The Influence of Acid and Cooking Methods on The Quality of Fish Meal Processed from by-products of Catfish Fillet Processing

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Abstract

Catfish fillet processing produces a by-product dominated by heads (25-28%) and bones (11-13%). This study aims to process fish meal from by-products of catfish using different treatments, namely soaking in a 4% formic acid solution (F) and a 4% hydrochloric acid solution (H), as well as cooking techniques involving boiling (R) and steaming (K). Observations were made on the yield, pH, proximate analysis, calcium content, NPN (non-protein nitrogen), TBA (thiobarbituric acid), microbiological tests, sensory evaluation, and amino acid profiles. The research showed that soaking in acid caused a decrease in pH, protein content, and TBA and increased the fat content of the resulting fish meal. The optimal treatment for the highest quality fish meal is steaming without acid treatment. In this treatment, the protein content meet the requirements of the SNI 01.2725.01.2013 as grade 3 fish meal, with a yield of 22.83%, a pH of 6.86, a moisture content of 6.2 ± 0.3%, an ash content of 27.33 ± 0.7%, and a protein content of 45.5 ± 1.3%. Analysis of the amino acid profile of fish meal from that treatment showed a better amino acid composition compared to commercial fish meal. The fish meal contained essential amino acids in sufficient quantities, with higher levels compared to the amino acid standards set by FAO/WHO 2013 for human nutritional needs, and met the requirements as a nutritional ingredient for fish and livestock feed.

Keywords: fish meal, by-product, quality of fish meal, amino acid profile

Introduction

Catfish is one of the fish commodities that is easy to cultivate in limited water sources with high stocking density and has a relatively short cultivation period, resulting in increased production annually (Anon, 2017). The cultivation of catfish has led to the development of the catfish fillet processing industry, which further processes it into catfish floss and minced meat-based products in several regions in Indonesia (Musyaddad et al., 2019). In processing catfish fillets for floss and minced meat, by-products such as heads, bones, fins, offal, and trimming are obtained, resulting in waste accounting for 65% of the fish's weight. The waste may negatively impact the environment if not managed properly (Mubarokah et al., 2021). The by-products of catfish fillets are predominantly composed of heads (25-28%) and bones (11-13%) (Suryaningrum et al., 2012). Fish bones contain approximately 30.54% organic compounds (dry basis), consisting of 28.04% protein, 1.94% lipid, and 0.56% carbohydrates, and approximately 69.49% inorganic materials (dry basis), primarily composed of calcium (Ca) and phosphorus (P) (Apitulay et al., 2020). The heads and bones of catfish can be utilized by processing them into fish meal to support government livestock farming or cultivation programs.

In fishery and livestock cultivation, feed requirements represent the most significant investment in business capital, reaching up to 70% of the total maintenance costs for fish (Suprayudi et al., 2012). The primary source of fish feed protein still relies on using fish meal as an unidentified growth factor (UGF), which is difficult to replace with other materials, especially for animal feed (Irene et al., 2021). Until now, fish meal has dominated feed requirements and needs to be imported. It is predicted that by 2024, the demand for fish meal as a raw material for feed will reach between 763.800 tons-1.2 million tons. Therefore, to reduce fish meal imports, the government has promoted policies related to self-sustaining feed programs (Puspaningtyas, 2022).

The by-products of catfish fillets can be used as raw materials in producing fish meal to support the autonomous feed program. Formulating feed using self-produced fish meal derived from the by-products of the catfish fillet processing industry can reduce production costs within a range of 60 -85% and generate greater farming profits (Amrullah et al., 2018). This study aims to process fish meal from the by-products of catfish fillet processing, such as heads and bones. The treatments involved using a weak acid solution (4% formic acid) and a robust acid solution (4% HCl) and heating techniques such as boiling and steaming. Formic acid is an organic acid with disinfectant properties due to an aldehyde group in its compound (Luisse et al., 2020). Research conducted by Safril and Lakahena (2015) on the red meat of tuna fish, extracted using a 3% acetic acid solution at 70°C for 30 minutes, showed a reduction in water content and produced a whiter fish meal. Boiling with an acid solution can produce a finer and brighter fish meal. Soaking in an acid solution prevents spoilage during drying, thus avoiding unpleasant odors (Sipayung et al., 2015).

In the processing of fish meal, a cooking process can be performed by steaming or boiling, which is carried out at 95–100°C to enhance the digestion of proteins and amino acids (Suharto et al., 2016). Cooking fish meal aims to achieve a better aroma and softer texture, eliminate microbes, and inactivate all enzymes (Candra et al., 2022). Research conducted by Dughita et al., (2021) showed that processing fish meal from the by-products of tilapia fillets using the steaming method resulted in fish meal with the best protein content, namely 58,80%. On the other hand, boiling causes soluble proteins, low-molecular-weight peptides, and free amino acids to dissolve in the boiling water (Assadad et al., 2015)

Fish meal is used in fish or livestock feed as a source of nutrition, and its nutritional value is determined by the completeness and balance of the amino acids contained (Hamid et al., 2020). The amino acids in the feed are continuously required by fish and livestock for cell growth and tissue formation (Pratama et al., 2019). Accurate knowledge of essential amino acid content in fish meal as a protein source is crucial. This information helps determine the levels of limiting amino acids such as lysine, methionine, cysteine, and tryptophan, which can serve as guidelines for formulating fish and livestock diets. The lysine, methionine, cysteine, and tryptophan levels should support optimal growth in livestock/poultry. (Andri et al., 2020). Therefore, this research aims to (1) study the processing of fish meal from the heads and bones of catfish using the soaking method with acid and cooking methods to assess the quality of the resulting fish meal and (2) determine the amino acid profile contained in the fish meal under the best treatment as a basis for formulating feed rations for poultry and livestock. Information regarding the processing of fish meal as a by-product of catfish fillet processing can be utilized by farmers for self-made feed materials.

Material and Method

Materials

The material used in this research consists of the head and bones obtained as a by-product of catfish fillet processing. Live catfish were purchased at a supplier in Cipayung with an average weight of 201,87 g ± 18,04 g. The fish were then euthanized by shock at a low temperature, subsequently slaughtered, and filleted by separating the flesh from the head and bones. The heads and bones, which are by-products of the catfish fillet, are further processed into fish meal. Other materials used include formic acid and hydrochloric acid, as well as a set of chemicals for chemical and microbiological analysis purchased from Merck Chemical agents CV Setia Makmur in Jakarta and commercial fish meal was purchased from CV Nitnot in Bogor by online.

Equipment

The equipment used in this study includes a manual hydraulic press, Tasin electric meat mincer made in Taiwan, disc mill Merck Getra, Sy 1200 made in China, a set of chemical and microbiological analysis tools, and HPLC (High-Performance Liquid Chromatography) Agilent 1260 infinity LC made in USA for amino acid profile analysis.

Research Method

This research used soaking in an acid solution and cooking methods. The variables used in the study are as follows: The type of acid used for soaking is 4% formic acid (F), 3% hydrochloric acid (H), and a control fish soaked in tap water (K). Cooking methods: boiling (R) and steaming (K).

Fish Meal Processing

The process of producing fish meal involves several stages, as follows: 1) The fish heads and bones as much as 5 kg are washed with clean water to remove blood, mucus, and other impurities; 2) Boiling the cleaned heads and bones was done in a solution of 4% formic acid, 3% hydrochloric acid, and water as a control; The boiling was done using a ratio of 2 parts water to 1 part heads and bones, at a boiling temperature of approximately 86°C-95°C; The boiling process is carried out for 30 minutes 3) Steaming: the steamed heads and bones are soaked in a solution of 4% formic acid and 3% HCl for 1 hour, then steamed at a temperature ranging from 86°C to 97°C; The steaming process is carried out for 30 minutes; 4) The boiled or steamed heads and bones were then pressed using a hydraulic press to remove excess water and obtain a press cake. 5) The press cake was crushed using a meat grinder and then dried under sunlight for two days; 6) The dried fish meal was ground using a disc mill to obtain a fine fish meal with a particle size of 80 mesh.

Observations

The observations on the fish meal were conducted for yield and pH (Sudarmadji et al., 1989). The quality of the fish meal was observed for moisture content (SNI 01-2354.2-2006) (BSN 2006a), ash content (SNI 01-2354.1-2006) (BSN 2006a), protein content (SNI 01-2354.4-2006) (BSN 2006[a]), and fat content (SNI 01-2354.3-2006) (BSN 2006[a]). Non-protein nitrogen (NPN) was determined using the Kjeldahl method, potassium content (SNI 01-2715-1996), and TBA (Sudarmaji et al., 1989). Microbiological tests were conducted for changes in total plate count (TPC) (SNI 01-2332.3-2006), Salmonella (SNI 01.2332.2.2006), and molds (SNI 2331-7.2096) (BSN 2006[b]). Sensory evaluation was performed using a semi-trained panelists from the Marine Research Center for Processing and Biotecnology following the guidelines of SNI 01-2346-2006 (organoleptic and sensory testing) (BSN, 2006[c]). The observation of amino acid profiles was conducted on the fish meal, which has the best treatment compared to commercial fish meal. The amino acid analysis was performed using HPLC with an Ultra Technosphere column. The experimental design used in this study is a factorial design with three treatments, two factors, and three replications. Post hoc testing with the Tukey test is conducted if the ANOVA shows a significant effect (Mattjik & Sumertajaya, 2013).

Results and Discussion

Analysis of Raw Materials

The proximate head and bones of catfish used as raw materials for fish meal have a moisture content of 75.66 ± 1.55%, an ash content of 1.06 ± 0.09%, a protein content of 14.98 ± 0.89%, and a fat content of 7.54 ± 0.46%. The protein content in this fish head is lower than in catfish meat, which is 17.09 ± 0.52%. Conversely, its fat content is higher than that of catfish meat, amounting to 2.75 ± 0.23% (Listryarini et al, 2018). The pH of the raw materials was within the normal range, measuring 7.04 ± 0.07. Based on their freshness, the heads and bones used as raw materials were still considered fresh, with a total volatile base (TVB) content of 13.19 ± 1.39 mgN%. TVB content is considered fresh when it falls within the range of 10–20 mgN% (Dalgraars, 2000, cited in Jinadasa, 2014).

Yield and pH of Fish Meal at Different Treatments

The yield and pH of fish meal processed from the heads and bones of catfish at different treatments are presented in Table 1. It shows that fish meal obtained through the treatment of formic acid and the steaming process yielded the highest value of 27.33 ± 0.48%, while the lowest yield was obtained from fish meal boiled with a 3% HCl acid solution, which amounted to 19.0 ± 0,31%. The analysis of variance results indicated that the treatments had a significant effect ($P < 0.05$) on the yield produced. The Turkey test revealed that fish meal processed with formic acid and then cooked by steaming resulted in the highest yield, significantly different ($P < 0.05$) from other treatments.

Meanwhile, the treatment of soaking in hydrochloric acid, both steamed and boiled, resulted in low yields and showed no significant difference ($P>0.05$) compared to boiled fish meal without acid treatment. On average, fish meal processed by steaming had a significantly higher yield ($P <0.05$) compared to the boiling process, except for the treatment of soaking in hydrochloric acid. Boiling in water can cause the dissolution of materials in contact with water, resulting in a decrease in the yield of fish meal produced.

On average, the yield of fish meal processed with formic acid is significantly higher ($P<0.05$) than those processed with hydrochloric acid. Boiling in hydrochloric acid solves with a pH of 4.4, which causes protein degradation from complex into simpler molecules and soluble during boiling (Amdanis et al., 2016), resulting in the lowest fish meal yield. On the other hand, boiling in formic acid solution, which is a weak acid, recorded a pH of 5.4, the isoelectric pH of fish protein. This is consistent with the findings of Margaretha (2017), who stated that the isoelectric pH of myofibril protein in nematophorus threadfin bream (*Nemipterus nematophorus*) is 5.4. The protein undergoes precipitation or coagulation at this pH and has low solubility (Novak & Havlicek, 2016). As a result, when steamed or boiled, the protein is not readily soluble leading to a higher yield compared to other treatments. The yield of the fish meal from catfish heads and bones is not significantly different from the yield of fish meal processed from trash fish, as found in the study by Assadad et al. (2015), which is 23.04 ± 0.63% when steamed and 19.95 ± 1.19% when boiled.

The pH of fish meal under different treatments can be seen in Table 1. Fish meal processed without acid (control) has a pH close to normal, while fish meal processed with acid yields a lower pH, indicating acidity. The variance analysis shows that fish meal processed without acid treatment has a significantly higher pH ($P<0.05$) than the acid-treated samples. The cooking process using boiling yields an average pH of fish meal that is not significantly different ($P>0.05$) from the fish meal cooked by steaming. Similarly, the pH of fish meal processed with formic acid does not significantly different ($P>0.05$) from those processed with HCl acid. Based on the pH values, fish meal processed with acid are suboptimal for fish meal use. For aquaculture feeding purposes, the pH of the fish meal should be normal or alkaline, ranging from 6.5 to 9. If the pH of the feed is low, the remaining feed may lead to an increase in H_2S concentration, which can be toxic to the organisms in the aquaculture system (Supriatna et al., 2020).

Table 1. Yield and pH of fish meal from heads and bones at different treatments

Treatments	Yield (%)	pH Fish Meal
Steaming (Control)	22,83 ± 0,33^{a}	6,76 ± 0,11^{a}
Boiling (Control)	19,5 + 0.8^{b}	6,86 ± 0,04^{a}
Formiat Acid Steaming	27,33 ± 0,48^{c}	5,66 ± 0,05^{b}
Formiat Acid Boiling	22,44 ± 0,71^{a}	5,78 ± 0,07^{b}
HCl Acid Steaming	19,5 ± 0,6^{b}	5,51 ± 0,06^{b}
HCl Acid Boiling	19,0 ± 0,31^{b}	5,82 ± 0,06^{b}

The results of the proximate analysis of fish meals are presented in Table 2. The chemical composition of the fish meal varies depending on the type of raw material, the quality of the raw material, the type of fish, and the processing method (Shaviklo et al., 2015). Table 2 shows that the moisture content of catfish meal processed from fish heads and fish bones ranges from 6.2 ± 0.3% to 7.9 ± 0.77%.

The variance analysis shows that fish meal processed with hydrochloric acid treatment followed by steaming resulted in the highest moisture content, significantly different from other treatments ($P<0.05$). Soaking in hydrochloric acid solution, a strong acid, causes more H^+ ions to bind to OH^+ groups, leading to more bound water (Fauziyah et al., 2017). The bonded water is difficult to evaporate after drying. Direct contact between the substance and the water-based heating medium occurs during the boiling phase. This facilitates better denaturation of proteins compared to steaming. Protein denaturation decreases water binding capacity and increases protein solubility, leading to structural changes in muscle proteins, particularly actin and myosin, resulting in the loss of their ability to bind water. Boiled fish have a lower water binding capacity than steamed fish (Lapase, 2016). During pressing, more water is released from the fish tissue cells, and when dried, the fish meal obtained from the boiling process is drier than the steamed one. According to Indonesian National Standard (SNI) No. 2715:2013, the fish meal produced from all treatments meets the SNI standards for moisture content classified as Grade 1, which is 10%.

The ash content of fish meal processed from fish heads and bones is relatively high, ranging from 27.33 ± 0.7% to 35.4 ± 0.96%. The ash content indicates the total minerals in the fish meal, such as calcium and phosphorus, which amount to 69.49 (bk) (Apitulay et al., 2020). According to Maulid et al., (2023), catfish heads and fish bones contain mineral salts such as calcium phosphate 58.3%, calcium carbonate 1.0%, calcium fluoride 1.9%, phosphate 2.1%, and protein 30.6%. These inorganic materials do not evaporate during processing, resulting in a fish meal with a high ash content. Analysis of variance results showed that fish meal processed without soaking (control) and soaked in a 4% HCl solution had significantly higher ash content ($P<0.05$) compared to other treatments. This is because boiling causes the meat attached to the bones and heads to dissolve into the boiling water. After pressing and drying, the remaining material is predominantly bone, leading to the fish flour category having a high ash content due to its ash content above 25%. However, fish meals boiled without acid treatment and soaked in hydrochloric acid exceed the quality threshold of grade 3, according to SNI 01-2715-2013, with ash content above 30%.

The protein content of the fish meal in this study ranged from 36.2 ± 0.73% to 45.5 ± 1.3%. The analysis of variance results showed that soaking the fish meal in an acid solution significantly reduced the meal's protein content ($P<0.05$) compared to the treatment without acid. The Turkey test results indicated that the treatment without acid, followed by steaming, had the highest protein content compared to other treatments. On the other hand, fish meal treated with acid showed no significant difference in protein content ($P>0.05$) whether it was cooked by boiling or steaming. Soaking in an acid solution accompanied by the heating process leads to the hydrolysis of proteins in the fish

Table 2. Proximate analysis of fish meal under various treatments

Treatments	Water Content (%)	Ash Content (%)	Protein Content (%)	Fat content (%)
Steaming (Control)	6.2 ± 0.3[b]	27.33 ± 0.7[a]	45.5 ± 1.3[a]	20.63 ± 0.58[a]
Boiling (Control)	6.5 ± 0.4[b]	31.39 ±0.14[b]	42.7 ± 1.44[b]	18.78 ± 0.31[a]
Formiat Acid Steaming	7.1 ± 0.22[b]	29.00 ± 0.15[a]	36.2 ± 0.73[c]	27.48 ± 0.79[b]
Formiat Acid Boiling	6.7 ± 0.18[b]	29.7 ± 0.3[a]	37,7 ± 0.79[c]	26.99 ± 0.56[b]
HCl Acid Steaming	7.9 ± 0,77[a]	29.43 ± 0.8[a]	39.3 ± 0.4[c]	26.36 ± 0.65[b]
HCl Acid Boiling	6.9 ± 0.27[b]	35.4 ±0.96[b]	36.33 ± 2.21[c]	22.92 ± 0.15[ab]
SNI Fish Meal 01-2715-2013				
Grade 1	Max 10	Maks 20	Maks 65	Maks 8
Grade 2	Mak 12	Maks 25	Maks55	Maks 10
Grade 3	Maks 12	MAKS 30	Maks 45	Maks 12

meat, causing them to dissolve in the acid solution used for boiling. This finding is consistent with the research by Sahril and Lakahena (2015) on the protein content of fish meal processed from the red meat of tuna fish. Fish meal boiled with water had a protein content of 81.46%, which decreased to 79.83% when boiled with 15% acetic acid. Soaking in an acid solution results in an excess of H^+ ions, which leads to the degradation of amino acids such as cysteine, tryptophan, serine, and threonine. Additionally, prolonged protein reactions with acid lead to the hydrolysis of peptide bonds, causing damage to the primary structure of the protein and resulting in a decrease in the protein content of the fish meal produced (Leninger, 1992, as cited in Sahril and Lakahena, 2015).

The protein content of the research findings is lower compared to the protein content of fish meal processed from trash fish in the study by Assadad et al., (2015), which had a protein content of 58.96%, and fish meal processed from red meat of tuna fish, which had a protein content of 81.46% (Safril & Lakahena, 2015). However, the protein content of the fish meal in this study is similar to the protein content of fish meal processed from tuna fish heads, as found in the study by Utomo & Setiawati (2013), which was 35.5%. The low protein content is in line with the research by Aman & Indarto (2018), which found that fish meal made from by-products of the fillet industry tends to have a lower protein content and a higher mineral content than fish flour made from whole fish. The raw materials of fish heads and bones in this study had a protein content of 14.98 ± 0.89%, which is lower compared to the protein content of whole catfish meat, as found in the study by Listyarini & Santoso (2019), which was 17.09%, and the study by Suryaningrum et al., (2012), which was 16.99 ± 0.78%. Based on the protein content referring to SNI-01-2715-2013 standards, the crude protein fish meal content is 65% (grade 1), 55% (grade 2) and 45% (grade 3). The fish meal produced in this study using the steaming treatment without acid treatment meets the grade 3 fish meal requirements.

The fat content of the fish meal from the treatments ranged from 18.78 ± 0.31% to 26.99 ± 0.56%. This fat content is much higher than the grade 3 fish meal requirement, which is 12%. Therefore, the fish meal processed from the heads and bones do not meet the fish meal standards according to SNI 01-2715-2013 (BSN, 2013). The high-fat content is due to the anatomical structure of the catfish head, which contains a lot of fatty tissue, significantly below the gill cover and the skin tissue beneath the head. The analysis of variance results showed that soaking the fish meal in an acid solution significantly increased the fat content ($P<0.05$) compared to the fish meal without acid treatment. Soaking in an acid solution, combined with heating, causes protein denaturation on the cell walls and breaks down these cell walls, allowing oil to quickly come out (Herman, 2015). The high-fat content of the fish meal processed from the by-products of the fillet industry leads to rancidity, making it unsuitable for long-term storage. Therefore, it is recommended to process the fish meal using the head and bone catfish when ready to used.

The calcium content of the fish meal processed from the heads and bones ranged from 2.8 ± 0.19% to 3.41 ± 0.16%. Referring to SNI 01-2715-2013 standards, the fish meal produced from various treatments meets the requirements for quality grade I regarding calcium content. The treatments applied did not have a significant effect on the calcium content. This calcium content is not significantly different from the calcium content of fish meal processed from tuna fish, which is approximately 2.92-3.03% (Irawati et al., 2014), but it is lower compared to fish meal processed from trash fish, which has a calcium content of approximately 4.13-4.36% when boiled and 4.66-5.24% when steamed (Assadad et al., 2015). According to Barus et al. (2022), calcium (Ca) is one of the essential macro minerals needed by the bodies of animals, playing a crucial role in bone and tooth formation and being involved in cellular and tissue development.

Non-protein nitrogen (NPN) refers to nitrogenous molecules in the feed that are not proteins but can be transformed into proteins by microorganisms in the animal's digestive tract. In aquaculture, non-protein nitrogen is used for protein synthesis, which can accelerate fish growth rates (Ahmad & Rusmaedi, 2005). The analysis results for non-protein nitrogen range from 0.84 ± 0.08% to 3.6 ± 0.25%. The analysis of variance showed that fish meal treated with 4% hydrochloric acid followed by steaming resulted in the highest non-protein nitrogen compound (3.6 ± 0.25%), and it was significantly different ($P<0.05$) from the other treatments. This indicates that the treatment with hydrochloric acid, combined with steaming (at a temperature of 86-97°C), causes the breakdown of proteins into compounds such as urea, peptides, amino acids, or ammonia (Fitrial, 2023).

The results of the TBA (thiobarbituric acid) analysis for fish meal under various treatments can be seen in Table 3. The TBA value measures secondary lipid oxidation products, especially those derived from polyunsaturated fatty acids (PUFA). It indicates the level of rancidity, particularly in fats containing high levels of PUFA. This rancid odor is caused by the formation of compounds resulting from the breakdown of hydroperoxides (Husain et al., 2017). In Table 3, it can be observed that fish meal processed without soaking in an acidic solution and cooked by boiling has a TBA content of 2.11 ± 0.25 mgMA/g while steaming the fish meal results in a TBA content of 2.62 ± 0.44 mgMA/g. The analysis of variance showed that the treatment without acid soaking yields fish meal with a higher TBA content. The Turkey test indicated that the cooking treatment does not significantly affect the TBA level in the resulting fish meal. This suggests that without soaking in an acidic solution, the fat in the fish meal is more readily hydrolyzed or oxidized, producing compounds such as hydrocarbons, ketones, epoxides, and monaldehydes, which contribute to the increased rancidity of the product (Gunsen et al., 2011).

On the other hand, the steaming process produces fish meal with a slightly higher TBA value than boiling, although this difference is not statistically significant. This can be attributed to the fat content obtained in the fish meal (Table 1). A product is considered rancid if it has a TBA value higher than 3 mgMDA/kg (Gunsen et al., 2011). This level is slightly higher when compared to the TBA content of a fish meal processed with 4% formic acid and soaked in hydrochloric acid (Table 3). Fish meal processed by boiling causes some fats to dissolve into the water medium during the boiling process.

Table 3. Calcium, Nitrogen non Protein, and Tribarbituric acid content of fish meal at various treatments

Treatments	Calcium (%)	NPN (%)	TBA (MgMA/kg)
Steaming (Control)	2.8 ± 0.19[a]	2.5 ± 0.3[a]	2.62 ± 0.44[a]
Boiling (Control)	3.32 ± 0.24[a]	1.69 ± 0.13[b]	2.11 ± 0.25[a]
Formic Acid Steaming	2.92 ± 0.04[a]	1.21 ± 0.09[b]	0.87 ± 0.11[b]
Formic Acid Boiling	3.22 ± 0.31[a]	0.84 ± 0.08[b]	0.71. ± 0.08[b]
HCl Acid Steaming	3.21 ± 0.02[a]	3.6 ±0.25[c]	1.46 ± 0.37[b]
HCl Acid Boiling	3.41 ± 0.16[a]	2.19 ± 0.06[c]	1.05 ± 0.17[b]
SNI Fish Meal 01-2715-2013 for Calsium			
Grade 1	2.5 – 5.0		
Grade 2	2.5 – 6.0		
Grade 3	2.5 – 7.0		

Microbiological Quality of Fish Meal

The results of the microbiological analysis for fish meals regarding TPC (total plate count), mold, and Salmonella are presented in Table 4. The total plate count of bacteria in fish meal processed from the head and bones of catfish from all treatments is relatively high, reaching 10^5 CFU/mL (log 5). The mold content ranges from 10^2 to 10^3 CFU/mL (log 2- log 3), while the presence of *Salmonella* sp. is negative. The analysis of variance shows that the acid treatment affects the decrease in the total plate count (TPC) of bacteria produced, except for the steamed acid-formiate treatment. The high bacterial content in the fish meal produced in this study is due to the cooking process for 30 minutes, which aims not only to kill microbes and inactivate enzymes but also to obtain a better fish meal aroma, a softer texture, and better protein digestibility (Candra et al., 2022). However, it was unable to kill heat-resistant bacterial spores.

During drying, these bacterial spores develop and recontaminate the sun-dried fish meal. Fish meal before

Table 4. The results of the total plate count (TPC) of the bacteria, Salmonella, and mold

Treatments	TPC	Salmonella	Mold
Steaming (Control)	log 5.852[a]	Negatif	Log 2.429[a]
Boiling (Control)	Log 5. 865[a]	Negatif	Log 2.255[a]
Formic Acid Steaming	Log 5.259[c]	Negatif	Log 3.253[b]
Formic Acid Boiling	Log 5.735[b]	Negatif	Log 2.328[a]
HCL Steaming	log 5.720[b]	Negatif	Log 3.785[b]
HCL Boiling	Log 5.712[b]	Negatif	Log 2.145[a]

drying is the semi-wet nature of the product, rich in protein with a high moisture content, which provides a suitable substrate for bacterial growth, especially spoilage bacteria from the environment (Rahmi et al., 2021). The relatively high bacterial content can cause the fish meal to spoil, thereby affecting its color, texture, and odor. According to the Indonesian National Standards, Fish products are safe if the total plate count (TPC) does not exceed $5x10^5$ CFU/gram. Fish Meal is considered spoiled if the bacterial content exceeds 10^6–10^8 CFU/gram (Arimbi, 2021).

The content of Salmonella bacteria in fish meal processed from the heads and bones of catfish under various treatments showed negative results. This is in line with the requirement for fish meal quality according to SNI 01-2715-2013, which states that fish meal should not be contaminated with Salmonella or have a negative Salmonella content. Salmonella is the leading cause of foodborne illnesses. Typically, Salmonella serotypes cause diseases in the digestive organs, leading to diarrhea in livestock within 8–72 hours after consuming food contaminated with Salmonella (Ekawati & Yusmiati, 2018).

The treatments applied affect the number of mold colonies in the fish meal. The analysis of variance showed that the treatments significantly influenced (P<0.05) the mold content in the resulting fish meal. The Turkey test results indicated that the highest mold content in the fish meal was obtained from fish meal processed with hydrochloric acid and cooked by steaming, which was significantly different (P<0.05) from other treatments and fish meal processed with formic acid and cooked by steaming. The high mold content in these two treatments is likely due to their growth at a pH suitable for molds. The pH of fish meal processed with formic acid and hydrochloric acid, then steamed, was measured as pH 5.66 and 5.51, respectively (Table 1). The pH range falls within the optimum pH for fungal growth, which is around 5.0 if nutritional requirements are met, and mycelium growth and spore formation occur at pH 5.5 (Deshmukh et al., 2012). The presence of mold is suspected to occur during the drying of fish meals in sunlight. An open environment, the nutritional content of fish meals, and a relatively high moisture content before drying trigger mold growth (Suryani et al., 2020). The presence of mold can break down organic matter into more stable compounds, affecting the aroma and color of the resulting fish meal. Additionally, there is concern that the presence of mold may lead to the presence of aflatoxins in fish meal, which are not allowed for use as animal feed (Anggraini et al., 2019).

Sensory Evaluation of Fish Meal

Sensory evaluation of fish meal was conducted based on color, texture, aroma, and the presence of foreign matter/mold. Good-quality fish meal, according to SNI 01-2715-2013, should have a bright color, clean brown to grayish, a strong and specific aroma, delicate texture, uniform particle size, absence of mold, and free from bone fragments and foreign objects (Sugiantoro & Gidajati, 2013). The color of fishmeals is typically influenced by the type of fish used and the processing method. Table 5 shows the sensory observations of appearance/color, aroma, and texture.

The panelists rated the appearance of fish meal in various treatments ranging from 6.88±0.71 to 7.56±0.51. The panelists delivered a higher rating for the appearance of boiled fish meal, which was significantly different (P<0.05) compared to fish meal processed by steaming. However, the acid treatment did not significantly affect the appearance of the resulting fish meal. Boiling will cause the black pigments in the fish skin to undergo hydrolysis, resulting in a lighter color. Additionally, the boiling process triggers a browning reaction, producing fish meal with a brighter brown color than fish meals processed by steaming. Fish meal processed by steaming yields a grayish-brown color. The grayish color of the fish meal comes from the black fish skin, which contains melanin pigments that are not lost when soaked in an acid solution. Meanwhile, the brown color arises from the browning reaction, which can affect the appearance of the products. This occurs due to the reaction between proteins, peptides, and amino acids with decomposed fat by-products (Sipayung et al., 2015).

Good-quality fish meal should have a strong and specific aroma. The panelists gave higher ratings for the aroma of fish meal treated with acid (7.25 ± 0.44 to 7.56 ± 0.51), which was significantly different (P<0.05) compared to fish meal processed without acid (control), which had an average rating of 6.93. The soaking in acid solution prevents the interaction of trimethylamine oxide with the double bonds of unsaturated fats, which produces a fishy odor. As a result, the pleasant aroma of fish meal becomes more pronounced (Andaka, 2009). This is consistent with the findings of Assadad et al. (2015), who found that acid treatment in processing fish meal from trash fish resulted in a less pungent fishy aroma (Assadad et al., 2015). Although this study had a high bacterial content in the fish meal, it did not affect the aroma of the resulting fish meal. The presence of microorganisms does affect aroma changes in fish meal, especially those with high moisture content (Solihin et al., 2015).

The texture of a fish meal should be dry, fine, non-clumping, and highly influenced by its moisture, protein, and fat content (Wirawan et al., 2018). The panelists gave higher ratings for fish meal processed without acid treatment, which was significantly different ($P<0.05$) compared to fish meal processed with acid. Fish meal without acid treatment exhibited a soft, fine-grained, dry texture that did not clump easily. The acid treatment in the processing resulted in a smooth texture with small clumps due to its high-fat content (Table 1). On average, the panelists rated the texture of boiled fish meal higher (7.08) than steamed fish meal (6.70). This is because boiled fish meal has finer grains and a lower moisture content, resulting in a drier and non-clumping texture (Anggriani et al., 2019).

Observations regarding the foreign matter, specifically the presence of mold, showed that the panelists rated 9 (nine) for all treatments, indicating the absence of mold and other foreign matter in the resulting fish meal. Although microbiological observations found the presence of mold with a count of 10^2-10^3 CFU/g, visually, no visible mycelium was observed, indicating that it did not cause sensory changes in the fish meal. The minimum value for sensory evaluation of fish meal, according to SNI 01-2715-2013, is 6 (six). Based on the assessment by the panelists, the fish meal processed from fish heads and bones under various treatments met the sensory requirements according to SNI 01-2715-2013.

Amino Acid Profile Fish Meal from a by-Product of Catfish

Fish meal was used as a source of nutrition for the growth of fish/livestock in their feed due to its protein content. The quality of protein in fish meal is determined by the completeness and balance of the amino acids it contains (Hamid et al., 2016). Fish and livestock continuously require the amino acids in the feed for cell growth and tissue formation (Pratama et al., 2019). The amino acid profile analysis of fish meal from fish heads and bones, processed without soaking and steamed, showed a protein content of 45.5±1.3%, compared to a commercial fish meal with a labeled protein content of 65%. Table 6 shows the amino acid profile of the fish meal. It can be seen fish meal processed from catfish heads and bones has a better amino acid composition than commercial fish meal. The fish meal processed from catfish heads and bones has a complete profile of essential amino acids with a higher quantity of 483.8 mg/g compared to only 39.4 mg/g of essential amino acids in commercial fish meal. Meanwhile, the amount of non-essential amino acids in the fish meal from catfish heads and bones is significantly higher at 709.6 mg/g compared to 63.28 mg/g in commercial fish meal. This amino acid profile is also superior to the amino acid composition of fresh catfish meat, as found in the study by Oluwaniyi et al., (2017), which reported an essential amino acid content of 292 mg/g. The lower quality of amino acids in commercial fish meal is likely due to the use of low quality raw materials, degradation during distribution and marketing, processing methods, and the presence of spoilage bacteria, which result in a significantly

Table 5. Sensory evaluation analysis of fish meal at various treatment

Treatments	Appearance	Aroma	Texture
Steaming (Control)	6.89 ± 0.72^b	7.0 ± 0.63^a	7.37 ± 0.5^a
Boiling (Control)	7,38 ± 0.61^a	6.87 ± 0.34^a	7.62 ± 0.5^a
Formic Acid -Steaming	6.75 ± 0.58^b	7.31 ± 0.47^b	6.25 ± 0.57^b
Formic Acid Boiling	7.38 ± 0.72^a	7.56 ± 0.51^b	6.31 ± 0.79^b
HCL Acid Steaming	6.88 ± 0.71^b	7.43 ± 0.51^b	6.35 ± 0 89^b
HCl Acid Boiling	7.56 ± 0.51^a	7.25 ± 0.44^b	7.35 ± 0.47^a

lower amino acid profile in commercial fish meal. Meanwhile, fish meal processed from fish heads and bones is processed from fresh fish (TVB 13.19±1.39 mgN%).

The catfish meal produced contains a complete profile of essential amino acids such as isoleucine, leucine, lysine, methionine, threonine, phenylalanine, valine, histidine, and tryptophan in sufficient quantities, with higher levels compared to the Amino acid standards set by FAO for human nutritional needs as seen in Table 6 (Egayanti et al., 2019).

The body cannot synthesize essential amino acids for metabolic purposes. Therefore, essential amino acids need to be supplied through feed to support optimal productivity of fish/livestock, as the completeness of essential amino acids greatly determines the protein quality of feed ingredients used in the diet (Andri et al., 2020). Similarly, the amount of non-essential amino acids, which account for 594.6 mg/g of the total, is higher than the essential amino acids, which account for 405.3 mg/g. In a feed ratio, approximately half of the required amino acids are non-essential, while the rest is supplied by essential amino acids (Nte & Gunn, 2021). Thus, the composition of essential and non-essential amino acids in the fish meal processed from catfish heads and bones is well-balanced. The fish meal from catfish heads and bones also meets the requirements for fish and livestock feed rations that require 10 Amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, as well as containing cysteine

Table 6. The amino acid profile of fish meal processed from catfish heads and bones was compared with commercial fish meal

No	Parameter	Fish meal from the project	Commercial fish meal	Standard of FAO/ WHO 2013
	Essential Amino Acid			
1	L- Phenilalanine (mg/g)	65.7 ± 0.12	5,89 ± 0,02	41*
2	L- Valine (mg/g)	61.0 ± 0.06	5.04 ± 0.00	40
3	L-Tryptophane (mg/g)	10.6 ± 0.03	0.73 ± 0.000	6.6
4	L Threonine (mg/g)	70.9 ± 0.17	5.63 ± 0.01	25
5	L- Isoleusine (mg/g)	54.2 ± 0.1	4.46 ± 0.01	30
6	L- Methionine (mg/g)	14.44 ± 0.01	0.31 ±0.00	23**
7	L Histidine (mg/g)	36.9 ± 0.03	3.28 ± 0.00	16
8	L- Lysine (mg/g)	74.1± 0.08	6.18 ± 0.00	48
9	L- Leucine (mg/g)	96.0 ± 0.25	7.63 ± 0.02	61
	Total (mg/g)	483,8	39.34	
	% Essential Amino Acid	40.53	38.65	
	Non Essential Amino Acid			
1	L- Alanine (mg/g)	23. 4 ± 0.12	6.62 ± 0.03	
2	L-Arginine (mg/g)	113.1 ± 0.44	7.95 ± 0.02	
3	L-Aspartat acid (mg/g)	90. 6 ± 0.12	9.31 ± 0.04	
4	L-Glutamic acid (mg/g)	142.2 ± 0,21	13.36 ±0.05	
5	L-Serine (mg/g)	64.78 ± 0.17	5.17 ± 0.05	
6	L-Glycine (g/mg)	123.83 ± 0.21	9.93 ± 0.04	
7	L-Proline (mg/g)	69.0 ± 0.10	5.79 ± 0.03	
8	L-Sistin (mg/g)	36.93± 0.00	1.85 ± 0.00	
9	L- Tyrosine (mg/g)	45.95 ± 0.05	2.46 ± 0.00	
	Total (mg/g)	709.60	62.38	
	% Non Esensial Amino Acid	59,46	61.34	

Note : * Phenilalanine +Tyrosin ** Metheonine + Serine and tyrosine according to the nutritional needs of fish that stimulate fish growth (NRC, 2011).

Lysine is used as an indicator to determine protein quality because it is one of the amino acids that are not heat-stable during cooking processes. Lysine is one of the four primary limiting amino acids in feed rations that promote growth and prevent fin decay and mortality in fish. Lysine and methionine provide an excellent response to specific growth rates (Salama et al, 2013). The other limiting amino acids are methionine, tryptophan, and cysteine. According to Agustono (2019), the lysine requirement for fish ranges from 4-6% of the dietary protein, while omnivorous fish require 2.07%. The lysine requirement for tilapia feed (*Sarotherodon mosambicus*) is 3.8%, African catfish is 5.7%, and channel catfish is 5.0-5.1% (Meilisza and Subamia, 2016). The addition of 3% lysine and methionine at a 1:1 ratio could improve fecundity, enhance immunity, and accelerate growth in mangrove crabs (Alissianto, 2017). Therefore, the fish meal processed from by-products such as catfish heads and bones, which have a lysine content of 7.4 g/100g, could fulfill the dietary requirements for cultured fish.

Methionine is an essential amino acid that is highly necessary for cultured fish and livestock growth rate. Methionine plays a crucial role in the growth rate and the overall well-being of all animals. It serves as a precursor for cysteine and other sulfide-containing compounds. Methionine is required for nucleic acid, tissue formation, and protein synthesis. It is also involved in the synthesis of other amino acids (such as cysteine) and vitamins (such as choline) (Andri et al., 2020). Research has shown that the formation of breast meat in chickens is highly influenced by lysine and methionine in their diet. Studies by Dita et al., (2021) demonstrated that supplementation of 1% lysine and 0.5% methionine can increase weight gain in various parts of the carcass, including the breast, wings, thighs, and back. In another study by Emu et al., (2022), the addition of 0.75% methionine to commercial feed resulted in a growth rate of 1.17% + 0.06% in *Nile tilapia* compared to the control group, which only achieved 0.85% + 0.06%. Therefore, fish meal processed from fish heads and bones containing 1.44 g/100 g of methionine can be used as an adequate ingredient to formulate feed rations for fish and livestock.

Tryptophan and the non-essential amino acid cysteine in catfish head and bone meal are at 1.06% and 6.47%, respectively. The addition of tryptophan to feed as a precursor for serotonin biosynthesis can help alleviate depression and aggressive behavior in fish, with a recommended dosage of 1-3 g/day (Rakhmawati et al., 2021). A study by Kumar et al. (2017) recommended 0.5% tryptophan in feed to reduce cannibalism and improve survival rates in Asian seabass (*Lates calcarifer*) juveniles. Cysteine is a non-essential amino acid that must be present in fish and livestock feed rations. Cysteine is an amino acid that contains sulfur and influences protein metabolism, similar to other amino acids. Insufficient cysteine in animal feed can reduce protein synthesis and slow animal growth. According to Nte and Gun (2021), the main ingredients used in poultry nutrition are the Amino acids methionine and cysteine. The optimum balance of the methionine-to-cysteine ratio in animal feed is between 52:43 and 49:45 from 3% used in the feed ration. This ratio ensures the efficient utilization of amino acids for proper growth and development, particularly in poultry. Based on the methionine and cysteine content, fish meal processed from fish heads and bones meets the requirements as a nutritional ingredient for poultry feed.

Conclusion

Based on the results of this study, among various acid soaking and cooking methods, the best treatment for fish meal processed from the heads and bones of catfish by-products of fish fillets is steaming without acid treatment. The fish flour produced complies the standards according to SNI 01-2715-2013 as grade 3 fish meal. The analysis of the amino acid profile of the best-treated fish meal showed that it has a higher composition of essential and non-essential amino acids than commercial fish meal. Therefore, it is suitable to be used as a material for fish/poultry feed as a source of nutrition for fish and livestock in sufficient quantities and contains better amino acid performance compared to commercial fish meal.

Supplementary Materials

Supplementary materials is not available for this article.

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