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AMINO ACIDS PROFILE OF HYDROLYSATE PROTEIN FROM NON-SHELLED SMALL CRAB (*PORTUNUS PELAGICUS*) WASTE

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ABSTRACT

Small crab by-product such as non-crab shells waste is a potential source of proteins and amino acids. Therefore, this research was to examine the non-shell small crab amino acid and determine the effect of hydrolysate processing on amino acid levels. The study used crab (*Portunus pelagicus*) *lemi* as part of the non-shell crab waste. *Lemi* was converted into hydrolysate using the alcalase enzyme. Amino acid profile was analyzed before and after the hydrolysis process. UHPLC (Ultra-High-Performance Liquid Chromatography) was used to determine the level of amino acids level. The use of hydrolysis technology increased the total of amino acids by 45.83%, from 21.32 g.100 g-1 to 31.09 g.100 g-1. The percentage of essential amino acids compared to non-essential amino acids is 58.06% of the total amino acid from the hydrolysate of a non-shell small crab waste. The hydrolysis technology also increases the chemical score. The amino acid histidine had a high chemical score after the hydrolysis process. The study suggested that by-products derived from small crab waste can be considered essential sources of proteins and amino acids for malnutrition disease.

Keywords: amino acids, non-shell small crab, waste, alcalase enzyme, stunting.

INTRODUCTION

Ministry of Marine Affairs and Fisheries (2020) records that the export value of small crabs and crabs in Indonesia has increased every year. In the first half of 2020, crab commodity ranks third after shrimp and tuna, with a total commodity of 25,900 tons with a US$ 393 million value and an export target of US$ 6.47 billion. Meanwhile, in Central Java Province, up to January 21, 2020, a total of 161 tons of small crabs were exported to the United States and Hong Kong [1].

An increase demands for crab will always follow increased production and export of small crabs in shells waste. Small crab waste can reach 40-60% of crabs total weight [2]. Therefore, based on small crab production data in 2019, the potential waste generated reaches 25,904 tons. The small crab has a large amount of untapped waste, shell, and non-shell waste. Shell waste is the head, skin, tail, and feet of the crab, which is generally 25-50% of the small crab weight, while non-shell waste is *lemi*, eggs, and gills 10-20% crab weight. Shell waste can be further processed into polysaccharides, chitosan, and glucosamine. While eggs can be processed into processed culinary ingredients, no one has used them for *lemi* and gills, so efforts must be made to add value for the *lemi* and gill waste. *Lemi* is a yellowish material found under the shell of a small crab that has been boiled. This material tastes like a slice of small crab meat. When removing the small crab meat, this *lemi* must be discarded because if it is mixed if it is mixed with the meat, it will change the color of the small crab meat, and it will reduce the quality of the small crab meat. Therefore, it must be discarded of and become waste.

One of the efforts to make use of fishery product waste is the use of hydrolysis technology. Hydrolysis technology breaks complex bonds into simple bonds using enzymes, acids, and bases [3]. The use of hydrolysis technology produces hydrolysate products with better nutritional and functional properties [4]. The hydrolysis process using acids or bases has drawbacks, such as damaging amino acid group. The enzymatic hydrolysis process is more profitable because of the high product quality [5], where enzymatic hydrolysis can convert protein into bioactive peptides. The purpose of protein hydrolysate from fish waste is to prevent fish deterioration and obtain easy digest food because protein has been converted into amino acids and peptides which possessed better functional properties.

The United Nations (UN) Standing Committee has identified malnutrition as the most significant factor affecting the global disease burden, including in Indonesia. The high prevalence of nutritional problems is stunting [6]; [7]. Stunting is defined as low height for age. It results from chronic or recurrent malnutrition, usually associated with poverty, poor maternal health and nutrition, frequent illness, and improper feeding and cares early in life. Basic Health Research 2018 shows that the prevalence of stunting in Indonesia is 30.8% [8]. Cases of failure to thrive or stunting and malnutrition in Brebes District, Tegal District, and Tegal City, Central Java, are concerning because they are relatively high. Brebes Regency, including the highest in Central Java, is even included in the top 10 in Indonesia [9]. According to WHO in 2010, the prevalence of stunting is problematic if the majority reaches> 20%, of which 20-29% is moderate, 30-39% high, and ≥ 40% is very high [10]. The UNICEF report states, 1 in 3 toddlers in Indonesia is stunted due to protein deficiency. Stunting is associated with reduced survival, impaired cognitive and motor development,

decreased economic productivity and increased likelihood of living in adult poverty [11]; [12]. Stunting also impacts low intelligence skills and non-communicable diseases in the future [13]. Protein requirements are also closely related to the incidence of stunting [14]. Research in Africa shows that stunted children have low levels of essential amino acids such as tryptophan and lysine from their diet [15]. Research on toddlers in Malawi, nine essential amino acids in the blood serum of stunted children, results in repression of protein and fat synthesis by the mTORC1 gene, inhibiting cell growth [16]. Children are more sensitive to malnutrition than adults because of the need for children's physiological functions to grow and develop rapidly [17].

In the form of hydrolysate, the protein of non-shell small crab will be broken down into short proteins or peptides and free amino acids that are more easily absorbed by the body than in the form of long-chain proteins. It is beneficial, especially if given to children who have indications of stunting. In the state of hydrolysate, which has been dried quickly, the hydrolysate will be safer and still have a high nutritional composition. Small crab by-product such as waste non-crab shells is an essential source of dietary amino acids for the human to sustain adequate protein nutrition and health. Small crab contains high amounts of protein and balanced proportions of all amino acids relative to human requirements [18]. Therefore, it is assumed that by-products extracted from small crab would also be an essential and potential source of proteins and amino acids. So that in this research will be carried out testing of non-shell small crab amino acid. And looking at the effect of hydrolysate processing on amino acid levels will be observed.

MATERIALS AND METHODS

Hydrolysate Preparation

The study used crab *lemi* as part of the non-shell crab waste. *Lemi* was obtained from the Small Crab Processing UKM in Demak Regency, Central Java Province. Protein hydrolysate was made by an enzymatic hydrolysis reaction using alcalase enzyme. The method of making protein hydrolysate used in this study followed the Riyadi method with modifications [19]. The crab *lemi* was homogenized with aquadest in a ratio of 1:2 using a blender. The addition of the Sigma-Aldrich brand of alcalase enzyme with a concentration of 1.5%. Hydrolysis using a water bath with a temperature of 55°C for 2 hours. Setting the pH during the hydrolysis process to neutral (pH = 7) using CH3COOH as a regulator of the acid atmosphere and NaOH as a regulator of the alkaline atmosphere. Enzyme inactivation was carried out at 80°C for 20 minutes. Centrifuge at four °C for 20 minutes at a speed of 5000 rpm to obtain a fraction of the crab protein hydrolysate solution.

Amino Acid Analysis and Chemical Score

Amino acid levels were determined using the UHPLC (Ultra - High - Performance Liquid Chromatography) method. Simultaneously, the chemical scoring method is based on the biological value of a protein limited by the relative proportion of essential amino acids in it [20]. This method was developed by comparing each amino acid in these proteins with those contained in egg protein as a standard protein. The most deficient essential amino acids express the nutritional quality of protein compared to the reference. In this study, the standard used is the need for amino acids for children and adults aged 4 to 50 years [21]. This method can be used to determine amino acid scores for both single protein and mixed products. However, protein digestibility which is an essential aspect in determining protein quality, is not considered. This method relies on amino acid analysis techniques and does not feel other components that can affect protein digestion and utilization.

The chemical scoring method was later simplified by Pyz-Lukasik & Paszkiewicz [22] because the limiting amino acids (limiting AA) in most foods are lysine, methionine (methionine + cystine), and sometimes tryptophan. The calculation of the chemical score is only carried out on these amino acids. In this method, the score for each essential amino acid is expressed as a percentage of concentration. The calculation is as follows:

$$\text{Amino Acid Score (\%)} = \frac{\text{Amino acid in test protein (g)}}{\text{Amino Acid in refrence protein (g)}} \times 100\% \quad (1)$$

The chemical score is represented by the number of the amino acid score with the lowest score.

Statistical analysis

The descriptive analysis test analyzed the amino acid profile of non-shell crab waste (*lemi*) before and after hydrolysis.

RESULTS AND DISCUSSIONS

Amino acids are divided into two classifications, namely essential and non-essential. Their analysis was carried out to determine the composition of amino acids in fresh non-shell crab waste (*lemi*) and hydrolysate of non-crab shell waste (*lemi)*. Table 1 shows an increase in essential amino acids by 45.09% after the hydrolysis process of non-crab shell waste hydrolysate while non-essential amino acids increased 46.85% after hydrolysis. Before hydrolysis, the amino acids were 21.32 g 100 g^{-1}, while after hydrolysis was 31.09 g.100 g^{-1}. There was an increase in essential and non-essential amino acids after being hydrolyzed by 45.83%.

The content of essential amino acids represent the protein's quality [23]. The ratio of essential amino acids in the non-essential amino acids of fresh crab waste protein was 1.4, whereas, after hydrolysis, it was 1.38. It shows that there are more essential amino acids than non-essential amino acids. The percentage of essential amino acids compared to non-essential amino acids is 58.06% of the total amino acid hydrolysate of non-shell crab waste. Essential amino acids are considered "essential" because they cannot be synthesized by the body and obtained from food [24]. Insufficient intake of essential amino acids can

affect various metabolic pathways as they play a diverse role in human health.

The hydrolysis process breaks down protein molecules into amino acid groups or by breaking the peptide chain. It usually contains a low molecular weight peptide consisting of 2 to 4 amino residues. The results shows that hydrolysis of non-crab shell waste produced 20 amino acids. It consists of 11 essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tyrosine, threonine, tryptophan, arginine, valine) and nine non-essential amino acids (asparagine, glutamine, serine, glycine, alanine, proline/hydroxyproline, cystine, aspartate, glutamate). The complete hydrolysis will produce hydrolysates consisting of approximately 18-20 amino acids [25]. The content of essential amino acids in non-shell crab waste is complete.

Table-1. The amino acid composition of *lemi* (g/100 g) and *lemi* hydrolysate.

Amino Acid	Quantity (g/100 g)		Percentage change (%)
	Lemi	*Lemi* hydrolysate	
Essential amino acids (E)			
Histidine	1.42	2.08	46.48
Isoleucine	1.03	1.52	47.57
Leucine	1.49	2.08	39.59
Lysine	1.99	2.87	44.22
Methionine	0.59	0.88	49.15
Phenyl alanine	0.74	1.06	43.24
Tyrosine	0.98	1.44	46.94
Threonine	0.68	1.01	48.53
Tryptophan	0.29	0.43	48.28
Arginine	1.32	1.94	46.97
Valine	1.91	2.74	43.46
Total essential amino acids	12.44	18.05	**45.09**
% E/(E+NE)	58.38	58.06	
Non-essential amino acids (NE)			
Asparagine	0.17	0.26	52.94
Glutamine	0.15	0.22	46.67
Serine	0.81	1.21	49.38
Glycine	0.85	1.26	48.24
Alanine	1.18	1.69	43.22
Proline/ hydroxy proline	0.69	1.01	46.38
Cystine	0.20	0.31	55.00
Aspartate	2.20	3.19	45.00
Glutamate	2.63	3.89	47.91
Total non-essential amino acids	8.88	13.04	46.85
% NE/(E+NE)	41.62	41.94	
Total E + NE	21.32	31.09	45.83
Total E/NE	1.40	1.38	

Isoleucine in non-crab shell waste hydrolysate increased by 47.57% from 1.03 g / 100 to 1.52 g 100 g^{-1} (Table-1). As one of the chain-branched amino acids, Isoleucine is also critical to the body's physiological functions as a whole, such as growth, immunity, protein metabolism, fatty acid metabolism, and glucose transport [26]. Isoleucine may improve the immune system, including immune organs, cells, and reactive substances.

Methionine in the hydrolysate of non-crab shell waste was 0.88 g.100 g^{-1}. Methionine increased 49.85% after hydrolysis. Methionine is a precursor to homocysteine, cysteine, taurine, and S-adenosylmethionine, the primary methyl donor to polyamine synthesis [24]. Phenylalanine in non-crab shell waste hydrolysate was 1.06 g 100 g^{-1}. Phenylalanine is a precursor to tyrosine, a substrate for catecholamine synthesis [24]. Arginine in non-crab shell waste hydrolysate was 1.94 g 100 g^{-1}. The proven anti-aging benefits of l-arginine have a more significant potential than any pharmaceutical or nutraceutical agent previously discovered [27].

Tyrosine in non-crab shell waste hydrolysate was 1.44 g.100 g^{-1}. Tyrosine increased 46.94% after hydrolysis. Tyrosine is a precursor to neurotransmitters and increases plasma neurotransmitter levels (particularly dopamine and norepinephrine) [28] but it has little effect on mood in normal human [29]. Threonine in non-crab shell waste hydrolysate was 1.01 g.100 g^{-1}. Threonine is the main component of secretory mucin two which forms the protective lining of intestinal mucus [24].

Tryptophan in the hydrolysate of non-crab shell waste is 0.43 g.100 g^{-1} and increased 48.28% after hydrolysis. Tryptophan is a precursor to niacin and serotonin, neuromodulators mainly found in intestinal enterochromaffin cells [24]. Arginine in non-crab shell waste hydrolysate was 1.94 g.100 g^{-1}. Arginine serves as a precursor for synthesizing symmetrical and asymmetrical nitric oxide, creatine, dimethylarginine, and polyamines and is interchangeable with proline and glutamate [24].

The content of asparagine in the hydrolysate of non-crab shell waste is 0.26 g.100 g^{-1}. It increased 52.94% after hydrolysis. Asparagine is needed for brain development and function [30]. It also plays an important role in ammonia synthesis. The availability of asparagine during the replication of poxviruses is also crucial for protein synthesis [31]. Glutamine in non-crab shell waste hydrolysate is 0.22 g.100 g^{-1}. Glutamine is an essential nutrient for lymphocyte proliferation and cytokine production, phagocytic macrophages plus secretive activity, and bacterial neutrophil killing [32]. Glutamine is

also crucial for enterocyte growth and intestinal barrier function [24].

Serine in non-crab shell waste hydrolysate 100 g^{-1}increased by 49.38% after hydrolysis. Serine is critical to the production of proteins, enzymes, and muscle tissue in the body. Serine is needed for proper fat and fatty acid metabolism. It also helps to produce antibodies. The glycine in the hydrolysate of non-crab shell waste is 1.26 g.100 g^{-1}. Glycine plays a role in protein synthesis, purine synthesis, bile acid conjugation, central nervous system neurotransmitter, and cytoprotectant [16].

Alanine in the hydrolysate of non-crab shell waste is 1.69 g.100 g^{-1}. Alanine increased 43.22% after hydrolysis. Alanine is an amino acid used to make proteins. It's used to break down tryptophan and vitamin B6. It's a source of energy for muscles and the central nervous system. The proline in the hydrolysate of non-crab shell waste was 1.01 g.100 g^{-1}. Proline plays essential roles in protein synthesis and structure, metabolism (especially arginine, polyamine, and glutamate synthesis via pyrroline-5-carboxylate), and nutrition, as well as wound healing, antioxidant and immune reactions [24].

The cystine in the hydrolysate of non-crab shell waste was 1.69 g 100 g^{-1}. Cystine increased 55.00% after hydrolysis. More recently, L-Cys has emerged as an essential molecule in the food supplement industry. Nutrition therapy and functional foods have therefore involved L-Cys as part of the treatment of several pathologies such as cirrhosis (in which L-Cys biosynthesis is compromised) [33], or only in the prevention of cancer or the promotion of good health [34].

High amino acids (more than 2 g.100 g^{-1}) in the non-crab shell waste hydrolysates (Table-1) were the amino acids histidine, leucine, lysine, valine, aspartate, glutamate. Leucine in non-crab shell waste hydrolysate was 2.08 g.100 g^{-1}. Leucine increases protein synthesis by activating the rapamycin (mTOR) mammalian target signaling pathway in the skeletal muscle, adipose tissue, and placental cells. Leucine promotes energy metabolism (glucose uptake, mitochondrial biogenesis, and fatty acid oxidation) to provide protein synthesis energy while inhibiting protein degradation [35].

Lysine in the hydrolysate of non-crab shell waste was 2.87 g.100 g^{-1}. The amino acid lysine tends to influence Insulin Growth Factor-1 (IGF-1) expression, promoting growth (predominantly linear growth). Lysine can increase growth through increased synthesis and secretion of IGF-1, affecting mammals Target of Rapamycin (mTOR). mTORC11 signaling can determine the mass of cell size and affect bone growth [36]. Also, lysine is a precursor to carnitine and is required to modify collagen [24].

The aspartate in the hydrolysate of non-crab shell waste was 3.19 g.100 g^{-1}. Aspartates are used to increase the absorption and enhance the athletic performance of the minerals. Glutamate in non-crab shell waste hydrolysate was 3.89 g.100 g^{-1}. Glutamic acid is an essential amino acid beneficial for accelerating wounds' healing in the intestine, improving mental health, and reducing depression. If there is a deficiency, it will negatively impact the intestine's integrity and result in immunosuppression.

The five amino acids that are often deficient in children's diets are lysine, methionine, cysteine, threonine, tryptophan [37]. *Lemi* is a good lysine source, which is very limited in cereals and nuts [38]. Table-1 showed that the hydrolysate of *lemi* contains the five amino acids mentioned above. So *lemi* hydrolysate can be used to add amino acid content in children's food.

Table-2. The amino acid composition of *lemi* (g / 100 g) and chemical score compared with FAO / WHO reference protein.

Amino Acid	Quantity (g.100 g^{-1})		Chemical score
	Lemi	Reference (FAO, 2013)	
Essential amino acids			
Histidine	1.42	1.60	88.75
Isoleucine	1.03	3.00	34.33
Leucine	1.49	6.10	24.43
Lysine	1.99	4.80	41.46
Methionine	0.59	2.30	25.65
Phenyl alanine	0.74	4.10	18.05
Tyrosine	0.98	-	
Threonine	0.68	2.50	27.20
Tryptophan	0.29	-	
Arginine	1.32	-	
Valine	1.91	4.00	47.75
Non-essential amino acids			
Asparagine	0.17	-	
Glutamine	0.15	-	
Serine	0.81	-	
Glycine	0.85	-	
Alanine	1.18	-	
Proline/ hydroxy proline	0.69	-	
Cystine	0.20	-	
Aspartate	2.20		
Glutamate	2.63		

*) If the calculation number is> 100, it is written as 100

Table-2 showed the chemical score of the non-shell fresh crab waste before the hydrolysis process. The protein standards used are amino acid requirements for children and adults aged 4 to 50 (FAO, 2013). Table-2 shows the chemical scores for the amino acids histidine, namely 88.75, isoleucine (34.33), leucine (24.33), lysine

(41.46), methionine (25.65), phenylalanine (18.05), threonine (27.20), valine (47.75).

Table-3. The amino acid composition of *lemi* protein hydrolysate (g/100 g) and chemical score in comparison with FAO/WHO reference protein.

Amino Acid	Quantity (g.100 g^{-1})		Chemical score
	Lemi	Reference (FAO, 2013)	
Essential amino acids			
Histidine	2.08	1.60	100.00
Isoleucine	1.52	3.00	50.67
Leucine	2.08	6.10	34.10
Lysine	2.87	4.80	59,79
Methionine	0.88	2.30	38.26
Phenyl alanine	1.06	4.10	25.85
Tyrosine	1.44	-	
Threonine	1.01	2.50	40.40
Tryptophan	0.43	-	
Arginine	1.94	-	
Valine	2.74	4.00	68.50
Non-essential amino acids			
Asparagine	0.26	-	
Glutamine	0.22	-	
Serine	1.21	-	
Glycine	1.26	-	
Alanine	1.69	-	
Proline/ hydroxy proline	1.01	-	
Cystine	0.31	-	
Aspartate	3.19		
Glutamate	3.89		

*) If the calculation number is> 100, it is written as 100

There is an increase in the chemical score after the hydrolysis process (Table-2, Table-3). Table-3 showed the higher nutrient content of non-crab shell waste amino acids after the hydrolysis based on a chemical score. Table-3 showed the amino acid chemical scores of isoleucine (50.67), leucine (34.10), lysine (59.79), methionine (38.26), phenylalanine (25.85), threonine (40.40), valine (68.50). The amino acids isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine are limiting, while histidine are abundantly occur after hydrolysis process. Limiting amino acids are amino acids that usually lack food ingredients [39]. Histidine plays a pivotal role in protein methylation, hemoglobin structure and function, and as the precursor of histamine and carnosine [24]. Histidine is an essential amino acid in mammals, fish, and poultry because it is not synthesized de novo and must be obtained through food [40]. Histidine functions in the growth and repair of body tissues and produce red blood cells [41]. Histidine is needed in infants because it acts as a precursor for histamine, a chemical released by the immune system during an allergic reaction. It stimulates hydrochloric acid production in the stomach, which is essential during the digestive process [42].

CONCLUSIONS

The use of hydrolysis technology increased the number of amino acids by 45.83%, from 21.32 g / 100 to 31.09 g.100 g^{-1}. The percentage of essential amino acids compared to non-essential amino acids is 58.06% of the total amino acid from the waste hydrolysate of a non-shell small crab. The hydrolysis technology also increases the chemical score. The amino acid histidine does not become a limiting amino acid after the hydrolysis process.

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