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Characteristic of Milkfish (*Chanos – Chanos* Forks) Bone Nanocalcium by Acids and Alkaline Extraction

Muhammad Fitri[1,2], Mursalim[3], Amran Laga [3], and Zainal [3]

*[1]Post-Graduate Student, Hasanuddin University, Makassar, South Sulawesi, Indonesia
[2]Department of Fishery Product Processing Technology, Pangkep State Polytechnic of Agricultural, Pangkep, South Sulawesi, Indonesia
[3]Department of Agricultural Engineering and Food Technology, Hasanuddin University Makassar, South Sulawesi, Indonesia

ABSTRACT

Milkfish is a fishery commodity in Pangkep regency of South Sulawesi Province. Milkfish production in 2011 was 585,242 tons Directorate General Ocean and Fishery (2012). Utilization of milkfish bone waste as a source of calcium is an alternative in providing a rich source of calcium as well as reducing the negative impact of environmental pollution. Calcium derived from animals such as fish bone waste so far has not been widely used for human needs. The purpose of this research is to study the quality of milkfish bone nano calcium with the acid and alkaline extraction method. The treatment in this study are alkaline solution (A) A_1 = 2 percentage, A_2 = 3 percentage and A_3 = 4 percentage, acid solution (B) B_1 = 2 percentage, B_2 = 3 percentage and B_3 = 4 percentage. The design used is complete randomized design with two factors. Each treatment was repeated three times. The data analyzed by variance analysis using SPSS V. 19 software. Research result of milkfish bone nano calcium By using alkaline and acid extraction the best milkfish bone nano calcium is alkaline extraction with 40 percent NaOH cooking at temperature 100°C for 60 minutes where the water content is 5.21 percentage. The ash content is 52.87 percentage, the calcium content is 46.13 percentage, the protein content is 2.04 percentage and the color sensory test is 3.9 (likes) and the aroma is 4.6 (very like).

Keywords : Extraction, Milkfish, Nano Calcium

I. INTRODUCTION

The production of milkfish has increased from 2007-2011 by 263,139 tons (2007), 277,471 tons (2008), 328,288 tons (2009), 421,757 (2010) and 585,242 tons (2011), with an increase of 38.76 percentage 2010-2011 Ocean and Fishery Ministery (2011). Milkfish is a fishery commodity of Pangkep regency in South Sulawesi Province. Increasing the value of milkfish production increased from year to year with a fairly sharp value. With the increase of milkfish consumption, there is also an increase in bone waste volume of milkfish who not much benefit . Utilization of milkfish bone waste as a source of calcium is an alternative in order to provide calcium rich food source as well as reduce the negative impact of environmental pollution (Hafiludin, 2015). Calcium derived from animals such as fish bone waste so far has not been widely used for human needs. Though fish bones contain tri calcium phosphate which is ideal for the human body (Basmal J, *et all* ,2000). Calcium requirement is 500 mg day $^{-1}$ for ages 1-9 years,700 mg day^{-1} for ages 10-15 years, 600 mg day^{-1} for ages 16-19 years and 500-800 mg day

$^{-1}$ for adults (age 20 to over 60 years) (Almatsier, 2004).

Bone is one form of waste generated from the fish processing industry that has the highest content of calcium in the fish body. From a food and nutritional standpoint, fish bones are rich calcium that needed human , because the main elements of fish bones are calcium, phosphorus and carbonate. Thus, fish bone waste has great potential to be utilized as a raw material of fish bone rich in calcium. Fish bones contain a lot of mineral salts such as calcium phosphate and creatine phosphate which has the potential to increase the nutrients of food products (Maulida, N. 2005).

The aim of this research is to study the quality of nanocalcium from bone of milkfish (*Chanos – Chanos* Forks), By using the alkaline and acid solution.

II. METHODS AND MATERIAL

A. Research Site

It was carried out at the Chemical Laboratory of State Agricultural Polytechnic of Pangkep and Plant Assessment Center of Makassar

B. Materials and Tools

Material

In this study the materials used were bone of milkfish, HCl, NaOH, pH paper and chemicals NaOH 0.1N, $MgSO_4$, HCl 0.01N, H_2BO_3, $Na_2S_2O_3$, HgO, Neutral Alcohol, PP Indicators, distilled water and some Zink, etc.

Tools

The tools used in the processing procedure are stoves, knives, blenders, pans, basins, grinders, digital scales, packaging. While the tools for chemical analysis are Erlenmeyer, Kjeldhal flask, test tube, condenser, oven, boiling rock, distillation flask, exicator, buret and others as needed in research

C. Sample Preparation

This research will be conducted for two years. The first year is to analyze the quality of milkfish bone nano calcium by using alkaline solution by treatment A_1 = 2 percentage, A_2 = 3 percentage and A_3 = 4 percentage and to analyze the quality of milkfish bone nano calcium by using acid solution by treatment.B_1 = 2 percentage, B_2 = 3 percentage and B_3 = 4 percentage.

The research design used was complete randomized design , With two factors. Each treatment was repeated three times. The observation data analyzed by variance analysis using SPSS V. 19 software. If the results of the variance analysis show a significant or very significant effect, then the middle value tested by using Honestly Significant Difference test. Parameters under observation is water, ash, protein, calcium and sensory test (color, aroma, and texture).

The tests conducted to determine the nano calcium quality, which are physicochemical characterization of each. Chemical composition test including water content with the oven method (AOAC,2000), ash content (AOAC,2000), protein content by Kjeldahl method (AOAC,2000), Calcium content (Sudarmadji1984)

Processing Procedures

Raw materials (milkfish bone powder), to produce raw materials of bones ready for extracted milkfish have gone through several stages of the process such as washing, boiling (80 ^{o}C, 30 minute), Washing, Autoclaving (121 ^{o}C, 1 atm, 1 hour), and reducing the size using a hammer mill ($\pm$5 cm). The second stage is

Boiling (100 °C, 30 minute, 1 frequency) the extraction of milkfish bones by using two different solvents, alkaline (NaOH) and acid (HCl) solution separately,Washing, drying and milling which aims to soften the fish bones so as to facilitate the process of flouring (Nabil, M. 2005).

Extraction with alkaline solution (NaOH)

Treatment A is 100 percentage rough powdered bone of milkfish was extracted with a 1 N NaOH (Merck) solution with A_1 = 2 percentage, A_2 = 3 percentage and A_3 = 4 percentage at 100°C for 60 minutes. This extraction process repeated 3 times. The extraction results are then cooled, filtered, neutralized to neutral pH and dried by oven at 50°C to a water content of <8 percentage.

Extraction with acid solution (HCl)

Treatment B is 100 percentage rough powdered milkfish bone is hydrolyzed using HCl (Merck) 1 N with B_1 = 2 percentage, B_2 = 3 percentage and B_3 = 4 percentage ,Then extracted at 100 ° C for 60 minutes. The extraction process was repeated 3 times and the extraction result was then cooled, filtered and neutralized using distilled water up to a neutral pH sample, then dried in oven at 50 ° C until it reaches the water content of <8 percentage. extracted Milkfish bone powder that extracted using NaOH and HCl then made into flour using a disc mill then sieved using 100 mesh size. Sterilization process carried out at 121 ° C for 15 minutes to maintain the quality and extend the shelf life of the resulting bone powder

Test Parameters

Water content of oven method (AOAC,2000)

Determination of water content based on the difference in weight for example before and after dried. At first the empty cup is dried in the oven for 30 minutes at 105 ° C. then cooled in the eksikator for 15 minutes, Then weighed. 3-5 gram of sample inserted into the cup then dried in 105°C oven for 6 hours. Cup cooled in eksikator for 30 minutes, then weighed. The water content determined by the formula

$$\text{Water Content (\%)} = \frac{B-C}{B-A}\ x\ 100\%$$

Ash content of gravimetric method (AOAC,2000)

3-5 grams of sample weighed and put into the cup, then burned in the Bunsen until no smoke. After it inserted in a furnace, burned to gray ash. Ash carried out in two stages, first at a temperature of 400°C and then a temperature of 550°C. After the weight of the cup is constant, the cup then cooled in a desiccator and weighed. Ash content determined by the formula:

$$\text{Ash Content (\%)} = \frac{A}{B}\ x\ 100\%$$

Calcium Content (Williard's way) in Sudarmadji1984

The residual solution obtained shall not contain calcium greater than 0.5 g, if more then dilute to the specified volume, then take as much as 70 ml for the determined K content contained in 70 ml of Solution, add 5 ml of per chlor acid solution ($HClO_4$) 20 % (Specific gravity: 1.12) steam above the water bath slowly.

Add 10 ml of hot distilled water and 5 ml (HClO4) and 20% more above the water bath until a thick dense acid vapor arises. Cool to below room temperature, then add the washing alcohol solution. Wash with 3 x 10 ml washing alcohol solution, dried in oven temperature 130°C for 1 hour, then weighed. The weighted residue is $KClO_4$

Weight (g) = 0,2821 x weight $KClO_4$ (g) .....(3)

Protein Content (AOAC,2000)

Take 10 ml of protein solution and diluted to 100 ml with the distilled water in the flask, the solution is then put into a 500 ml Kjeldahl flask and 10 ml of H_2SO_4 (93% - 98% free N) add 5 grams of a mixture of H_2BO_3, Na_2SO_4-HgO for catalyst. Boil until clear and continued for another 30 minutes. After a cold washed in a Kjeldahl flask with distilled water then boiled again for 30 minutes.

Once cool add 140 ml of distilled water, and added 35 ml NaOH-Na2S2O3 and a few grains of zinc. Then it was distilled, 100 ml of distillate accommodated in an erlenmeyer containing 25 ml of boric acid saturated solution and a few drops of PP indicator. Solution obtained with 0.02 N HCl. The total N or percent (%) of protein in the sample is calculated by the following equation

$$\text{N Total} = \frac{\text{ml HCl x N HCl}}{\text{ml sample solution}} \quad (4)$$

Sensory Test (Soekarto S.T. 1985)

Sensory tests include color, aroma and protein hydrolysate by "Consumer preference test" method in which the material is presented randomly after coded on a hedonic scale. The panelist is selected as much as possible, at least 15 persons to provide an assessment based on the preference degree to the sample presented and declare the judgment in the list. Panelist ratings range from Very like (5), likes (4), regular / Neutral (3), rather dislike (2), dislikes (1). Each panelist has a list of work procedures to be filled in accordance with the statement and panelist preferences.

D. Data Analysis

The research design used was complete randomized design , With two factors. Each treatment was repeated three times. The observation data analyzed by variance analysis using SPSS V. 19 software. If the results of the variance analysis show a significant or very significant effect, then the middle value tested by using Honestly Significant Difference test. Parameters under observation is water, ash, protein, calcium and sensory test (color, aroma, and texture).

III. RESULTS AND DISCUSSION

a. Water Content Analysis Result of Milkfish Bone Nano Calcium

Water content of food ingredients also determine the acceptability, freshness, and durability of food. The water content of food affects the durability of the food against microbial attack expressed by which is the amount of free water that can be used by microorganisms for growth (Sokartono.S.T., 1985).

Water is a major component in foodstuffs that greatly influence texture, appearance, and taste in food. The durability of processed materials also strongly related to the water content because it greatly affects the proliferation of microorganisms in processed products (Wardani.*et all* , 2012). The results of the water content of milkfish bone nano calcium with the treatment of alkaline and acid extraction ranged from 5.21 to 5.47 percentage with the average moisture content of fish bone nano calcium showed 5.34 percentage. .The highest water content obtained in the treatment of acid extraction method with 2 percentage HCl cooking with 100^{o}C temperature for 60 minutes with value 5.47 percentage and the lowest water content obtained at alkaline extraction treatment with NaOH 1 N 4 percentage with temperature 100^{o}C for 60 minutes with value 5.21 percentage.

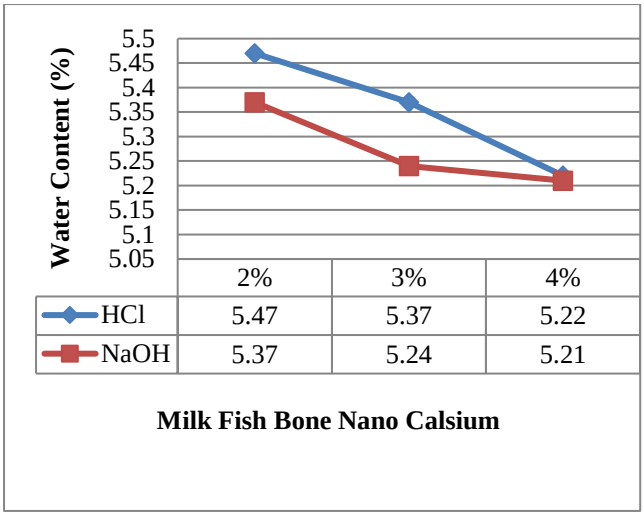

Figure 1. Water Content Analysis Result of Milkfish Bone Nano Calcium by Acid Alkaline Method

Figure 1 shows that the moisture content of milkfish bone nano calcium from all treatments still meets the water content standard specified by SNI 01-3158-1992 (DSN, 1992) that maximum 8 percentage, One of the methods that affect the water content in the material is drying process that drying with long sunlight because the temperature, humidity, and airflow rate can not be regulated in (Nabil, M. 2005)

Variance analysis result (Anova) on water content of milkfish bone nano calcium showed a significant difference between treatment P<0,05 Variance analysis result of water content of milkfish bone nano calcium With treatment of acid-alkaline extraction significant value less than 5 percentage, Then H_1 is accepted and H_0 is rejected. This means there is a difference between the treatments tested.

b. Ash Content Analysis Result of Milkfish Bone Nano Calcium

Ash is one component in food ingredients. This component consists of minerals such as potassium, phosphorus, sodium, magnesium, calcium, iron, manganese, and copper (Wardani *et all*, 2012). The results of ash content of milkfish bone nano calcium with acid-alkaline extract treatment ranged from 48.94 to 55.56 percentage with the average value of 52.25 percentage.

Figure 2 shows ash content of all treatment increases. According to (Elfauziah R.2003) , the main part in bone powder is the ash content that found 75 percentage. The highest ash content obtained on the treatment of alkaline extraction method by cooking NaOH 4 percentage at temperature 100°C for 60 minutes with value 55,56 percentage and the lowest ash content obtained at treatment of acid extraction method with 2 percentage HCl cooking at temperature 100°C for 60 minute with a value of 48.94 percentage. The ash content of milkfish bone nano calcium in this research higher than the bone powder standard based on ISA standards (International Seafood of Alaska) 33.1 percentage But lower than the results of research conducted (Mulia. 2004) 63,5 percentage and (Elfauziah R.2003) 79,14 percentage.

The variance analysis result of ash content with treatment of acid-alkaline extraction method is significantly less than 5 percentage, H_1 accepted and H_0 rejected. This means that there is a difference between the treatments tested. The high ash content obtained from milkfish bone contains calcium elements that needed by the body. This is caused more and more high concentration alkaline before through the saturated point more and more high result the ash content, and kinds and much mineral be contained in the milkfish bone . The main ingredients of fish bone are calcium, sodium, strontium, chloride, hydroxide, and sulfate. Increased ash content influenced by the amount of burnt minerals into volatile substances. The highest ash content of a foodstuff means the highest mineral the food contains. While result research (Anggraeni N.,

et all ,2016) show the ash content nano calcium who produced 78.82 percentage and result research [11] (Lekahena V., *et all*. 2014.) is 85.44 percentage where both higher compared nanocalcium this research

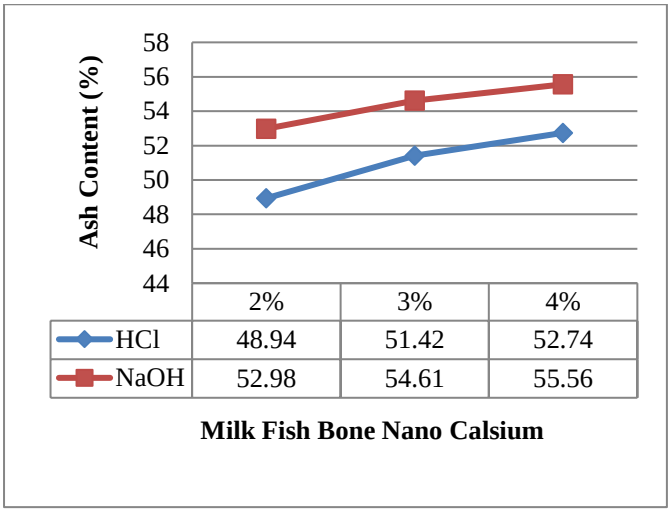

Figure 2. Ash Content Analysis Result of Milkfish Bone Nano Calcium by Acid Alkaline Method

This is caused content mineral of milkfish is low caused brackish fish . content ash on foodstuff influenced amount mineral who are in fish of the example mineral is calcium opinion [8] (Ferazumma H., *et all*, 2011) .These many are influence content of mineral ,content ash influenced time duration autoclaving and frequency boiling the significant influence of ash content, protein and fat bone flour. . bone before processing content cell alive and intracellular matrics in form the salt mineral. Ash content who high in the bone flour caused by main component manage bone is mineral and process it's making had occur hydrolysis protein who almost total that produced protein the lowest.

c. Calcium Content Analysis Result of Milkfish Bone Nano Calcium

Calcium derived from animals such as fish bone waste so far has not been widely used for human needs. However, fish bones contain tri calcium phosphate, which is ideal for the human body (AOAC. 2000.)

Fish bone is one form of waste generated from the fish processing industry that has the highest content of calcium in the fish body. From a food and nutritional standpoint, fish bones are rich in calcium that humans need, because the main elements of fish bones are calcium, phosphorus and carbonate. Fish bones contain many mineral salts such as calcium phosphate and creatine-phosphate, which has the potential to increase the nutrients of food products (Maulida, N. 2005)

The calcium content analysis result of with acid-alkaline extract treatment ranged from 42.94 to 49.89 percentage with the average 46.13 percentage .The highest calcium content obtained on the treatment of alkaline extraction method with 4 percentage NaOH cooking at 100°C temperature for 60 minutes with value 49.63 percentage and the lowest calcium content obtained in the treatment of acid extraction method with 2 percentage HCl cooking at 100°C temperature for 60 Minute with value 43.06 percentage.

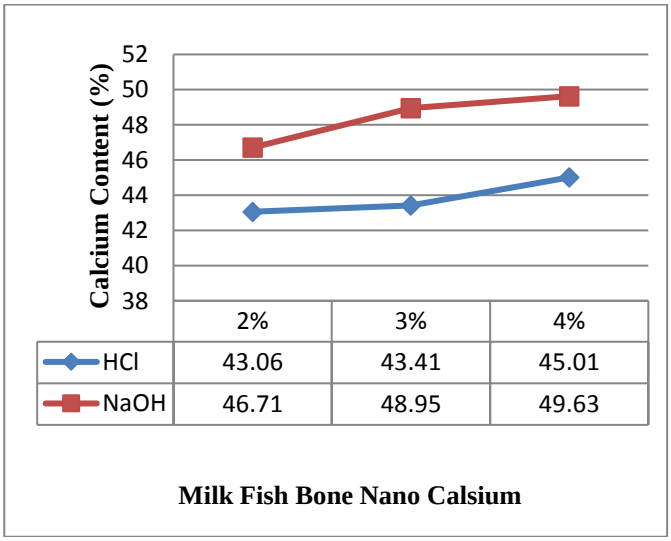

Figure 3. Calcium Content Analysis Result of Milkfish Bone Nano Calcium by Acid Alkaline Method

Figure 3 shows the calcium content of milkfish bone nano calcium from all treatments increase. According to calcium consumption should not exceed 2500 mg day $^{-1}$ (Almatsier. 2004).The results also showed that the higher the concentration of HCl and NaOH used, the higher the calcium content in the fish bone powder. Calcium is one of the most mineral in bone attached to collagen protein (Wardani, *et all*, 2012). In base condition, calcium in bone along with phosphorus forms calcium phosphate. Calcium phosphate is a mineral crystal that has insoluble properties in alkaline pH (Almatsier. 2004). Insoluble calcium during boiling will be left behind and settle in the matrices of the shell so as to increase the proportion of calcium in the Milkfish bone material.

The variance analysis result of calcium content with treatment of acid base extraction method significantly less than 5 percentage , then the H_1 accepted and H_0 rejected. This means that there are differences between treatments tested. This is presumably the higher the temperature the higher the calcium content. The best calcium content is by the NaOH method made possible by the high temperature extraction used in the NaOH solution, which will allow the amount of calcium to settle in bone matrices, so that the calcium powder of fish bones will increase. This is reinforced by the statement of Harrow and Mazur,1961 in (Nabil, M. 2005) . States that extraction with an alkaline solution at high temperatures causes a denatured protein. The protein denatured at alkaline pH then the molecule is present as a soluble protein (Nabil, M. 2005)

This is suspected in acid-alkaline conditions during boiling with high temperatures to increase solubility of protein in bone so that insoluble calcium under alkaline conditions will be left behind and settle in bone matrices so as to increase the proportion of calcium in the material (fish bone powder). According to Karmas (1982), the effectiveness of the alkaline solution depends on the concentration of the alkaline solution, the temperature used, duration extraction .

d. Protein Content Analysis Result of Milkfish Bone Nano Calcium

The results of protein content analysis with acid-alkaline extract treatment ranged from 0.67 to 3.90 percentage with an average value of 2.04 percentage. The highest protein content was obtained in the treatment of acid extraction method with 20 percentage HCl cooking at 100 ° C for 60 minutes with a value of 3.56 percentage and the lowest protein content was obtained in the treatment of alkaline extraction methods by cooking NaOH 4 percentage at 100 ° C for 60 minutes with Value of 0.80 percentage.

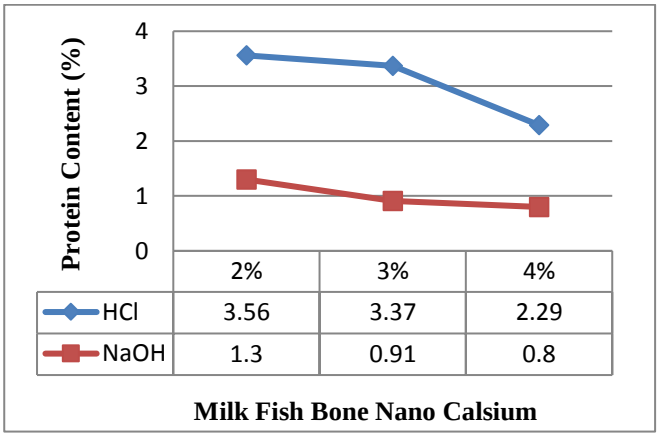

Figure 4. Protein Content Analysis Result of Milkfish Bone Nano Calcium by Acid Alkaline Method

Figure 4 shows that the protein content of fishbone nano calcium protein from all treatments decreased, both treatment of acid and alkaline extraction methods. According to (Nabil. M, 2005) states that in making fish bone powder, protein content is removed as much as possible by the process of protein

hydrolysis using acid and alkaline solutions. This protein removal intended to increase the mineral / ash content contained in the flour. This is presumably because there is still a protein content of bone powder caused by the lack of perfect deproteinize process in making of milkfish bone nano calcium . While result research (Anggraeni N., *et all* , 2016) with alkaline method is 0,21 percentage lower from this research .

This could proved at treatment autoclaving for 1 hour . Calcium content of Tuna fish bone flour more and more increasing along with decreasing protein content This could proved at treatment autoclaving for 1 hour . protein content of milkfish bone flour with hydrolysis the alkaline (NaOH)) lower because NaOH can hydrolysis protein in fish bone. For increasing content calcium at the "Tuna" fish bone flour took separation protein with calcium through deproteinase (Wardani, *et all*, 2012). Calcium content of Tuna fish bone flour more and more increasing along with decreasing protein content

Variance analysis result of protein content with treatment of acid-alkaline extraction method significant value less than 5 percentage H_1 accepted and H_0 rejected. This means there is a difference between the treatments tested.

e. Sensory Test Analysis (Color) of Milkfish Bone Nano Calcium

The average analysis of Sensory Test (Color) of milkfish bone nano calcium shows panelist value 3,9 (like). The highest level of Sensory Test (Color) obtained on the treatment of base extraction method with 4 percentage NaOH cooking at 100 ° C for 60 minutes. The panelist gave the likes of 4.5 and the lowest Sensory (Color) test obtained on the treatment of acid extraction methods HCl 2 percentage with temperature 100°C for 60 minutes panelists give the normal value / neutral value is 3.3. Sensory Test Result (Color) The resulting milkfish bone nano calcium is the preferred criterion. This is thought to be due to a Millard reaction that can cause brown color and high mineral content in milkfish bone nano calcium. The high content of minerals in a foodstuff will affect the color of the resulting product

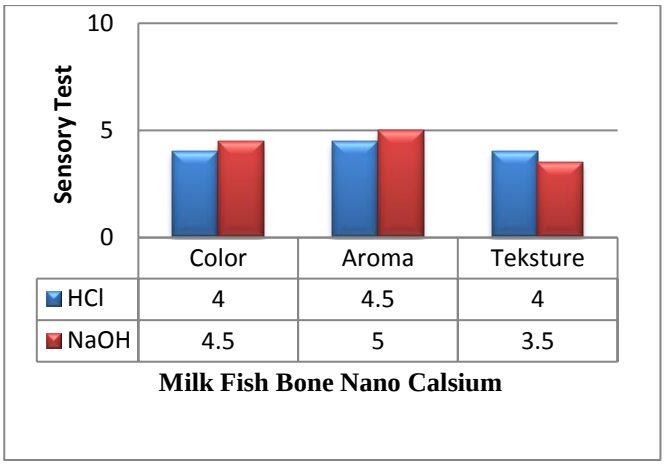

Figure 5. Sensory Test Analysis of Milkfish Bone Nano Calcium

Figure 5 shows that the Sensory Test (Color) of the milkfish bone nano calcium from all treatment levels of consumer acceptance increases. The colors received by the panelists are at a value of 3 (normal) to 4.5 (very like). Differences in the color of the flour produced is also caused by the treatment of heating during the drying process.

The results also show that the higher concentration of NaOH used tends to increase the degree of white. The higher the concentration of NaOH used, the more fat and protein content lost so it will tend to increase the degree of white in fish bone. the use of alkaline is more advantageous than the acid (Nabil, M. 2005). The use of a base as a hydrolysis agent produces whiter soluble fraction and insoluble fraction. The weakness of acid use is the formation of blackish brownish or blackish substance called humin or melanin formed from the content of the

indole core tryptophan with aldehyde derived from carbohydrates contained in the material.

Result of average analysis of Sensory Test (Aroma) milkfish bone nano calcium show panelist value 4,6 (very like). The highest concentration of Sensory Test (Aroma) was obtained on the treatment of base method with 40 percentage NaOH cooking at 100°C for 60 minutes. The panelist gave a very like value of 5 and the lowest Sensory (Aroma) test was obtained on the treatment of acidic method with 20 percentage HCl cooking At a temperature of 100°C for 60 minutes the panelist gives a liking value of 4.2. This is because the resulting aroma is very distinctive., the fragrance-forming components in fishery products are proteins, polysaccharides, pigments and vitamins. added that the flavor of fishy odor is a distinctive odor of fish caused by nitrogen components other than fish protein

Figure 5 shows that the Sensory Test (Aroma) of milkfish bone nano calcium from all treatments increased. The aroma received by the panelist is on the value of 4 (Likes) to 5 (very like).

VI. CONCLUSIONS

The research result conclusions of milkfish bone nano calcium by using alkaline extraction and acid extraction are the following:

1. Base extraction with NaOH has a significant effect on water content, ash content, calcium levels, protein content
2. The best milkfish bone powder nano calcium is alkaline extraction with 4 percentage NaOH cooking at 100°C for 60 minutes where the water content is 5.21 percentage. The ash content is 52.87 percentage, the calcium content is 46.13 percentage, the protein content is 2.04 percentage and the color sensory test is 3.9 (Likes), aroma 4,6 (very like) and texture3,6 (likes).

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