

The Effect of Acid Solvent on the Physicochemical Characteristics of Tuna Dark Meat Fish Meal

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ABSTRACT

Tuna dark meat can be processed into fish meal to increase its economic value and shelf life. Fish meal is a high-protein, low-moisture product derived from fish parts or a fish grinding mill. This study was organized to discover the effect of an acid solvent on the physicochemical properties of tuna dark meat in fish meal. The results of the study revealed that the extraction of tuna dark meat using acid solvent types affected the physicochemical characteristics of the produced fish meal in all test parameters, including yield value, bulk density, whiteness, pH, moisture content, ash, protein, fat, and total plate count. The best characteristics of fish meal were those extracted using lime acid (AJ); with a yield value of 26.230%, bulk density of 0.937 g.ml⁻¹, whiteness of 33.967%, pH of 3.6, moisture content of 6.257%, ash of 4.674%, protein of 78.077%, fat of 1.938%, and total plate count of 1.79 x 10³ cfu.g⁻¹. This finding serves as a model for the processing of tuna meat as an innovation in providing healthy and nutritious food for fish consumption.

INTRODUCTION

The different methods of fish processing may result in different waste potential and quality (Bragadottir *et al.*, 2007). Solid waste resulted from tuna fish processing in the form of fish bones, fins, scales, and dark meat of about 20-30% from the total fish was not optimally utilized. Fish dark meat is a meat layer found in the whole fish lower body part under scales, approximately 2-20% depending on the fish species and size, containing myoglobin, hemoglobin and high in fat. Stone (2007) stated that tuna loin processing resulted in waste of approximately 37.1% and dark meat of 17.9%. That waste was not commonly utilized or was merely used for animal feed making, yet in fact fish dark meat has essential components that might be used as nutritional sources in food processing.

Karunaratha and Attygalle (2010) stated that tuna dark meat had the moisture content of 70.83%, protein of 20.22%, fat of 1.01%, and ash of 0.92%, besides other compositions, such as SFA of 22.74%, MUFA of 21.11% and PUFA of 55.85%.

Meanwhile, **Sánchez-Zapata *et al.* (2011)** mentioned that mineral content of Fe was 32.11 mg or 73.38% of the total mineral content with high bio-viability, water binding power of 8.37 g water.g⁻¹, and oil holding capacity of 8.11 g oil.g⁻¹.

Tuna dark meat had peroxide compounds of 80% in the form of myoglobin and hemoglobin composed of myosin, globin, and heme structure (**Okada, 1990**). Myoglobin content in tuna dark meat was approximately 9650.12 mg/kg (**Sánchez-Zapata *et al.*, 2011**) that it might easily result in rancidity, decreasing quality, and when processed, it was not well accepted by some individuals due to its taste, color, and odor (**Okada, 1990** ; **Bertoldi *et al.*, 2004**). The utilization of fish dark meat into fish meal is one alternative solution to improve the economic value, storage time, and meat quality that the society may well accept and have the potential as animal protein source in food processing.

Fish meal is one utilization form of fish waste or fish parts which fat is separated, dried, and grinded to produce protein, mineral, and vitamin-rich fish meal. Good quality fish meal is that with the same particle size, free from fish bones, fish eyes, foreign materials, brighter color, and fish-like odor (**Afrianto & Liviawaty, 2005**).

Fish meal making can be done by using wet, dry, and distillation methods. Dry method is the simplest method with low cost, yet the fish meal quality is also poor. Wet method may be conducted with extraction through boiling process in either acid or alkali solution using autoclave, dried, and flouring. This method may result in better quality of fish meal when compared to that made with dry method. The extraction process in this research was conducted using acid solvent, namely acetic acid and lime acid since according to the research conducted by **Immaculate *et al.* (2013)**, that extraction using acetic acid solvent at the temperature of 70 °C for 30 minutes had the function to lose the fat and whitens the dark meat.

This research aimed at revealing the effect of acid solvent on the physicochemical characteristics of fish meal from tuna dark meat, while its utilization is achieved by minimizing the waste resulted from tuna processing in the form of processed product diversification in the form of fish meal.

MATERIALS AND METHODS

Materials and Equipment

The material used was tuna dark meat already cleaned from its scales, bones, and other unused parts from the tuna loin industrial processing in Bastiong market, Ternate, Indonesia. The other additional materials were technical NaCl, distilled water, acetic acid, citric acid and lime acid with the concentration of 5%. The materials used for analysis were buffer solution to measure pH, solution and other chemical materials to test the content of protein, fat and total plate count.

The equipment used in this research consisted of knife, grinder, plastic containers, digital scale, boiling pot, stirring spatula, measuring glass, stove, drying oven, thermometer, timer, disc mill and stationaries for the fish meal making process. The equipment used for analysis included vacuum oven, furnace oven, pH meter, whiteness meter, analytic scale, destruction equipment, titration, soxhlet flask, glass equipment, desiccator, petri disk and other equipment for proximate and microbiological analysis.

Fish meal processing

The fish meal making process was conducted through some steps. First, the tuna dark meat was washed using clean running water to get rid of the fish dirt, slime, and blood. Second, the cleaned tuna dark meat was then grinded using grinder and washed once again using the solution of NaCl 2%. Third, the tuna dark meat was then extracted using acid solvent types as follows: AA (acetic acid 5%); AS (citric acid 5%); AJ (lime acid 5%) and AQ (distilled water, control), with the solvent and tuna dark meat ratio of 3:1, in the temperature of 100 °C for \pm 20 minutes. The tuna dark meat resulted from the extraction was then filtered, cold, dried using a drying oven in the temperature of 50 °C for \pm 48 hours up to the water content that was < 12%, and then grinded using disc mill and sieved with the mesh size of 100.

Characterization of fish meal

The characterization of fish meal procedures included yield, bulk density, whiteness level, pH value, proximate and total plate count test. Yield was based on the simultaneous weight ratio of fish meal and fish dark meat. Bulk density was the sample weight per material volume (Singh *et al.*, 2005). Whiteness level was measured with whiteness meter (Lanier *et al.* 1991). The pH value was determined with pH meter, while the proximate analysis included the moisture content measured with gravimetric method (AOAC, 2005); ash content measured with gravimetric method (AOAC, 2005); protein content measured with ash micro method of kjeldahl (AOAC, 2005), fat content measured with fat shoxlet (AOAC, 2005), and total plate count (TPC) was determined using BSN (2015).

Experimental design and statistical analysis

This research was conducted using a single-factor completely randomized design with three repetitions in each treatment. The data resulted from the physicochemical test was analyzed using SPSS 22.0 program for single-factor Anova at significant range of 95% and if the test result was significantly different, Duncan Multiple Range Test (DMRT) was further conducted.

RESULTS AND DISCUSSIONS

Fish meal is a product containing protein, mineral and vitamin, produced from the fish meat by extracting its fat and liquid. The fish meal's nutritional content depended on the fish type used as the raw material (Martinez *et al.*, 1998). The fish meal's characterization in this research included: determining yield, bulk density, whiteness, pH value, proximate (moisture content, ash, protein and fat) and total plate count, as presented in Table (1). The utilization of SNI 01-2715-1992 on fish meal quality was to compare the fish meal making stages and its quality to meet the commercial fish meal quality standard as presented in Table (2).

Yield

Calculation of the yield was conducted to determine the economic value and effectiveness of a material determined based on the percentage ratio between the fish meal initial and final weight. The greater the yield value of a product, the higher is the economic value of that product. The yield values of fish meal were as follows: 22.193%

(AQ); 21.910% (AS); 26.230% (AJ) and 27.077% (AA), with the highest fish meal value of AA and the lowest one of AS (Table 1).

Table 1. Physicochemical characteristics of fish meal (g/100 g sample)

Physicochemical characteristics	Treatment			
	AQ	AS	AJ	AA
Yield (%)	22.193 ^a	21.910 ^a	26.230 ^b	27.077 ^b
Bulk density (g/mL)	0.867 ^b	0.037 ^a	0.937 ^c	0.933 ^c
Whiteness (%)	22.333 ^a	29.900 ^b	33.967 ^c	30.200 ^b
pH value	5.267 ^c	2.500 ^a	3.600 ^b	3.867 ^b
Moisture (%)	8.346 ^b	7.800 ^{ab}	6.257 ^a	11.450 ^c
Ash (%)	4.218 ^a	6.884 ^a	4.674 ^a	4.294 ^a
Protein (%)	81.851 ^c	67.987 ^a	78.077 ^b	79.144 ^b
Fat (%)	5.148 ^c	3.438 ^b	1.938 ^a	4.461 ^c
Total plate count (cfu/g)	4.20 x 10 ^{3b}	2.76 x 10 ^{3ab}	1.79 x 10 ^{3a}	1.24 x 10 ^{3a}

Remarks AQ (distilled water), AS (citric acid), AJ (lime acid), AA (acetic acid)

The numbers followed by the same letter in the same line are not significantly different at $\alpha_{0.05}$

Table 2. Grade quality of fish meal (SNI 01-2715-1992)

Chemical Composition	Grade I	Grade II	Grade III
Moisture content (%) max	10	12	12
Crude protein (%) min	65	55	45
Crude fiber (%) max	1,5	2,5	3
Ash (%) max	20	25	30
Fat (%) max	8	10	12
Ca (%)	2,5-5,0	2,5-6,0	2,5-7,0
P (%)	1,6-3,2	1,6-4,0	1,6-4,7
NaCl (%) max	2	3	-
Salmonella (in 25 g/sampel)	Negatif	Negatif	Negatif
Organoleptic, minimum value	7	6	6

The acid solvent types used in the extraction process statistically affected the yield value of fish meal. The yield value of fish meal showed that the solvent played an important role on the yield value of products resulted from the extraction. The higher the yield value showed, the more the solvent used showed the same polarity with most material biomass (Sani *et al.*, 2014).

Litaay & Santoso (2013) reported that, the yield of skipjack fish meal with the treatment method and immersing period between 38.79 - 43.95 % and explained that the fish meal immersed in water had a higher yield, when compared to that immersed in acid and alkali. That result had a higher yield value than that in the present research which was only 21.910-27.077%, while the fish meal extracted using acid solvent had a higher yield value when compared to that extracted with the distilled water.

Bulk density

Bulk density was the size of material mass amount per the occupied volume including empty room between materials (Rusmono *et al.*, 2016). Bulk density might be used as the characterization parameter of powder material particles, since single particle significantly influenced the product characters, such as particle size, shape, surface,

density, hardness and adsorptive character. Bulk density of powder food product depended to a great extent on its particle size and distribution (**Barbosa-Cánovas *et al.*, 2005**). A very small change in powder bulk density might result in a great flow-ability (**Levy & Kalman, 2001**). Bulk density might also be utilized as the completeness parameter of drying process or material shape and size uniformity. The fast drying process might result in smaller bulk density when compared to the slow drying process.

The bulk density values of fish meal (Table 1) were 0.867 g/ml (AQ); 0.037 g/ml (AS); 0.937 g/ml (AJ); and 0.933 g/ml (AA), with the highest value in fish meal AJ and the lowest in fish meal AS. The bulk density of fish meal statistically showed that acid solvent types affected the bulk density of the product. Bulk density describes a material's particle shape and size (**Atmaka & Sigit, 2010**). Based on the average value of bulk density, it showed that the fish meal AJ particle size was smaller when compared to the others with big bulk density. Meanwhile, the fish meal AS particle was bigger and had spores that resulted in small bulk density. Bulk density always paid attention to the material porosity since the smaller the bulk density, the more pores the resulted powder had indicating that the cavities between the material particles were filled with air (**Jufri *et al.*, 2006**).

Whiteness

Color is an important attribute characteristic of food materials (**Dewi *et al.*, 2012**), since the material color might influence the acceptance level of a material. The whiteness level measurement of a flour product was a parameter made to determine the brightness of flour color since flour generally had different brightness levels (**Hutching, 1999**). The whiteness level measurement scale was 0-100% from black (0%) to white (100%).

The values of fish meal's whiteness level (Table 1) were as follows: 22.333% (AQ); 29.900% (AS); 33.967% (AJ); and 30.200% (AA) with the highest value in fish meal AJ and the lowest in the control flour (AQ). The acid solvent types used in the extraction process affected the whiteness level of fish meal.

The whiteness level of a product was affected by the raw material color and solvent type used. The whiteness level of fish meal in this research ranged between 22.333-33.967%, meaning that the resulted fish meal had low whiteness level. The extraction made with distilled water resulted in fish meal with the whiteness level of 22.333%. This showed that the heating and watering process might reduce the tuna red meat color, with lower effectiveness when compared with extraction using acid solvent. The result of this research was in accordance with the research conducted by **Dewi *et al.* (2012)**, stating that the extraction using acid solvent significantly affected the control of whiteness level.

The pH value

The pH value was the value stating the acidity or alkalinity level of a solution. Product was considered acid if H^+ ion was dissolved in greater amount than OH^- ion within a solution (**Nugroho *et al.*, 2018**). The pH testing of fish meal was conducted to reveal the effect of acid solvent types used for extraction process on the resulted pH value of product.

The pH value of fish meal in this research respectively ranged between 2.50 - 5.27 as follows: 5.267 (AQ); 2.500 (AS); 3.600 (AJ); and 3.867 (AA) with the highest value in fish meal control (AQ) and the lowest in fish meal AS (Table 1). The acid

solvent types used effected the resulted pH of fish meal since effected by the acidity level of used solvent.

Proximate Analysis

Proximate analysis was the examination conducted to reveal the nutritional composition and energy from the food material or certain product by estimating the relative amount of food materials' nutritional substances, such as moisture content, ash, protein, and fat (Table 1).

a. Moisture content

The moisture content of food varies depending on the water contained in the food products. Furthermore, food moisture content determines the food quality and endurance. Food products with low water content have longer endurance when compared to those with higher water content. Hence, food water measurement is greatly required to uniformly calculate the composition of other substances in the dried weight or known as total solids (Nielsen, 2009).

Results showed that, the moisture content of fish meal ranged between 6.257-11.450%, with the value of 8.346% (AQ); 7.800% (AS); 6.257% (AJ); and 11.450% (AA), while the highest level was recorded in flour AA and the lowest in flour AJ (Table 1). The acid solvent types affected the moisture content fish meal. The extraction process using the acid solvent caused a decline in the water extracted from the fish meat's cellular tissues, hence, the fish meal's moisture content was reduced. The extraction using acid solvent might reduce the ability of gel formation in the fish meat, that when getting dried in the easily-to-lose water from the fish meat tissues. The good quality of fish meal that had moisture content was 6-10%, since in that moisture content, damage rarely happened which might be resulted from the microbial activities (Kurnia & Purwani, 2008). The resulted moisture content of fish meal in this research had met the best quality of fish meal standard ranging between 6.25-11.45%.

b. Ash content

The ash content is usually utilized to estimate the total minerals in a food material. The minerals in ash are in the form of sulfate, phosphate, nitrate, chloride and others (Fennema, 1996). Food material are consisted of the combination of organic and inorganic components, yet when burned the organic elements (C, H, O and N) would be lost, and the remaining was the inorganic components in ash called mineral.

The ash content (Table 1) of fish meal respectively ranged between 4.218-6.884%, as follows: 4.218% (AQ); 6.884% (AS); 4.674% (AJ); and 4.294% (AA), while the highest value was in fish meal AS, and the lowest was in control fish meal (AQ). The acid solvent types affected the ash content of fish meal.

The fish meal resulted from the extraction using citric acid solvent (AS) had a high ash content when compared to that in control fish meal, yet it was not in accordance with the result of research conducted by Sundari (2015) who stated that, the product resulted from the boiling process has a lowering ash content since heating process during extraction causes most minerals to dissolve in the solvent used, and in turn, the ash content becomes low.

c. Protein

Protein content consisted of macro-nutritional energy sources has an essential role in bimolecular formation. Protein is a complex polymer composed of some amino acids (Fennema, 1996). The protein content of fish meal respectively ranged between 67.987-81.851%, with the following values: 81.851% (AQ); 67.987% (AS); 78.077% (AJ); and 79.144% (AA), while the highest value was recorded in fish meal AQ and the lowest in fish meal AS (Table 1). The utilization of acid solvent in the extraction process decreased the protein content of fish meal since acid had the ability to hydrolyze protein in the fish meat that might dissolve in the acid solvent and be wasted in the filtering process.

The protein content of fish meal increased being influenced by the pH level of solvent used for extraction, as the lower the solvent's pH is, the lower the fish meal's protein content might be resulted. Besides, there were some other factors influencing the fish meal's protein content, such as temperature, length of time, and processing method. Puwaningsih (2015) stated that the processing that utilizes steaming method result in products with higher protein content when compared to those resulted from boiling method.

The acid solvent types significantly affected the fish meal's protein content as its resulted protein contents were different as presented in Table (1). The fish meal's protein content showed that the hydrolysis ability of citric acid was stronger than that of the other acid solvents. Meanwhile, the hydrolysis ability of lime acid solvent and acetate acid showed no difference when extraction process was conducted with distilled water.

d. Fat

The fat content of fish meal respectively ranged between 1.938-5.148%, as follows: 5.148% (AQ); 3.438% (AS); 1.938% (AJ); and 4.461% (AA), with the highest value in control fish meal AQ and the lowest in fish meal AJ (Table 1). The fat content of fish meal in this research was adequately high since assumed that the red meat used had experienced fat oxidation during the distribution process and frozen storage. Bragadottir *et al.* (2007) stated that the oxidized raw materials may result in low quality end-product since the fish meal's stability and quality are influenced by temperature and storage duration.

The acid solvent types used for extraction affected the fat content of fish meal that the resulted fat contents were various. This result showed that the lime acid solvent had stronger ability to reduce the fat content than the others. The extraction using the acid solvent types in high temperature might hydrolyze the fat content found in the fish meal extracted using acetate acid solvent (flour AA), since its fat content was not different compared to that in the control fish meal (AQ).

Good fish meal has fat content of $\leq 12\%$ since fat content greatly affect the quality (Kurnia & Purwani, 2008). The fish meal with low fat content was uneasy to experience quality decrease and rancidity. High fat content could make the fish meal easy to rancid caused by the fat oxidation. Drying in high temperature might result in higher fat oxidation in food material when compared to that in low temperature.

Total Plate Count

Total plate count (TPC) testing was one method indirectly used to determine the number of microbes in food product by calculating the living microbes within media. This test parameter was commonly used as the general indicator explaining the food contamination level. TPC was defined as the number of colony forming unit (cfu) of bacteria in each gram or each food milliliter (**Puspandari & Isnawati, 2015**). Based on SNI 7388-2009, what was meant by TPC was the number of mesophilic aerobic microbes found in per gram or per milliliter sample determined through standard method (**BSN, 2009**).

The TPC value of fish meal ranged between 1.24×10^3 - 4.20×10^3 cfu/g, respectively with the following values: 4.20×10^3 cfu/g (AQ), 2.76×10^3 cfu/g (AS), 1.79×10^3 cfu/g (AJ) and 1.24×10^3 cfu/g (AA). The extraction using different acid solvent types effected the TPC values of fish meal. The TPC value of resulted fish meal in Table (1) indicate that the acetate acid, lime acid and citric acid solvents had the same ability in inhibiting the bacterial growth in fish meal when compared to that resulted from the distilled water solvent.

Acid solvent types used in the extraction process might inhibit the bacterial growth found in tuna dark meat shown with the TPC value of fish meal which still met the standard of **BSN (2006)**, with the minimum standard of <2500-65.000 cfu/g. The microbial growth in food content is influenced by the moisture content of food, water activity (aw) and pH (**Atma, 2016**). The pH of fish meal and moisture content values respectively ranged between 2.500-5.267 and 6.257-11.450% that were able to inhibit the microbial growth in the fish meal products.

CONCLUSION

The fish meal making process using extraction method in acid solvent showed that acid solvent types affected the physicochemical characteristics of fish meal resulted in all test parameters including yield value, bulk density, whiteness level, pH, water content, ash, protein, fat and total plate count.

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