Amino acid content in *black soldier fly* maggot with trypsin and acid hydrolysis method

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Abstract. The Black Soldier Fly (*Hermetia illucens*) maggot is one of the abundant insect commodities in nature, with high protein content and diverse amino acids required by livestock. This research aimed to identify the amino acid content of BSF maggots using different hydrolysis methods, namely, chemical and enzymatic. The analysis included the identification of maggot nutritional content through proximate analysis, enzymatic hydrolysis with trypsin, chemical hydrolysis with HCl solution and amino acid identification using HPLC analysis. The research results indicated that the BSF maggot used in this study contained 29.36% protein. The extraction and hydrolysis processes broke down the protein into amino acids that were detectable in the HPLC instrument. Hydrolysis using trypsin produced amino acids with glutamic acid (1.54 mg/g) being the most abundant, followed by serine (0.86 mg/g) and aspartic acid (0.75 mg/g). Hydrolysis using HCl solution yielded amino acids with the highest concentration, where glutamic acid (10 mg/g) was the most abundant, followed by leucine (8.1 mg/g) and aspartic acid (7.5 mg/g). The highest total concentration of amino acids was obtained from HCl hydrolysis, amounting to 74.2 mg/g of maggots. In conclusion, the chemical hydrolysis process using HCl resulted in a higher quantity of amino acids compared to enzymatic hydrolysis with trypsin.

1 Introduction

One of the high operational cost components in livestock farming is related to feed provision. According to Tumion *et al* [1], more than 70% of the operational costs in livestock farming are allocated to raw materials for animal feed formulation. The availability of quality and nutritious feed is a key factor in the success of the livestock sector [2]. In addition to utilizing natural forage, farmers have been relying on manufactured feed, the cost of which continues to rise. Therefore, farmers must be more intelligent in selecting high-quality feed ingredients at affordable costs to maintain economic stability in their livestock products. Efforts to discover and use alternative feeds are continuously being developed, one of which involves utilizing insect commodities.

Maggots, also known as the Black Soldier Fly (BSF) (*Hermetia illucens*) larvae, are insect commodity that is currently studied due to the nutritional potencies. According to Rachmawati *et al* [3], maggots have all the criteria necessary for animal feed: they have

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fulfilled nutritional composition, competitive pricing, and are abundantly available due to their metamorphic life cycle, making them relatively easy and inexpensive to cultivate. One essential component of high-quality animal feed formulation is protein. Mokolensang *et al* [4] reported that maggots contain a relatively high protein content, approximately 44%, making them suitable as feed ingredients.

The protein obtained from the feed is digested by livestock bodies into simpler forms, namely amino acids. Some types of amino acids are essential that required by livestock bodies, critical yet cannot be synthesized by their bodies, thus must be available in the provided feed. The balance of amino acid intake in livestock has an impact on the quality of the livestock products, such as egg quality in poultry [5] and fish [6], milk production in dairy animals [7], and the quality or flavor of the livestock meat [8]. Feed deficient in essential amino acids can result in poor feather growth and increased carcass fat accumulation in livestock animals [9].

BSF maggots are known contain a diverse range of amino acids, some of which are essential amino acids that crucial for livestock growth and metabolism, such as lysine, methionine, threonine, and tryptophan [8]. Studies by Dita *et al* [10] respectively indicated that supplementing the feed ration with lysine and methionine significantly influenced the weight gain of broiler and village chickens. Threonine, a key essential amino acid, plays an important role in the synthesis of muscle proteins and impacts livestock growth. Tryptophan is a precursor for serotonin synthesis, reducing livestock stress levels and consequently impacting meat quality [8].

One way to obtain amino acids from BSF maggots is through protein hydrolysis. This process utilizes acid, base, or proteolytic enzymes to break proteins down into amino acids and peptide fragments. Identifying the best hydrolysis method is necessary to determine which way proteins can be broken down into amino acids efficiently, because the advantages and disadvantages of each method can produce final products with a different balance of quality and quantity. Enzymatic hydrolysis is based on the proteolytic enzyme ability like trypsin to digest and break down proteins into amino acids in the digestive [11], while chemical hydrolysis generally involves the use of strong acid solutions heated at high temperatures to accelerate the breakdown of chemical bonds between compounds [12]. Enzymatic hydrolysis is generally preferred in terms of safety and environmental impact, but chemical hydrolysis also holds similar potential for success [12]. Hence, the present study aimed to isolate the amino acid content in BSF maggots with different hydrolysis treatments, namely enzymatic and chemical (acid) hydrolysis.

2 Method

2.1 Sample Preparation

The samples used were maggots or BSF larvae. First, the maggots were sorted, selecting those with aged 10 days and still white in color that indicate the traits of the larval stage in BSF. The maggots were then thoroughly washed, drained on tissue paper until residual water was absorbed, and subsequently frozen in a freezer at -20°C for 24 hours. Afterward, the ice-covered maggots were thawed at room temperature and dried in an oven at 55°C for 24 hours. The dried maggots were then pulverized using a blender to obtain maggots powder, which would be utilized for the subsequent analysis [13].

2.2 Proximate Analysis

Proximate analysis on the sample was assessed to determine the content of moisture, ash, total protein, total fat, total carbohydrate, and total calories by following the AOAC procedure [14]. In brief, the determination of moisture content involved heating the sample in an oven at 105°C for 6 hours. The moisture content was obtained from the difference in sample weight before and after heating. The determination of ash content involved burning the sample in a furnace at 500°C for 3 hours. The ash content was obtained from the ratio of the final sample weight to the initial sample weight. The total protein content was assessed by employing the Kjeldahl method, involving several procedural steps such as digestion, distillation, and titration. The protein content was obtained from the percentage of nitrogen obtained during the titration process. The fat content was measured using an extraction procedure with Soxhlet to obtain the weight of dried fat. The amount of total carbohydrates was computed by subtracting the sum of the percentages of moisture, ash, protein, and fat from 100 percent. The measurement of total calories was determined by the formula summing 4x the protein content, 9x the fat content, and 4x the carbohydrate content.

2.3 Protein Extraction

A total of 10 grams of maggot powder were dissolved in 100 mL of distilled water, then incubated at 4°C and homogenized at a speed of 100 rpm for 10 minutes. Subsequently, the sample was centrifuged at 3000 rpm for 10 minutes at 4°C. The obtained supernatant was centrifuged again at 14000 rpm for 10 minutes at 4°C produced filtrate or supernatant that analyzed for its amino acid content using HPLC and pellet that resuspended in 100 µL of distilled water, resulting in the protein extraction product for use in trypsin hydrolysis [15-17].

2.4 Trypsin Hydrolysis

The pellets obtained from the previous centrifugation were further subjected to trypsin digestion. The sample was supplemented with 200 µg/L trypsin, dissolved in the pellet along with distilled water to reach a volume of 50 mL, and homogenized. Then, the sample was incubated at 37°C for 2 hours with a speed of 120 rpm. Furthermore, Na_2CO_3 was added with a weight ratio of 1:1 (trypsin: Na_2CO_3). Subsequently, the sample was centrifuged at 10000 rpm for 15 minutes at 4°C. The obtained filtrate was analyzed for the amino acid profile using HPLC [18].

2.5 Acid Hydrolysis

Prepared maggot samples were hydrolyzed using a 500 mL Florence flask and 400 mL of 6 M HCl solution in a steam bath at temperature of 110°C. Approximately 10 grams of samples, were added sequentially until the desired amount (100 grams) was reached. Heating was continued until the sample dissolved. After the maggot sample dissolved, further high-temperature heating (reflux) was performed for approximately 5 hours. The entire sample heating process was conducted in an acidic environment. A small portion of the sample was taken for the biuret test. If the biuret test yielded a negative result, activated carbon was added to the sample (6 grams) to remove the color, and it was allowed to sit for 24 hours. After 24 hours, the sample was decanted to separate the activated carbon from the clean sample. The sample was then filtered using a Buchner funnel, and the insoluble solid residue was rinsed twice with a small amount of distilled water.

The formed solid was dissolved in 20 mL of 40% NaOH while stirring. The solution was then added to the Congo red indicator and titrated with 6 M HCl until the solution turned blue. The sample was stored for 3 days at room temperature (27°C). The solid and liquid were separated using a Buchner funnel, and the solid was dissolved in 150 mL of 1 M HCl. The solution was added to 4 grams of activated carbon and left to sit for 24 hours. The solution was filtered using a Buchner funnel, and the filtrate was collected. The solution was tested with Congo red. If the result was negative (blue), the solution was subjected to isoelectric pH separation to determine the ratio of HCl and NaOH used. After titration, the solution was stored at room temperature for 24 hours. The precipitated amino acids were filtered, rinsed, and dried [19].

2.6 Amino Acid Analysis using HPLC

The maggot powder was dissolved in 5 mL of 0.01 N HCl, and combined with a 10.4 pH potassium borate solution at a 1:1 ratio. The solution was then placed in an empty vial (50 µL) and added with 250 µL of ortho-phthalaldehyde (OPA) reagent, then left for 1 minute. The sample was introduced into the HPLC column with a 5 µL injection, and the amino acids were allowed to separate for about 25 minutes with fluorescence detector and an amino acid column [14]. Concentration (µmol amino acid) and percentage of amino acid were calculated using the following formula:

$$\text{Concentration of amino acid} = \frac{\text{Sample Peak Area x Standard concentration Peak}}{\text{Peak area of standard}} \quad (1)$$

$$= \frac{\text{Sample Peak Area x 0.5 µmol/mL x 5 mL}}{\text{Standard Peak Area}}$$

Amino acid Percentage = µmol of amino acid x Amino Acid Mr Value x 100 µg of test sample (2)

3 Results

3.1 Nutrient Content of BSF Maggot

According to the proximate analysis, the maggots powder in this study had a low moisture content of 4.76% and a notably high ash content of 11.66%. The largest nutritional content of the maggot was fat (50.47%) followed by protein 29.36% and carbohydrates 3.74%. The total calories in 100 grams of maggot amounted to 604.63 calories, (Table 1).

Table 1. Nutrional content of BSF maggot

Testing	Mean
Moisture	4.76 ± 0.10 %
Ash	11.66 ± 0.48 %
Total Protein	29.36 ± 1.91 %
Total Fat	50.47 ± 1.06 %
Total Carbohydrate	3.74 ± 3.74 %
Total Calories	**604.63 calories / 100 gram**

3.2 Amino Acid Content of BSF Maggot from Protein Extraction and Trypsin Hydrolysis

The first group of amino acids is obtained from the extraction process which is the first step of the isolation process. Seventeen types of amino acids were identified, comprising 8 non-essential amino acids that can be synthesized by the body and 9 essential amino acids that need to be supplied through the feed [20]. Glutamic acid exhibited the highest concentration of non-essential amino acids at 1.4 mg/g in maggots, with serine and aspartic acid following at 0.8 mg/g and 0.7 mg/g, respectively. For essential amino acids, arginine and leucine had the highest concentrations, both at 0.5 mg/g, while valine, phenylalanine, and isoleucine each had a concentration of 0.4 mg/g. The total amount of amino acids detected from the protein extraction was 8.1 mg/g. (Table 2).

The second group of amino acids is obtained from enzymatic hydrolysis using trypsin. In this group, the amino acid identification results indicated that glutamic acid was present in the highest concentration of 0.14 mg/g among non-essential amino acids, followed by serine at 0.06 mg/g, and aspartic acid and tyrosine both at 0.05 mg/g. On the other hand, leucine was the most concentrated essential amino acid at 0.05 mg/g, with arginine, valine, and phenylalanine each showing a concentration of 0.03 mg/g. The total amount of amino acids identified in this process was 0.63 mg/g (Table 2).

Table 2. Amino Acid Content of BSF Maggot from Protein Extraction and Trypsin Hydrolysis

Amino Acid Group	Amino Acid Types	Concentration (mg/g)	
		Protein Extraction	Trypsin Hydrolysis
Non-Essential	**Glutamic Acid**	**1,40**	**0,14**
	Serine	**0,80**	**0,06**
	Aspartic Acid	**0,70**	**0,05**
	Alanine	0,50	0,03
	Tyrosine	0,50	0,05
	Glysine	0,40	0,03
	Proline	0,40	0,03
	Cysteine	0,20	0,02
Essential	**Arginine**	**0,50**	**0,03**
	Leucine	**0,50**	**0,05**
	Valine	**0,40**	**0,03**
	Phenylalanine	**0,40**	**0,03**
	Isoleucine	0,40	0,02
	Lysine	0,30	0,02
	Methionine	0,30	0,02
	Histidine	0,20	0,01
	Threonine	0,20	0,01
	Total	**8,10**	**0.63**

3.3 Amino Acid Content of BSF Maggot from Acid Hydrolysis

The third amino acid group of this study was obtained from acid hydrolysis using 6 M HCl solution. Amino acid analysis from this acid hydrolysis process indicated that glutamic acid was present at the highest concentration of 10 mg/g, with aspartic acid and alanine following at 7.5 mg/g and 6.8 mg/g, respectively, in the group of non-essential amino acids. Meanwhile, leucine at 8.1 mg/g, lysine at 5 mg/g and threonine at 4.6 mg/g were the three amino acids with the highest concentration in the essential amino acid group. The total amino acids identified in this process reached 74.2 mg/g (Table 3), which was higher than in the protein extraction and enzyme hydrolysis processes.

Table 3. Amino Acid Content of BSF Maggot from Acid Hydrolysis

Amino Acid Group	Amino Acid Types	Concentration (mg/g)
Non-Essential	**Glutamic Acid**	**10,00**
	Aspartic Acid	**7,50**
	Alanine	**6,80**
	Glycine	4,80
	Serine	4,40
	Proline	3,10
	Tyrosine	1,80
	Cysteine	0,40
Essential	**Leucine**	**8,10**
	Lysine	**5,00**
	Threonine	**4,60**
	Isoleucine	3,90
	Valine	3,80
	Phenylalanine	3,30
	Arginine	3,00
	Histidine	2,50
	Methionine	1,20
	Total	**74,20**

4 Discussion

4.1 Nutrient Content of BSF Maggot

Proximate analysis was used to evaluate the characteristics and nutritional content of BSF maggots, focusing on their moisture, ash, protein, fat, carbohydrate levels, and total caloric content. Moisture and ash content of maggot powder in this study almost same as reported by Saputra and Lee [21] who obtained results of 6.56% and 11.97% respectively. Measurement of moisture and ash content applies the gravimetric method with the principle of drying to remove water molecules and organic minerals in simplisia. Moisture content and ash content in a material indicate the shelf life and characteristics of a material [22]. The tolerable standards for moisture content and ash content of simplisia materials are less than 10% and 17%, respectively [23]. High moisture content can accelerate the growth of microorganisms that can damage the physical and chemical properties of the material, while high ash content indicates the content of inorganic minerals and foreign materials in a simplisia [24].

Previous studies reported that BSF maggot contains 4.8% - 36.2% fat with protein content ranging from 30% - 48.2% [21]. As the age of maggot increases, the protein content of maggot will decrease while the fat content will increase [25]. The carbohydrate content of maggot in previous studies ranged from less than 1% [26]. The total calories in this study are smaller than the results of research by Suparman *et al* [27] which reported total maggot calories of more than 2000 calories/100g maggot. The nutritional content of maggot varies depending on the feed media and the age of the maggot [28].

Protein plays an important role in the nutritional needs of livestock and fish. Pas *et al* [29] stated that at least livestock need 20% protein from the total feed given, while according to Manik *et al* [30], about 50% of the calories needed by fish come from protein, especially for young fish.

4.2 Amino Acid Content of BSF Maggot

Amino acids in this study were obtained from three separate processes. The first of these processes involved the extraction of maggot protein using centrifugation. This process involves the mechanical breaking of cells to release proteins from their biological material, then homogenized through a centrifugation process for sedimentation separation between proteins and other molecules based on their molecular weight and density [31]. The second sample used in this process is the precipitate or pellet from the second centrifugation step in the protein extraction process. The precipitate was resuspended and centrifuged again to obtain the supernatant, then assessed for their amino acid breakdown through the trypsin enzyme activity on the maggot protein.

This study uses trypsin derived from the pancreas of cattle with the brand Himedia, as an enzyme that replaces the role of digestive enzymes in the body of livestock to break down protein molecules into amino acids, so that they can be digested by the body [32]. Trypsin is produced in the form of inactive trypsinogen or often called zymogen, which is the basis of the proteolytic process. Trypsin has a very specific mode of action by breaking peptide bonds on the C-terminal side of lysine (K) or arginine (R) residues, except when followed by proline (P) [33]. Enzyme hydrolysis in terms of health and environment is preferred because it does not produce harmful residues, but with a very specific mechanism of action and reaction, making the results of enzyme hydrolysis vary greatly [34]. The amino acid content of the protein extraction results and after trypsin hydrolysis were compared to identify the free amino acid content in BSF maggot. According to this study, BSF maggots have a higher content of amino acids that are readily digestible by the body when only extraction is performed, rather than using enzymatic trypsin treatment [35].

The third amino acid group of this study was obtained from acid hydrolysis using 6 M HCl solution heated at high temperature using the reflux method. The principle of hydrolysis using acidic solutions refers to the ability of acidic solutions to cut compound bonds randomly with the help of high temperatures. The acid hydrolysis process is easier to control in terms of temperature, pH and time, making it easier to modify the process as needed [34]. However, wise action is still needed to treat the residue or waste solution produced.

The highest amino acid content obtained from all three processes, be it extraction, enzyme hydrolysis and acid hydrolysis, was glutamic acid. This is in accordance with what was reported by Magalhães *et al* [36] and Widianingrum *et al* [37] which stated that the highest amino acids in maggot flour are glutamate and aspartate from the non-essential amino acid group as well as leucine and arginine from the essential amino acid group.

The diversity of amino acids possessed by maggots has the potential to be an alternative to additional nutrition for livestock and fish. Maslami *et al* [17] reported that feeding glutamic acid to broiler chickens reduced abdominal fat and improved meat texture by reducing red bruising on carcasses. The high content of glutamic acid in chicken meat can also increase the umami flavor of broiler meat [39]. Aspartic acid, which is also highly concentrated in maggot, can improve animal growth performance [40]. Meanwhile, leucine deficiency in livestock can result in growth retardation as leucine is required to stimulate protein synthesis in muscles [41]. Feed supplementation with lysine and methionine significantly affects weight gain in chickens [10], Vaname shrimp growth [3] as well as energy retention in pomfret [42]. Threonine plays an important role in protein synthesis in muscle, thus can affect livestock growth. Tryptophan is a precursor for serotonin synthesis, reducing stress levels in livestock and ultimately affecting meat quality [8]. Arginine plays a role in improving beta (β) cell function and energy metabolism as well as insulin sensitivity for poultry [37].

5 Conclusion

The amino acids from BSF maggot were successfully isolated through enzymatic and chemical hydrolysis. In terms of quantity, chemical hydrolysis using HCl solution produces a greater yield of amino acids of 74.2 mg/g maggot compared to trypsin hydrolysis which produces amino acid yield of 8.73 mg/g. Amino acid analysis from both hydrolysis methods revealed a diverse amino acid profile in Black Soldier Fly maggot, including amino acids commonly used in animal feed and fish supplementation, such as glutamate, aspartic acid, lysine, methionine, arginine, leucine, and threonine.

Acknowledgement

Our sincere thanks to The Indonesian Government's Endowment Fund for Education or LPDP RI for the financial backing provided for this study.

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