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Physico-chemical properties of *Madidihang* (*Thunnus albacares* Bonnaterre) fish bone flour in Ternate, North Moluccas

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Abstract

Fish bone flour of *Madidihang* - yellowfin tuna (*Thunnus albacares* Bonnaterre) contains minerals, especially high calcium and phosphorus, and properly used in food products, as feed or supplement. This study aimed to determine the physico-chemical characteristics of *Madidihang* fish bone flour processed with water and acetic acid. The method used in the study is an experimental method by boiling the bones of *Madidihang* using water and acetic acid. Observed physic parameters are rate of whiteness, absorbency and camba density while chemical parameters are content of moisture, ash, fat, protein, levels and solubility of calcium and phosphor. The results show that Ca and P parameters were indifferences but solubility of Ca and P are very influential.

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Introduction

Calcium and phosphor needs will be fufilled when consuming foods with a balanced daily diet and supplemented with vitamin D (Diane *et al.,* 2003). The best recommended calcium and phosphor sources is milk, but milk prices still comparatively expensive for some communities. Therefore it is necessary to find less expensive alternative sources of calcium, easily available and absorbed naturally by the body (Trilaksani *et al.,* 2006). Calcium and phosphor obtained from various sources, but not all sources have the same solubility characteristics. Solubility will affect the bioavailability of minerals in the body. Terms of *bioavailable* nutrition is in the form of dissolved (soluble) mineral (Clydesdale, 1988; Santoso *et al.,* 2006). Minerals in the body will be *bioavailable* if the minerals is dissolved, but not all dissolved minerals are *bioavailable*. Conditions of dissolved minerals needed to facilitate the absorption of minerals in the body (O'Dell, 1984). Fish bone mineral will be absorbed by the body if the fish bone flour has a high degree of solubility and resulting a bigger absorption of calcium in the body. Therefore fortification into another comestibles is necessary, especially the amino acids lysine, arginine and lactose with a balanced high intake of vitamin D (Weaver, 2006).

High-fat food products may interfere with the metabolic processes in the body because the foods that contain calcium and phosphor can be tied so that the metabolism process is not running perfectly and can lead to osteoporosis. Osteoporosis is a systemic skeletal disease characterized by low bone density and microarchitectural deterioration of bone tissue resulting brittle bones (WHO, 2003). Osteoporosis is a bone disease that has typical attributes of low bone mass, accompanied by bone micro-architecture and quality deterioration of bone tissue that can lead to bone fragility (Dawson and Hughes, 1996). Predispose to osteoporosis begins right of childhood and adolescence period. Therefore, prevention of osteoporosis is emphasized in early age through the improvement of physiological processes such as an increase in bone mass during growth to reach the peak of bone mass (Karlson *et al.*, 1995). Higher bone density in premenopausal period can maintain deposits of bone calcium thus reducing calcium loss or decrease during menopause. Individuals with high bone density during growth to premenopausal will avoid osteoporosis in postmenopausal period (Compston *et al.,* 1993). Calcium deficiency at an early age, may cause fracture at the age of 57-58 years (Nguyen *et al.,* 1995). Presenger *et al.* (1995) explained osteoporosis prevention is to maintain a good balance of calcium in the bones to avoid excessive loss of calcium in the kidneys and avoid excessive intestinal absorption disorders.

Fish bone flour has a high mineral content as a suitable ingridient of natural calcium source (Malde *et al.,* 2010). Fishbone that generated from fishing industry after filleted is about 30% of the total weight of fish (Shahidi, 1994). Generated fish processing waste - the head, bones and skin scales – are discarded and not used by the public or the fishing industry, thus negatively impacted the environment. This waste, if managed well will have an important economic value because wastes of the head bones and scales have quite high mineral content; especially calcium and phosphor (Thalib *et al.*, 2009). *Madidihang* – yellowfin tuna – is one of major fishery catch. *Madidihang* bones were processed into fish bones flour that contains high calcium and phosphor, especially in the form of the most important inorganic elements in the fish body and in greatest number.

Components of calcium and phosphor in the bones of *Madidihang* fish is very high (Thalib *et al.*, 2009) which contains 16,48% calcium and 6,25% phosphor. Fish bone also contain high enough fat and protein. Fat of fish bones are in simplest form of fat, i.e. triglycerides of fatty acids. The simple fatty acids classified into neutral fat. Besides, there is a complex fat phosphatides (phospholipids) and sterols. This type of fat can be hydrolyzed when heated in alkali. One effort to eliminate or minimize the fat of fish bones is to use high temperature and boiling acid (Soeparno and Susana, 1984). Along with this

Nurhayati (1994) reported that acetic acid may also be used to facilitate fat expenditure. Acetic acid (vinegar) is an organic acid known for sour flavoring and aroma in food. Acetic acid is a chemical reagent and industrial raw materials, especially in the food industry, used to set the acidity. High use of acid in food products can eliminate the fat content.

Researches on fish bone flour of *Madidihang* have been studied previously and the results stated that the flour contained high calcium and phosphorus, which also implied the high fat content. This high fat of the flour can be eliminated using acid and high heating. Therefore, further research needs to be done about the *Madidihang* bone flour treated with water heating and acetic acid. This study then aimed to determine the physico-chemical characteristics of *Madidihang* fish bone flour processed with the water and acetic acid.

Materials and methods

Raw materials that used in the manufacture of *Madidihang* fish bone flour is wet bone obtained from tuna filleting units in Ternate, North Maluccas. Chemicals used to make fish bone flour is H_2SO_4, alcohol, NaOH, $Na_2S_2O_3$, HNO_3, $HClO_4$, distilled water, kjeltab tablets, buffer pH 7 and pH 4, KH_2PO_4 (phosphor standard) and 1000 ppm of Ca salt solution (Ca standard) (AOAC, 1995).

The tools used for the manufacture of fish bone flour is tray, knives, pans, stove, oven, autoclave, disc mill, 100 mesh sieve and analytical scales. For physical and chemical analysis, we used oven, analytical balance, beaker glass, Kjeldhal beaker, water bath, homogenizer – Nissei AM-3, AAS (*Atomic Absorpstion Spectrophotometer*) – Shimadzu AA-680, Rheoner – 3350 RE Yamaden, porcelain cup, filter paper, *Whiteness meter* – Kett electric C-100-3, measuring cup, erlenmeyer, soxhlet, fat-free cotton, pipettes, electric stove, furnace, pH meters, funnel, 42 Whatman filter paper and test tube centrifuge (AOAC, 1995).

Boiling of Madidihang (T. albacares) Fish Bone with Water and Acetic Acid

Madidihang fish bone was obtained from the tuna filleting central in Ternate. The process of making fish bone flour from *Madidihang* – yellowfin tuna (*Thunnus albacares*) starts by chopping the 60-100 cm waste fish bones into 30-35 cm length. Next is washing of the sticky dirt and blood, followed by boiling. Boiling is repeated 3 times, 4 hours each; total 12 hours at 100°C. After each phase of boiling, bones then washed again with running water. The next stage is the fish bone boiling treatment of water and acetic acid by 1:3 ration for 30 minutes. Post-boiling fish bones were washed three times and aotoclaved for 2 hours at 121°C with subsequent oven with 60°C for 8 hours. The next process is grinding with disc mill and sieved to 100 mesh size (Thalib *et al.,* 2009).

Observed Parameters

Physico-chemical characteristics of *Madidihang* fish bone flour is observed as follows: rate of whiteness, absorption of water (Fardiaz *et al.,* 1992) and camba density (Wirakartakusumah *et al.,* 1992). Camba Density is the ratio of weight materials with volume that they occupied, including the empty space between the grains of the material (Syarif and Halid, 1992). Camba density measured by mass per unit volume. The higher the density of an object, the greater the mass of each volume. While observed chemical characteristics include moisture content, ash, protein and fat (AOAC, 1995), calcium and phosphor (Raitz *et al.,* 1987). Solubility of calcium and phosphor and pH were according to Santoso *et al.* (2006).

Result and discussion

Physico-chemical characteristics of Madidihang Fish Bone Flour (T. albacares)

Analysis results on whiteness rate of yellowfin tuna bone flour produced by using water and acetic acid are 46,45% and 46,33%, respectively (Table 1). Results of previous studies (Trilaksani *et al.,* 2006) on the whiteness rate of bone powder with hydrolysis method is 59,3 - 74,8%. Both researches are less white compared to 80-90% whiteness on fish bone

flour produced by *Bogasari Cap Kunci Biru*. This is because the yellowfin tuna fish bone flour produced without the use of bleach that is commonly sold in the market – benzoyl peroxide (Winarno, 1997).

Rate of whiteness on flour organoleptically is a factor that indicates the quality of the flour. The higher the rate then the flour is better quality (Buckle *et al.,* 1987). Whiteness of fish bone flour produced with water and acetic acid were insignificantly different (p> 0,05) (Table 1). It is presumably because the slight concentration of acetic acid, so it's difficult to degrade the fat of bone fish caused less whiteness. Fat of fish bones are thought to be degraded only by using high concentrations of acetic acid.

Table 1. Characteristic of *Madidihang* (*T. albacares)* Fish Bone Flour.

Parameter	Boiling Media	
	Water	Acetic acid
Rate of whiteness (%)	46,45 ± 0,56[a]	46,33 ± 0,703[a]
Water absorption (g/ml)	1,05 ± 0,11[a]	1,08 ± 0,00[a]
Camba density (g/ml)	0,76 ± 0,04[a]	0,74 ± 0,01[a]

Description: The numbers in the same row followed by different superscript letters shows significant indifferent (p> 0.05).

Water absorption is the unoccupied part off particles or solids (Wirakartakusumah *et al*., 1992). One factor that affects the water absorption is porosity. The porosity of material indicated by camba density of the material; the greater the porosity then the smaller is the camba density. Water absorption on *Madidihang* fish bone flour produced with water and acetic acid is 1,05 g/ml and 1,04 g/ml respectively (Table 1), smaller than the absorption of water on previous research (Trilaksani *et al*., 2006) with hydrolysis method that ranged 14 – 14,7. Water absorption of *Madidihang* fish bone flour with two different treatments were statistically not different (p > 0,05). This is presumably due to the hydrolysis of fish bones is not running perfectly. Hydrolysis is a chemical process that uses H_2O as breaking compound including the inversion of sugar, fat and ester saponification and proteins breaker. When the hydrolysis was carried out perfectly then hydrolyzate obtained, which is consisted of 18-20 amino acids mixture and the final product can be a hygroscopic liquid, paste or powder (Paustina, 2001).

Table 2. Chemical Characteristic of *Madidihang* (*T. albacares)* Fish Bone Flour.

Parameter's Level (%)	Boiling Media	
	Water	Acetic acid
Water	2,98 ± 0,14[a]	2,54 ± 0,09[b]
Ashes	56,65 ± 2,26[a]	58,21 ± 0,08[a]
Fat	6,36 ± 0,61[a]	6,31 ± 0,56[a]
Protein	20,98 ± 2,98[a]	17,21 ± 2,58[a]
Ca	23,61 ± 3,86[a]	16,64 ± 2,52[a]
P	15,25 ± 2,23[a]	16,62 ± 2,50[a]

Description: The numbers in the same row followed by different superscript letters shows significant indifferent (p> 0.05).

Camba density (*bulk density*) is the particles mass that occupy a certain volume of units that are affected by the size, shape, material, container and water content (Wirakartakusumah *et al*., 1992). Camba density of fish bone flour that generated with water and acetic acid are 0,76 g/m and 0,74 g/m respectively (Table 1) and were not significantly different (p>0,05). This camba density is lower

compared with Trilaksani *et al.* (2006), with hydrolysis method that ranging from 7,42 g/m to 9,42 g/m. This is presumably because the autoclave process before is done shorter than in this study.

Chemical characteristics of Madidihang Fish Bone Flour (T. albacares)

The results analysis of variance shows water content in the boiling media (water and acetic acid) gave significantly different effect ($p < 0,05$), whereas for the parameters of the ash content, fat, protein, levels of calcium and phosphor in both boiling medium show no difference significantly ($p > 0,05$). Experiments on water content was significantly different (Table 2). Acetic acid absorbs more water than water as boiling media. Water content in foodstuffs present between the cells and within the cells. Water is freely contained in the tissues, whereas bound water normally in the cell. Conventionally, water can be divided into three types, i.e. chemically bound water, physically bound water and free water (Syarif and Halid, 1992).

Table 3. Ca and P Solubility Characters of *Madidihang* (*T. albacares)* Fish Bone Flour to pH.

pH	Ca (%)		P (%)	
	Water	Acetic acid	Water	Acetic acid
2	6,35 ± 0,57[b]	13,02 ± 0,62 [a]	7,81± 1,02[a]	2,95 ± 0,18[b]
4	2,92 ± 0,55[b]	7,92 ± 0,54 [a]	8,62± 2,93[a]	2,25 ± 1,11[b]
6	2,12 ± 0,55[b]	5,88 ± 0,21[a]	1,05±0,20[b]	4,30 ± 1,03[a]

Description: The numbers in the same row followed by different superscript letters shows significant indifferent (p> 0.05).

Water content of yellowfin tuna fish bone flour with medium boiling water and acetic acid are 2,98 and 2,54 g/ml respectively, showing a significant difference ($p<0,05$). Compare to previous research which showed 5,60 - 8,30% water content (Trilaksani *et al.,* 2006), water content in this study was much smaller. The difference is presumably due to the autoclave time in previous studies was only one hour but our study was conducted for two hours. Water and acetic acid boiling media produce much lower water content so it is more durable in storage because it inhibit the growth of bacteria and mold. The water content of yellowfin tuna fish bone flour in this study is still in range of Indonesian National Standard 10-15% (SNI, 1996).

Ash content is one components in foodstuffs which consists of minerals such as potassium, phosphor, sodium, magnesium, calcium, iron, manganese and copper (Winarno, 1997). Ashes content of water and acetic acid boiling medium 56,65% and 58.21% (Table 2) were not significantly different from previous research by hydrolysis method 77,54 – 84,22%. Different ash content due to the length of autoclaving. Autoclaving time of previous studies was one hour whereas in this study was two hours. It makes the materials lost much protein and some fats that can ultimately increase the ash content of the final material (Thalib *et al.,* 2009). When compared with standard fish bone flour's ash content of 20-30%, then the ash content of fish bone flour in this study did not qualified the standard set (SNI, 1996). The higher ash content, the higher content of calcium and phosphorus in the material (Thalib *et al.,* 2009).

Protein levels obtained in this study were 20,98% with water and 17,21% with acetic acid, and they are not significantly different ($p>0,05$) (Table 2), whereas previous studies with hydrolysis method is 0,48-1.29% (Trilaksani *et al.,* 2006). Levels of proteins in this study is smaller compared to Trilaksani *et al.* (2006) study. Standard fish flour protein set 45-65%, then the fish bone flour produced in this study have not been entered on the standard set. The low protein in this study presumably because the boiling process is done repeatedly. Protein content in each treatment decreased allegedly, affected by the length of boiling and concentration of used acid. The longer the

ingredients in acid environment, the greater the the acid breaks down protein (Lee and Kanis, 1994).

Fat does not dissolve in water, which is the medium in the cell, interstitial fluid and blood circulation system. Fat must be returned in such soluble in water prior to biological function (Piliang and Djojosoebagjo, 2006). Fat in the two boiling media (wáter and acetic acid) are 6,36% and 6,31% respectively (Table 2). Compared with fat content in previous studies 8,49 for water media, 8,22 for acetic acid media and 7,58 for chloride acid media (Thalib *et al.,* 2009), this study is lower although not significantly different (p>0,05). The higher levels of fat found in acetic acid boiled fish bones. This is because the fat composition of the acid hydrolyzed producing glycerol fatty acid. Due to the boiling process, fat content in the fih bone reduced. The decrease in the fat content affects durable of materials; high- fat ingredients will accelerate rancidity due to oxidation of fat (Kataren, 1986).

Calcium is the most important and the highest amounts inorganic elements in the body (Djojosoebagjo, 1991). Calcium content in fish bone flour are 23,62% of water boiling media and 16,64% of acetic acid media. The results of this study were not significantly different (p>0,05). Previous studies with the hydrolysis method resulted 23,72 – 39,24 mg/g dry weight (Trilaksani *et al.,* 2006). Besides influenced by different boiling media, bone's mineral is also influenced by ecological catching factors, i.e. season, nutrients availability, temperature and salinity (Sediaoetama, 2006).

Phosphor is important for muscle contraction, bone formation (ossification) and secretory activity (Piliang and Djojosoebagjo, 2006). Phosphor content of fish bone in water media is 15,25% and in acetic acid is 16,62%. It was not significantly different (p>0,05), but the levels of phosphor is higher compared to hydrolysis method (11,34 to 14,25 mg/g dry weight). The ratio of Ca and P closely effect the process of absorption. For a good Ca absorption, we need Ca : P comparison 1 : 1 to 1 : 3 in the intestinal cavity (in a dish), with intestinal pH < 6. When Ca : P ratio is greater than 1 : 3, it will inhibit the absorption of Ca (Sediaoetama, 2006). Calcium and phosphor content in this study comply the Indonesian National Standard of fish flour, with a range of 2,5 to 7,0 for calcium and 1,6 to 4,7 for phosphor (SNI, 1996).

Calcium and Phosphor Solubility of Madidihang Fish Bone Flour to pH

Comestibles with high amounts of calcium is not the only requirement to be a useful source of calcium for the body. Another requirement is the calcium must be *bioavailable* to be utilized by the body. One *bioavailable* indicator is have a high solubility in a solution with pH below 6 (Santoso *et al.*, 2006).

Based on ANOVA, the interaction between water and acetic acid boiling media with pH variation showed significantly different results (p <0.05) on the solubility of calcium and phosphor (Table 3). Boiling with pH 2 acetic acid has a high Ca solubility (13,02%) whereas at pH 2 water is 6,35%. Ca and P of pH 4 and 6 is lower due to the use of acid facilitates calcium exit from fish bone flour which is easier to eventually be absorbed. The lower the pH, the higher calcium solubility of fish bone flour yellowfin is (Thalib *et al.,* 2009).

Solubility of phosphor were highest at pH 4 with medium boiling water (8,62%) and in the acetic acid was 2,95%. Both showed a statistically significant difference (p< 0,05). The lower the pH, the higher calcium solubility is. Conversely, the higher the pH then the lower solubility of phosphor. Driving factors that affecting the absorption of minerals is acidic pH, whereas the inhibiting factor is alkaline pH conditions, the presence of fiber and phytic acid (Sediaoetama, 2006; Almatsier, 2003). It is because of boiling with acetic acid facilitates calcium out of fish bone flour that make it easier to absorbed. Bone cooked with pressure (*steam*) has a high calcium levels compared with bone cooked with open panic. This is in line with Kaya *et al.* (2008) that mentioned the calcium solubility percent of catfish bone had the highest value to 1,25% of dry method and 1,07% of wet method at pH 2. While the highest percent

solubility of phosphor of dry method is 0,81% and wet method of 0,11%. The best phosphor solubility of yellowfin tuna fish bone powder was at pH 4 with water media and the lowest was at pH 6 also with water.

Ca and P solubility of yellowfin tuna fish bone flour with different media, were significantly increased along with increasing of acidity (low pH), which produced the highest solubility at pH 4 with water as boiling media. Yoshie *et al.* (1997) and Santoso *et al.* (2006) was studied the minerals solubility of seafood and seaweeds in different acidity. The results also showed that the solubility of minerals (Ca, Mg, Fe, Zn) were highest in acidic and will decrease in line with the decrease of acidity, as well as the absorption percent.

Physico-chemical characteristics of *Madidihang* fish bone flour which are processed both with water and acetic acid was high but has no affect. Calcium and phosphor solubility at pH 2 is high, i.e. 13,02% and 6,35% better than treatment at pH 4 and 6 which has a low value.

References

Almatsier S. 2003. Basic principles of nutrition science. Jakarta: Gramedia Pustaka.

AOAC – Associattion of Official Analiytical Chemist. 1995. Official methods of analiysis. Washingthon DC: AOAC.

Buckle KA, Edward RA, Fleet GH, Wootton N. 1987. Food science, 2nd Edition. In: Purnomo H, Adiono, transl. Jakarta: University of Indonesia Press.

Clydesdale FM. 1988. Minerals: their chemistry and fate in food. In: Smith KT, ed.Trace mineral in foods, New York: Marcel Dekker Inc.

Compston JE, Garraham NJ, Croucher PI, Wright CDP, Yamaguchi K. 1993. Quantitative analysis of trabecular bone structure. Bone **14,** 187-192.

Dawson, Hughes B. 1996. Calcium and vitamin D nutritional needs of elderly women. Journal of Nutrition **126,** 1165-1167.

Diane FC, Walter W, Graham AC. 2003. Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women[1–3]. American Journal of Clinical Nutrition *77*, 504-511.

Djojosoebagio S. 1991. Physiology of nutrition, Vol. I and II. Bogor: Bogor Agricultural University Press.

Fardiaz D, Andarwulan N, Wijaya H, Puspitasari LN. 1992. Mechanical Analysis of chemistry and functional properties of food components. Inter - University Center of Food and Nutrition, Bogor: Bogor Agricultural University.

Karlson MK, Vergnauld P, Delmos PD, Obrant KJ. 1995. Indicates of bone formation in weight lifters. Calcified Tissue International **56(3),** 177-180. http://dx.doi.org/10.1007/BF00298605

Kataren S. 1986. Introduction: oil and fat food technology, 1st Edition. Jakarta: University of Indonesia Press.

Kaya A, Santoso, Salamah. 2008. Utilization of catfish (*Pangasius* sp.) bone flour as calcium and phosphor sources in biscuit manufacturing. Master thesis, Bogor Agricultural University, Indonesia.

Lee CJ, Kanis JA. 1994. An association between osteoporosis and premenstrual symptoms and postmenopausal symptoms. Journal of Bone Mineral **24,** 127-134.

Malde ML, Graff IE, Resi HS, Venäläinen E, Julshamn K, Pedersen II, Valaja. 2010. Fish bones – a highly available calcium source for growing pigs. Journal of Animal Physiololgy and Animal Nutrition **94,** 66–76.

http://dx.doi.org/10.1111/j.1439-0396.2009.00979.x.

Nurhayati T. 1994. Effect of acid and bleaching to the quality of fish flour. Thesis, Department of Fisheries Product Technology, Bogor Agricultural University, Indonesia.

Nyuyen TVC, Jones G, Sambrook PN, White GP, Kelly PJ, Eisman JA. 1995. Effects of estrogen exposure and reproduction factor and bone mineral density and osteoporosis fractures. Journal of Clinical Endocrin Metabolism ***80*(9),** 2709-2714. http://dx.doi.org/10.1210/jcem.80.9.7673413.

O'Dell BL. 1984. Bioavailability of trace elements. Nutrition Review **42,** 301-308. http://dx.doi.org/10.1111/j.17534887.1984.tb02370.x.

Paustina T. 2001. Protein Structure. Medison: University of Wisconsin.

Piliang WG, Djojosoebagio S. 2006. Physiology of nutrition, Vol. II. Bogor: Bogor Agricultural University Press.

Presenger E, Alaclamiolu Y, Pils K, Saradetand T, Scheider B. 1995. Therapeutic exercise in the prevention of bone loss. American Journal of Physiology Medicine Rehabilitation **74**, 120-123.

Raitz LL, Smith WH, Plumlee MP. 1987. A simple wet oxidation procedure for biological material. West Lafayee: Animal Science Purdue University.

Santoso J, Gunji S, Yoshie-Strark Y, Suzuki T. 2006. Mineral contents of Indonesian seaweeds and mineral solubility affected by basic cooking. Food Science Technology Research **12**, 59-66. http://dx.doi.org/10.3136/fstr.12.59.

Sediaoetama AD. 2006. Science of nutrition: for students and professionals in Indonesia, Vol. I. Jakarta: Dian Rakyat.

Shahidi F. 1994. Seafood processing by-products. In: Shahidi F, Botta JR, Eds. Seafoods chemistry, processing technology and quality, Glasgow: Blackie Academic and Professional, 320–334 p.

SNI (National Standard ofIndonesia). 1996. Fish flour/raw food. No. 01-2715-1996. Jakarta: Department of National Standarization.

Soeparno, Susana. 1984. Science and technology of meat. Yogyakarta: Gajah Mada University Press.

Syarief, Halid. 1992. Food storage technology. Inter University Center of Food and Nutrition, Bogor: Bogor Agricultural University.

Thalib A, Santoso, Ibarahim. 2009. Utilization of Madidihang (*Thunnus albacores*) fish bone flour as calcium and phosphor sources to improve makron walnuts nutritional value. Jurnal Sains ***1*** (3). Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor: UMMU Press.

Trilaksani W, Salamah E, Nabil M. 2006. Utilization of tuna (*Thunus* sp*)* bone flour waste as calcium sources with hydrolysis method. Buletin Teknologi Hasil Perikanan ***IX*** (2), 38-43.

Weaver MC. 2006. Vitamin D and calcium metabolisme in adolescents. Journal Elsevier International Congress Series ***1297***, 32-38. http://dx.doi.org/10.1016/j.ics.2006.08.013.

WHO. 2003. Prevention and management of osteoporosis. WHO Technical Report Series **921**. Geneva: Report of a WHO Scientific Group.

Winarno B. 1997. Bone mineral density of breastfeeding women. Spesialist thesis, Faculty of Medicine, University of Indonesia, Indonesia.

Wirakartakusumah MA, Abdullah K, Syarief AM. 1992. Physical properties of food materials. Inter - University Center of Food and Nutrition, Bogor: Bogor Agricultural University.

Yoshie Y, Suzuki T, Clydesdale FM. 1997. Iron solubility from seafoods with added iron and organic acid under stimulated gastrointestinal conditional. Journal of Food Quality **20,** 235-246.