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Extraction and Purification of Yellowfin Tuna *Fishbone* Flour as an Ingredient of Future Traditional Medicine

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ABSTRACT*: Yellowfintunafishbone flour contains high macro and micro minerals from its bone which can be used as an alternative ingredients to supply minerals for those who are allergic to dairy products. High quality extraction of fishbone will produce high quality medicine ingredients in the future. Medicine refers to any ingredients that can be used to influence and modify the physiological and pathological state to determine the diagnose, prevention, medicine, and health improvement of the patients in pharmaceutical field. The objective of this study is to perform extraction and purification procedures to fishbone flour as the future ingredient of natural medicine. This study employed an experiment design by trying out different solvents in boiling the fishbone to produce high quality fishbone flour. The result of this research shows that the best quality fishbone powder was obtained from the process that used ascorbic acid at the lowest level and acetate acid at (0.91-0.83 g/ml) and the highest absorbance ability found by adding NaOH and the lowest ability with acetate acid at (81,7;44.71 g/ml), while the highest whiteness level was also obtained from the use of NaOh and the lowest one was obtained by using acetate acid at (44;21.2 g/ml). The highest water content was obtained from the use of of HCL while the lowest was found using acetate acid at (10.80;5.61%). The highest ash content was produced from the use of NaOH and the lowest at (56.7;55.1 g/ml). Meanwhile,the highest fat level was obtained from the use of acetate acid and the lowest one from NaOH (56.7;55.1 g/ml). Lastly, the highest protein was obtained from the treatment using NaOH and the lowest one using acetate acid (23.1-20.4 g/ml).*

Keywords: *extraction, main ingredient, natural medicine, yellowfin tuna fishbone flour*

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I. INTRODUCTION

Fisheries potential in North Maluku is quite high, making the North Maluku Province chosen as the national fish barn in 2015. The outstanding production of tuna fish and skipjack fish has improved tuna fish industries to export their product. The production of loin tuna that tends to increase each time leaves fish litters such as fish head, fishbone and fish scales which become a huge environmental issue. It has been confirmed that tuna fishbone litters keeps increasing each year, giving negative impact to the environment[1]. Fishbone has 10% of proportion out of the total fish body and it contains high calcium. Fishbone contains various minerals especially calcium and phosphor[2] .

Lack of calcium and health problems related to the low ability to absorb calcium in the intestines have caused severe diseases such as rickets in children and osteomalacia in adults[3]. Around 98-99% calcium is filtrated which later is reabsorbed by the body[4] . 65% re-absorbance of calcium happens in proximal tubule, and the rest is wasted.

Calcium in the form of ion is needed in the physiological and biochemical processes such as the neuromuscular excitability, blood coagulation, and other secretive process, membrane integration, plasma membrane transportation, enzyme reaction, hormone and neurotransmitter release as well as intra-cell process of various hormone[5] .

Biological activities as mentioned above are able to occur normally when the calcium is at the ideal level. The level of ionic calcium is maintained by homeostatic mechanism[6] and around 1-5% of calcium change in the blood allows the homeostatic mechanism to return the calcium at the normal level[4.]

Osteoporosis is a disease related to the deficiency of bone solidity, causing decrease on the power of the bone and fractures as the impact of porous bone. Osteoporosis usually occurs in women after menopause as the result of bone metabolism degradation. Physiologically, women have their ovary function decreased which caused the less production of estrogen called menopause state, in which women loses minerals in their bones

rapidly (3% per year in the first 5 years, and 1%-2% in the following years). This rapid decrease caused the bone to lose its mass and its ability to absorb minerals, causing osteoporosis to happen[6].

For women after menopause, the decrease of estrogen production causes balance problem among bone destructive cells (Masyitha, 2006)[7] .The decreased bone mass is caused by absorbance ability which is stronger than the bone formation ability (Magetsari, 1999) [8.] Bone mineralization depends on genetic factors and nutrients, including endocrine, metabolic, and mechanical factors. Some research show that the tendency of osteoporosis starts from the nutrients fulfillment in younger ages, especially the fulfillment of calcium and vitamin D (Gonzlez et al. 2011)[9] .

It has been proven that fishbone contains high minerals. Unfortunately, fishbone has not yet been utilized as medicine ingredients in osteoporosis therapy. Fishbone has been empirically believed to increase the amount of minerals in the body, even people consume fishbone of small fish.

According to *BadanPengawasanObatdanMakanan*(BPOM) which refers to an institution that controls medicine and food production, traditional medicine refers to the products which are made using natural ingredients that contain various function. Therefore, production of traditional medicine should be maintained by improving the production process right from the supply of ingredients to ensure the quality of the medicine. *Cara PembuatanObatTradisional yang Baik (CPOTB)* or the procedure to produce good traditional medicine covers all aspects in the process of traditional medicine production to make sure that the products meet certain quality standards. Regarding to those facts, this research is intended to see how fishbone is used as an ingredient of traditional medicine to cure osteoporosis in the future.

Research Objective

The objective of this research is to perform extraction and purification procedures to produce yellowfintunafishbone flour as an ingredient of traditional medicine to prevent osteoporosis.

II. RESEARCH METHOD

This research was administered by analyzing the water content, ash content, fat level, protein and carbohydrate using the AOAC 1995, level of whiteness, and water absorption ability (Fardiaz*etal*, 1992)[10] , as well as whiteness level, bulk density and water absorption (Wirakartakusumah*etal*,1992). Meanwhile, for the analysis of the total mineral, ICP-MS was employed and the analysis on the vitamin D was done using the HPLC Hewlett Packard model 1084 B completed with automatic injectors done in the Angler BiochamLab Laboratory in Surabaya, East Java, Indonesia.

The boiling process of yellowfin tuna fishbone using various solvents

Yellowfin tuna fishbone was collected from the central market of fish filleting in Bastiong, Ternate City. The process of the production was started by cutting off the size of big fish from 60-85 cm to leave the diameter of around 10-15 cm. The next step is cleansing the bones from dirt such as blood and other dirt, followed by boiling the bones. The boiling process was done three times which lasted for 4 hours per step in 12 hours at 100°C. After the first boiling process, the bones were washed using water and then put into the boiler again. After that, the fishbone were boiled using certain solvent at certain proportion NaOH1:3*b/v*, HCL1:3*b/v*, CH3COOH1:3*b/v*, C6H8O61:3*b/v* *and* H2O 1:3*b/v* in 40 minutes. After the boiling process, the bones were washed three times which were then being autoclaved for 2 hours at 121°C and being put into an oven at 60°C for 8 hours. The next step was done by grinding the bones using the disc mill, and filtering it within 100 mesh size (modification of Thalib et al'smethod., 2009) [11] .

The analysis of physio-chemical characteristic of *madidigang*fishbone flour

An analysis was done to see the nutrients contained in yellowfintunafishbone flour including its physical and chemical characteristics. The physical analysis of yellowfintunafishbone flour included analysis on its whiteness level, water absorption ability and bulk density, while the chemical analysis included the analysis on its water content, ash content, fat level, protein, and total mineral as well as the vitamin D and crude fiber.

III. RESULTS AND DISCUSSIONS

The Physio-Chemical Analysis

Bulk density refers to the proportion of a material's mass and its volume, including the empty spaces among the food grain. Acidity during the boiling process is assumed to give no influence to the bulk denisity, in which the higher the acidity, the lower the bulk density and vice versa. The result of the bulk density analysis is illustrated in Figure 1.

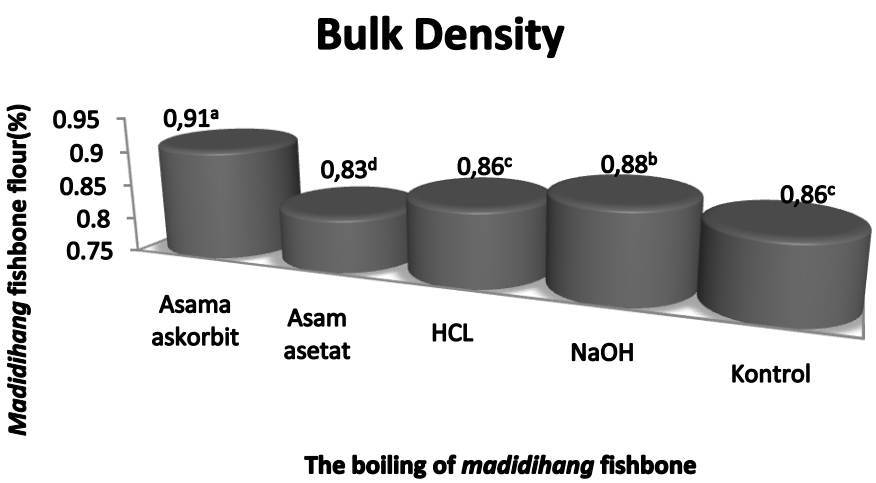

Figure 1. Bulk density of yellowfintunafishbone flour

The bulk density resulted from the boiling process of yellowfintunafishbone using ascorbic acid, acetate acid, HCL, NaOH and control showed obviously different levels. It is found in this research that the highest bulk density was obtained from the boiling using ascorbic acid at 0.91 g/ml, and the lowest one was obtained from the boiling using acetate acid at 0.83 g/ml, whilst the other treatments showed relatively low level including the control treatment. Compared to research[12] , the density was obtained from the control at 0.76 g/ml and the acetate acid at 0.74 g/ml, while the result shows that the boiling using acetate acid at 0.96; NaOH at 0.92 and 1.55 using lime juice[13].

The high bulk density is assumed to be affected by the use of different solvents such as acetate acid, HCL, NaOH which belong to strong weak acids and strong acids that are powerful enough in degrading more fat and protein compared to other substances.

The high bulk density in ascorbic acid is caused by the characteristic of this acid which has less ability in degrading fat, making the porosity of the fishbone flour higher. Whereas, in other treatment, the porosity of the fishbone flour is hampered which makes the bulk density lower. Thus, the result of this research shows statistically different value at ($p<0.05$). Furthermore, bulk density of yellowfin tuna fishbone flour has a significant influence toward its water absorption. The graph on water absorption is presented in Figure 2.

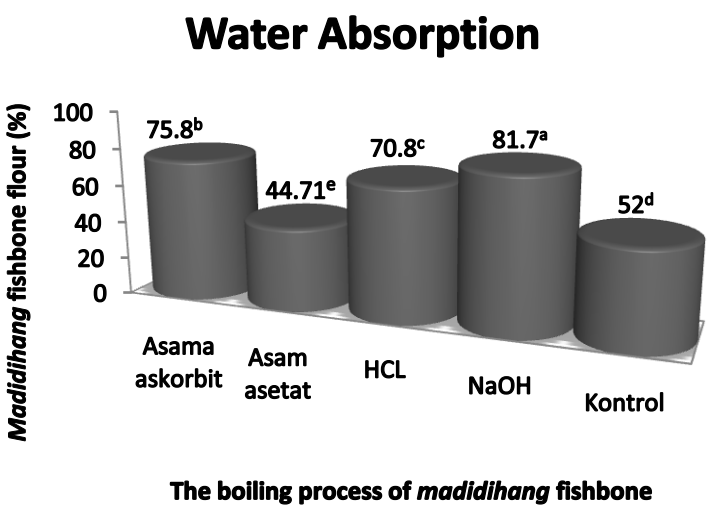

Figure 2. The water absorption of yellowfin tuna fishbone flour

Porosity of yellowfintunafishbone flour refers to parts of the flour which are not filled with any particle or solid materials[14] . Porosity is one of factors that influence the water absorption. Material porosity is shown by the bulk density value. The higher the porosity of a material, the lower the bulk density. The result of an analysis in the water absorption of yellowfintunafishbone flour from five treatments (ascorbic acid, acetate acid, HCL, NaOH, and control) shows that the higher value is obtained from the treatment using ascorbic acid at 75.8 g/ml

and the lowest one from the acetate acid treatment at 44.71 g/ml. Whereas, the treatments using water and acetate acid show value of 1.05 g/ml and 1.04 g/ml[15] . Compared to other research, the value of water absorption found in this research is relatively higher.

Other researchers stated that the higher the protein level of a certain substance, the higher its water absorption level[16] . Water absorption level also has a significant influence on the level of whiteness. The higher the level of whiteness, the higher the water absorption level. The whiteness level of yellowfintunafishbone flour is presented in Figure 3.

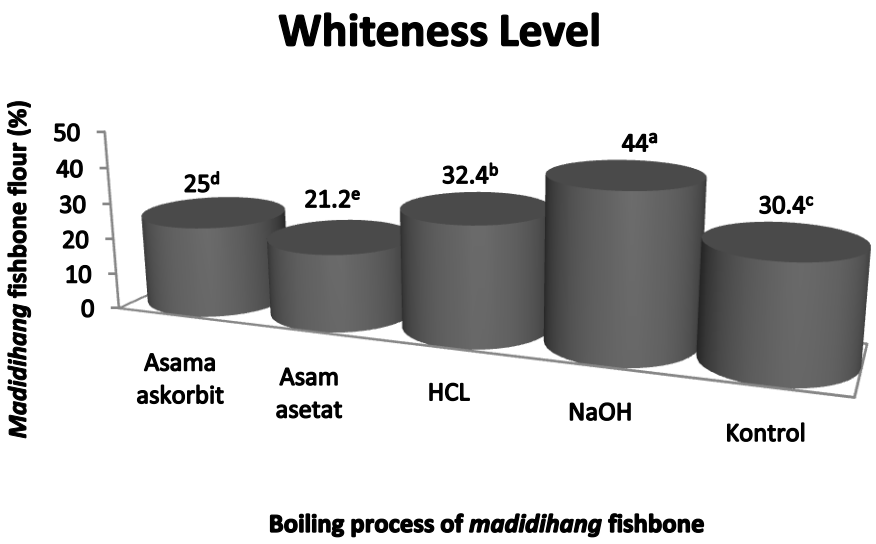

Figure 3. Whiteness level of yellowfintunafishbone flour

The result of this study shows that the highest whiteness level of yellowfintunafishbone flour was found in the treatment using NaOH at 44%, and the lowest one was obtained from the treatment using acetate acid at 21.2%.

The whiteness level of yellowfintunafishbone flour using water and acetate acid show value of 46.45 and 46.33[1] respectively. Compared to the whiteness level of wheat flour of around 80-90%, the whiteness level of yellowfintunafishbone flour is relatively low. It might be caused by the high protein and fat contents in the flour which cannot be degraded during the boiling process. This result goes in line with the theory proposed by some researchers in which it is stated that higher whiteness shows better flour quality[17.] However, some flour sold in the market contains high bleacher such as benzoyl peroxide[2] .

Chemical Analysis

An alisiskimiatepungtulangikanmadidihangdenganperlakuanperebusanasamcitrat, asamasetat, HCL, NaOHdankontroldisajikanpadaTabel.2.

Table 2. Chemical analysis on yellowfintunaflour

Perameter	PerlakuanPerebusan				
	Citric acid	**Acetate acid**	**HCL**	**NaOH**	**Control**
Water content	7.24 ± 0.55^{b}	5.61 ± 0.02^{e}	10.80 ± 0.01^{a}	6.83 ± 0.02^{c}	5.96 ± 0.02^{d}
Ash content	55.7 ± 0.01^{c}	55.1 ± 0.05^{d}	52.3 ± 0.0^{e}	56.7 ± 0.03^{a}	56.1 ± 0.09^{b}
Fat level	5.22 ± 0.34^{c}	8.73 ± 0.34^{a}	$6,24 \pm 0.34^{b}$	4.49 ± 0.00^{c}	6.73 ± 0.36^{b}
Protein	21.3 ± 0.20^{c}	20.4 ± 0.33^{d}	22.7 ± 0.20^{a}	23.1 ± 0.38^{a}	22.1 ± 0.19^{c}
Carbohydrate	10.53 ± 0.12^{a}	10.0 ± 0.64^{ab}	7.81 ± 0.65^{c}	8.80 ± 0.37^{bc}	9.02 ± 0.68^{bc}
Vitamin D	0.17 ± 0.12^{a}	0.12 ± 0.12^{b}	0.10 ± 0.12^{c}	0.11 ± 0.12^{c}	0.06 ± 0.12^{d}
Crude fiber	0.07 ± 0.12^{bc}	0.05 ± 0.12^{d}	0.08 ± 0.12^{b}	0.03 ± 0.12^{e}	0.12 ± 0.12^{a}

Note: Similar letters in the same row followed by different superscripts letter (a,b,c,d.e) show significant different result *($p<0,05$)*

The boiling process of yellowfintunafishbone using (citric acid, acetate acid, HCL, NaOH, and control) toward (water content, ash content, fat, protein and carbohydrate) shows that the lowest water level was obtained from the treatment using acetate acid (5.61%) and the highest from HCL treatment (10.80%).

Water content shows the wáter consisted in certain substance. Higher water content has certain influence on the preservation of the material since the water is usually absorbed within cells. Compared to the maximum standard water content of wheat flour around 14.5%, the water content of yellowfintunafishbone flour is considered appropirate with the national standard of Indonesia[18] .

The highest result of the analysis on the ash content was found in the treatment using NaOH (56.7%) and the lowest one was from the control treatment (55.1%). Ash content is one of the components in food that indicates the amount of minerals. This component consists of various minerals such as potassium, phosphor, sodium, magnesium, calcium, iron, manganese, and copper. (Winarno, 1997)[2] . Based on the result of the research done by (Talib*et al.,* 2017), the result of analysis on boiling treatment using water and boiling treatment using acetate acid and its ash content (56.65% and 58.21%) are not significantly different from the previous research that employed water, acetate acid and hydrochloric acid which obtained lowe ash content at 51.45, 44.95 and 45/21%[19] .

The different ash content found in this research might be caused by the different length of autoclaving process. In previous research, autoclaving processes were set for an hour, while in this study, the researcher set the autoclaving process for two hours. As an implication, the material lost some of its protein and fat during the autoclaving process, making the ash content of yellowfintunafishbone flour higher[11].

The highest fat content was obtained from the treatment using acetate acid (8.73%) and the lowest from the use of NaOH (4.49%). The amount of the fat has certain influences on other parameters. Following previous research on the making of yellowfintunafishbone flour, the water content can be seen from the boiling process using water and aceate acid at (6.3 and 6.31%)[13]. Results of previous research showed higher value which might be influenced by the use of different chemical solvents and less advanced method.

The boiling process was done for 12 hours, followed by the autoclave process and then finished by the boiling process using chemical substances that is believed to be the phase in which the material lost its fat content. The decreased fat amount is highly influential to the preservation of the product. Higher fat amount makes the product become easily pungent as the impact of fat oxidation[20] .

The highest amount of protein was obtained from the treatment using NaOH (23.1%) and the lowest one from the treatment using acetate acid (20.4%). Previous research on the same treatment showed that the protein amount obtained from the boiling using water was 20.98% and 17.21% using acetate acid (Talib*et al.,* 2014)[1]. Compared to the standard of Indonesia, the standard protein content of wheat flour is at 7.0. Thus, yellowfintunafishbone flour produced in this study has not yet met the national standard of Indonesia.

It is found in this study that the highest carbohydrate was obtained from the boiling process using citric acid (10.53%) and the lowest amount was obtained from the use of HCL (7.81%). This result is similar to a research conducted on catfish fishbone flour which showed value of 14.40%. Generally, carbohydrate refers to certain organic compound which contains carbon atoms, hydrogen atoms and oxygen atoms[21].

Vitamin D and Crude Fiber

From the result of the boiling treatment using different solvents (citric acid, acetate acid, HCL, NaOH, and control) to see the vitamin D content in the material show that the highest and the lowest content were found in the treatment using citric acid and control treatment respectively (0.17-0.06.mg/kg). Similar research was conducted by (Talib*et al.,* 2016) which result showed that the vitamin D content of *skipjack tuna*fishbone flour obtained from boiling process using NaOH was 4/6 mg/g bk[13] .

Vitamin D can be produced at ideal amount in the body by the help of ultra-violet rays from the sunshine that is received by the skin. The vitamin D can be synthesized by the body and it is transported by the blood circulation to the whole part of the body. Seen from the phylogeny point of view, vitamin D is older than parathyroid glands. For instance, parathyroid glands are not found in fish, while vitamin D is found at a high amount in the species to control the metabolism of calcium. Lower order animals which have less complex body organs are able to control the calcium exchange between the environment and their internal milieu using vitamin D which is stored in the liver (PilangWiranda, 1990)[22] .

Vitamin D obtained from plants is called calypherol or vitamin D2. Meanwhile, the vitamin D from the sunshine is called ergosterol which is usually found in the liver of cod fish and human's skin that is produced by the pro vitamin D3 or 7 –dehydrocholesterol. The term calciferol refers to two types of sterol; cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2).

Measurement on the crude fiber as one of the parameter shows that the highest value was obtained from the control treatment (0.12%) and the lowest one from the treatment using NaOH (0.03%). Previous research done by (Widyasari*et al.,* 2013) on the analysis of the fishbone flour of sidat fish showed a percentage of (1,11%), whilst on the fish's head bone was (1.33%). Those different results might be caused by the different

types of the bones. Yellowfintunafish has bigger size, while eel has smaller size with softer fiber. In addition, crude fiber is an organic material which comes from a part of carbohydrate that is less soluble into the water.

The fulfillment of vitamin D and calcium rather depend on the fulfillment of other nutrients. The interdependence of vitamin D and calcium has been confirmed in adults. In a cross-sectional study conducted to adults, it is estimated that the increase of calcium necessity is accociated with the effect of PTH serum to the deficiency of vitamin D. However, the same pattern is not found in children.

Bones are formed by minerals such as calcium and phosphate to make them strong and solid. To maintain the solidity of the bone, enough supply of calcium and other minerals should be fulfilled in order to enable the body to produce enough amount of various hormones (parathyroid hormone, growth hormone, calcitonin, estrogen in women and testosterone in men). In addition, adequate supply of vitamin D is necessary for the body to absorb calcium from food to be transported to the bones. Generally, bones progressively improve their solidity until they reach the maximum solidity (around the age of 30). After that, the bones gradually lose the solidity. Hence, if the body is unable to control the supply of minerals in the bones, the bones will become weaker and will be more vulnerable to osteoporosis (Heike, 2007)[23.]

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IV. CONCLUSIONS

The result of this study shows the best extraction of fishbone flour is obtained from the treatment using ascorbic acid, and the worst one is produced from the use of acetate acid in the treatment (0.91-0.83 g/ml). Meanwhile, the highest water absorption is obtained from the use of NaOH and the lowest one is obtained from the use of acetate acid (81.7; 44.71 g/ml). Furthermore, the highest whiteness level can be obtained from the use of NaOH, while the lowest whiteness level is produced by the treatment using acetate acid (44;21.2 g/ml). The highest water content is obtained from the use of HCL, while the lowest one is obtained from the use of acetate acid (10.80;5.61%). The highest ash content is produced from the treatment using NaOH and the lowest ash content is found in the treatment using acetate (56.7;55.1 g/ml). The highest fat level is produced from the use of acetate acid in the treatment, while the lowest fat level can be obtained from the use of NaOH(8.73;4.49 g/ml). Similarly, the amount of protein is found at the highest level from the treatment using NaOH and the lowest from the use of acetate acid in the treatment (23.1-20.4 g/ml). Based on the empirical data in this research, it can be concluded that yellowfintunafishbone flour contains high amout of nutrients which can be used as an ingredient of medicine in the future.

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