design used was a Completely Randomized Design (CRD) with 5 treatment levels with different concentrations of green mussel shells, namely P1 (0), P2 (16.5%), P3 (25%), P4 (33.5%), P5 (50%).The research results showed that the best treatment was toothpaste with a combination of clam shell powder and lime peel extract in the P5 treatment in the antibacterial test with an inhibitory power obtained of 156.67 ± 15.28. Treatment P5 with a concentration of 50% addition of green mussel shell flour was the best treatment for toothpaste preparations.

Keywords: Antibacterial; dental health; fishery waste; green mussel

INTRODUCTION

Green mussels are a commodity that is often found in coastal areas with the Latin name Perna viridis. Green mussels are usually used as a delicious dish because green mussels have high nutritional value and are economically priced. However, there is a problem regarding waste from green mussel shells that occurs in Domas Village, Pontang District, Serang Regency, Banten Province. Green mussels only use their meat for consumption without thinking about the waste (Nisa *et al*., 2021). As a result, environmental pollution occurs due to the accumulation of green mussel shell waste, therefore it is necessary to utilize green mussel shell waste to prevent environmental pollution. Green mussel shells have a high calcium carbonate (CaCO3) content, namely 98% (Awang *et al*., 2005). Calcium carbonate has benefits as an abrasive material which can be used as a cosmetic preparation, namely toothpaste.

Toothpaste is a product commonly used to clean teeth, which consists of a mixture of cleaning ingredients without damaging teeth or the mucous membranes of the mouth (SNI 8861-2020). There are 2 types of toothpaste on the market, namely natural and synthetic toothpaste, but people prefer toothpaste made from natural ingredients (Oroh *et al*., 2015). Therefore, there is a need to innovate toothpaste preparations made from natural ingredients, such as shellfish waste which can be used as flour and can be processed to become an ingredient for making toothpaste. because shellfish flour contains various minerals such as phosphorus, calcium and carbohydrates. The highest content found in green mussel shell flour is calcium carbonate at 98%. Calcium carbonate has benefits as an abrasive for toothpaste (Awang *et al*., 2005). Apart from that, antibacterial substances are needed to prevent dental problems, therefore it is necessary to add natural ingredients that can be used as antibacterial substances. One natural ingredient that has high antibacterial properties is lime peels.

Lime peel has various useful active substances such as flavonoids, citric acid, vitamin B1, vitamin C, amino acids, and saponins (Putri *et al*., 2010). One of the compounds that has antibacterial, antidiabetic, anticancer and antiseptic properties is flavonoids. In lime peel there is an essential oil which also contains flavonoid compounds which can inhibit the growth of pathogenic bacteria such as *Escherichia coli*, *Salmonella* sp., *Streptococcus aureus* and *Streptococcus mutans* (Putri *et al*., 2010). Therefore, it is necessary to carry out this research to determine the effectiveness of toothpaste preparations by combining green mussel shell flour and lime peel extract as an antibacterial.

The aim of this research is to determine effectiveness and find out the best treatment for toothpaste with the addition of green mussel shells and lime peel extract. The benefit of this research is that it can provide information to the public that green mussel shells and lime peels can be used as ingredients for making toothpaste, as well as a solution to reduce green mussel shell waste and lime peel waste.

Green mussels with the Latin name *Perna viridis* are included in the biota that is easy to develop, green mussels can adapt to various environments (Rajagopal *et al*., 2013). Green mussels are easy to find and can be processed into delicious and nutritious food. Green mussels consist of meat and shells. The part that can be processed into food is green mussel meat and green mussel shells often become waste that accumulates and can even cause environmental pollution. Clam shells have the potential to be used as handicrafts, accessories, and even as cosmetic preparations. Green mussel shells contain 98% calcium carbonate (Awang *et al*., 2005). This high content can be used as a cosmetic formulation, namely toothpaste, calcium carbonate has the benefit of forming bones and teeth (Agustini *et al*., 2011). Consuming calcium carbonate can prevent tooth and bone loss and prevent osteoporosis in old age (Khairil, 2012).

Lime has a Latin name *(Citrus aurantifolia)* this plant is a shrub, which is easily found in Indonesia. The distribution area of this plant is very wide, this plant can bear fruit all year round, this plant is often used for cooking spices, med-

icines and fresh drinks. The use of lime as medicine can increase appetite, reduce fever, anti-inflammatory, antibacterial and antioxidant (Okwu 2008). The lime plant consists of several parts, namely lime leaves, fruit, stems and lime peel. The part that is often consumed is the flesh of lime which is used by squeezing it. Apart from that, lime peel also has unexpected benefits. When extracted, lime peel has bioactive substances, namely flavonoids such as naringin, hesperidin, naringenin, esperitin, routine, nobiletin and tangeretin which has the benefit of inhibiting bacterial activity or what is known as antibacterial (Putra *et al*., 2014). Flavonoids are polyphenolic compounds that can be used as antibacterials and antioxidants. In toothpaste preparations, the flavonoids in lime peel extract can inhibit plaque-forming bacterial cells on teeth (Okwu, 2008). Flavonoids can also inhibit the bacterial activity of Streptococcus mutans in teeth (Zenia *et al*., 2013).

Toothpaste is a cosmetic product consisting of a mixture of scrubbing agents, cleaning agents and additional ingredients that can be used to clean teeth without damaging teeth or mucous membranes.(SNI 8861-2020). The function of toothpaste is to reduce the formation of plaque or color that sticks to the teeth, clean teeth, strengthen protection against dental caries and eliminate bad breath (Ilmi, 2017). Public awareness about dental and oral health has increased, resulting in demand for cleaning and dental care products also increasing. Toothpaste is an oral cleaning product that is useful for cleaning, improving the appearance of teeth and, eliminating bad breath, there are abrasive ingredients in toothpaste. Toothpaste contains an abrasive ingredient to clean plaque or dirt on teeth, namely calcium in the form of CaCO3, namely calcium carbonate (Ilmi, 2017).

Calcium carbonate ($CaCO_3$) contained in toothpaste is abrasive, whose function is to remove dental plaque and prevent dental problems. There are 2 types of toothpaste on the market, namely natural and synthetic toothpaste. Increasing public interest in the use of natural ingredients is because the use of natural ingredients in toothpaste preparations can reduce side effects such as irritation, itching and redness, so there is a need for natural toothpaste preparations (Sabir, 2005). Natural toothpaste does not contain synthetic ingredients such as fluoride. Natural ingredients that can be used to make cosmetic preparations include essential oils, aloe vera and strawberries which are widely used in the world of health, but not many people use toothpaste products that use green mussel shells as an abrasive with the addition of lime peel extract which works. as an antibacterial (Marlina *et al*., 2017).

Table 1. SNI 8861-2020 toothpaste quality requirements.

No.	Criteria	Unit	Condition	
			Adult toothpaste	Children's toothpaste
1	Carbohydrate	-	0	0
2	pH	-	6-10	6-9
3	Formaldehyde	g/mol	0.1	0.1
4	Fluorine free	g/mol	0.15	0.15
5	Arsen	Mg/kg	5	5
6	Lead	Mg/kg	20	20
7	Cadmium	Mg/kg	5	5
8	Mercury	Mg/kg	1	1
9	Total Plate Numbers	Colonies/g	5 x 102	5 x 102
10	Mold Numbers	Colonies/g	5 x 102	5 x 102
11	Streptococcus mutans	Colonies/g	-	-
12	Candida albicans	Colonies/g	-	-

MATERIALS AND METHODS

The tools used were a blender (Philips), (Miyako) mixer, (Iwaki) beaker, (Iwaki) measuring cup, stirrer, (Osuka) digital scale, and (Omron) thermometer. The ingredients used are green mussel shell flour obtained from the Domas green mussel shell production house, lime peel obtained from the Rau market in Serang City, phenoxyethanol, sodium chloride, gum arabic, glycerin, Na-lauryl sulfate, texapon, Na-saccharin, menthol, calcium carbonate, dye, distilled water, 96% ethanol, vinegar water, and packaging.

Methods

The research design used in this study was a one-factor Completely Randomized Design (CRD) with two replications. This research was carried out in four stages, namely making green mussel shell flour, making lime peel extract, making toothpaste formulations and testing which included organoleptic, carbohydrate, pH, antibacterial, calcium, viscosity, centrifugation and stability testing.

Green mussel shell flour (Perna viridis)

Ahmad (*2017*) washed the shells clean, then soaked the shells in 500 ml of vinegar solution for 24 hours. Then the shells are washed again and the drying process is carried out for 1 day by drying them in the sun, then the green mussel shells are soaked again using 50% Hydrogen Peroxide (H_2O_2) solution, then the shells are subjected to a drying process again for 1 day, then the shells are The dried ones will be ground using a blender, then sifted until you get shellfish flour which is ready to be used as a formula for making toothpaste.

Lime peel extraction (Citrus aurantifolia)

Dried lime peel was extracted using the maceration method using 50 ml of 96% ethanol for two days and stirring twice a day. The macerate was first filtered and then

extracted again with new 96% ethanol, repeated until clear, then thickened using a vacuum rotary evaporator at a temperature of 50 °C (Zenia *et al*., 2013).

Toothpaste

The ingredients are weighed according to the formulation, then heat the distilled water solution with a magnetic stirrer until it reaches a temperature of 80 °C, then dissolve the phenoxyethanol in the distilled water solution, add sodium chloride according to the toothpaste formulation, in another container add gum arabic moistened with glycerin then stir until homogeneous, after that the ingredients are mixed and then mixed until it becomes a mass of gel points. Then add sodium chloride, Na-lauryl sulfate, texapon, green mussel shell flour, lime peel extract, Na-saccharin, menthol, coloring, then mix until homogeneous to form a soft paste base.

Table 2. Toothpaste formulation (Ahmad, 2017) with modifications.

No.	Material	Quantity (%)					
		P1	P2	P3	P4	P5	Utility
1.	Shell Flour Green Shellfish	0	16.5	25	33.5	50	Active ingredients
2.	Lime Extract	5	5	5	5	5	Active ingredients
3.	Phenoxyethanol	0.5	0.5	0.5	0.5	0.5	Preservative
4.	Sodium Chloride	1.5	1.5	1.5	1.5	1.5	Preservative
5.	Gum Arabic	12	12	12	12	12	Binder
6.	Glycerin	14	14	14	14	14	Moisturizing
7.	Na-Lauryl Sulfate	1	1	1	1	1	Binder
8.	Texapon	3	3	3	3	3	Foaming agent
9.	Na-Saccharin	1	1	1	1	1	Sweetener
10.	Menthol	1	1	1	1	1	Flavor
11.	Aquades	10	10	10	10	10	Solvent
12.	Calcium carbonate	50	33.5	25	16.5	0	Active Ingredients
13.	Food Coloring	1	1	1	1	1	Dye

Organoleptic test

Organoleptic testing is carried out using a score sheet which includes hedonic testing. Organoleptic testing of toothpaste, hedonic test parameters in this study include color, aroma and texture. The hedonic organoleptic test will be carried out at the Aquatic Products Technology Laboratory, Department of Fisheries Science, Sultan Ageng Tirtayasa University, involving 30 panelists.

Carbohydrate test

According to SNI 8861-2020 (BSN, 2020), the quality requirement for good toothpaste is that it does not contain fermentable carbohydrates. Therefore, it is necessary to test carbohydrates in toothpaste to determine whether there is carbohydrate content in the toothpaste preparation. Carbohydrate testing uses the Molisch method, the Molisch method is a test carried out qualitatively to determine the presence or absence of carbohydrate content (Desyanti, 2013). This test uses Molisch reagent by liquefying 5 grams of sample using 10 ml of distilled water, then inserting the thawed sample into a test tube, then inserting Molisch reagent through the wall of the test tube, then look to see whether a purple ring forms on the sample. If the test result is positive, a purple ring will appear in the Molisch reagent (Desyanti, 2013).

Antibacterial power test (Handayani et al., 2017)

The resulting mouthwash formulation was tested against Streptococcus mutans bacteria using the disk diffusion method, The advantages of using the disc diffusion method are that it is economical, does not require a lot of equipment and is easy to use. The antibacterial test process includes the following stages, Sterilization Process; The tools and materials to be used must be sterile, therefore sterilization is carried out with the aim of killing microorganisms on the tools and materials. The sterilization process uses an autoclave at a temperature of 121 °C for 15 minutes. Making Agar Media; Agar media is used for bacterial inoculation. A total of 5 ml of MHA agar media was poured into each sterile test tube and covered with aluminum foil. Next, it was sterilized for 15 minutes in an autoclave at 121 °C, then left at room temperature for 30 minutes until the medium solidified at a slope of 30 °C. Creation of Mueller Hinton Agar (MHA) Media; Dissolve 3.8 grams of MHA media with 100 ml of distilled water, then carry out a heating and stirring process, after that carry out the sterilization process again for 15 minutes using an autoclave at a temperature of 121 °C. Inoculation of Bacteria on Slanted Agar Media; The test bacteria were taken using a sterilized loop needle, then scratched and embedded on slanted agar media, then the incubation process was carried out at a temperature of 37 °C for 24 hours. Preparation of Turbidity Standards Using Mc Solution Farland; Mix the H_2SO_4 solution into the Erlenmeyer flask with the BaCl2 solution, then stir until homogeneous so that a cloudy solution is formed. Turbidity is used as a standard for bacterial suspension turbidity. Preparation of Test Bacterial Suspension; The test bacteria that have gone through the inoculation process are taken with a sterile tube needle, then suspended in a tube containing 2 ml of 0.9% NaCl solution until the standard Mc solution turbidity is obtained. Paper Disk Soaking; The paper disks were soaked for 15-30 minutes in treatment 1, treatment 2, treatment 3, treatment 4, and treatment 5. Antibacterial Test with Agar Diffusion Method; Prepare a petri dish that has been divided into 5 parts, 15 mL of MHA media is poured into each part of the cup until it is homogeneous and allowed to solidify. Then a sterile cotton bud is dipped in the test bacteria until it is absorbed. Then remove the cotton bud, then rub all parts of the surface of the media for 15 minutes to absorb. Then attach the soaked paper disk. Next, incubation was carried out for 24 hours at 37 °C. Inhibition Zone Measurement; The diameter of the inhibition zone is calculated using the following formula:

Zone of Inhibition= A-B

Note: A = Calculated Obstacle Zone Diameter (mm).

B = Paper Disk Diameter.

Test calcium levels

Calcium carbonate ($CaCO_3$) functions as an abrasive material that can polish and remove stains and plaque. Testing calcium levels involves preparing a sample of 10 grams of toothpaste, then adding n-hexane, stirring and filtering until it is separated from the residue. The residue is then oven-treated at a temperature of 100 °C, the oven-baked samples are ready to be tested for calcium levels using an Atomic Absorption Spectrophotometer (AAS).

Viscosity test

Viscosity test to determine the thickness of the toothpaste produced. The higher the viscosity, the greater the resistance. The standard viscosity for toothpaste ranges from 2.000-50.000 cPs (Aprilianti *et al*., 2020). The viscosity value of toothpaste must be balanced because if the viscosity is too high, the toothpaste will be runny and difficult to apply, whereas if it is too low the texture will be hard and difficult to remove from the tube.

Centrifugation test

According to Freddy *et al*. (2012) the centrifugation test is an indicator of the physical stability of a preparation and aims to determine the occurrence of phase separation in preparation. Centrifugation test on toothpaste, namely a 10 g sample, is put into a centrifuge tube, then the device is set at a speed of 3.000 rpm for 30 minutes. The toothpaste sample must not change shape and phase separation occurs.

Physical stability test

There are physical stability tests, namely cycling tests, sample storage at low temperatures, sample storage at room temperature, and storage at high temperatures. The cycling test is an accelerated test that evaluates toothpaste preparations. The toothpaste preparation was placed at a temperature of 4 °C for 2x24 hours then followed by placing the preparation at a temperature of 40 °C for 2x24 hours (1 cycle) and the test was carried out for 6 cycles, and the physical changes in the preparation were observed at the beginning and end of each cycle which included pH and physical stability (Ahmad, 2017).

Based on SNI 8861-2020 (BSN 2020), the pH requirement for toothpaste is 6-10. This pH test aims to determine the acid and alkaline conditions in toothpaste preparations; therefore, it is necessary to carry out pH testing on toothpaste preparations. The working procedure for this test is to calibrate the pH meter so that it is sterile, dip the pH meter into the toothpaste sample, note the scale on the pH meter (Ahmad, 2017).

Bayes test

The research data was analyzed using IBM SPSS Statistics 25 software, followed by determining the best treatment using the Bayes method. The Bayes method is a method used to determine the best decision making from treatment by considering the criteria. The panelists used in testing the Bayes method were 20 semi-trained panelists (Asyari *et al*., 2016). In this test, semi-trained panelists fill in the values for each parameter listed in the score sheet in Appendix 3. The following is the Bayes test formula.

Data analysis

Data analysis used in this research, namely the organoleptic test, was analyzed using Kruskal-wallis if the results were significantly different and continued with the Mann Whitney test. The research data has been analyzed using the Mann Whitney test, then for stability and physical activity tests using One Way (ANOVA) followed by the Duncan test for normal and homogeneous data distribution, then it will be analyzed using the Bayes test to determine the best treatment. in this research. The data obtained is displayed in the form of graphs and tables explained descriptively.

RESULTS AND DISCUSSION

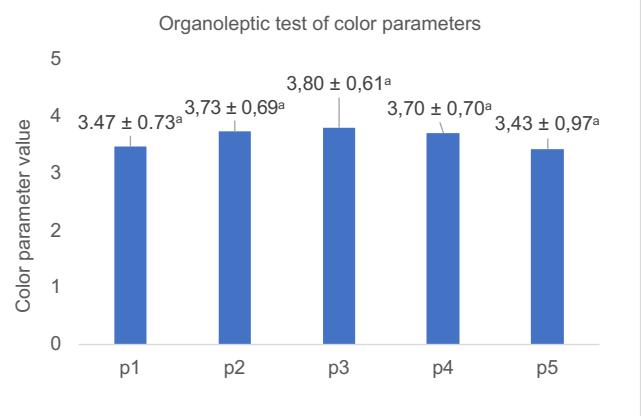

Figure 1. Color parameter results.

The results of analysis of variance (ANOVA) showed that the differences in the concentration of green mussel shells in making toothpaste were not significantly different to the color value ($p>0.05$). Based on Figure 1 The highest color of toothpaste was in treatment P3 and the lowest was in treatment P5, in treatment P3 the toothpaste composition contained 25% green mussel shells, while for treatment P5 the toothpaste composition contained 50% green mussel shells.

Factors that influence the color of toothpaste products are the combination of ingredients used with the addition of coloring, the use of coloring in toothpaste preparations aims to increase the attractiveness of consumers or panelists. The results of this study show that the color of the resulting toothpaste is yellowish white which is caused by the combination of a combination of lime peel extract used in toothpaste. The addition of lime peel extract aims to inhibit plaque-forming bacterial cells on teeth (Okwu, 2008), apart from that, lime peel extract also contains flavonoids which play a role in inhibiting the activity of *Streptococcus mutans* bacteria on teeth (Zenia *et al*., 2013).

Aroma is one of the parameters contained in the organoleptic test, aroma is one of the factors that influence the attractiveness to consumers or panelists, the aroma of a product is a subjective assessment. Testing aroma parameters is one of the important tests because it can provide assessment results on the effectiveness receive the product (Setyaningsih *et al*., 2008).

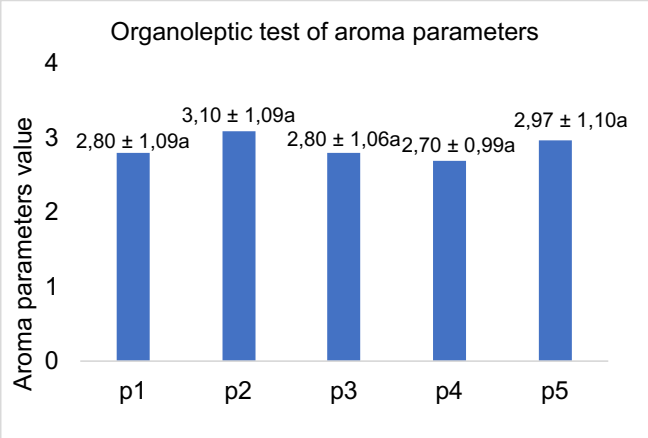

Figure 2. Aroma parameter results.

The results of analysis of variance (ANOVA) showed that the differences in the concentration of green mussel shells in making toothpaste were not significantly different to the aroma value (P>0.05). Based on Figure 2 The highest aroma of toothpaste was in treatment P2 and the lowest was in treatment P4, in treatment P2 the toothpaste composition contained 16.5% green mussel shells, while for treatment P4 the toothpaste composition contained 33.5% green mussel shells. In this study the aroma the resulting product is a combination of the ingredients used with fragrance, namely menthol, the addition of fragrance aims to increase the attractiveness of consumers or panelists, the use of menthol aims to make the aroma produced in the toothpaste preparation have a fresh impression (Hanum & Siti, 2018), a strong fragrance is produced. From the use of menthol, the resulting combination of toothpaste preparations can be mixed homogeneously, and does not produce a disturbing aroma (Diana, 2014).

One of the organoleptic tests on toothpaste preparations is texture parameters. Texture is one of the most important parameters used by panelists to determine whether a product is good or not (Midayanto & Sudaminto, 2014). The results of analysis of variance (ANOVA) showed that there was a significant difference in the concentration of green mussel shells in making toothpaste (P>0.05).

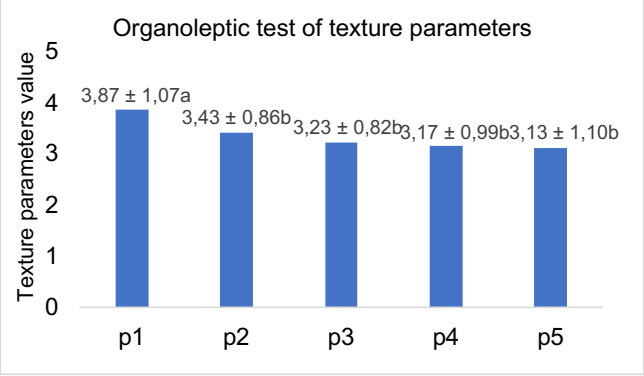

Figure 3. Texture parameter results.

Based on Figure 3 The highest texture was in treatment P1, without the addition of green mussel shell flour, in treatment P1 there was a high addition of calcium carbonate of 50%, this is thought to make the texture of the toothpaste softer, compared to other treatments which used the addition of mussel shell flour, use High concentrations of calcium carbonate can help form viscosity in toothpaste preparations (Ahmad, 2017). The concentration of added shellfish flour affects the texture produced in toothpaste preparations.

The carbohydrate test carried out in this research used the Molisch method. The molisch method is a qualitative chemical test to determine the presence or absence of carbohydrates. This test is to determine all types of carbohydrates, such as monosaccharides, disaccharides and polysaccharides. If the test result is positive, a purple ring will appear which is the condensation between furfural or hydroxymethyl furfural and a-naphthol in the molish reagent (Desyanti, 2013).

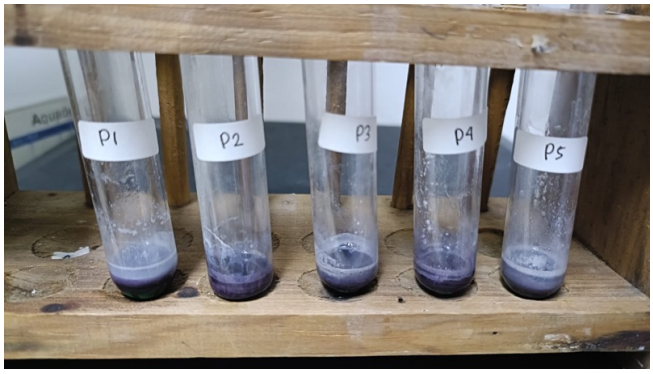

Figure 4. Carbohydrate test results.

Research carbohydrate testing uses a qualitative type of test, to determine whether there is carbohydrate content in the toothpaste preparation. The results obtained in the study of toothpaste with the addition of clam shell flour and lime peel extract contained carbohydrates, which were marked by the presence of a purple ring as attached in Figure 4. The toothpaste product in this study contained carbohydrates, it is suspected from the combination of ingredients used. namely gum arabic, the gum arabic used in toothpaste products contains a sugar compound, namely arabinose, and this compound is what triggers the presence of carbohydrates in the preparation. Arabinose is a carbohydrate compound that is included in the aldopentose group according to Praseptiangga (2016). Lime peel extract is thought to contain carbohydrates. In this study, the type of carbohydrate in lime peel extract is glucose, in line with research (Putri, 2021) that lime peel contains glucose. A good toothpaste product according to SNI 8861-2020 is toothpaste that does not contain carbohydrates.

The antibacterial test in this study used the type of bacteria Streptococcus mutans, this bacteria is a bacteria found in the mouth, the type of bacteria Streptococcus mutans is the cause of problems in the mouth, namely the growth of dental caries caused by infection from bacteria (Yumas, 2017).

Table 3. Inhibitory power of antibacterial test.

Type	Treatment				
Bacteria	P1	P2	P3	P4	P5
S. mutans	96.67±15.28	73.33±5.77	76.67±28.87	130.00±20.00	156.67±15.28

The results of analysis of variance (ANOVA) showed that there was a significant difference in the concentration of green mussel shells in making toothpaste from the concentration of green mussel shells in making toothpaste (P>0.05). The combination of treatment with green mussel shell flour and lime peel extract can inhibit microbial growth, because lime peel extract contains bioactive substances, namely flavonoids such as naringin, hesperidin, naringenin, esperitin, routine, nobiletin, and tangeretin which have the benefit of inhibiting activity. bacteria or what is known as antibacterial (Putra *et al*., 2015). Factors inhibiting microbial growth can be seen from the microbe-free areas that form around the petri dish. Flavonoids are polyphenolic compounds that can be used as antibacterials and antioxidants. In toothpaste preparations, the flavonoids in lime peel extract can inhibit plaque-forming bacterial cells on teeth (Okwu, 2008). Flavonoids can also inhibit the bacterial activity of Streptococcus mutans in teeth (Zenia *et al*., 2013).

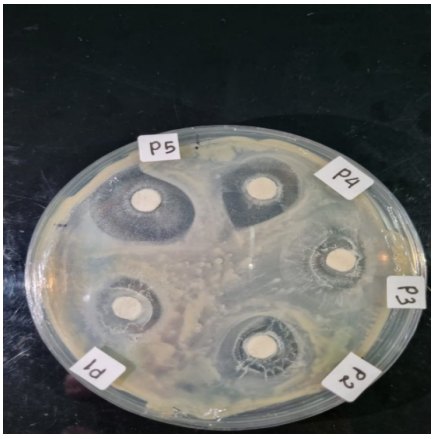

Figure 5. Antibacterial test results.

Based on research, each concentration has bacterial inhibitory power, there are several categories of inhibitory zones, inhibitory zones with a diameter of >20 mm are in the very strong category, 10-20 mm are in the strong category, diameters of 5-10 mm are in the medium category, and diameters <5 included in the weak category (Aziza *et al*., 2021). It can be seen in Figure 6 that the antibacterial test results for each treatment have different inhibitory power. In treatment P1 has an average resistance of 96.67mm, P2 has an average resistance of 73.33mm, P3 has an average resistance of 76.67mm, P4 has an average resistance of 130.00mm, The P5 has an average resistance of 156.67mm. The results obtained for bacterial inhibition from each concentration are included in the strong category, according to Pertiwi *et al*. (2022) the greater the resulting resistance, the larger the clear zone that can be seen. Compounds that act as antibacterials will cause osmotic pressure within the cells to increase and cause them to rupture.

Testing calcium levels in toothpaste preparations aims to find out how much calcium is contained in toothpaste preparations. Calcium is a mineral needed by the human body which has the main function of filling bone density. Calcium also plays a role in tooth formation (Oktaviani, 2023). Calcium is the fifth element and the most abundant cation in the body, amounting to 1.5-2% of the entire body. More than 99% of the calcium contained in cartilage and teeth is in body fluids and soft tissue.

Table 4. Calcium level test results.

Formulation	Ca (mg/kg)
P1	41.04
P2	40.46
P3	41.55
P4	40.83
P5	40.96

The results of testing calcium levels in toothpaste preparations showed that the P3 treatment value received the highest calcium test value of 41.65 (mg/kg), with the formulation adding 25% green mussel shell flour and 25% calcium carbonate. The factor that causes P3 to have the highest calcium content test value is thought to be a balanced concentration between the addition of green mussel shell flour and calcium carbonate. The use of an equivalent combination of toothpaste preparations is the treatment with the highest score in the calcium content test in toothpaste preparations (Ahmad, 2017).

Viscosity testing is one test that influences the level of toothpaste preparation when used. Tests were carried out using a Brookfield viscometer. The purpose of testing the viscosity of toothpaste preparations is to determine the amount of viscosity in the preparation.

Table 5. Viscosity test results.

Formulation	Viscosity (cPs)
P1	4.583 ± 1.283[a]
P2	4.229 ± 1.213[a]
P3	3.330 ± 1.531[a]
P4	2.411 ± 1.866[a]
P5	2.283 ± 1.120[a]

The results of the viscosity test on the formula preparation P1 have a viscosity value of 4583 cPs, P2 has a viscosity value of 4229 cPs, P3 has a viscosity value of 3330 cPs, P4 has a viscosity value of 2411 cPs, P5 has a viscosity value of 2283 cPs. The highest value was obtained by P1 with the addition of 50% calcium carbonate. The factor that influences the viscosity test value in toothpaste preparations is thought to be due to the influence of high levels of calcium carbonate addition because calcium carbonate besides functions to increase viscosity and strengthen the walls of pore cavities in toothpaste preparations according to Aprilianti *et al*. (2020). The viscosity test results of toothpaste preparations are

still within the SNI criteria, namely in the range of 2.000–50.000 cPs or 2-50 cPs (Aprilianti *et al*., 2020). The viscosity value of toothpaste must be balanced because if the viscosity is too high, the toothpaste will be liquid and difficult to apply.

Centrifugation testing is one of the parameters to see the stability of preparation. This test was carried out using a centrifuge for 30 minutes at a speed of 3.000 rpm, after two repetitions observations were made. Figure 6 shows the results of the centrifugation test as below.

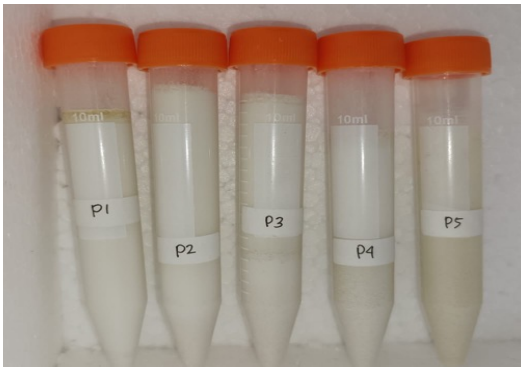

Figure 6. Centrifugation test results.

The results of centrifugation tests on toothpaste preparations showed that in the five formulations there was no phase separation that occurred in the toothpaste preparations. This shows that the five formulations of toothpaste preparations are in accordance with SNI 8861-2020, where the physical criteria for toothpaste preparations are indicated by the absence of separation after centrifugation testing. The absence of phase separation can indicate consistency, which is very important in the quality of a product. Preparations that have good stability will not experience phase separation because it can affect the properties, characteristics and storage period of a product (Maharani *et al*., 2021). It can be concluded that the preparation of toothpaste formulations P1, P2, P3, P4, and P5 does not occur. phase separation which shows that the toothpaste preparation is physically stable.

The physical stability test uses the cycling test method. The cycling test is an accelerated test that evaluates toothpaste preparations. The toothpaste preparation was placed at a temperature of 4 °C for 2x24 hours then followed by placing the preparation at a temperature of 40 °C for 2x24 hours. The test was carried out in 6 cycles, and the physical changes in the preparation were observed at the beginning and end of each cycle which included pH and physical stability. The results of analysis of variance (ANOVA) showed that differences in the concentration of green mussel shell flour had no significant effect on the cycling test ($P<0.05$). The results obtained were that the pH value of the preparation decreased after the cycling test was carried out, however the resulting pH

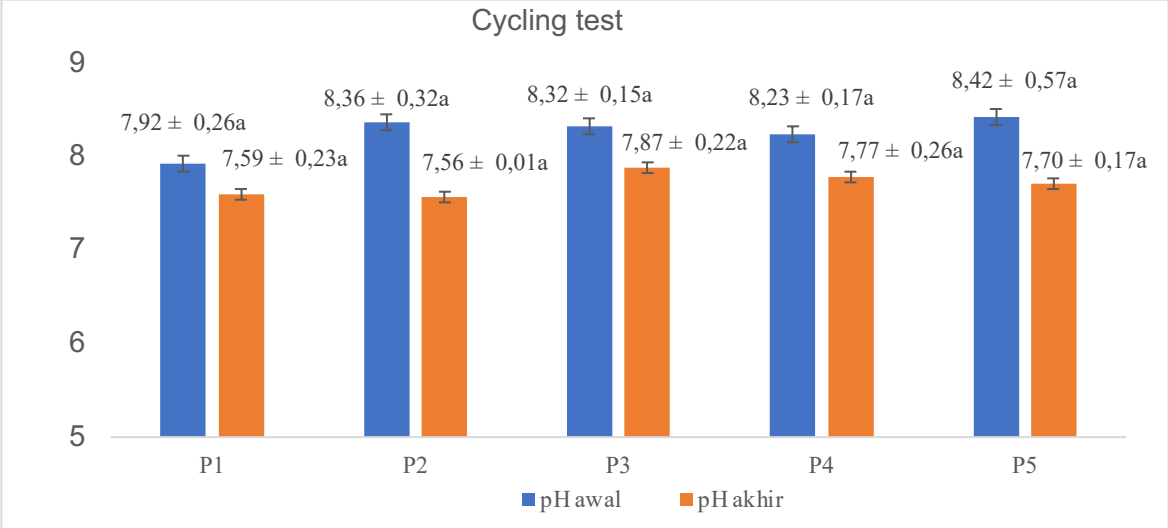

Figure 7. Physical stability test results.

range value was still within the normal standard limit of SNI 8861-2020, namely 6-10.

Factors that influence the decrease in pH are due to the influence of CO_2, where CO_2 reacts with the water phase to form acid (Rabima & Marshall, 2017). The decrease in the pH value of the preparation can also be influenced by ingredients that are decomposed at high temperatures which produce acidic compounds and can also be caused by environmental factors such as temperature

Table 6. Organoleptic testing cycling test for 6 cycles.

		Cycle					
Formulation	Observation	1	2	3	4	5	6
P1	Smell	Typical	Typical	Typical	Typical	Typical	Typical
	Color	White	White	White	White	White	White
P2	Smell	Typical	Typical	Typical	Typical	Typical	Typical
	Color	White	White	White	White	White	White
P3	Smell	Typical	Typical	Typical	Typical	Typical	Typical

	Color	White	White	White	White	White	White
P4	Smell	Typical	Typical	Typical	Typical	Typical	Typical
	Color	White	White	White	White	White	White
P5	Smell	Typical	Typical	Typical	Typical	Typical	Typical
	Color	Yellowish White	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white

and poor storage (Putra *et al*., 2014).

The results of the organoleptic test on the toothpaste preparation which was carried out for 6 cycles showed no change in the odor and color of the preparation. The results showed that the entire toothpaste did not experience a change in color or odor, this shows that the toothpaste preparation is stable in storage at extreme temperatures (Sona, 2018). The resulting toothpaste is white, which is a combination of a formulation of green mussel shell flour, lime peel extract and coloring. The purpose of using dyes in toothpaste preparations is that the resulting color aims to increase the appeal of consumers or panelists. Toothpaste generally has a white preparation, the attractiveness of toothpaste products can be seen from their color, color is the main sensor that can be seen directly by panelists, attractive colors can influence the panelists' level of preference for toothpaste products (Negara *et al*., 2016).

Decision making on the best toothpaste treatment was carried out using the Bayes method. The Bayes method is a technique that can be used to carry out analysis in making the best decision from several alternatives with the aim of producing optimal value (Rachmansyah *et al*., 2016).

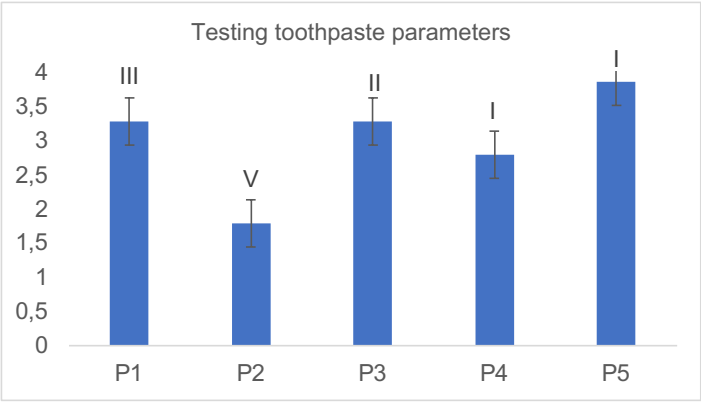

Figure 8. Bayes test results.

The weight values in the Bayes test of the toothpaste testing parameters. The results of the best method analysis using the Bayes method for antibacterial, calcium and viscosity testing showed that the P5 treatment with the addition of 50% green mussel shell flour got the highest weight value (first rank), so P5 was the best treatment in this study. The organoleptic test using the Bayes method showed that the texture parameter was the most important parameter in the toothpaste product with the highest weight value (first rank).

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

Based on research results, toothpaste with the addition of shellfish flour and lime peel extract can be used as an alternative ingredient in the pharmaceutical sector. Based on research, there is an effect of the combination of green mussel shell flour and lime peel extract on organoleptic tests on texture, aroma and color parameters, then toothpaste is proven to be effective in antibacterial, calcium and viscosity tests, there is an influence of the composition of each treatment, then in the test physical stability, the pH resulting from all preparation concentrations shows that all toothpaste preparations are stable and the resulting changes in pH range values are still within the normal standard limits set by SNI of6-10 (SNI 8861-2020).Treatment P5 with a concentration of 50% addition of green mussel shell flour was the best treatment for toothpaste preparations.

Recommendations

This research requires development in determining the composition of the formulation used in toothpaste preparations so that there is no carbohydrate content in it and further analysis is needed.

AUTHORS' CONTRIBUTIONS

Each author's contribution to the manuscript's analysis technique, English grammar check, and proofreading.

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