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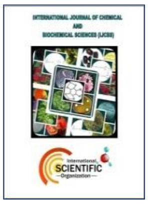


Panelist Acceptance Analyses of Tooth Remineralization Paste Formulation: Adding the Bone Meal of Channa Striata Fish Hydroxyapatite

Sri Wahyuni[1], Masayu Nurhayati[2], Mujiyati[3], Listrianah[4], Ocktariyana[5]

[1,2,3,4]*Department of Dental Health, Poltekkes Kemenkes Palembang, Indonesia*
[5]*Department of Midwifery, Poltekkes Kemenkes Palembang, Indonesia*

Abstract

Caries are caused by demineralization or a loss of minerals from teeth. Demineralization can be reduced by remineralization by applying minerals to the tooth surface. In the fish processing industry, fish bones are considered waste but contain a high calcium content (the main element of fish bones are calcium, phosphorus, and carbonate). Snakehead fish bone (Canna Striata) has great potential as a base for tooth remineralization pastes because it is rich in calcium. Calcium from snakehead fish bones can be used to form hydroxyapatite. 92% of Enamel is composed of hydroxyapatite crystals. Hydroxyapatites can combine with human bones and can also be absorbed by the body to form new bone tissue. This study aimed to develop a remineralization paste and assess its acceptability via organoleptic tests. The study included 65 panelists in 4 groups. One-way ANOVA and the least significant difference test (LSD) were applied to analyze the data. The results demonstrated the successful synthesis of hydroxyapatites from snakehead fish bone meal. The resulting crystals averaged 43.91 nm in size and 32.957% calcium. The tooth remineralization paste made from snakehead fish bone meal was assessed to be acceptable in terms of taste, color, texture, and aroma. It was the second most preferred formulation.

Keywords: Tooth, Remineralization Paste, Hydroxyapatite, Snakehead.

Full length article **Corresponding Author*, email: ocktariyana@gmail.com

1. Introduction

The biggest dental problem faced by Indonesians and other developing countries is dental caries. Attempts to reduce dental problems are inadequate when measured by community dental health indicators [1]. According to data from Indonesian basic health research (RISKESDAS), in 2018, 57.6% of Indonesian people had dental and oral health problems, and only 10.2% received services from personnel in dental clinical care. In South Sumatra, 52.41% with dental and oral health problems, and only 5.27% received dental treatment from medical personnel [2]. The demineralization process can be explained by the oral pH demineralization occurs when the oral pH decreases below the critical point of 5.5, resulting in more and more acid ions reacting with the teeth [3]. Excess acid ions lead to the dissolution of enamel crystals, which in turn, causes the tooth enamel surface to soften. Substances that have a higher pH than the critical pH of 5.5, such as calcium, phosphate, and other materials, are needed to suppress or reverse the demineralization process. Adding ions with a high pH can change the critical pH and improve the balance between demineralization and remineralization. This keeps the mouth stable and stops natural minerals from leaving dental hard tissues [4]. Caries are the most common dental and oral health problem, especially in children. According to the World Health Organization (WHO), approximately 60%–90% of kindergarteners experience caries. Therefore, efforts must be made to prevent dental caries. Caries involve the loss of tooth minerals, that is, demineralization. Remineralization is necessary to reverse or prevent demineralization [5]. Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) is a milk derivative that can remineralize teeth by replenishing the lost minerals. Specifically, CPP-ACP incorporates calcium and phosphate ions into the surface enamel of teeth. This paste is commercially available but costs ten times as much as toothpaste [6]. Snakehead fish bone (Channa striata) is a waste generated by Palembang, that traditional food, which is Pempek. Fish bones are very rich in calcium. The main elements of fish bones are calcium, phosphorus, and carbonate [7]. Snakehead fish bone has great potential as a basic ingredient for a tooth remineralization paste because the fish bone meal is rich in calcium. Fish bones contain a lot of mineral salts, such as calcium phosphate and creatine phosphate [8]. Remineralization is a process of partially forming minerals in enamel and dentine. The remineralization process occurs when a combination of saliva and external minerals creates amorphous mineral deposits in the inter-crystal and interred spaces [9]. The remineralization process involves mainly calcium and phosphate from the saliva [10]. Remineralization can also occur via externally sourced minerals. Results from in vitro and in vivo studies have demonstrated that several materials can result in the growth of hydroxyapatite and increase the crystal size. Moreover, novel remineralizing agents are continually being developed. Toothpaste containing synthetic hydroxyapatite crystals can remineralize human teeth based on crystal structural acidity [11]. Ninety-two percent of the enamel component is hydroxyapatite $Ca_{10}(PO_4)_6OH_2$. The properties of hydroxyapatite can combine with human bones and can be absorbed by the body to form new bone tissue [12]. In addition, calcium from snakehead fish bones can be used for hydroxyapatite formation [13]. The authors proposed a low-cost novel tooth remineralization paste with hydroxyapatite made from snakehead fish bone meal.

2. Materials and methods

2.1. Equipment

Beaker glass, measuring cup, porcelain cup, pH paper, spatula, 200 mesh sieve, measuring pipette, dropping pipette, fuel flask, Erlenmeyer, ball milling, oven, furnace, analytical balance, mortar, burette, stative, magnetic stirrer, hotplate, atomic absorption spectrophotometer (AAS; Shimadzu AA-7000), and X-ray diffractometer (XRD; Rigaku-Miniflex 600) [14].

2.2. Research Materials

We used many materials such as Snakehead fish bones, filter paper (Whatman), distilled water, demineralized water, acetone (CH_3COCH_3; UNIVAR), $CaCl_2.2H_2O$ (UNIVAR), diammonium hydrogen phosphate ($(NH_4)2HPO_4$; UNIVAR), nitric acid (HNO_3; Merck), ammonium hydroxide (NH_4OH; Merck) and acetic acid.

2.3. Snakehead Fish Bone Preparation

One kilogram of fish bones was boiled at 100°C for 10 minutes. After that, the meat still attached to the bones was removed, and the bones were washed with water and then washed again with distilled water. Subsequently, the washed bones were soaked in an acetone solution for 3 x 24 hours, with solvent changes every 24 hours. Snakehead fish bones were dried in an oven for approximately 100 minutes at a temperature of 100°C and then crushed into powder. The fishbone powder was crushed using a ball-milling machine for 24 hours to obtain a fine powder [15]. The $CaCO_3$ of the snakehead fish bone power was changed to CaO by calcination in a furnace at 900°C for 4 hours. Then the CaO powder products were sieved with a 100-mesh sieve and weighed [16].

2.4. Calcium Standard Solution Preparation

2.4.1. Preparation of the Calcium Mains Solution (1000 ppm)

A thousand ppm calcium mother liquor was prepared by adding 0.9178 g of $CaCl_2.2H_2O$ to a 250 mL volumetric flask and diluting it with demineralized water up to the mark.

2.4.2. Preparation of the Basic Calcium Solution (100 ppm)

To make a basic solution of 100 ppm, 10 mL of 1000 ppm calcium mother liquor was put into a 50 mL volumetric flask, and demineralized water was added until the mark was reached.

2.4.3. Determination of the Calcium Content from the CaO Powder

Many 0.1244 grams of CaO powder were put into the digestion flask, and then 5 mL of HNO_3 and 50 mL of demineralized water were added. The mixture was digested for 30 minutes at 180°C. The sample was then cooled and filtered using filter paper. The filtrate was put into a 50 mL measuring flask and diluted to the mark. The dilution was carried out 100 times. Specifically, the dilution was carried out by taking 0.1 mL of filtrate, putting it in a 10 mL measuring flask, and diluting it to the mark. The filtrate from the 100-time dilution was measured using AAS at a wavelength of 421.76 nm (SNI 01-3158-1992).

2.4.4. Hydroxyapatite Synthesis

The snakehead fish bone powder (CaO) samples were weighed (1.25 grams) and mixed with 25 mL of distilled water. The mixture obtained was added to 25 mL of $(NH_4)2HPO_4$ (0.6 M) at 90°C. Then, a solution of 1 M NH_4OH was added little by little, up to 11 mL, and stirred for ± 1 hour using a 120-rpm magnetic stirrer until completely mixed. The suspension formed was precipitated for 24 hours. The precipitate formed was filtered and heated at 110°C for 2 hours [17]. The characteristics were analyzed using XRD. From the XRD, the 2Θ angle position, intensity, and size of the crystals were obtained.

2.5. Ethical approval

This study was approved by the Health Research Ethics Committee of the Health Polytechnic University of the Ministry of Health of Palembang (Number: 1219/KEPK/ adm2/IX/2021). Ethical approval covered the period from November 30, 2021, to November 30, 2022. Informed consent was obtained from each respondent before participation.

2.6. Statistical analysis

Data analyses were performed using the one-way ANOVA and the least significant difference test (LSD).

3. Results and Discussions

3.1. Formulation

This study made a tooth remineralization paste product containing hydroxyapatite from snakehead fish bone meal (Channa striata) and measured its acceptability. Hydroxyapatite has been successfully synthesized from snakehead fish bone meal through the precipitation method.

The visible white color indicates the formation of hydroxyapatite [18]. Measurement of hydroxyapatite calcium levels from snakehead fish (Channa striata) bone meal using the AAS technique yielded 32.957%. Characterization with X-ray diffraction (XRD) was carried out to ensure that the results obtained from this calcination process contained hydroxyapatite. The XRD test is also used to determine the particle size of hydroxyapatite. The XRD results from the manufacture of hydroxyapatite show the highest peaks on the diffractogram with an angle (2θ) for hydroxyapatite. The results of the hydroxyapatite diffractogram from snakehead fish bone meal are shown in Figure 1. The highest peak from the XRD test results for hydroxyapatite samples from snakehead fish bone meal showed a diffraction angle of 31,918 with an intensity of 382. This can be seen from the high peaks of the hydroxyapatite diffractogram and high intensity. The crystal size from Hydroxyapatite XRD examination of snakehead fish (*Channa striata*) bones was 43.76 nm. Characterization of the hydroxyapatite using SEM-EDS to determine the surface morphology of hydroxyapatite from snakehead fish bone meal (*Channa striata*) at 10,000 times magnification (Figure 2). Figure 2 shows agglomeration with the characteristics of single particles that tend to be elliptical-like bars and appear to form pores, the grains are distributed evenly, and the grains stick together to produce a more homogeneous shape.

3.2. Participants Acceptance

Minerals must diffuse into the tooth enamel to enhance hardness and acid resistance to prevent or reduce dental caries. Hydroxyapatite crystal sizes of 43.76 nm, 47.14 nm, and 40.83 nm allowed this process. Hydroxyapatites are biocompatible, meaning that they are bioactive and can adapt to the recipient's body and blend with human bones. Hydroxyapatites are also bioresorbable and can be absorbed by the body to form new bone tissue. Tooth and bone tissues can be used for teeth. 92% of teeth are composed of inorganic materials, namely hydroxyapatites. Hence, increasing the hydroxyapatite level can enhance tooth remineralization. Measuring the concentration of calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) in snakehead fish bones used by AAS yielded a result of 32.957%. The calcium content affects the mechanical properties of hydroxyapatite [19]. Organoleptic tests were performed to determine the acceptability of the novel hydroxyapatite remineralization paste made from snakehead fish bone meal.

3.2.1.Teste

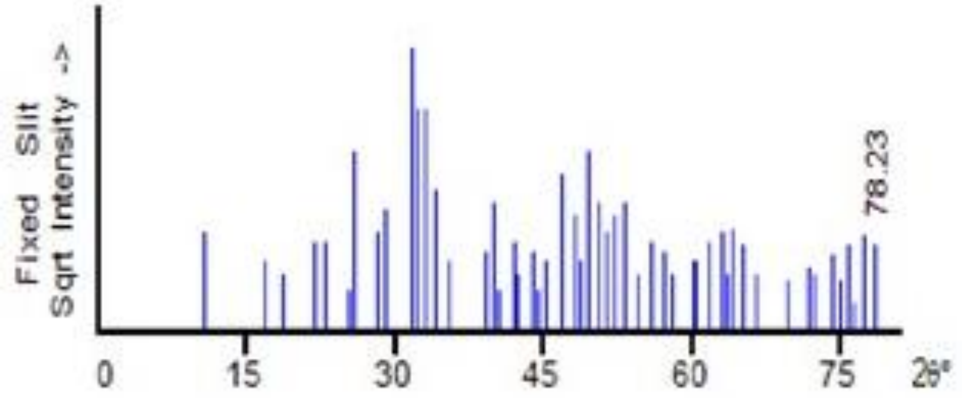
Figure 3(A) shows the results of the taste analysis. The p-value was <0.005, meaning there was at least one significant difference in taste between the two groups. The taste is different between Formula 0 and Formula 1, Formula 0 and Formula 2, Formula 1 and Formula 3, as well as Formula 2 and Formula 3. Conversely, the taste between Formula 0 and Formula 3 and Formula 1 and Formula 2 was not significantly different. The taste of a product is a key determining factor for consumer acceptance. In assessing flavor, multiple senses are utilized. Taste is produced when something is placed in the mouth and interpreted by the senses of taste, smell, and temperature. The taste tests we did on the new tooth remineralization paste with hydroxyapatite from snakehead fish bone meal showed that the average score for how good the paste tasted was 4.06 for Formula 2 and 2.84 for Formula 3. Formula 2 contained 30% hydroxyapatite and tasted good to the panelists. There was menthol added to Formula 2, and it may have enhanced the taste. In contrast, the panelists found the taste of Formula 3, which contained 50% hydroxyapatite, to be the least acceptable. They reported that the menthol taste was not obvious and that the paste appeared bland. The results of the LSD Post Hoc Test analysis (a significance level (α) of 0.05 indicated significance) demonstrated a p-value < 0.05, which meant a significant difference in the acceptance of the taste of the remineralization paste. When we compared Formula 0 with Formula 1 and Formula 1 with Formula 2, we found no significant difference in the concentration of hydroxyapatite [20].

3.2.2. Texture

Figure 3(B) shows the texture analysis results. Among the results from the LSD test, there was a significant difference in textural (p-value = 0.003), such as between Formula 0 and Formula 2, and between Formula 2 and Formula 3. Conversely, there were no significant differences between Formula 0 and Formula 3, Formula 1 and Formula 2, and Formula 1 and Formula 3. The texture was measured with organoleptic tests. The texture of the tooth remineralization pastes, which contain hydroxide, was described in terms of softness and roughness. According to the panelists, the texture of the tooth remineralization paste containing hydroxyapatite from snakehead fish bone meal (Channa striata) ranged from 4.1 to 3.52. The most preferred texture was Formula 2, and the least preferred was Formula 3. It was due to the higher hydroxyapatite content in Formula 3 (50%). The panelists described Formula 3 as being less soft and rather dense. This result is consistent with the findings of Sidoretno's 2020 study. The author of that study reported that the higher the concentration of catfish bone meal, the denser the toothpaste was. The results of the LSD post-hoc test analysis (a significance level (α) of 0.05 was set) indicated that the p-value was 0.001. This study showed a significant difference in the texture of the remineralization paste. Formula 0 and Formula 1, Formula 1 and Formula 2, and Formula 2 and Formula 3 found a significant difference in texture. Conversely, Formula 0 and Formula 3, Formula 1 and Formula 2, and Formula 1 and Formula 3 were not significantly different in texture. The insignificance may be due to only minor variations in the hydroxyapatite concentration between these formulations [21].

3.2.3. Aroma

Figure 3(C) shows the aroma test results. A p-value of 0.002 was found. According to the LSD test, there were no statistically significant differences between Formula 0 and Formula 3 and Formula 1 and Formula 2. In contrast, statistical significance was detected between Formula 0 and Formula 1, Formula 0 and Formula 2, Formula 1 and Formula 3, and Formula 2 and Formula 3. According to Kartika et al. (1998), the aroma determines the quality of tooth pasta. Testing on aroma can be used as a criterion for whether a product is acceptable for marketing. Additionally, the aroma can be used as an indicator of issues with a product. This scent



2θ	int-f	h	k	l	2θ	int-f	h	k	l	2θ	int-f	h	k	l
10.820	12	1	0	0	35.480	6	3	0	1	50.493	20	3	2	1
16.841	6	1	0	1	39.204	8	2	1	2	51.283	12	4	1	0
18.785	4	1	1	0	39.818	20	3	1	0	52.100	16	4	0	2
21.819	10	2	0	0	40.452	2	2	2	1	53.143	20	0	0	4
22.902	10	1	1	1	42.029	10	3	1	1	54.440	4	1	0	4
25.354	2	2	0	1	42.318	4	3	0	2	55.879	10	3	2	2
25.879	40	0	0	2	43.804	8	1	1	3	57.128	8	3	1	3
28.126	12	1	0	2	44.369	2	4	0	0	58.073	4	5	0	1
28.966	18	2	1	0	45.305	6	2	0	3	59.938	6	4	2	0
31.773	100	2	1	1	46.711	30	2	2	2	60.457	6	3	3	1
32.196	60	1	1	2	48.103	16	3	1	2	61.660	10	2	1	4
32.902	60	3	0	0	48.623	6	3	2	0	63.011	12	5	0	2
34.048	25	2	0	2	49.468	40	2	1	3	63.443	4	5	1	0

Figure 1. Hydroxyapatite diffractogram

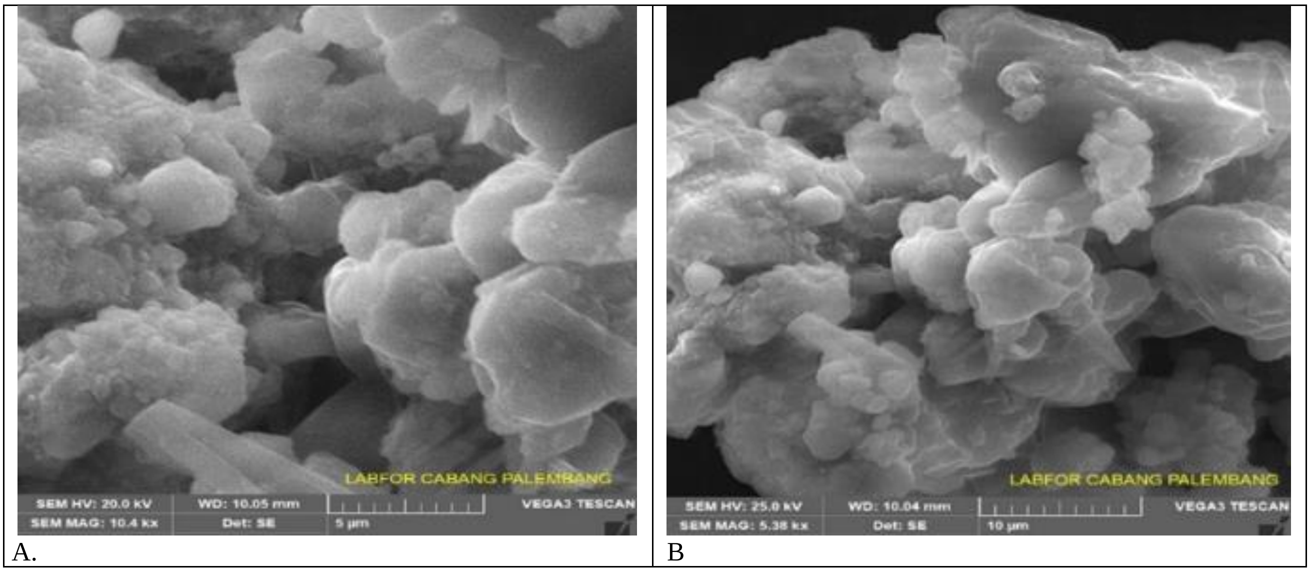


Figure 2. Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX) Hydroxyapatite *Channa striata* powder.

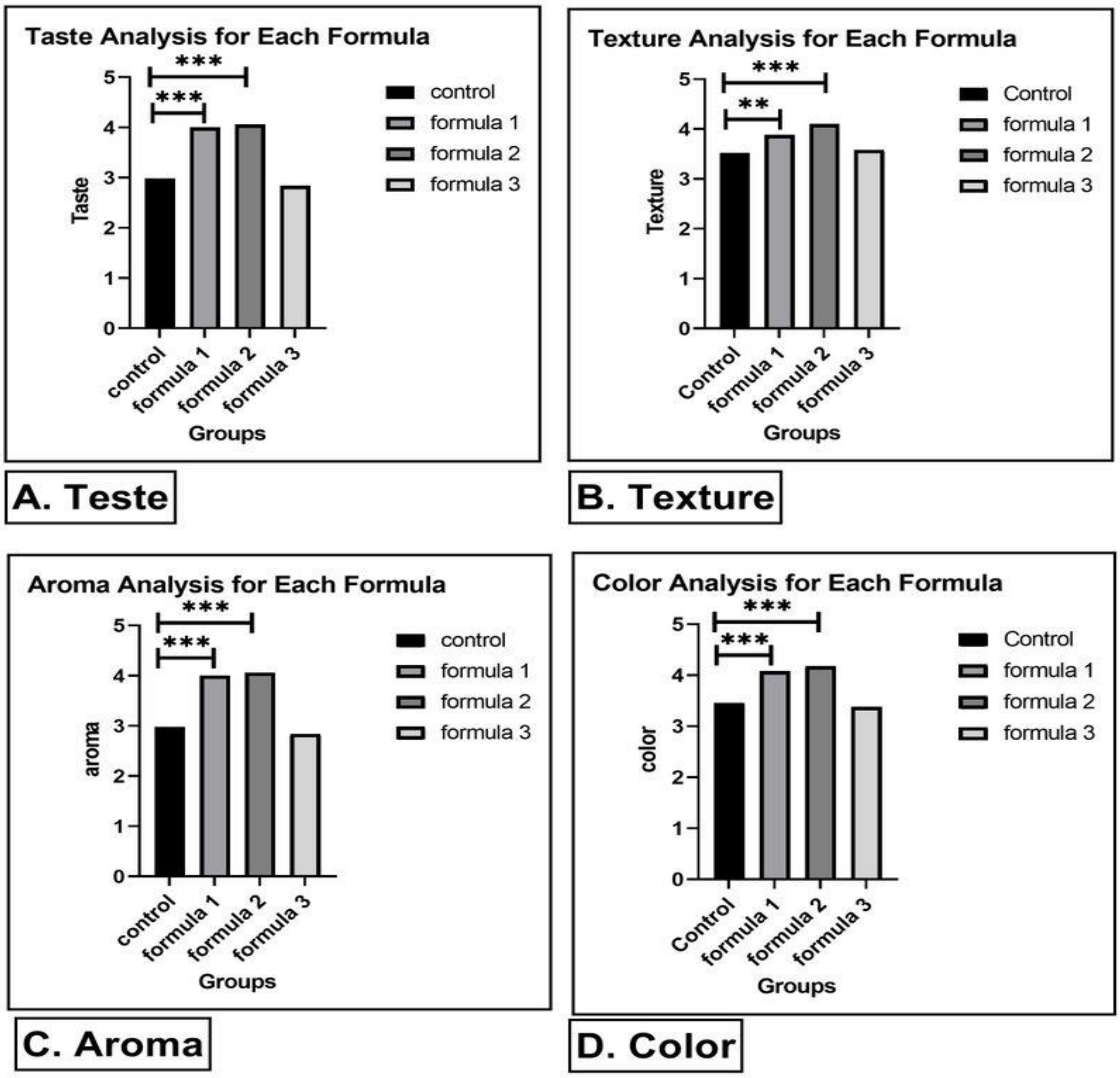


Figure 3. Analysis of Organoleptic of remineralize paste from snakehead fish (Channa striata) bones based on taste, texture, aroma, and color

test makes extensive use of the sense of smell [22]. From the results of organoleptic tests on the aroma produced by the tooth remineralization paste containing hydroxyapatite from snakehead fish bone meal, the average acceptance of panelists ranged from 4.34 to 3.84. The aroma of Formula 2 was most preferred, and Formula 4 was least preferred. It indicated a significant difference in the aroma of tooth remineralization paste containing hydroxyapatite from snakehead fish bone meal [23]. In contrast, Formula 0 to Formula 1, Formula 0 to Formula 3, Formula 1 to Formula 2, and Formula 2 to Formula 3 showed no significant differences. Furthermore, the aroma was very similar in these formulations. Namely, the smell of menthol was perceived. Furthermore, the aroma was very similar in these formulations. Namely, the smell of menthol was perceived.

3.2.4. Color

Figure 3(D) shows the color acceptance test results (p-value < 0.05) than indicated significance. According to the LSD test, there were no statistically significant differences between Formula 0 and Formula 3 also Formula 2 and Formula 2. In contrast, statistically significant differences were demonstrated between Formula 0 and Formula 1, Formula 0 and Formula 2, Formula 1 and Formula 3, and Formula 2 compared to Formula 3 (p-value < 0.05). Color is also the first factor that attracts consumer attention. The appearance of a product affects the level of consumer preference [24]. According to organoleptic tests on the color of the tooth remineralization paste, the average acceptance of the panelists ranged from 4.18 to 3.38. Formula 2 was accepted (4.18) than Formula 3 (3.38). It may cause hydroxyapatite concentration. Specifically, Formula 3, that contained 50% hydroxyapatite, so the color appeared slightly grayish, which caused the panelists to dislike its appearance. This study showed that there was a significant difference in the acceptability of the color of the paste. Formula 0 and Formula 3 were similar. In contrast, Formula 3 contained 50% hydroxyapatite, so the color was not white but gray. Moreover, Formula 1 and Formula 2 were not significantly different due to only minor variations in the hydroxyapatite content, so the color was almost the same and preferred by the panelists. Formula 0 than Formula 1, Formula 0 than Formula 2, Formula 1 than Formula 3, and Formula 2 than Formula 3 demonstrated significant color differences. The most preferred color was white. White was identical to normal toothpaste, so the panelists did not hesitate to put the paste in their mouths. Our result concurred with the findings of Sidoretno et al. (2020). In that study, the authors stated that the color of toothpaste greatly affected its acceptability and that the color of toothpaste must comply with SNI standards 12-3524.1995 [25].

The main constituents in fish bones are calcium and phosphorus (as much as 14% of the total bone constituent). Fish bones are very rich in calcium because the main elements of fish bones are calcium, phosphorus, and carbonate [26]. Calcium from snakehead fish bones has been widely used to make fishbone meals [28]. For example, Cucikodana et al. demonstrated an average calcium content of 16.86% -22.77% in snakehead fish bones. Apart from being the main ingredient for making snakehead fish bone meal, the calcium in snakehead fish bones may also be used to form hydroxyapatites [28]. Hydroxyapatite has a size, morphology, and chemical composition comparable to dentine, increasing the acceptability of a tooth remineralization paste. A 10% concentration of hydroxyapatite is optimal for enamel remineralization. Hydroxyapatite is added to toothpaste as well as pit and fissure sealants. In addition to its remineralization property, the hydroxyapatite content of these products can effectively penetrate dentinal tubules, thereby obtruding and sealing the exposed tubules.

4. Conclusions

The Snakehead fish bone meal can be used in a tooth remineralization paste. According to the organoleptic tests, formula 2 containing 30% hydroxyapatite is the most acceptable.

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