Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 9 No. 3 December 2022, 279-289
DOI: 10.20473/jfiki.v9i32022.279-289
Available online at https://e-journal.unair.ac.id/JFIKI/

Jelly Candy Hydroxyapatite from Mackerel Fish Bone

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Submitted: 7 May 2022
Accepted: 22 November 2022
Published: 9 December 2022

Abstract
Background*: Calcium is a mineral that is needed by bones and teeth. Calcium needs have not been achieved evenly among children, about 1000mg/day. Hydroxyapatite contains Ca and apatite which is good for the maintenance of bones and teeth. Hydroxyapatite is made from the bones of mackerel, where the bones of mackerel have a high source of calcium. For easy being consumed for children, the hydroxyapatite made in the form of jelly candy.* ***Objective****: The aim of this research was Hydroxyapatite Mackerel Fish Bone can be made as Jelly Candy.* ***Methods****: Hydroxyapatite from mackerel bones is made by the precipitation method. Then, hydroxyapatite was formulated into jelly candy with concentrations of hydroxyapatite was 18%, 19% and 20% respectively for Formula I, II and III. Jelly candy evaluation includes organoleptic test, gel strength, khamir and ALT ochre, weight uniformity, pH, hedonics, homogeneity and storage.* ***Results****: The evaluation showed that all jelly candy formulas are safe for consumption according to SNI 3547.2-2008.* ***Conclusion****: In conclusion, hydroxyapatite mackerel fish bone can be made as Jelly Candy with the best concentration in Formula 1.*

Keywords*: calcium, hydroxyapatite, jelly candy, mackerel fish bone, precipitation*

How to cite this article:
Anggresani, L., Perawati, S. Afandi, R. & Rahmadevi. (2022). Jelly Candy Hydroxyapatite from Mackerel Fish Bone. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 9(3), 279-289. http://doi.org/10.20473/jfiki.v9i32022.279-289

INTRODUCTION

Calcium is an important mineral in the human body, which can affect the development and growth of bones and teeth in humans, where 99% of calcium in the human body is found in bones (Shita & Sulistiyani, 2010). Age 9 to 18 years is the age that most need calcium with an average amount of calcium of 1000 - 1200 mg/day compared to other ages (Jauhari *et al*., 2019). Unfortunately, many children in Indonesia do not have enough calcium intake every day. Calcium sources are divided between animals and vegetables (Shita & Sulistiyani, 2010).

Hydroxyapatite (HAp) with molecular formula $Ca_5(PO_4)_3(OH)$ or better known as Formula $Ca_{10}(PO_4)_6(OH)_2$ is bio ceramic calcium apatite that can be found in human teeth and bones. Hydroxyapatite is the most similar to the mineral part of the bone, since the salt calcium phosphate (CaP) is the main mineral that composes bones and teeth (Mozartha, 2000). It is widely used as an adsorbent for removing heavy metals due to high-efficiency ion exchange between ion Ca and metal, purification of wastewater treatment and biomedical applications with excellent physical and mechanical properties. Many studies investigated the preparation and characterization of Hydroxyapatite from natural sources, such as cockle shells, cattle bones, and fish bones with various methods (Stötzel *et al.*, 2009).

One source of hydroxyapatite is mackerel bones. Fish bones have the most calcium content among other parts of the fish body because the main elements of fish bones are calcium, phosphorus and carbonate (Chadijah *et al*., 2018). Mackerel bones (Scomberomorus guttatus) can be processed into products that have a high economic value in the form of hydroxyapatite ($Ca_5(PO_4)_3(OH)$) (Anggresani *et al*., 2018). Aside from mackerel bones, hydroxyapatite can be found in egg shells (Noviyanti *et al*., 2017), blood shells (Ahmad, 2017), and tuna fish bones (Mutmainnah *et al*., 2017).

The development of science and technology in the field of pharmacy encourages pharmacists to make the right formulations to process hydroxyapatite into a form of preparation that is easily accepted by the community (Andasari, Zukhri and Nurjanah, no date). Pharmaceutical preparations made from hydroxyapatite active that has been made into a pharmaceutical product are the form of toothpaste (Anggresani *et al*., 2021; Hernawan *et al*., 2021; Wadu *et al.*, 2015), biomaterials (Hanura *et al*., 2017), and injectable bone substitutes (Budiatin *et al.*, 2016). There are also jelly preparations made from active calcium (Lesmana *et al*., 2008) and there are preparations of calcium jelly candy in the market. However, because hydroxyapatite has not been made in candy preparations, there needs to be efforts to develop hydroxyapatite preparations in a form that can be consumed more easily by the community, especially children. Children become unacceptable when directly contact with the tongue taste receptor, which has partially dissolved in it. Jelly candy is a kind of oral drug that many pharmaceuticals have been used nowadays for patients who lost their primary teeth (such as children), and patients who have Parkinson's, stroke, nausea, and thyroid disorder disease (Sunil *et al*., 2020). Jellies are translucent and transparent and can be used for internal and external application. Jellies offers efficacy, safety, low cost of treatment. In general, jelly candy reveals pleasant taste, magnificent appearance and convenient to handle.

Based on the description, researchers were interested in making hydroxyapatite jelly candy from mackerel fish bone due to the easy to be consumed. Jelly candy is made from water or juice of plants and gel-forming materials (Alridho *et al*., 2017).

MATERIALS AND METHODS

Materials

Reagents and chemicals used in this research were mackerel bone waste (*Scomberomorus guttatus*) were collected from Traditional Market in Jambi City, distilled water (Brataco® Indonesia), phosphoric acid (Merck® Germany), 85% 0.1 M, Ethanol (Merck® Germany) 50%, hydrochloric acid (Merck® Germany) 37% 1 M, diammonium hydrogen phosphate (Merck® Germany) 10 M, sodium hydroxide 0.1 %, and acetone (Merck® Germany), coco pandan syrup, gelatin, water, citric acid, benzoic acid, sodium benzoate, menthol and HAp.

Equipment

The equipment were used in this study were furnace (Sh scientiac® Canada), oven, balance analytics (Shimadzu® Japan), stirrer hot plate (Ika c-mag hs7® Germany), soaking container, sift mesh 80, porcelain cup, beaker glass, spatula, measuring pipette, burette, measuring glass, X-ray Analysis Fluorescence Spectrometer (XRF) UK, United Kingdom, X-Ray Diffraction (XRD) (Xpert pro analytical® United Kingdom, United Kingdom), Scanning Electron Microscopy (SEM) (Tabletop Microscope tm3000 United States).

Method

The powder formation of mackerel fish bone waste

Mackerel fish bones (*Scomberomorus guttatus*) 1 Kg was cleaned and boiled. Then washed it, immersed

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E-ISSN: 2580-8303

in 10 L of 0.1% NaOH solution for 7 hours, drained then soaked in a container containing 50% acetone for 8 hours. Then, the bones are drained and dried for 7 days. Then crush it into powder. The powder was calcinated for 3 hours at 800°C. Then the powder is crushed then sieved with 80 mesh. Powder of mackerel fish bone (CaO) was formed and then analyzed using XRF (Anggresani *et al*., 2021).

The synthesis of hydroxyapatite

CaO powder 180 g was diluted with 1 L distilled water and then stirrer at speed 300 rpm for 30 minutes until the suspension $Ca(OH)_2$ was formed. Add diammonium hydrogen phosphate $(NH_4)_2HPO_4$ to the suspension $Ca(OH)_2$ using the variation of mol Ca/P was 1,67 and for 1 hour heated at 90^{o}C. Adjust pH using NaOH 1 M until it reaches 12. Aging the solution for 24 hours at room temperature. Then the formed deposition was filtered and dried using an oven at 120°C for 5 hours. The dry precipitated was calcinated at 900°C for 5 hours. Then analyzed with XRD and SEM (Anggresani *et al*., 2021).

The formulation of jelly candy hydroxyapatite from mackerel fish bone waste

Hydroxyapatite from Mackarel Fish Bone Waste. The formulation of jelly candy can be seen in Table 1.

The evaluation of jelly candy hydroxyapatite from mackerel fish bone waste

The evaluation of raw material

The raw material evaluation includes organoleptic and the solubility of hydroxyapatite, sodium benzoate, citric acid, gelatin, syrup, and menthol. All materials used in this formulation conform to the standard.

The evaluation of jelly candy

The evaluation of jelly candy includes a weight uniformity test, organoleptic test, pH test, and hedonic test with ethical clearance No. LB.02.06/2/007/2021, kapang khamir test and total plate number test, gel strength, homogeneity test and storage test (Badan Standarisasi Nasional, 2008) (from SNI Standard No. 3547-2-2008).

Weight uniformity test. Thirty pieces of jellies, each of them was weight and the average weight from each formula was calculated. *Organoleptic test*. Organoleptic tests included the observed of the color, smell, texture, and taste. *pH test*. This test used pH meter. Jelly candy hydroxyapatite mackerel fish bone was diluted with hot distilled water 10 mL. Then measure the pH using pH meter. *Hedonic test*. This test included color, taste and smell of the jelly candy hydroxyapatite mackerel fish bone made by the panelists. *Kapang khamir test.* This test was carried out to determine the shelf life of the product. Kapang khamir test was carried out by counting the colonies of kapang growth on jelly candy. According to the National Standardization Agency, the limit of kapang maximum was 1 x 10^2 colony/gram. *Total plate number test*. This test refers to National Standardization Agency (SNI) 3557.2-2008. *Gel strength*. Gel strength was measured using a texture analyzer with a load strength of 4500 grams and a cylindrical TA5 probe with a length of 40.25 mm. Gel strength was measured as the force required by the probe to compress a thick gel 5 mm at a speed 2.5 mm/second. *Homogeneity test*. This test was observed using a piece of glass, and then jelly candy was smeared in it. *Storage test*. The test was carried out by storing jelly candy at room temperature for 21 days. Then, jelly candy was observed on the 7th day, 14th day and 21st day.

RESULTS AND DISCUSSION

Calcium oxide (CaO) formation from mackerel fish bone

Mackerel fish bones was boiled for 45 min to cleanse the bones from the meat. After that, the bones were soaked with NaOH 0.1% and acetone 50% to remove the fat compound. Then crusher the bones and calcinated using a furnace at 800^{o}C for 3 hours to get CaO powder. The function of calcination is to remove calcium carbonate ($CaCO_3$) and becomes calcium oxide (CaO) (Mutmainnah *et al*., 2017).

Table 1. The formulation of jelly candy

Formula	Concentration (%)			
	Formula 0	Formula 1	Formula 2	Formula 3
Hydroxyapatite	-	18	19	20
Sodium Benzoate	0.15	0.15	0.15	0.15
Benzoic Acid	0.1	0.1	0.1	0.1
Citrate Acid	0.8	0.8	0.8	0.8
Gelatin	19	19	19	19
Syrup	35	35	35	35
Menthol	1	1	1	1
Distilled water	Ad 100	Ad 100	Ad 100	Ad 100

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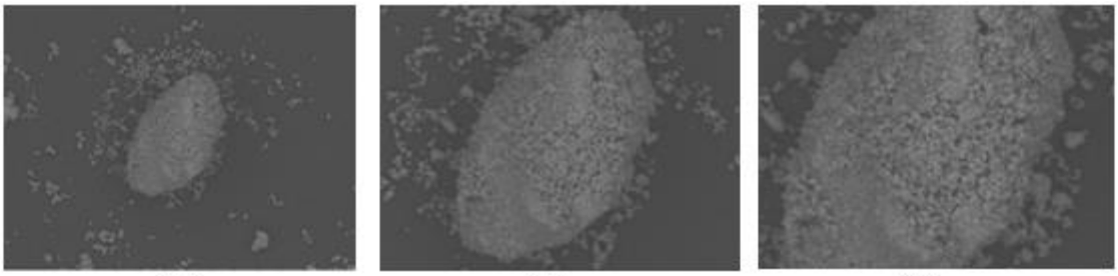

Figure 2. SEM analysis of hydroxyapatite with magnification (a) 500x, (b) 1000x, (c) 1500x

$$CaCO_3 \rightarrow CaO + CO_2$$

Then the powder analyzed with XRF (X-Ray Diffraction) to determined calcium oxide content. XRF analysis obtained CaO content of 49.846%. In Tuna Bones (Mutmainnah *et al*., 2017), the CaO content as much as 62.31%, cow bones the CaO content of 31.48% (Yuliana *et al*., 2017), and mackerel fish bones (Anggresani *et al*., 2018) as 50.814%. In this study has smaller CaO content from the previous studies. It is because when calcination occurs there is an organic material degradation process (Mutmainnah *et al*., 2017).

The synthesis of hydroxyapatite

Hydroxyapatite synthesis was made by calcium precursor and phosphate precursor in precipitation methods. In this study, the calcium precursor is CaO from mackerel fish bones and the phosphate precursor is $(NH_4)_2HPO_4$. Precipitation methods is an alkaline acid reaction that produces crystalline solids as well as water. This process is simpler, using cheap raw materials and homogeneity (Haris *et al*., 2016).

In synthesis, CaO from mackerel fish bones was dissolved in distilled water to get $Ca(OH)_2$ calcium hydroxide. Reactions that occur as follows:

$$CaO + H_2O \rightarrow Ca(OH)_2$$

Further, to synthesize hydroxyapatite, needed phosphate as a source of phosphate. In this study, we used diammonium hydrogen phosphate $(NH_4)_2HPO_4$ with a mole ratio of Ca/P 1.67. Hydroxyapatite with this mole ratio has the same crystalline arrangement as animal/human bones (Rana *et al.*, 2017). The reaction that occurs in the process of hydroxyapatite synthesis is:

$$10Ca(OH)_2 + 6(NH_4)_2HPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6H_2O + 12\ NH_4OH$$

The hydroxyapatite was analyzed using X-Ray Diffraction (XRD). High intensity was found in 2ϴ: 32.58; 33.71; 33.14; 23.13; and 31.82 where the peak of this 2ϴ was in accordance with ICSD standard of hydroxyapatite No. 96-900-3549 (Figure 1).

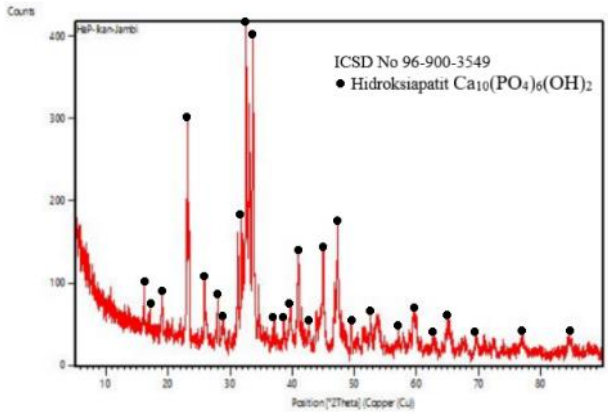

Figure 1. XRD analysis of hydroxyapatite

XRD analysis was conducted to see the composition of atoms in a crystalline material so that it can be known the structure, orientation and size of crystals (Munasir *et al.*, 2012). The crystal size of the XRD analysis can be known using the Scherrer method, the crystal size in this synthesis was 77.47 nm. Products that have a low FWHM will result in a larger crystal size (Warna *et al.*, 2015).

SEM analysis was conducted to look at the morphology of particle surfaces of hydroxyapatite compounds. SEM analysis was done at magnification 500x, 1000x and 1500x (Figure 2).

This study with a mole ratio of Ca/P 1.67 particles formed granular like a sphere with an even particle distribution and the particle size is in the range of 0.1 to 0.3 µm. Particles shaped like spheres with a size of 20 – 30 µm will form at pH 10, while most hydroxyapatite synthesized at pH 8 are needle-like with a length of 0.25 µm (Wang *et al.*, 2010), but if there is the interaction between substances, it can affect the crystal morphology of a substance (Fadhila *et al*., 2020).

Using calcination temperatures of 800°C and 1,000°C for 5 hours impacts the morphological changes of hydroxyapatite particles that bond between particle granules and form irregular rods (Setiawan & Basit, 2012). The particle size obtained is 0.19433 µm, which is in the range of 0.1 to 0.3 µm. This particle size is

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E-ISSN: 2580-8303

almost the same as the previous research conducted by researchers who obtained particle sizes of 0.127 - 0.538 µm with an average of 0.396 µm (Siregar & Sulistyawati, 2019).

Jelly candy evaluation

a) Raw Material Inspection Results

Examination of raw materials carried out organoleptic and solubility in hydroxyapatite as an active substance with white powder and practically insoluble in aquadest and ethanol 95%. Sodium benzoate as a preservative, white in color, odorless and soluble in aquadest. Benzoic acid as a preservative, hablur powder, colorless, and odorless and soluble in aquadest. Citric acid as a taster of acid, white powder, odorless and very easily soluble in aquadest. Gelatin as a gel shaper and candy base, pale yellowish in color and soluble in hot water. Cocopandan syrup as a sweetener and dye, red liquid, and menthol as a flavor giver and mint aroma.

b) Jelly Candy Evaluation

Evaluation on Jelly Candy preparations was conducted to see which formula was best and meets the requirements of Jelly Candy in the National Agency Standard (SNI) No. 3547.2-2008 including weight uniformity test, organoleptic test, pH test, hedonic test, kapang khamir test, total plate number test, gel strength, homogeneity test and stability test.

A weight uniformity test was conducted to see the uniformity of the size of gummy candy preparations made, all formulas of hydroxyapatite jelly candy preparations 6 candies deviate from the condition of weight uniformity that should not be more than 5%. Factors that affect the uniformity of weights are shape, print and temperature (Firdaus *et al*., 2014). In addition, during the heating process causes the amount of water that evaporates in each formula is not the same and at the time of pouring printed or printing techniques. If the diameter and thickness are not uniform it will affect the number of doses of active substances (Syukri *et al*., 2018). Weight uniformity test in all formulation summarizes in Table 2.

Jelly candy without hydroxyapatite has a very striking red color, for jelly candy that uses hydroxyapatite has a pink color, this is due to the addition of hydroxyapatite substances affect the color quality of jelly candy (Figure 3). The addition of calcium carbonate to milk jelly candy affects the Brightness of milk jelly candy (Lesmana *et al.*, 2008) which means the addition of calcium carbonate affects the Brightness of milk jelly candy which is getting murky due to insoluble calcium. The observation result of organoleptic test present in Table 3.

Table 3. Organoleptic test

Formulation	The Observation Result			
	Smell	Color	Taste	Texture
F0	Mint	Red	Sweet Mint	Chewy
F1	Mint	Pink	Sweet Mint	Chewy
F2	Mint	Pink	Sweet Mint	Chewy
F3	Mint	Pink	Sweet Mint	Chewy

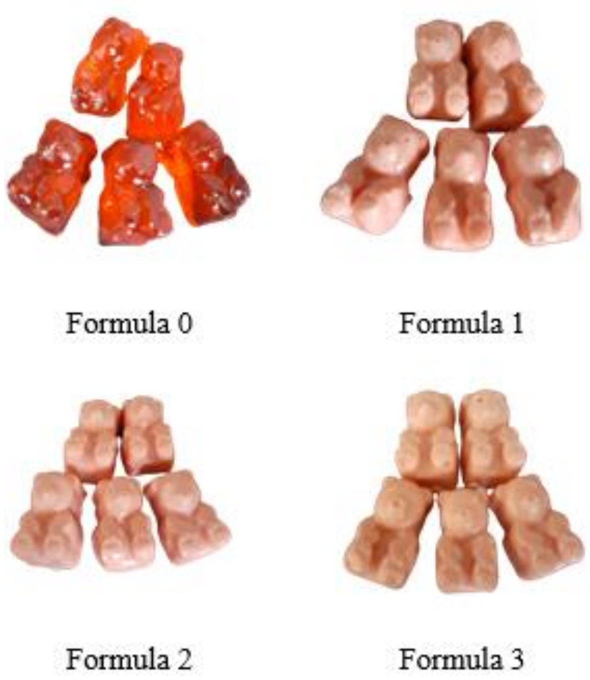

Figure 3. Jelly candy hydroxyapatite fish bone

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Table 2. Weight uniformity test in all formulation

No	F0	%D	F1	%D	F2	%D	F3	%D
1	1.6021	5.5309%*	1.9212	4.5745%	1.7285	4.7133%	1.9934	8.6084%*
2	1.6594	2.1522%	1.9242	4.4255%	1.7685	2.5082%	1.9647	7.0447%*
3	1.6675	1.6746%	1.9133	4.9669%	1.7866	1.5104%	1.8205	0.8118%
4	1.6369	3.4789%	1.9937	0.9735%	1.7743	2.1888%	1.8421	0.3650%
5	1.6325	3.7384%	1.9258	4.3460%	1.8216	0.4189%	1.8737	2.0867%
6	1.6028	5.4897%*	1.9649	2.4040%	1.8525	2.1223%	1.8174	0.9807%
7	1.6203	4.4878%	2.0653	2.5828%	1.7039	6.0694%*	1.8252	0.5557%
8	1.6228	4.3103%	2.0227	0.4668%	1.8645	2.7839%	1.8058	1.6127%
9	1.7554	3.5084%	2.0562	2.1308%	1.8223	0.4575%	1.8347	0.0381%
10	1.6664	1.7394%	2.0445	1.5496%	1.7851	1.5931%	1.8093	1.4220%
11	1.7098	0.8196%	2.0164	0.1539%	1.7627	2.8280%	1.8694	1.8524%
12	1.6817	0.8373%	2.0586	2.2500%	1.8951	4.4707%	1.8139	1.1714%
13	1.7474	3.0367%	2.0518	1.9122%	1.7317	4.5369%	1.8240	0.6211%
14	1.7140	1.0672%	2.0403	1.3410%	1.8611	2.5964%	1.8764	2.2338%
15	1.6892	0.3950%	2.0972	4.1672%	1.8436	1.6328%	1.8043	1.6944%
16	1.7081	0.7193%	2.0458	1.6142%	1.7583	3.0705%	1.8341	0.0708%
17	1.7414	2.6829%	2.0813	3.3775%	1.8087	0.2921%	1.8217	0.7464%
18	1.6485	2.7949%	2.0099	0.1688%	1.8503	2.0011%	1.8065	1.5745%
19	1.7941	5.7904%*	1.9156	4.8527%	1.8360	1.2127%	1.8173	0.9861%
20	1.7546	3.4612%	1.9824	1.5347%	1.8171	0.1708%	1.8273	0.4413%
21	1.6654	1.7984%	2.0436	1.5049%	1.8437	1.6372%	1.8176	0.9698%
22	1.7446	2.8716%	2.0538	2.0116%	1.8382	1.3340%	1.8007	1.8905%
23	1.7655	4.1040%	2.0111	0.1092%	1.8071	0.3803%	1.8325	0.1580%
24	1.7196	1.3974%	2.0788	3.2533%	1.8749	3.3572%	1.8089	1.4438%
25	1.7344	2.2701%	2.0470	1.6738%	1.8468	1.8081%	1.8263	0.4958%
26	1.7673	4.2101%	2.0081	0.2582%	1.8215	0.4134%	1.8223	0.7137%
27	1.7438	2.8244%	2.0354	1.0977%	1.8067	0.4024%	1.8150	1.1114%
28	1.7408	2.6475%	1.9817	1.5695%	1.8156	0.0882%	1.8139	1.1714%
29	1.6993	0.2004%	2.0933	3.9735%	1.8285	0.7993%	1.8173	0.9861%
30	1.6427	3.1369%	1.9138	4.9421%	1.8642	2.7673%	1.8260	0.5121%
Average	1.6959 ± 0.0544		2.0133 ± 0.0574		1.8140 ± 0.0462		1.8354 ± 0.0435	

Note : F0 : Formulation jelly candy without hydroxyapatite
F1 : Formulation jelly candy with hydroxyapatite 18%
F2 : Formulation jelly candy with hydroxyapatite 19%
F3 : Formulation jelly candy with hydroxyapatite 20%
%D : % deviation
* : Deviation > 5%

The test results of each hydroxyapatite jelly candy formula have a pH value range between 4.8 - 6.7 (Figure 4). The pH test is conducted to determine the pH value of the preparations that have been made. pH testing is important to know the acidity level of a product because acidity levels can affect consumer preference (Susanti *et al.*, 2019).

pH testing in F1 obtained a more acidic pH compared to formulas that use hydroxyapatite, this is thought to be influenced by hydroxyapatite, because hydroxyapatite produced is made in a state of pH 12 which means more alkaline and can increase the pH value in hydroxyapatite jelly candy. The pH value of jelly candy is pH 4.3 to pH 6 (Lees, 1973), which means that hydroxyapatite jelly candy preparations enter the criteria.

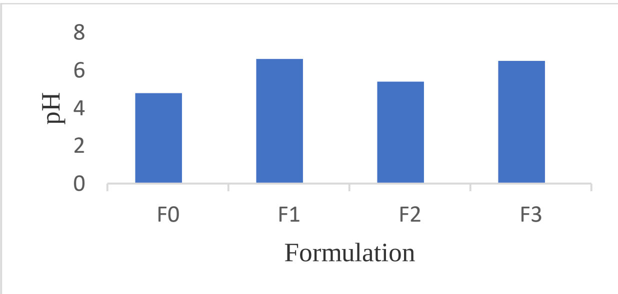

Figure 4. pH test of jelly candy hydroxyapatite mackerel fish bone

The hedonic test due to observe the respondent's preference in jelly candy hydroxyapatite mackerel fish bone. The hedonic test in smell indicated that F2 has the highest percentage in like jelly candy around 40%. On the other hand, F1 has the most significant percentage in

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dislike jelly candy hydroxyapatite, about 30% (Figure 5).

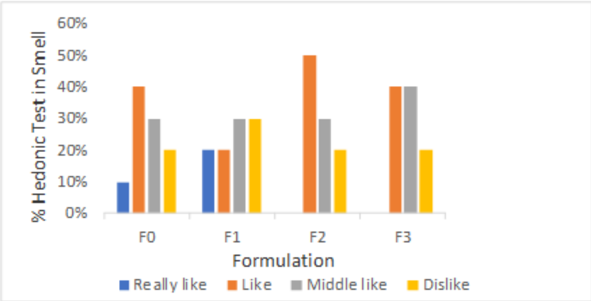

Figure 5. The result of the hedonic test in smell

Based on the result of hedonic test in color, the really like respondents were in F0 (formulation without hydroxyapatite) and the middle like respondents of the jelly candy were in F2 and F3 about 40%. The more hydroxyapatite added in the formulation, the jelly candy become soft pink and not eye-catching anymore like F0. However, the percentage of respondents dislike jelly candy hydroxyapatite were in F1 (Formulation 1) around 20% (Figure 6).

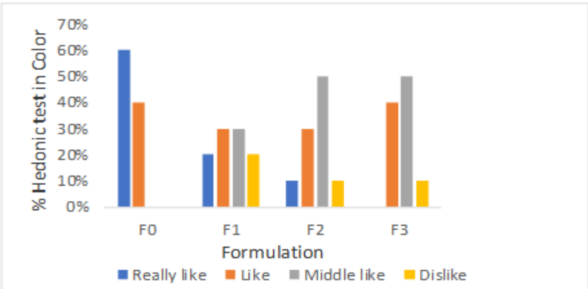

Figure 6. The result of hedonic test in color

Regarding this result of hedonic test in taste (Figure 7), the respondents like the taste of formulation 3 (F3) around 40%. However, the taste of jelly candy that the respondents disliked was in F1 and F2, about 40%. Meanwhile, the really like respondents in the texture of jelly candy (Figure 8) were in F0 by 40% and the dislike respondents was in F2 and F3 around 30%. Based on the result of hedonic test, F0 was the best formulation in color, taste, and texture.

The test of the kapang khamir test and total plate figures (Table 4) were obtained that all formulas of hydroxyapatite jelly candy are still below the standard limit of the time according to SNI 3547.2-2008 (Badan Standarisasi Nasional, 2008).

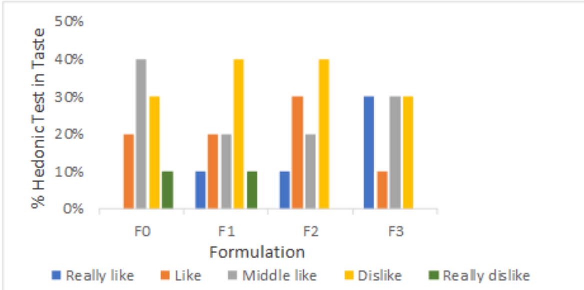

Figure 7. The result of hedonic test in taste

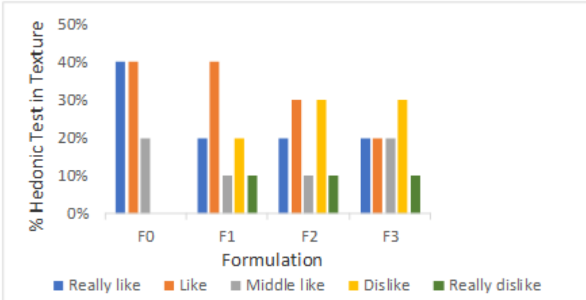

Figure 8. The result of hedonic test in texture

Regarding the results of the test of khamir time, it turns out that the sample produced the number of colonies that met the limit of khamir cup contamination on jelly candy preparations. Based on SNI, a jelly candy was safe if the total contamination of khamir time is not more than 1 x 10^2 colonies/g and the maximum ALT is 5 x 10^4 colonies/g, while in the test results of weak contamination and ALT is in the range of 1 x 10^1 to 2 x 10^1 and for ALT ranges from 2x10^1 to 8.5 x 10^2 then from the test results obtained shows that the preparation of hydroxyapatite jelly candy was not dangerous to be consumed because it does not exceed the standard limit of SNI 3547.2-2008 (Badan Standarisasi Nasional, 2008). Based on gel strength test results, the more hydroxyapatite added the higher the strength of the gel (Table 5).

Table 5. Gel strength test result

No	Formula	Gel Strength (N)
1.	F0	0.2173 N
2.	F1	1.8689 N
3.	F2	5.0647 N
4.	F3	6.3446 N

Table 4. Kapang Khamir Test and Total Plate Number Test Result

Formula	Kapang Khamir Result		Total Plate Number Test	
	Test Result (koloni/g)	Standard (koloni/g)	Test Result (koloni/g)	Standard (koloni/g)
F0	0	1 x 10^2	2 x 10^1	5 x 10^4
F1	1 x 10^1	1 x 10^2	10 x 10^1	5 x 10^4
F2	0	1 x 10^2	3 x 10^1	5 x 10^4
F3	2 x 10^1	1 x 10^2	85 x 10^1	5 x 10^4

P-ISSN: 2406-9388
E-ISSN: 2580-8303

The addition of different calcium concentrations may increase the strength value of the gel (Dewi *et al*., 2020). Increased moisture content can decrease hardness, where water will diffuse into the gel (Muhandri & Subarna, 2009). So that the gel formed becomes softer and causes the hardness to decrease.

A homogeneity test (in Figure 9) is done to find out whether the spread evenly or not hydroxyapatite in jelly candy. The test results were obtained that hydroxyapatite is distributed evenly in jelly candy even though jelly candy.

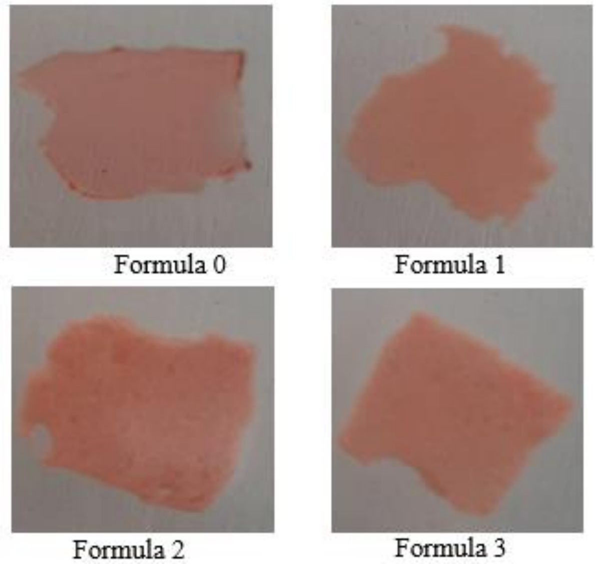

Figure 9. Homogeneity test

Jelly candy hydroxyapatite conducted observations stored at room temperature for three weeks, the testing of which included organoleptic examination of the taste, color, aroma, texture, and pH of jelly candies. Hydroxyapatite jelly candy changes every week. Storage of jelly candy changes on the 7th to 2nd day, where the change occurs in aroma, shape and taste while for the color in the jelly candy hydroxyapatite does not change from the 0th day to the 21st day. On the 14th day the aroma of mint begins to fade because the mint derived from menthol evaporates with increasing days. Formula 2 and formula 3 on the 7th and 21st days undergo changes in texture, this is because formulas 2 and 3 have more hydroxyapatite additions compared to formula 1. This is in line with the research that has been done that mentions the addition of calcium has an effect on the hardness of jelly candy (Lesmana *et al*., 2008).

The addition of calcium concentration impacts the gel's strength (Dewi et al., 2020), and the moisture content increases the gel's hardness (Muhandri & Subarna, 2009). In this study, formulas 2 and 3 have less moisture content than the formula 0 and 1, causing the jelly candy to harden on the 7th and 21st days. For the taste of hydroxyapatite jelly candy obtained changes on the 14th and 21st days in formulas 2 and 3; the change is the decrease in the taste of mint in preparations that cause the taste of sweet candy only; this is thought to be due to the addition of highly relevant hydroxyapatite resulting in the fading of mint on preparations on days 2 - 14 and 21. On the pH examination of hydroxyapatite, jelly candy obtained a stable pH value at pH 4 to 6.

The storage test of jelly candy hydroxyapatite was observed in the stability of the formulation, which stored at room temperature for 3 weeks and tested every week; where the observations consisted of several parameters are, including organoleptic test color, smell, texture and taste) and pH test (Figure 10). This is examined to guarantee that the jelly candy before and after the preparation was still in good parameters during the storage time. Based on Table 6, the changes occurred from the smell, texture and taste after the 7th day until the 21st day. However, there were no differences in the color from the 0 day until the 21st day. Formulations 2 and 3 were altered on the 7th day and 21st day, respectively, due to the more hydroxyapatite quantity in this formula. This research is in accordance with Lesmana *et al*. (2008), who stated that the addition of calcium affects the hardness of jelly candy.

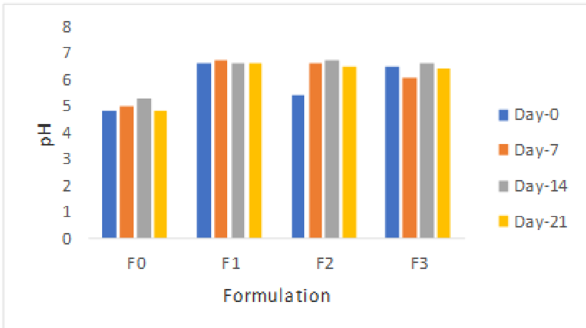

Figure 10. Storage test in pH

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 6. Storage test in organoleptic

Temperature	Observation	Formulation	Day 0	Day 7	Day 14	Day 21
Room Temperature	Color	F0	Red	Red	Red	Red
		F1	Pink	Pink	Pink	Pink
		F2	Pink	Pink	Pink	Pink
		F3	Pink	Pink	Pink	Pink
	Smell	F0	Mint	Mint	Mint	Syrup
		F1	Mint	Mint	Mint	Syrup
		F2	Mint	Mint	Syrup	Syrup
		F3	Mint	Mint	Syrup	Syrup
	Texture	F0	Chewy	Chewy	Chewy	Chewy
		F1	Chewy	Chewy	Chewy	Chewy
		F2	Chewy	Chewy	Chewy	Chewy
		F3	Chewy	Chewy	Chewy	Chewy
	Taste	F0	Sweet Mint	Sweet Mint	Sweet Mint	Sweet Mint
		F1	Sweet Mint	Sweet Mint	Sweet Mint	Sweet Mint
		F2	Sweet Mint	Sweet Mint	Sweet	Sweet
		F3	Sweet Mint	Sweet Mint	Sweet	Sweet

CONCLUSION

Based on the results of this study, we can conclude that Hydroxyapatite Mackerel Fish Bone can be made as Jelly Candy, with the best concentration of Hydroxyapatite was 18%.

AUTHOR CONTRIBUTIONS

Conceptualization, L. A., S. P., R. A., R.; Methodology, L. A.; Validation, L. A., S. P., R.; Formal Analysis, L. A., S. P., R. A., R.; Investigation, L. A., S. P., R. A., R.; Resources, L. A., S. P., R. A., R.; Data Curation, L. A., S. P., R. A., R.; Writing - Original Draft, L. A., S. P., R. A., R.; Writing - Review & Editing, L. A., S. P., R. A., R.; Visualization, R.A.; Supervision, L. A., S. P.; Project Administration, L. A., S. P., R. A., R.; Funding acquisition, L. A., S. P., R. A., R.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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P-ISSN: 2406-9388
E-ISSN: 2580-8303