

Determination of Nutrient Contents and Amino acid Composition of Pasuruan *Channa striata* Extract

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Abstract. *Channa striata* is considered as a potential food, because it is known to be used as the treatment of wounds, reduced pain, and as additional energy during illness. This fish is always consumed by rural peoples to having good medicinal qualities. But why this can happen need to be explored. This study was undertaken to determine the nutrients content and amino acid composition in Pasurua *Channa striata* extract (CSE). The Pasuruan CSE contained copper, iron, calcium, zinc and total protein with values: 0.01 ± 0.001 mg/100 ml, 5.78 ± 0.015 mg/100 ml, 2.26 ± 0.020 mg/100 ml, 0.41 ± 0.010 mg/100 ml, 5.524 ± 0.020 g/100 ml, respectively. This extract was found contained at least 17 amino acids. The major amino acids are glutamat, lysine, leucine, asparagin, alanin, arginine and valin making up 1.494 ± 0.004 g/100g, 1.152 ± 0.0006 g/100g, 0.956 ± 0.0002 g/100g, 0.911 ± 0.010 g/100g, 0.725 ± 0.0005 g/100g, 0.624 ± 0.0002 g/100g and 0.606 ± 0.0009 g/100g extract, respectively. It is concluded that the CSE is a potential food containing complete and balanced of essential and non-essential amino acids.

Keywords: *Channa striata* extract, Nutrient content, amino acid composition

1. Introduction.

Fish is vertebrate that has the greatest population and types. Fish species has been known to be more than 25,000 species. One of the freshwater fish species which has been commercialized is *Channa*. There are approximately 30 species from *Channa* family, in Malaysia there are seven of them, namely: *Channa bankanensis*, *Channa gachua*, *Channa lucius*, *Channa maruloides*, *Channa melasoma*, *Channa micropeltes*, and *Channa striatus* but there are many more which have not been identified yet [1]. Among this type of fish *Channa*, *Channa striata* is one of the widely studied. In the Asian community, particularly Indonesia, Malaysia, Brunei, Thailand *Channa striata* is believed to provide many benefits for health [2].

Channa striata is originated from India and also found in Myanmar, Bangladesh, Laos, Vietnam, Kambodia, Malaysia and some part of Indonesia. It has spread to Madagaskar and Phillipines [3,4,5]. *Channa striata* is a carnivore and predator species of freshwater fish, found in a variety of water area in parts of Indonesia, and has not been cultivated [6,7]. *Channa striata* is known to have many name, among others are Kutuk (Java), Aruan, Haruan (Malaysia and Banjarmasin). In a foreign language is also known as *Common Snakehead*, *Snakehead Murrel*, *Chevron snakehead*, *Striped snakehead*, while the binomial name is *Channa striata* [8]. In Malaysia, the different regional names give different fish names [9]. While in India, the names of the different areas could be used for the same types of fish [10].

Extract of *Channa striata* (CSE) is one of the refined products that considered to be a potential food because it is known to be used as the treatment of wounds, reduce pain and inner inflammation, as additional energy during illness for the elderly as well as to accelerate the recovery of postpartum mothers either in normal or sectio caesarea delivery [11,12]. In West Sumatra, community has utilized this fish as a commodity to support the family income and as a source of animal protein [13]. *Channa striata* also contain some fatty acids as a lipophilic antioxidant [4,14].

Channa striata have been proven containing monounsaturated, polyunsaturated fatty acid and good quality of protein together with vitamins and minerals composition. This fish has been successfully demonstrated in alleviating rheumatoid arthritis and inflammatory disorders [15]. A study of *Channa striatus* were collected from cultivated ponds from Naning-Melaka and Rawang-Selangor, has been known that Haruan Traditional Extract (HTE) contains the major amino acids especially glutamic acid, glycine, leucine,

aspartic acid. All the amino acids present in this extract are suggested to play an important role to human body [14].

Adequate protein intake is essential in good wound healing. If the body is in a state of protein deficiency it will lead to longer-inflammatory phase. This is caused by inhibition of the formation of fibroblasts, collagen and proteoglycan synthesis and inhibition of neoangiogenesis and wound remodeling, thus the wound healing process is delayed [16,17].

Adults need nine essential amino acids, ranging from 0.5 g/day (tryptophan) to 2 g/day (leucine and phenylalanine). While babies and children need 10 amino acids, with addition of Arginine. Although the Arginine included into amino acid group that can be synthesized by the liver, but the children cannot make arginine fast enough to support the synthesis of urea and body protein [18]. Administration of fish extract through menu has positively correlated to postoperative wound healing [19]. and shortened the duration of wound healing [20].

Utilization of Pasuruan *Channa striata* in Malang has been started since 1999, with the design of the extraction tool. The process of extraction began in 2001 and in 2003 it was used to support applications in hospital diet. The purpose of this study to explore the nutrients content and amino acids composition of Pasuruan CSE.

2. Methods

2.1 Identification the type of *Channa*

Two alive fishes are taken randomized from Pasuruan river East Java-Indonesia. Identification of the type of fish that will be used is done in Laboratory of Faculty of Fisheries and Marine Sciences Brawijaya University. The identification includes: physical observation, anatomy and classification with literature references: Fish base and FAO 2004: 1974-2004

2.2 *Channa striata* Extraction

Extraction process uses a plain steaming water/distilled water, according to the principles that are common fish protein soluble proteins (sarcoplasmic) situated between the myofibrils and easily removed by extraction [21]. In the extraction process that we do without adding anything to the mix of fish meat, with the following procedures [22, modification]:

- a. Choose fish weighing 300-400 grams/fish which is alive.
- b. Kill the fish by hitting the neck (oblongata) and the fish has to be immediately died and cannot move, then cut in two veins of the head with a slice removed from the bottom of the gills to the top, left and right.
- c. Clean up of scales, fins, guts and digestive tract. Wash thoroughly.
- d. Cut across of ½ -1 cm thickness
- e. Put it in the extractor with a temperature of $70^{\circ} \pm 2.5^{\circ}$ C. Discard the extract in the first half hour, after that take the remaining extract. The extraction of 5 kg fish is maximum 2 hours. Extracts obtained 1150-1250 ml, with a mean of 240 ml of 1 kg of fish meat.
- f. The extract then subsequently analyzed.

2.2 Nutrients Analysis and Amino Acid Levels in *Channa striata* Extract

Total protein content was analyzed using Kjeldahl distillation method, and the mineral contents (Cu, Fe, Zn and Ca) using Atomic Absorption spectrophotometry (AAS). Amino acid analysis was performed by the method of High Performance Liquid Chromatography (HPLC) [23]. (Shimadzu LC driver open LAB CDS EZchrom Edition), requires a fluorescence detector (Shimadzu RF 20A) using a column: Ultra techsphere C 18, flow rate: 1 ml/min, at wavelength of 280 nm. Mobile phase were used buffer A: Na-Acetate (pH 6.5) 0.025 M; Na-EDTA 0.005%; Methanol 9.00%; THF 1.00%. Buffer B: Methanol 95%. The total time between injections to the end of the analysis was 25 minutes. Before injection into HPLC, CSE samples were filtered using a millipore paper 0.45µm. Detection with Ophthaldialdehyde solution (OPA). Procedure of sample preparation and analysis of amino acids using the guidelines of AOAC, 1988. [24].

2.3 Statistical Analysis

Descriptive statistics were used in analysing mean and standard deviation (SD). The levels of Nutrients and amino acid content were presented as mean±SD.

3. Results

Based on the observation and identification included physical and anatomy observations, morphometric measurements, and literature reference of laboratory of the Faculty of Fisheries and Marine Sciences University of Brawijaya, the fish samples identified is:

Phylum :Chordata
 Class :Actinopterygii
 Order :Perciformes
 Family :Channidae
 Genus :Channa
 Species :*Channa striata* [8].

The analysis of fish extract /100 ml has nutritional values as follows:

Table 1 Nutritional Content of *Channa Striata* Extract

No	Nutrients	level /100 mL (mean±SD)
1	Copper (Cu)	0.01±0.001 mg
2	Iron (Fe)	5.78±0.015 mg
3	Calcium (Ca)	2.26±0.020 mg
4	Zinc (Zn)	0.41±0.010 mg
5	Total Protein	5.524±0.020 g

Table 2 Amino Acid Content of *Channa Striata* Extract

No	Level of Amino acid (g/100g)			
	Essensial (mean±SD)		Non essensial (mean±SD)	
1	Leucine	0.956±0.0002	Proline	0.312±0.003
2	Isoleucine	0.558±0.0003	Serine	0.447±0.002
3	Valine	0.606±0.0009	Arginine	0.624±0.0002
4	Tryptophan	0.159±0.0002	Tyrosine	0.414±0.0004
5	Phenylalanine	0.453±0.0004	Glycine	0.567±0.001
6	Methionine	0.343±0.0008	Glutamat	1.494±0.004
7	Threonine	0.551±0.003	Alanine	0.725±0.0005
8	Lysine	1.152±0.0006	Asparagin	0.911±0.010
9	Histidine	0.405±0.0006	T Total	10.677±3.79

The table above shows that in 100 grams of ECS there are 10.677 total amino acids, consisting of 5.183 grams of essential amino acids and 5.494 grams of non-essential amino acids. Lysine and leucine constitute a dominant essential amino acid, while the glutamat and Asparagine are non essential amino acids that have the highest content in the *Channa striata* extract. From the HPLC analysis, the appearance of amino acids is proceeded by aspartic and ended by Tryptophan. There is a high peak on the amino acid Glutamate, Lysine, Leucine and Arginine (Figure. 1).

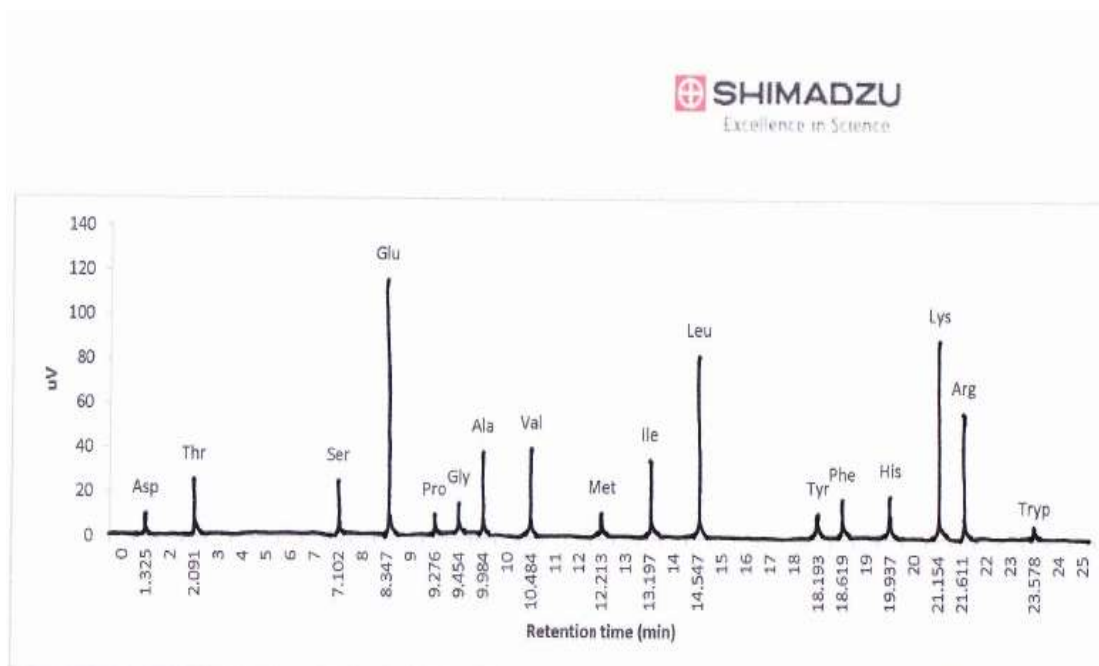


Figure 1 The chromatographic profile of CSE Amino Acid

4. Discussion

Channa striata collected from the rivers in Pasuruan have been used to determine the nutrients and amino acids compositions present in this extract. This fish has black colour on its back and white on abdomen. The fish is chosen because the supply is stable and has more supple flesh compare to the other area. CSE is a good source of animal protein (5,524±0.020 g/100 ml), also contains Copper, Zinc and Calcium. Extract of *Channa striata* has a good nutritional content, aside from protein and albumin which is the dominant nutrient content, this extract also contains Fe, Ca and Zn which are quite high.

The major fraction of CSE from several studies were presented in table 3.

Table 3. Nutritional Composition of *Channa striata* Extract in 100 milliliters

Nutrient	Value [25] (mean±SD)	Value * (mean±SD)
Protein (g)	3.36 ± 0.29	5.524±0.020 g
Zn (mg)	3.34± 0.08	0.41 ±0.010 mg
Cu (mg)	2.34 ± 0.98	0.01±0.001 mg
Fe (mg)	0.20 ± 0.09	5.78±0.015 mg

* Recent study

Two CSE analysis results showed different levels, protein and iron in this study is higher, whereas Zn and Cu levels lower than in previous studies. This difference is likely to occur due to the type of fish used in this study was obtained from the river, which has a more chewy meat and dense compared with those obtained from the dam. Previous studies using fish from the dam.

The presence of Zn in CSE is likely to be key factor that plays role in wound healing. Zinc are important for wound healing since the protein is able to bind Zn and transports it in the blood plasma [26]. Zn is an essential mineral in the structure and function of cell membranes. As metalloenzim component, in the body Zn is required in almost all aspects of cellular metabolism. Zn supplementation may limit membrane damage caused by free radicals during inflammation. Furthermore, Zn also involves in the immune system, ranging from defense systems by the skin to gene regulation in lymphocytes [27]. Administration of this extract may trigger formation of Endothelial Progenitor Cell (EPC) and accelerate wound healing [28].

In physiology minerals copper (Cu) affect spending Vascular Endothelial Growth Factor (VEGF) as a change in human keratinocytes. Immunohistochemical study also showed that the copper-treatment VEGF

production fix. Effect of Cu^{2+} at the expense of VEGF is not mediated by H_2O_2 but bound in thiol-redox status in the cell. Histological analyzes were performed on the edge of the wound tissue is known that the CuSO_4 treatment not only speed up the process of wound closure but also the quality of different tissue regeneration clearly. CuSO_4 treatment hyperproliferatif associated with epithelial tissue, and saturation in the cell membrane of granulation of copper-treatment on wound into a higher expression of VEGF [29].

Glycyl-L-histidyl-L-lysine (GHK) is a tripeptide that is paired with the copper ions in the form of GHK-Cu^{2+} that has been able to be isolated from human plasma [30,31].

This Complex copper tripeptide physiology suggests a role as an activator in wound healing in several animal and in vitro studies [32,33,34,35,36].

There are two functions that can be demonstrated by the GHK-Cu^{2+} , namely: the first function to maintain a network, as an anti-inflammatory in a way to minimize the occurrence of oxidative damage after tissue damage. The second function is to activate the process of remodeling a way memersihkan damaged proteins and replacing the scar tissue and normal tissue. This ability raises the postulate that the increased tissue damage and decreased ability to repair tissue during the aging process is the impact of a decrease in the concentration of GHK-Cu^{2+} [37].

Channa striata extract have a good source of protein with amino acids content of leucine, arginine and glutamate in high levels and important amino acid in our body. Amino acids play a role in regulating a variety of processes, including activation P70S6K (70 kDalton ribosomal protein S6-Kinase), is in the process of translation initiation factor mRNA, gene expression and cellular uptake of amino acids. One of the essential amino acids which have a branched chain, namely Leucine, has been shown to play an important role in mediating these effects, although the precise mechanism of this effect is not known [38].

Research by Greiwe *et al.*, 2001 found that the granting of leucine alone, or leucine + Insulin in adults may increase plasma leucine 116-343 $\mu\text{mol/L}$. Giving leucine alone can increase the phosphorylation P70S6K 4x as much, and increased to 18x as much if leucine + Insulin. This means that the physiological concentration of leucine and insulin is a key mediator in order to activate protein synthesis in humans. Leucine stimulates protein synthesis through a signaling mechanism that is not dependent on the presence of insulin. Provision of branched-chain amino acids such as leucine allows for increased protein synthesis in insulin-resistant conditions [39].

One of the non-essential amino acid that has a key role in the synthesis of proteins and amino acids are arginine. The amino acids found in food and is produced endogenously as a result of derivative Citrulin on the reaction catalyzed by the enzyme arginine synthetase. Arginine can be a material essential amino acid in adults who experience stress. More modern nutritional therapy currently Arginine supplementation leads to the use of specific, so that the occurrence of protein synthesis and cell communication via the production of Nitric Oxide (NO) and cell proliferation. This is due to the metabolism of Ornithine and Polyamines [40].

Arginine adequacy of scar tissue would seem to be important for the occurrence of wound repair and immune function [41]. Arginine supplementation of 17 g/day were given to 30 elderly patients (> 65 years) who suffered injuries surgery, showed results in patients with an increase in hydroxyproline supplementation as a sign of collagen deposition. Hydroxyproline is a major component of collagen and there is accumulation of proteins in the wound site, compared with the group who were not given the supplement. Lymphocyte response as immune activity also increased significantly, look at supplementation group, as well as insulin-like growth factor-1, a molecule in the control of wound repair are also increased [42,43,44].

Arginine is also known to increase insulin sensitivity [45]. Arginine is the substrate for two enzymes that play an important role in wound healing, namely nitric oxide synthase (NOS) and arginase. Arginine is a precursor of nitric oxide (NO), a substance that transmission has a direct influence on insulin sensitivity [46]. Arginine and NO are known to accelerate wound healing [47,48]. Impaired wound healing in patients with diabetes, followed by NO reduction in the wound. Amino acid L- arginine is the only material used in the synthesis of NO. NO can affect processes in the body, both physiological and pathological conditions [49].

L-arginine is also an ingredient for Arginase enzyme that metabolizes L-arginine to ornithine and subsequently became Proline and Polyamines are both going to interact on the proliferation and collagen synthesis in cells, but the functional implications of this pathway still requires further research [50]. Administration of L-arginine increases the levels of nitrite / nitrate significantly in diabetic rat wound fluid. This study proves that impaired wound healing that occurs in diabetes can be partially corrected by supplementation of L-arginine, and this effect was followed by increased synthesis of nitric oxide in wound [51].

Leucine plays an important role in stimulating protein synthesis and for growth. Leucine is very helpful in healing the wound of the skin and helps in regulating blood glucose levels, because it can produce energy (gluconeogenesis). Supply of Arginine and Leucine from CSE administration in diabetic rats could

potentially hurt to accelerate the process of wound healing. Arginine and Leucine is an amino acid that plays a role in neutralizing the blood glucose levels and stimulates wound healing [52].

Glutamine is found naturally in the body as free amino acids, which are the basic ingredients of endogenous protein synthesis. In the body, Glutamine is produced by muscle and distributed by the blood to the body parts that need. In the absence of a state of stress due to surgery or injury, the need for Glutamine increases, particularly for energy production, especially Nitrogen and Carbon, but the insufficient production of muscle, it causes the activity of cytokines and growth factors to be disrupted, and will have an impact on the delay wound healing. This condition should be improved by providing the basic material for a protein of cells. Provision of this protein is required for activation of growth factors that have been clinically proven to accelerate the healing of wounds [53].

Glutamine is an amino acid that is used by inflammatory cells in the wound site for the proliferation, and which is a source of energy [54,55]. Fibroblasts using glutamine for the same purposes as well as for the synthesis of proteins and nucleic acids. Optimal function of inflammatory cells that are the primary healing process, glutamine is an important component in the process of tissue repair. Glutamine is a non-essential amino acids, but in certain circumstances, for example there is an injury, glutamine becomes an essential amino acid [56].

The main material used to produce Adenosine Triphosphate (ATP) on neutrophils are known glucose. The results of the new study states that the neutrophils in mice using glutamine is higher than glucose [57]. Lymphocytes and macrophages also use more Glutamine [58]. Glutamine has been shown to be very important in the production of cytokines such as IL-1 β , IL-6, TNF- α and IL-8 from macrophages and monocytes immunostimulation. Similarly, glutamine metabolism is needed in the production of ATP and biosynthetic proteins (cytokines) [59,60,61].

Amino acids arginine and glutamine becomes critical for the wound. some studies found that the specific role of arginine is known to result in the disposition of collagen and accumulation of proteins in the wound, as the activity of lymphocyte immune response, and the presence of arginine supplementation may also increase insulin-like growth factor-1 [42,43,44]. It is known that the presence of collagen disposition and protein accumulation in the wound, lymphocyte responses and increased insulin-like growth factor-1 (IGF-1) is an indicator of wound healing. The success of wound healing is highly dependent on angiogenesis. Angiogenesis is the process of forming capillaries through a process of intussusception or sprouting of capillaries This growth of new capillary blood vessels which improves the supply of nutrients and accelerate the elimination of metabolic waste products [62,63,64].

It is known there are about 20 kinds of growth factors that stimulate angiogenesis. Among the various growth factors, 7 of which are the focus of angiogenesis therapy is platelet-derived growth factor (PDGF-BB), vascular endothelial growth factor (VEGF), fibroblast growth factor 1, 2 and 4 (FGF1, FGF2 and FGF4), Keratinocyte growth factor-2 (KGF-2) and Transforming growth factor-beta (TGF- β). Whole of these growth factors is a protein, each having a receptor present in wound tissue [65].

5. Conclusion and Further Direction

Pasuruan Channa striata Extract is one source of protein that has a high quality, with essential amino acids such as glutamine, lysine, leucine, asparagine, alanine and arginine. The content of essential and non-essential amino acids in the CSE in balanced proportions. The research that has been done, giving confidence that CSE is an additional food source that can be used to help accelerate wound healing patients who had tissue damage. It can be concluded that the CSE potential in diabetic wound healing. Good research and premeditated needed to know the benefits as well as the interaction of these natural ingredients in some therapies, especially in the treatment of diabetic wounds.

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