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Amino Acid Profiles of Crude Albumin Extracted from Several Marine and Brackish Water Fish in South Sulawesi, Indonesia

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Abstract
Fish are an important source of food that is rich in protein. The determinants of protein quality are amino acids, which can be divided into non-essential and essential amino acids. Non-essential amino acids can be produced by the body, while essential amino acids cannot be produced by the body and must be obtained from food. The purpose of this study was to analyse the amino acid profile of crude albumin from four fish species: the Indian scad *(Decapterus ruselli),* Indian mackerel *(Rastrelliger kanagurta,* Japanese threadfin bream *(Nemipterus japonicus),* and milkfish *(Chanos chanos)*. The method used for analysing amino acids was High-Performance Liquid Chromatography (HPLC). The results showed that Indian scad had the highest proportion of two non-essential amino acids (aspartic acid and serine) and three essential amino acids (isoleucine, leucine, and phenylalanine). Indian mackerel did not have the highest content of any non-essential amino acids but was highest in the essential amino-acid histidine. Japanese threadfin bream had the highest content of three non-essential amino acids (proline, alanine, and cysteine) and two essential amino acids (methionine and lysine). Milkfish had the highest content of three non-essential amino acids (proline, glycine, and tyrosine) and four essential amino acids (valine, arginine, threonine, and tryptophan).
Keywords: Amino Acid Profile, Fish, HPLC

1. Introduction

Fish are widely regarded as a highly nutritious food with substantial health benefits. One freshwater fish known to contain albumin which is beneficial to human health to accelerate the wound healing process is the striped snakehead *Channa striata* [1, 2]. There is an increasing demand for snakehead albumin as a substitute for Human Serum Albumin (HSA) which has resulted in high and still rising prices for this fish [2, 3, 4]. After administration of snakehead fish extract, almost all patients had low albumin levels, their albumin rose faster than the administration of albumin by infusion. Even patients with low albumin levels followed by complications of diseases such as hepatitis, tuberculosis (lung infection), nephrotic syndrome, tonsillitis, typhoid, diabetes, bone fractures, gastritis, poor nutrition, sepsis, stroke, ITP (Idiopathic Thrombocytopenia tupnia purpura), HIV , Thalassemia Minor, Autism, can be better with the administration of snakehead fish extract albumin [6].

The results of study, in postoperative patients with low albumin levels (1.8 g/100ml) at the General Hospital dr. Saiful Anwar Malang showed that the patient's blood albumin level became normal (3.5-5.5 g/100 ml) after being given snakehead fish extract 2 kg per day from steaming [6]. Snakehead fish extract has the potential to increase postoperative serum albumin because it has an antioxidant capacity that reacts and suppresses the production of free radicals [7]. Hypoalbuminemia patients who were given Fish Serum Albumin (FSA) supplementation of snakehead fish in clinical trials showed an increase in their albumin content [8]

Extracts from steamed snakehead fish that are drunk to postoperative patients are able to heal their surgical wounds [9,10,11]. Snakehead fish extract which contains high albumin is used to treat wounds because in the body albumin plays an important role in the process of forming new cell tissue [12]. In addition, children under the age of five who consumed biscuits enriched with snakehead fish

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albumin had higher albumin levels compared to children who were only given milk biscuits [13]. To fulfil this demand, it is necessary to seek alternative sources of fish albumin. However, there is still a lack of information on the potential of other fish to promote wound healing, including other members of the snakehead family Channidae [4,10].

In South Sulawesi, striped snakeheads are often consumed by women who have just given birth. Local people believe that by consuming snakehead fish, it is hoped that postpartum women will recover faster and have plenty of breast milk for their babies needs. In the Tanah Toraja and Enrekang areas, snakehead fish are given to children because they are believed to increase children's immunity [10]. A study on the albumin content of several marine and brackish water fish found in South Sulawesi, Indonesia found that the fishes studied contained relatively high levels of albumin [14]. However, the amino acid profile of these fish with high albumin contents was not known. This research is part of a research by Fatma et al., 2020. In this study, an exploration of the albumin content of seawater and brackish water fish was carried out which has abundant stocks in nature and is easy to obtain, so it is hoped that a source of fish albumin other than snakehead fish will be found. have pharmacological properties.

Therefore, the aim of this study was to determine the amino acid profile of crude albumin from four fish species: the Indian scad *(Decapterus ruselli),* Indian mackerel *(Rastrelliger kanagurta),* Japanese threadfin bream *(Nemipterus japonicus)*, and milkfish *(Chanos chanos)*.

2. Materials and Methods

Materials. Four species of fish were used in this study, comprised of three marine fishes and one brackish water fish. The marine fishes were the Indian scad *Decapterus ruselli*, Indian mackerel *Rastrelliger kanagurta*, and Japanese threadfin bream *Nemipterus japonicus*; specimens were obtained from fish traders at the Makassar Paotere Fish Landing Base, South Sulawesi, Indonesia. The brackish water fish was the milkfish *Chanos chanos*, obtained from aquaculture ponds in Barru Regency, South Sulawesi Indonesia.

Sample preparation and extraction. Before extraction of the soluble fraction, the sampled fish were thoroughly washed under running tap water, and filleted to remove the bone and skin. The fillets were then pre-homogenized using a commercial blender, transferred into zipped high-density polyethylene (HDPE) plastic bags, and frozen for storage until used for further analyses. Crude albumin were extracted by homogenizing 50 g of the pre-homogenized fish meat with 4 portions of distilled water (1:4 w/v) using a laboratory homogenizer (WiseTis® HG-15D Homogenizer). Each sample was heated in a water bath at 50ºC for 1 h, then cooled and filtered through Whatman no. 1 filter paper to separate the filtrate.

To determine the amino acid content and the types of amino acids contained in the crude albumin extracted from *D. ruselli*, *R. kanagurta*, *N. japonicus*, and *C. chanos*, the amino acid profile was determined using a High-Performance Liquid Chromatography (HPLC) method. This method works by breaking the hydrogen bonds in proteins through acid hydrolysis [15].

Data analysis

Research data from High-Performance Liquid Chromatography (HPLC) analysis is described in tabular form.

3. Results and Discussion.

Characteristics of Indian scad, Indian mackerel, Japanese threadfin bream and Milkfish

Morphometric measurements were carried out on indian scad, indian mackerel, Japanese threadfin bream and milkfish using three fish in each species. Parameters observed were total length and weight. The morphometrics of indian scad, indian mackerel, Japanese threadfin and milkfish can be seen in table 1.

Differences in size and weight are indian scad, indian mackerel, japanese threadfin and milkfish are influenced by internal and external factors including growth, gender, food, and an environment that supports growth. Growth is a change in size, either in length or weight. The average total length of indian scad caught in the waters of the Malacca strait ranged from 99-224 mm with a weight ranging from 10.23-114.7 g [16]. Indian mackerel has a length ranging from 150-220 mm with a weight ranging from 40-116.87 g in the waters of Central Tapanuli [17]. The average total length of female japanese threadfin is 16.97 cm and average body weight is 62.20 g and the average total length of male japanese threadfin is 17.57 cm and average body weight is 71.81 g in the waters of Malang Village. Meeting of Bintan Regency, Riau Archipelago [18]. The average total length of milkfish is between 270-490 mm while the average body weight is 147-680 g in Larea Rea ponds, Lappa Village, Sinjai Regency, South Sulawesi [19].

The difference in the proportion of body parts in fish is caused by the size, weight, and type of fish.

Fish with different weight sizes have different body lengths and body proportions because fish body length is directly proportional to fish body weight [20]. The proportion of fish body parts also increases from the head to the viscera [21].

Amino Acid Profiles of Decapterus ruselli, Rastrelliger kanagurta, Nemipterus japonicus, and Chanos chanos. Protein quality can be determined from the constituent amino acids [22]. Amino acids have various important functions and are needed by humans [23]. Some of these functions include repairing damaged tissue after injury, protecting the liver from various toxic substances, lowering blood pressure, regulating cholesterol metabolism, encouraging growth hormone secretion, and breaking down ammonia in the blood [24]. The amino acid content obtained from the crude albumin extraction of *D. ruselli*, *R. kanagurta*, *N. japonicus*, and *C. chanos* is shown in Table 2 (mg/kg) and Table 3 (percentage, %).

The data in Tables 1 and 2 show that 18 amino acids were detected in the four fish species analysed, .comprising eight non-essential amino acids and ten essential amino acids. The amino acid profile differed between the four species, in terms of the overall content as well as the proportion of each essential and non-essential amino-acid. The non-essential amino acids aspartic acid and serine were both highest in *D. ruselli* and lowest in *R. kanagurta*. Glutamate and alanine content were highest in *N. japonicus* and lowest in *R. kanagurta*. Both proline and glycine content were highest in *C. chanos* and lowest in *D. ruselli*. Tyrosine content was highest was in *C. chanos* and lowest in *R. kanagurta*, while cysteine was highest in *N. japonicus* and lowest in *C. chanos*.

With respect to essential amino acids, valine content was highest in *C. chanos* and lowest in *R. kanagurta*, while methionine was highest in *N. japonicus* and lowest in *R. kanagurta*. Both leucine and phenylalanine were highest in *D. ruselli* and lowest in *R. kanagurta*. Histidine was highest in *R. kanagurta* and lowest in *N. japonicus*; conversely, lysine was highest in N. japonicus and lowest in *R. kanagurta*. Arginine and threonine were highest in *C. chanos* and lowest in *R. kanagurta* while tryptophan was highest in *C. chanos* and lowest in *N. japonicus*.

Overall, *D. ruselli* had the highest content of two non-essential amino acids (aspartic acid and serine) and three essential amino acids (isoleucine, leucine, and phenylalanine) while *R. kanagurta* was not highest in any non-essential amino acids but did have the highest content of the essential amino acid histidine. N. japonicus had the highest levels of three non-essential amino acids (proline, alanine, and cysteine) and two essential amino acids (methionine and lysine), while *C. chanos* has the highest levels of three non-essential amino acids (proline, glycine, and tyrosine) and four essential amino acids (valine, arginine, threonine, and tryptophan).

Table 1 Morphometrics of Decapterus ruselli, Rastrelliger kanagurta, Nemipterus japonicus, Chanos chanos

Parameter	Decapterus ruselli	Rastrelliger kanagurta	Nemipterus japonicus	Chanos chanos
Total length (cm)	20	18	16	37
Weight (g)	282	149	258	488

Table 2. Amino acid profiles of crude albumin extracted from *Decapterus ruselli*, *Rastrelliger kanagurta*, *Nemipterus japonicus*, and *Chanos chanos* by weight (mg/kg)

Amino Acid	Fish species *Decapterus ruselli* (mg/kg)	*Rastrelliger kanagurta* (mg/kg)	*Nemipterus japonicus* (mg/kg)	*Chanos chanos* (mg/kg)
		Non-Essential		
Aspartic acid	759.34	370.70	602.26	502.07
Serine	239.42	150.13	173.49	201.47
Glutamate	925.48	596.43	1068.00	693.94
Proline	138.85	195.10	203.92	219.81
Glycine	401.72	476.98	478.63	586.01
Alanine	453.27	377.35	609.22	430.04
Tyrosine	68.51	42.15	66.25	99.79
Cysteine	55.36	94.38	148.68	38.55
		Essential		
Valine	341.38	260.27	339.90	361.47
Methionine	138.46	86.51	155.08	119.79
Isoleucine	319.35	204.26	264.03	252.99
Leucine	601.49	393.51	478.40	437.20
Phenylalanine	468.11	205.66	252.71	244.76
Histidine	221.26	1795.76	170.43	1740.43
Lysine	660.55	414.14	709.44	541.10
Arginine	489.45	77.90	428.58	508.28
Threonine	238.36	142.36	189.29	246.57
Tryptophan	24.24	21.20	19.65	25.07
Total	6544.59	5904.79	6357.97	7249.33

Table 3. Amino acid profiles of crude albumin extracted from *Decapterus ruselli*, *Rastrelliger kanagurta*, *Nemipterus japonicus*, and *Chanos chanos* as a percentage (%) of total amino-acid content

Amino acid (%)	Fish species			
	Decapterus ruselli	*Rastrelliger kanagurta*	*Nemipterus japonicus*	*Chanos chanos*
		Non-Essential amino-acid %		
Aspartic acid	11.60	6.28	9.47	6.93
Serine	3.66	2.54	2.73	2.78
Glutamate	14.14	10.10	16.80	9.57
Proline	2.12	3.30	3.21	3.03
Glycine	6.14	8.08	7.53	8.08
Alanine	6.93	6.39	9.58	5.93
Tyrosine	1.05	0.71	1.04	1.38
Cysteine	0.85	1.60	2.34	0.53
		Essential amino acid %		
Valine	5.22	4.41	5.35	4.99
Methionine	2.12	1.47	2.44	1.65
Isoleucine	4.88	3.46	4.15	3.49
Leucine	9.19	6.66	7.52	6.03
Phenylalanine	7.15	3.48	3.97	3.38
Histidine	3.38	30.41	2.68	24.01
Lysine	10.09	7.01	11.16	7.46
Arginine	7.48	1.32	6.74	7.01
Threonine	3.64	2.41	2.98	3.40
Tryptophan	0.37	0.36	0.31	0.35
Total	100%	100%	100%	100%

The amino acid profiles expressed as a percentage of the total amino acid content (Table 2) show that, while each of the four fishes had the same amino-acids, these were present in different proportions in each species. The amino acid composition of *D. ruselli* from highest to lowest proportion was glutamate, aspartic acid and lysine contributing over 10% each, followed by leucine, arginine, phenylalanine, alanine, glycine, and valine each contributing 5-10%; isoleucine, serine, threonine, histidine, methionine and proline, and tyrosine contributing 1-5% each; while cysteine and tryptophan each contributed less than 1%. The amino acid composition of *R. kanagurta* comprised just over 30% histidine and 10% glutamate followed by glycine, lysine, leucine, alanine and aspartic acid contributing 5-10%; valine, phenylalanine, isoleucine, proline, serine, threonine, cysteine, methionine and arginine contributing 1-5%; while tyrosine and tryptophan each contributed less than 1%. The amino acid composition of *N. japonicus* comprised glutamate and lysine (> 10%) followed by alanine, aspartic acid, glycine, leucine, arginine, and valine contributing 5-10%; isoleucine, phenylalanine, proline, threonine, serine, histidine, methionine, cysteine, and tyrosine contributing 1-5%; while tryptophan contributed less than 1%. Histidine comprised nearly a quarter of the amino acid composition of *C. chanos*, followed by glutamate, glycine, lysine, arginine, aspartic acid, leucine, and alanine contributing 5-10%; valine, isoleucine, threonine, phenylalanine, proline, serine, methionine, and tyrosine contributing 1-5%; while cysteine and tryptophan each contributed less than 1%.

Amino acids are a determinant of food protein quality [25], and varied substantially between the four types of fish studied. Amino acids are mono protein molecules that have an important role in living organisms; while non-essential amino acids are amino acids that can be produced by the body itself, essential amino acids must be obtained from food [23,25,26]. Functional amino acids are those which play key roles in metabolic pathway regulation for maintenance, growth, reproduction, and immunity; they include leucine, arginine, glutamine, proline, cysteine and tryptophan [23]. Leucine is needed for growth and as a trigger for brain function development in children and plays a role in regulating nitrogen balance and muscle degradation in adults while Alanine plays a role in the prevention and healing process of skin wounds [23,27,28]. Arginine is particularly effective in maintaining immune system functions and decreasing post-surgical infections [23,29]. Arginine content is also very important for children as it can affect wound healing, tumour growth, and the secretion of growth hormone and the hormones prolactin and insulin [30]. Glutamic acid has an important role in sugar and fat metabolism; furthermore, glutamic acid from animals or plants can be used as a medicinal agent in treating epilepsy, mental retardation, muscular dystrophy, ulcers, hypoglycaemic coma, and to reduce the side effects of insulin medication for diabetes [31]. Arginine and glutamic acid can be used as anti-inflammatory agents, while the amino acid histidine functions as a limiting amino acid [32].

Although the wound healing process is complex and not fully understood, amino acids that have been reported as having wound healing properties include arginine [23, 33-35], glycine [36], lysine, proline, and glucosamine [37], D acid-glucoronates [38] and carnosine, a dipeptide of alanine and histidine [39]. While other amino acids reported to be involved in wound healing (valine, histidine, glycine, proline, and alanine) are only present on low quantities in the striped snakehead, this fish is particularly rich in arginine [40].

The quantity of arginine may be important because this amino acid plays many roles in the wound healing process, including stimulating the release of the most important hormones for wound healing, such as growth hormone from the pituitary gland and insulin from the pancreas [41]. In the post-injury catabolic state, arginine has also been shown to reduce urinary nitrogen loss to promote positive nitrogen balance [42]. Arginine is also a substrate for two enzymes needed for wound healing, nitric oxide synthase (NOS) and arginase; arginine is metabolized in wounds via arginase, an enzyme that is abundant in wound fluid [43]. During connective tissue formation, arginine is used to produce hydroxyproline, which is important in collagen formation because it contains about 9.1% of the total amino acid residues in the collagen sequence [44]. Arginine is also an amino acid that is required for the manufacture of seminal fluid (semen) and strengthens the immune system [45]; it is useful in increasing body resistance or lymphocyte production, increasing growth hormone production, and increasing male fertility [46].

The high glutamate content in meat is due to deamination between the amino acids glutamine and asparagine which forms glutamic acid and thereby increases glutamate levels [47]. Glutamate plays a role in supporting brain function, making learning easier and strengthening memory [24], as well as helping to increase muscle mass [48].

4. Conclusions

The crude albumin extracted from *Rastrelliger kanagurta*, *Decapterus ruselli*, *Nemipterus japonicus*, and *Chanos chanos* contained 18 amino acids comprising eight non-essential and ten essential amino acids. These amino acids were: aspartic acid, serine, glutamate, proline, glycine, alanine, tyrosine, cysteine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, arginine, threonine, and tryptophan. *Decapterus ruselli* had the highest levels of two non-essential amino acids (aspartic acid and serine) and three essential amino acids (isoleucine, leucine, and phenylalanine). *Rastrelliger kanagurta* had the highest content of the essential amino acid histidine. *Nemipterus japonicus* had the highest content of thee non-essential amino acids (proline, alanine, and cysteine), and two essential amino acids (methionine and lysine). *Chanos chanos* had the highest content of 3 non-essential amino acids (proline, glycine, and tyrosine) and four essential amino acids (valine, arginine, threonine, and tryptophan).

5. Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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