

*Research Article*

# Effect of Lime Solution Soaking on Physicochemical Characteristics of Catfish Bone Powder (*Arius thalassinus*)

Winda Erliana Putri, Ima Wijayanti\*, Apri Dwi Anggo

Department of Fishery Products  
Technology, Faculty of Fisheries and  
Marine Science, Universitas  
Diponegoro, Semarang, 50275,  
Indonesia.

\*Corresponding Author  
ima.wijayanti@live.undip.ac.id

**Abstract**

Fish bone powder is one of the fisheries by product processing that has a high nutritional content, especially calcium and phosphorus content. In the processing of fish bone powder, the fishy odor is still detected due to organic compound such as protein and lipid which produce volatile compounds yielded unpleasant odor. The use of lime can eliminate fishy odors, improve color, shape, taste, texture, and extend shelf life. This study aimed to determine the difference of lime solution concentration on the physicochemical characteristics of catfish bone powder and to determine the best lime concentration used on the volatile compound content of catfish bone powder. The method carried out in this study was a laboratory experimental method and used a Complete Random Design (CRD) with 4 treatments (0%, 15%, 20%, 25%) and carried out in triplicate. Data analysis from physical and chemical tests were analyzed using ANOVA, while hedonic tests were performed using the *Kruskal-Wallis* test followed by *the Mann-Whitney* test. The results showed that soaking lime solution had a significant effect ( $P < 5\%$ ) on moisture content, protein content, fat content, ash content, calcium content and color. Catfish bone powder with the best treatment of soaking in lime solution of 20% contained a calcium of 25.17%. The results of the hedonic test showed that the best treatment (20%) had a confidence interval of  $3.95 < \mu < 4.19$  from 5 scale. The volatile compound of 4-[4-(dimethylamine) Benzaldehyde associated with fishy odor was detected in fish bone powder without lime solution, but it could not be detected in sample soaked in 20% lime solution showing the capability of lime solution to reduce fishy odor in fishbone powder.

**Keywords:** Calcium, Volatile compounds, Fish bones, Fish bone powders

**1. Introduction**

Catfish (*Arius thalassinus*) is one of the groups of offshore demersal fish located in the waters of the Java Sea. Processed products with catfish basic ingredients include smoked fish and salted fish. The body parts of the catfish that are used for the smoked product include meat, head and tail. That process yields the waste, including fish bone. Fish bones contain high calcium and phosphorus so they are good for health [1]. According to Ahmil et al. [2], bones are a form of waste produced by the fish processing industry that has the most calcium content in the body of fish. Marsaoly and Mahmud [3] added that fish bones have the potential as a source of calcium that is easily available to the community and can be used as an alternative diet to prevent a calcium deficiency.

One of the uses of fish bone waste is to process it into flour. Fish bone flour is made from fish waste in the form of dry

preservation, which is then processed into flour. The processing of fishbone flour is determined by its chemical composition and the availability of existing equipment. The procedure of creating different fish bone flour will influence the qualities and quality of the resultant fish bone flour [4]. The application of the use of fish bone powder in the form of food products has been carried out by several researchers, including Suarsa et al. [5] on the processing of Tuna fish bone powder in the manufacture of biscuits using the proximate testing method. The high calcium content in fish bone powder is one of the advantages of being used as a food additive. However, behind these advantages, fish bone powder is still not in demand because there is still a fishy smell in the flour. Lime (*Citrus aurantifolia*) is known as a fruit that has a fresh taste and contains many vitamins and minerals. There are many benefits obtained from the use of lime, one of which is that it can reduce

the fishy smell of meat or fish that is to be cooked [6]. Fitri et al. [7] reported that the use of lime solution for milkfish bone powder production could reduce its fishy smell, hence it could be fortified at some level in the fish stick product without unpleasant odor. However, the use of different concentrations of lime solution in the catfish bone production has not been studied.

The purpose of this study was to determine the physicochemical characteristics and volatile compound levels in catfish bone powder soaked with lime solution to minimize the fishy smell in the flour.

## 2. Materials and methods

### 2.1. Materials

The raw materials used in making fish bone powder is fresh catfish bones (*Arius thalassinus*) which are obtained from fish smoking production waste located in Doropayung Village, Juwana DistrFPLS-0, Pati Regency. The supporting materials used include ripe lime or yellowish-green and aquaous solution.

### 2.2. The process of lime solution and fish bone powder

The acid extraction process of fish bone powder using lime solution was carried out by preparing lime solution first with concentrations of 15%, 20% and 25% (v/v). The lime solution production was started with peeling the lime, then mashing it with a blender without adding water. Next, the lime fruit extract (juice) was filtered using a cheese cloth to separate the juice and pulp. The filtered lime juice was diluted using distilled water (v/v) as percentage used for extraction.

Fish bone powder then produced as following the method of Wijayanti et al. [8] with modification of additional extraction process using lime solution. The bone was boiled for 60 min. The boiled bones that have been cleaned, then cut smaller and continued with the second boiling stage. The boiling process in phase 2 was carried out for approximately 45 min. Further, the bone was autoclaved at 121°C for 2 hours. Then, the autoclaved sample was extracted using lime solution with different concentrations (0%, 15%, 20%, and 25%) for 2 h at a temperature of 60°C, at a ratio of fish bone powder:lime

solution of 1:3. Extracted catfish bones were then rinsed with running water to neutralize pH. The neutralized sample was dried using an oven for 5 hours at a temperature of 100°C and continued with grinding and sieving process using an 80-mesh sieve. The autoclaved bone without lime solution extraction was dried and grinded at the same manner namely as FPLS-0 (0% concentration). The resulted powder extracted using 15%, 20% and 25% lime solution were named as FPLS-15, FPLS-20, and FPLS-25, respectively.

### 2.3. Proximate testing

The proximate testing carried out on the fish bone powder produced includes moisture content [9], protein content [10], ash content [10], and fat content [9].

### 2.4. Calcium levels [11]

The analysis of calcium levels in the study used a series of ICP-OES (*Inductively Coupled Plasma-Optical Emission Spectrometry*) instruments which began by weighing a sample of 1 g and then put it into a *vessel*. The sample was added with HNO<sub>3</sub> and H<sub>2</sub>O solution in a ratio of 1:3, then let it sit for 15 minutes. The solution in the *vessel* is destructed by inserting the *vessel* into the *microwave digester*. The sample solution that had been decomposed was cooled and then put into a 50 mL measuring flask with the internal addition of a standard itrium of 100 mg/L of 0.50 mL and homogenized using aqueous to the specified limit. In the next stage, the sample solution is filtered using filter paper. The resulting filtrate can be measured for calcium content using the ICP-OES instrument. The wavelength used to measure calcium levels is 317.933 nm, then calcium levels are calculated using the formula:

$$\text{Calcium content (\%)} = \frac{A_{spl-a} \times V_{fp}}{W_{spl} \text{ at } a \times V_{spl}}$$

Where:

- A<sub>spl</sub> = Sample intercept
- A = *Intercept* from the standard calibration curve
- B = Slope of the standard calibration curve
- Fp = Dilution factor
- V = Volume of the final flask of the sample (mL)

$W_{spl}$  = Test sample weighing weight (g)

$V_{spl}$  = Test sample pipetting volume (mL)

## 2.5. Color test

Color testing was carried out using the WR-10 *colorimeter* as per method Wijayanti et al. [8]. The first step in using this tool is to press the *on* button on the tool, then calibrated first with the standard white color and recorded. The sample is inserted into a clear plastic clip then the receptor sensor is placed on the plastic containing the sample and the results obtained are recorded. Measurements were carried out 3 times.

## 2.6. Hedonic testing [12]

The parameters in this hedonic test include color, odor, texture. In determining the quality of a food ingredient in general, it is greatly influenced by several factors, including taste, color, texture and nutritional value. The scores used for the assessment of a product include 1 (strongly disliked), 2 (disliked), 3 (neutral), 4 (liked), 5 (strongly liked). Sensory testing was carried out by 30 semi-trained panelists by scoring on product quality attributes. The hedonic test was carried out by presenting samples in small containers to each panelist.

## 2.7. Levels of Volatile Compounds [14]

The analysis of volatile compounds was carried out using *Gas Chromatography* (GC-MS QP2010S Shimadzu) and *dichloro methane* (DCM) solvent with Rtx-5MS column conditions measuring 30mm with an internal diameter of 0.22mm. Helium carrier gas with injector parameters in the form of an initial temperature of 70°C (*hold* 5 minutes), pressure of 13.7 kPa, total flow of 40 mL/min, column flow of 0.50 mL/min, linear velocity of 25.90 cm/s, purge flow of 3 mL/min, split ratio of 73.0, final temperature of 320°C (*hold* 52 minutes), temperature increase of 10°C/min. The temperature at the ion is 250 °C and the interface temperature is 320°C. On the chromatog, the number of compounds in the extract is indicated by *the peak*, while the name/type of compound is interpreted based on the *spectrum data* at each peak using the library approach database GC-MS.

## 2.8. Particle size test [14]

Particle measurements are carried out using *the Laser Particle*

*Size Analyzer* (LPSA) tool. The specification of the tool used is *Malvern Zetasizer series 7.01* which can find out the particle size up to 0.6 nm.

## 2.9. Data analysis

The data analysis used in this study consisted of parametric and non-parametric analysis. Non-parametric analysis was used for data with hedonic test results. Parametric data analysis used a Complete Random Design (CRD) which was carried out a homogeneity test, the smallest significant difference test used the *Analysis of Variance* (ANOVA) and continued with the Tukey test to determine the significant differences ( $p < .05$ ). The non-parametric analysis (sensory data) was analyzed using *the crucial Wallis-Dunn's Multiple Comparison* and continued with *the Mann-Whitney* test to find out the significant difference between treatments.

## 3. Results and discussions

### 3.1. Moisture content

The moisture content of fish bone powder extracted using lime solution is shown in Table 1. The average value of the moisture content in fish bone powder ranged from 4.35 – 6,83%. The decrease in moisture content in catfish bone powder is possible from the concentration of lime used. The higher the concentration of lime used, the lower the moisture content in fish bone powder. According to Setiani et al. [15] the higher the strength of the acid that can clump proteins, the greater the moisture content released. This is in accordance with Sa'adah [16] research, soaking catfish bone powder in lime juice solution can cause a decrease in moisture content of 7.38%, 7.28%, and 7.15% which is likely due to the rapid evaporation of lime juice so that a lot of moisture content in the bone powder was lost. The water compound's H+ bond in the fishbone powder was broken during the reaction with the acid of lime solution, resulting the weaker bond and more susceptible to evaporation during the drying process [17]. As a result, the moisture content of the resulted powder decreased as shown in Table 1.

### 3.2. Protein content

The protein content of catfish bone powder extracted using lime solution at different concentrations is shown in Table 2.

The average value of protein content ranged from 14.42 – 15.96%.

**Table 1.** Results of Catfish Bone Powder Moisture Content Test

No.	Treatment	Moisture content (%)
1	FPLS-0	6.83 ± 0.08 <sup>d</sup>
2	FPLS-15	5.99 ± 0.10 <sup>c</sup>
3	FPLS-20	5.22 ± 0.18 <sup>b</sup>
4	FPLS-25	4.35 ± 0.11 <sup>a</sup>

Note: Data followed by different superscript letters in the same column shows a significant difference ( $P < 0.05$ ).

FPLS-0 (0% lime solution), FPLS-15 (15% lime solution), FPLS-20 (20% lime solution) and FPLS-25 (25% lime solution).

The lime-soaking treatment with a concentration of 0% (FPLS-0) had the lowest average protein content in catfish bone powder of 14.42%. The highest average was obtained by soaking lime concentration of 25% (FPLS-25) by 15.96%. In the treatment of 15% and 20%, respectively, they were 14.50% and 15.58. Lime soaking has a great effect on the protein content in catfish bone powder. The increase in protein levels can be caused by the long soaking and concentration of lime juice because the acids contained in lime can inhibit protein damage. This is in line with the results of research conducted by Liana et al. [18] the increase in protein levels in lime juice soaking at a concentration of 15% is due to the length of soaking and will decrease due to the addition of acid.

**Table 2.** Results of protein content test of Catfish Bone Powder

No	Treatment	Protein Content (% ,db)
1	FPLS-0	14.42 ± 0.21 <sup>a</sup>
2	FPLS-15	14.50 ± 0.14 <sup>a</sup>
3	FPLS-20	15.58 ± 0.32 <sup>b</sup>
4	FPLS-25	15.96 ± 0.37 <sup>b</sup>

Note: Data followed by different superscript letters in the same column shows a significant difference ( $P < 0.05$ ).

The factor that affects the increase in protein is the processing method, which includes the entire process of making fish bone

powder. The cooking process using an autoclave can release free water into the food. The evaporation of these substances causes the moisture content to decrease, so it can increase protein levels [19]. FPLS-0 (0% lime solution), FPLS-15 (15% lime solution), FPLS-20 (20% lime solution) and FPLS-25 (25% lime solution).

db: dry base

### 3.3. Ash content

The ash content of catfish bone powder extracted using lime solution is displayed in Table 1. The average value of ash content in Catfish bone powder with lime soaking ranged from 64.92 – 68.26%. Immersion of lime solution with a concentration of 0% has the highest ash content and a concentration of 25% has the lowest ash content. The higher the lime solution, the lower the ash content was observed. The decrease of ash content with the increase of lime solution might be due to the mineral content in the fish bone powder that was leached during extraction using lime solution. The lime solution contains organic acid, such as citric acid, around t (34–39 g/L) [20]. The citric acid in the lime solution can leach several minerals in the fishbone powder during extraction, resulting in ash reduction. Yahya et al. [21] showed that citric acid can be used as a leaching treatment to reduce the heavy metal element of the coal. Moreover, citric acid can also be a chelating agent of several minerals. Hence, several minerals in the fishbone powder could also be chelated during extraction. Abano et al. [22] reported that citric acid could be as chelating agent that can decrease the ash content of garlic slices during citric acid pre-treatment.

**Table 3.** Test Results of Ash Content of Catfish Bone Powder

No	Treatment	Ash Content (% ,db)
1	FPLS-0	68.26 ± 0.84 <sup>b</sup>
2	FPLS-15	68.54 ± 1.05 <sup>b</sup>
3	FPLS-20	66.93 ± 0.51 <sup>ab</sup>
4	FPLS-25	64.92 ± 0.22 <sup>a</sup>

Note: Data followed by different superscript letters in the same column shows a significant difference ( $P < 0.05$ ).

FPLS-0 (0% lime solution), FPLS-15 (15% lime solution),

FPLS-20 (20% lime solution) and FPLS-25 (25% lime solution).

db: dry base

### 3.4. Fat content

The fat content of catfish bone powder extracted using lime solution at different concentration is shown in Table 4. The average value of fat content in catfish bone powder ranges from 3.79 – 6.80%. The results decreased in accordance with the immersion of lime solution at concentrations of 0%, 15%, 20% and 25%. Higher fat content can cause the aroma in fish bone powder to become more intense. According to Wahyuni et al. [23], the lime soaking method is the process of soaking ingredients in a lime solution before cooking. It is generally done by soaking in an acidic solution to reduce the pungent aroma of the material. This is in line with the research conducted by Litaay and Santoso [24] regarding soaking using acetic acid solution in skipjack fish bone powder. The decrease in fat content is influenced by the use of the soaking method, where the acidic properties that can break down fat result in a decrease in fat content compared to soaking with water and alkali. Acidic solutions with their properties that tend to be stronger in opening the bond structure in proteins, can cause more proteins to be dissolved which will bind fat molecules so that fat levels become lower. The results of the fat content test can be seen in Table 4.

**Table 4.** Test Results of Fat Content of Catfish Bone Powder

No	Treatment	Fat Content(% ,db)
1	FPLS-0	6.80 ± 0.8 <sup>c</sup>
2	FPLS-15	5.59 ± 0.23 <sup>b</sup>
3	FPLS-20	5.03 ± 0.39 <sup>b</sup>
4	FPLS-25	3.79 ± 0.27 <sup>a</sup>

Note: Data followed by different superscript letters in the same column shows a significant difference (P<0.05).

FPLS-0 (0% lime solution), FPLS-15 (15% lime solution), FPLS-20 (20% lime solution) and FPLS-25 (25% lime solution).

db: dry base

### 3.5. Calcium levels

The calcium levels of catfish bone powder extracted using lime solution at different concentration is displayed in Table 5. The average value of calcium levels in Catfish bone powder ranged from 23.48 – 25.17%. Catfish bone powder without soaking in lime solution had the lowest calcium content value of 23.48%, compared to Catfish bone flour with lime solution soaking. This is in accordance with the research of Lekahena et al. [25], the calcium level of fish bone powder in the control was 18.70%, while in the immersion of the acid solution there was an increase in calcium content of 21.48%. The difference in calcium content is suspected to be due to the extraction treatment using an acidic or alkaline solution that results in hydrolyzed proteins and fats contained in the bone matrix. The calcium content in fish bone powder can be related to the ash content. The higher the calcium content, the lower the ash content.

**Table 5.** Test Results of Calcium of Catfish Bone Powder

No	Treatment	Calcium Content (% ,db)
1	FPLS-0	23.48 ± 0.26 <sup>a</sup>
2	FPLS-15	23.51 ± 0.10 <sup>a</sup>
3	FPLS-20	25.17 ± 0.06 <sup>b</sup>
4	FPLS-25	24.80 ± 0.12 <sup>b</sup>

Note: Data followed by different superscript letters in the same column shows a significant difference (P<0.05).

FPLS-0 (0% lime solution), FPLS-15 (15% lime solution), FPLS-20 (20% lime solution) and FPLS-25 (25% lime solution).

db: dry base

### 3.6. Color

The color characteristics of catfish bone powder extracted using lime solution at different concentration is shown in Table 6. Color analysis of Catfish bone flour was carried out using a *colorimeter*. The tool produces L\*, a\* and b\* values on the screen. This value will show that the higher the value on L\* (*Lightness*) indicates that the bone powder is brighter, the higher the value of b\* the color of fish bones is yellower, the higher the value of a\* the color of fish bones is redder. The brightness value (L\*) of Catfish bone powder ranges from

92.94 – 93.82. The  $L^*$  value in the control had the lowest value of 92.82, while the highest was at a concentration of 25% with a value of 93.95. The results showed that there was an effect on the soaking of lime solution on the brightness value ( $L^*$ ) of catfish bone powder. The higher the concentration in the lime solution added, the lower the brightness level in the flour. The value of  $a^*$  is positive with a range of 3.02 – 3.78. This shows that the effect of lime soaking has a significant effect ( $P < 0.05$ ) on Catfish bone powder. The reddish color can come from organic compounds found in the bones. According to Suryaningrum et al. [26], soaking in an acidic solution produces degrees of redness and yellowness. Immersion in the solution is possible to suppress the *Maillard* reaction rate which is indicated by the low absorbance of reddish and yellowish colors in the resulting product. The yellowish value of fish bone powder has increased due to the soaking of lime solution. Limes contain flavonoids that can cause the onset of yellow color. According to Jayani et al. [27], limes contain flavonoids, one of which is

hesperidin which gives the juice its yellow color.

FPLS-0 (0% lime solution), FPLS-15 (15% lime solution), FPLS-20 (20% lime solution) and FPLS-25 (25% lime solution).

db: dry base

### 3.7. Hedonic value

The hedonic value of fishbone powder extracted using lime solution at different concentration is shown in Table 7. The hedonic value of catfish bone powder showed that the existence of different concentrations had a significant effect ( $P < 0.05$ ) on aroma and texture, and had no significant effect ( $P < 0.05$ ) on the color of catfish bone powder.

The average result of the color value of catfish bone powder ranged from 3.50 – 4.13. The highest average score of treatment was 0% so that it was preferred by the panelists with a brilliant white bone powder color. The 25% concentration treatment got the lowest average result with a yellowish flour color due to the lime soaking treatment.

**Table 6.** Results of the Color Test of Catfish Bone Powder

No	Treatment	$L^*$	$a^*$	$b^*$
1	FPLS-0	92.82 ± 0.09 <sup>a</sup>	3.78 ± 0.05 <sup>c</sup>	16.99 ± 0.20 <sup>b</sup>
2	FPLS-15	93.78 ± 0.02 <sup>b</sup>	3.02 ± 0.04 <sup>A</sup>	15.46 ± 0.04 <sup>A</sup>
3	FPLS-20	92.94 ± 0.04 <sup>a</sup>	3.82 ± 0.09 <sup>c</sup>	16.70 ± 0.13 <sup>b</sup>
4	FPLS-25	93.95 ± 0.06 <sup>b</sup>	3.36 ± 0.01 <sup>b</sup>	15.88 ± 0.07 <sup>A</sup>

Note: Data followed by different superscript letters in the same column shows a significant difference ( $P < 0.05$ ).

**Table 7.** Results of Hedonic Test of Catfish Bone Powder

Treatment	Parameters		
	Color	Aroma	Texture
FPLS-0	4.13 ± 0.67 <sup>b</sup>	3.20 ± 0.40 <sup>A</sup>	4.20 ± 0.65 <sup>A</sup>
FPLS-15	4.03 ± 0.75 <sup>b</sup>	3.40 ± 0.49 <sup>A</sup>	4.23 ± 0.56 <sup>A</sup>
FPLS-20	3.70 ± 0.64 <sup>A</sup>	4.17 ± 0.69 <sup>b</sup>	4.20 ± 0.75 <sup>A</sup>
FPLS-25	3.50 ± 0.62 <sup>A</sup>	4.20 ± 0.60 <sup>b</sup>	4.33 ± 0.65 <sup>A</sup>

Note: Data followed by different superscript letters in the same column shows a significant difference ( $P < 0.05$ ).

FPLS-0 (0% lime solution), FPLS-15 (15% lime solution), FPLS-20 (20% lime solution) and FPLS-25 (25% lime solution). Lime contains high flavonoids so it will be able to cause the onset of yellow color in fish bone powder. According to Mufidah and Hendrawati [28], soaking lime extract can produce a brownish-yellow color. The discoloration is caused by flavonoid compounds in limes. The color indicates that the flavonoid compounds in lime have dissolved in the extract. The average result of the aroma parameter of catfish bone powder ranged from 3.20 – 4.20. The aroma value of FPLS-0 sample was lower than FPLS-20 and FPLS-25 samples, but FPLS-0 showed similar odor value with FPLS-15. In the sample of FPLS-25 had a fresh aroma due to the presence of acid soaking using lime solution with a concentration of 25%. The aroma can be reduced because the lime solution is acidic so that it can inhibit the growth of bacteria and inhibit the formation of TMA compounds that cause fishy odors. According to Nurmalasari and Zaenab [29], lime juice contains a lot of chemical compounds such as citric acid, amino acids (tryptophan, lysine), carbonic acid, essential oils, glycosides, fats, calcium, phosphorus, iron, sulfur vitamins B1 and C. Citric acid contained in lime is 7-7.6%. It is this citric acid that can dissolve both polar compounds such as and non-polar compounds such as oil and other elements.

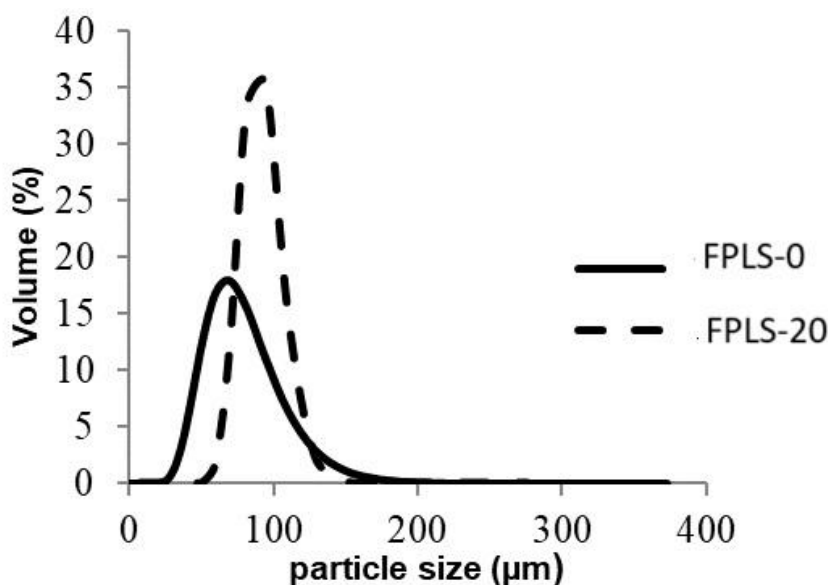
The average results on the texture parameters of fish bone powder ranged from 4.20 – 4.33. The texture of the catfish bone flour produced is smooth and not *grittiness*. The texture can be affected by the moisture content contained in the catfish bone powder. When the moisture content in the flour is high, then the texture of the fish bone flour will be somewhat clumpy, and vice versa. According to Handajani et al. [30], the criteria for good fishpowder are smooth texture and not clumpy. This can be caused by the high moisture content in the feed. Other factors that can affect the texture are the type of fish used, the moisture content and fat content of the fish used. Overall, the hedonic value of FPLS-20 was not significant different to FPLS-25, for the efficiency the FPLS-20 was selected for further characterizations.

### 3.8. Levels of volatile compounds

The results of estimating the content of volatile compounds in Catfish bone powder with soaking in lime solution showed a decrease in the number of volatile compounds contained in bone powder. Volatile compound component groups such as hydrocarbons, esters, aldehydes, hydrocarbons and fatty acids were detected in control fish bone powder (FPLS-0) and 20% concentration treatment (FPLS-20).

**Table 8.** Test Results of Volatile Compounds of Catfish Bone Powder.

No	Compound Names and Formulas	Quantity (% area)	
		FPLS-0	FPLS-20
1.	Hydrocarbon Class:		
	5-Hydroxy-4-cyclohexane	27	ND
	1-methyl-2-(p-nitrophenyl)-benzimi	ND	38
	1-methyl-4-[4,5-dihydroxyphenyl]	ND	14
	5-methyl-2-Cyclohexanol	ND	25
2.	Fatty Acid Group:		
	n-Hexadecanoic acid	91	ND
	9-Octadecenoic acid	49	ND
3.	Esther Group :		
	1-nitrophenanthro[4,5-bcd]thiopen	ND	36
4.	Aldehyde Class:		
	4-[[4-(dimethylamino)Benzaldehyde	22	ND
5.	Aromatic Hydrocarbons :		
	1-Fluoro-4-Nitrobenzene	ND	14



**Figure 1.** Distribution of Particle Sizes of Catfish Bone Powder. FPLS-0 (0% lime solution), FPLS-20 (20% lime solution)

One of the compounds contained in the control fish bone powder (FPLS-0) aldehyde compound is 4-[[4-(dimethylamino) - Benzaldehyde which is suspected to cause fishy odors. The volatile compounds found in fish bone powder are *n-Hexadecanoic acid* and *9-Octadecenoic acid* which are included in the group of fatty acid compounds. Compounds with shorter chains include fatty acids, aldehydes, and ketones. These compounds cause rancid and fishy odors in products [31]. The hydrocarbon compounds detected in fish bone powder products are *5-Hydroxy-4-cyclohexane*, *1-methyl-2-(p-nitrophenyl)-benzimidazole*, *1-methyl-4-[4,5-dihydroxyphenyl]* and *5-methyl-2-Cyclohexanol*. Hydrocarbon compounds are mostly found in fish bone powder with a concentration treatment of 20%. The aromatic hydrocarbon compound found in 20% treated fish bone powder (FPLS-20) is *1-Fluoro-4-Nitrobenzene*. The compound detected in the control fish bone powder is *1-nitrophenanthro[4,5-bcd]thiopen*, which belongs to the ester compound group. According to Widyasanti and Hanif [32], esters are one of the compounds that provide aroma and taste to food products. This compound can be obtained through the process of esterification obtained from the reaction between carboxylic acid and alcohol. Esters can be hydrolyzed into acid and alcohol components. The ester is hydrolyzed by a fast-acting

acid catalyst due to the presence of a hydrogen ion catalyst [33]. The results of the test for estimating the content of volatile compounds can be seen in Table 7.

### 3.9. Particle size

Soaking the acid solution using natural acid, namely lime extract, has a different effect on the particle size of Catfish bone powder. The particle size of Catfish bone powder in the control treatment was 66.77 µm, while in the 20% concentration immersion treatment was 83.03 µm. This is because the use of acid solvents in the extraction process can hydrolyze organic compounds contained in fish bones. According to Prinaldi et al. [34], in the extraction process using an acidic solution, there is a control of pH, temperature, and solvents that are able to change organic compounds such as proteins and fats dissolved in the extraction process so that the solution becomes saturated and produces particle deposits. The formation of particle size can be caused by a precipitation process that produces fine, small-sized calcium deposits. This is possible from the process of softening bones using solvents at high temperatures can change the texture of the bones. Other factors that can cause the variety of particle sizes of the types of fish bones used. The image of the distribution of the particle size of catfish bone powder can be seen in Figure 1.



The curve at the control (FPLS-0) and the 20% concentration (FPLS-20) showed a significant difference. The FPLS-0 curve has a tall and small shape, while the FPLS-20 curve is short and wide. Based on the results of research conducted by Mujamilah and Sulungbudi [35], the particle size distribution with an average of 80  $\mu\text{m}$ , the size of the curve will tend to shrink slightly in proportion to the addition of a solution of solution compounds so that the size distribution narrows. The wider the particle size distribution, the less uniform it will be. The processing process in fish bone powder also affects the size of the particles produced. According to Admi et al. [36] smaller particle sizes will be very advantageous if used as catalysts. This is because the smaller the particle size, the larger the surface area so that it will be able to affect the reaction speed in the sample used.

#### 4. Conclusions

Soaking lime solution with different concentrations has a significant effect on moisture content, protein content, ash content, fat content, color in catfish bone powder. The catfish bone powder extracted with lime solution were sensorial accepted by the panelists in all concentrations. The volatile compounds of catfish bone powder extracted using lime solution decrease compared to that without lime solution extraction. The groups of compounds identified in catfish bone powder showed that lime solution could reduce the volatile compound that have responsible for fishy odor.

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#### Data availability statement

Data available within the article.

#### Conflicts of Interest

All authors declare that they have no conflicts of interest.

#### Authors contribution

Winda Erliana Putri, conducted the research, collected, and analyzed the data, and wrote the manuscript. Ima Wijayanti, supervised, wrote, edited, analyzed and reviewed. Apri Dwi Anggo reviewed and edited.

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#### REFERENCES

1. Febriani, H.N., Rochima, E., Rostini, I. and Pratama, R.I., 2021. Pangasius bone powder (definition, production, analysis physicochemical characteristics and potency): A review. *Asian Journal of Fisheries and Aquatic Research*, 13(6), pp.1-9.
2. Ahmil, A., H. Mulyati., and O. Mananta. 2021. Analysis of Nutrient Content of Eel Fish Bone Powder (*Anguila sp.*). *Ghidza: Journal of Nutrition and Health*, 5(1): 36-44.
3. Marsaoly, M., and M. Mahmud, M. 2020. Making Plate Sago with Tuna Fish Bone Powder Substitution (*Thunnus albacores*). *Global Health Science*, 5(1) : 28-33.
4. Yusrina, A., Rochima, E., Handaka, A.A. and Rostini, I., 2021. Fishbone flour (definition, analysis of quality characteristics, manufacture): A review. *Asian Journal of Fisheries and Aquatic Research*, 13(4), pp.18-24.
5. Suarsa, I.W., A. A. B. Putra., S. R. Santo and A. Faruk. 2020. Production of Tuna Fish Bone Flour (*Thunnus sp*) by Dry Method as a Source of Calcium and Phosphorus for Biscuit Making. *Indonesian Journal of Education*, 8(1) : 19-28.
6. Fitriyana, R. A. 2017. Comparison of Vitamin C Levels in Limes (*Citrus x aurantiifolia*) and Lemons (*Citrus x Lemon*) Sold at Linggapura Market, Brebes Regency. *Publicity*, 2(2) : 1-16.
7. Fitri, A., R. B. K. Anandito and S. Siswanti. 2016. The Use of Milkfish Meat and Bones (*Chanos Chanos*) in Fish Steaks as a Snack High in Calcium and Protein. *Journal of Agricultural Product Technology*, 9(2): 65-77.
8. Wijayanti, I., Benjakul, S. and Sookchoo, P. 2021. Preheat-treatment and bleaching agents affect characteristics of bio-calcium from Asian sea bass (*Lates calcarifer*) backbone. *Waste and Biomass Valorization*, 12: 3371-3382.

9. AOAC. 2007. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Washington DC.
10. AOAC. 2005. Association Official Analytical Chemistry. Official Methods of Analysis. Arlington. York.
11. AOAC. 2011. Association Official Analytical Chemistry. 14 Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Product (Microwave digestion and ICP OES).
12. National Standardization Agency [BSN]. 2006a. SNI 01-2346-2006. Instructions for Organoleptic and or Sensory Testing. National Standardization Agency. Jakarta.
13. Hasanah, H., A. Jannah and A. G. Fasya. 2012. Effect of Fermentation Time on Alcohol Content of Cassava Tape (Manihot utilissima Pohl). *Alchemy*, 2(1) : 68-79.
14. Saputra, A. H., A. Haryono., J. A. Laksmono and M. H. Anshari. 2011. Colloidal Silver Nanoparticles Preparation Using Various Types of Reducing Agent as Antibacterial Material. *Indonesian Journal of Materials Science*, 12(3) : 202-208.
15. Setiani, B. E., V. P. Bintoro and R. N. Fauzi. 2021. Effect of the Addition of Lime Juice (*Citrus Aurantifolia*) as a Natural Coagulant on the Physical and Chemical Characteristics of Green Bean Tofu (*Vigna Radiata*). *Journal of Food Technology and Agricultural Products*, 16(1), 18-34.
16. Sa'adah, U. (2013). Acceptability and Composition of Proximate Catfish Bone Powder Undergoing Soaking Process in Lime Solution (Doctoral dissertation, University of Muhammadiyah Surakarta).
17. Suryono, S., Syarifuddin, H., Maranata, J., Musyid, M. and Nizori, A., 2023. Development of high-calcium chicken nuggets fortified with various citric acid-extracted chicken eggshells powder. *Journal of Applied Agricultural Science and Technology*, 7(1): 36-44.
18. Liana, G. L., D. A. Wati., A. R. Pratiwi and L. A. Lestari. 2023. Effect of Lime Juice Soaking Duration on Proximate Levels of Toaya Jackfruit Seed Flour. *Journal of Health*, 16(1) : 104-114.
19. Adwyah, R., S. K. Khotiffah., Wahyudinur and F. Puspitasari. 2020. Effect of Cooking Time on Protein, Fat, Asama Amino Profiles, and Fatty Acids of Swamp Fish Powder (*Trichogaster trichopterus*). *Jurnal Pengolahan Hasil Perikanan Indonesia (Indonesia Journal of Aquatic Product Technology)*, 23(2) : 286 – 294.
20. Penniston, K.L., Nakada, S.Y., Holmes, R.P. and Assimos, D.G., 2008. Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products. *Journal of Endourology*, 22(3), pp.567-570.
21. Yahya, A.A., Ali, N., Samad, A.A.A., Kamal, N.L.M., Shahidan, S. and Abdullah, S.R., 2024. Mortar Containing Coal Bottom Ash (CBA) Treated with Citric Acid as Partial Cement Replacement. *International Journal of Integrated Engineering*, 16(9), pp.118-129.
22. Abano, E.E., Ma, H. and Qu, W. 2011. Effects of Pretreatments on The Drying Characteristics and Chemical Composition of Garlic Slices in A Convective Hot Air Dryer. *Journal of Agriculture and Food Technology*, 1(5): 50-58.
23. Wahyuni, D. T and S. B. Widjanarko. 2015. Effect of Solvent Type and Extraction Duration on Yellow Pumpkin Carotenoid Extract by Ultrasonic Wave Method. *Journal of Food and Agroindustry*, 3(2) : 390-401
24. Litaay, C and J. Santoso. 2013. The Effect of Different Soaking Methods and Soaking Duration on the Physico-Chemical Characteristics of Skipjack Fish Powder (*Katsuwonus pelamis*). *Journal of Tropical Marine Science and Technology*, 5(1) : 85-92.
25. Lekahena, V., D. N. Faridah., R. Syarief, and R. Peranginangin. 2014. Physicochemical Characterization of Nanocalcium from Tilapia Bone Extraction Using Base and Acid Solutions. *Journal of Food Technology and Industry*. 25(1) : 57-64.
26. Suryaningrum, T. D., Suryanti., R. N. Sari., E. Hastarini and D. A. Lestari. 2022. Effect of Soaking with Vinegar Acid and Sodium Bicarbonate, and Blansing Treatment on the Characteristics of Catfish Skin Chips (*Pangasius hypophthalmus*). *JPB Marine and Fisheries*, 17(1) : 63-76.

27. Jayani, N. I. E., Kartini and N. Basirah. 2017. Formulation of Hand Soap Preparation of Lime Extract (*Citrus aurantifolia*) and Its Effectiveness as an Antiseptic. *Media Pharmaceutica Indonesiana*, 1(4) : 222-229.
28. Mufidah, H and N. Hendrawati. 2022. Effect of Lime Peel Extract Concentration on the Making of Hand Sanitizer Gel. *Disstilat: Journal of Separasi Technology*, 8(4): 965-973.
29. Nurmallasari, N., and Z. Zaenab. 2015. Utilization of Lime Juice (*Citrus aurantifolia* swingle) in Reducing the Pb Heavy Metal Levels Contained in Shellfish Flesh. *HIGIENE: Journal of Environmental Health*, 1(3) : 168-174.
30. Handajani, H., S. D. Hastuti and Sujono. 2013. The Use of Various Organic Acids and Lactic Acid Bacteria on the Nutritional Value of Fish Waste. *A. Aquatic Sciences*. 2 (3): 126- 132.
31. Burhan, A. H., Y. P. Rini., E. Faramudika and R. Widiastuti. 2018. Determination of Peroxide Figures of Palm Oil Bulk Cooking Oil in Repeated Dredging of Catfish. *Journal of Science Education*, 6(2) : 48-53.
32. Widyasanti, D and A. Hanif. 2022. Identification of Oleoresin Components of Kuweni Mango Peel Obtained from Microwave-Assisted Extraction by Gas Chromatography-Mass Spectrometry (GC-MS) Method. *Journal of Tropical Agriculture and Biosystems Engineering*, 10(2) : 166-123.
33. Saragih, S. W. 2015. Effect of Base Concentration on Ester Hydrolysis Products. *BioLink*, 1(2) : 81-88.
34. Prinaldi, W. V., P. SupFPLS-15h and Uju. 2018. Characteristics of Physicochemical Properties of NanoCalcium Bone Extract of Yellowfin Tuna Fish (*Thunnus albacares*). *Journal of Indonesian Fishery Product Processing*, 21(3): 385-395.
35. Mujamilah, M and G. T. Sulungbudi. 2013. Dynamic Characteristics of Magnetic Colloidal Systems Based on Fe-Chitosan Oxide Nanoparticles. *Journal of Chemistry and Packaging*, 35(1), 65-70.
36. Admi., E. Widi and Syukri. 2015. Synthesis and Characterization of Mobilized Cu(II) Catalysts in Modified Silica. *Journal of Chemistry Unand*, 4(1) : 116-122.

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