

Inventory and Extraction of Taurine from Deep Sea Fishes: Escolar (*Lepidocybium flavobrunneum*) and Lobster

Sugeng Heri Suseno, Sri Hayati, Raja Roza Tirta Faradila, Nurjanah,
Pipih Suptijah, Roni Nugraha and Saraswati
Department of Aquatic Products Technology, Bogor Agricultural University,
Jl. Agatis, Dramaga, Bogor 16680, West Java-Indonesia

Abstract: Today the utilization of deep sea fishes for consumption as food supplements and pharmaceuticals is being developed. Escolar (*Lepidocybium flavobrunneum*) and deep sea lobster, originating from South Sea of Java-Indonesia can be used for product development as a source of taurine. The purpose of this study was to determine the characteristics of escolar and lobster as raw materials and to determine the best extraction treatment to produce crude taurine powder. Analysis of taurine level in fresh samples indicated that taurine level of escolar flesh was higher than the lobster. Escolar and lobster had yield of flesh at 59.81 and 20.47%. The yield of escolar and lobster viscera were 11.41 and 4.62%, respectively. Protein level which was calculated as the sum of free nitrogen in escolar was higher than the lobster. Highest amino acid content in escolar flesh was glutamic acid (2.04%) for non-essential amino acid and lysine (1.21%) for essential amino acid. Highest taurine level in escolar crude taurine powder produced from the extraction treatment at 30°C for 60 min with taurine levels of 83.71 mg/100 g.

Keywords: Escolar, extraction, lobster, taurine, temperature, time

INTRODUCTION

Deep sea is an alternative fishing ground. In Australia, deep sea fishing of *Beryx splendens* has been exploited to such an extent that there is a danger from over-fishing (Anonymous, 2004). Soselia and Rustam (1993) predicted that another deep sea fish, *Cubiceps whiteleggi*, has the potential of becoming an economically important fish in the future.

In Indonesia, research conduction related to deep sea fishes had been done by Baruna Jaya IV expedition in 2004-2005 which did research in the Indian Ocean, from the South of Java to the west of Sumatra. The expedition found various species of deep sea fishes, which 529 species were newly identified and had not have a taxonomic name. But this expedition study is limited to a review of biological aspects and nutritional aspects as well as bioactive content, but the product development of its commodities has not been researched and created.

Suseno *et al.* (2008) showed that deep sea fishes contained 64.38 to 86.10% water, 0.17 to 3.92% ash, 0.01 to 13.30% fat and 11.18 to 24.80% protein, respectively. Amino acids of its fish which were caught from west of Sumatra to south of Java were dominated by glutamic acid, aspartic acid, alanine, valine, cysteine, isoleucine, leucine and phenylalanine. Based on the analysis of mineral content, it was known that phosphorus was highest macro-mineral content in that samples which were observed and its value was ranged

from 196.26 to 339.67 ppm. Iodine was the highest micro-mineral content of deep sea fishes and its value was ranged from 11.89 to 10.27 ppm. Phosphorus, iron, iodine, zinc, cadmium, mercury in some deep sea fishes which were observed, were still below standard provision that allowed, except lead content of some deep-sea fishes. Several species of deep sea fishes had lauric acid content of 0.017 to 22.54 mg/g fat, myristic acid content of 0.122 to 137.67 mg/g of fat, palmitic acid content of 0.020 to 363.00 mg/g fat, stearic acid content of 0.417 to 157.30 mg/g fat, oleic acid content of 0.290 to 467.62 mg/g fat, linoleic acid content of 0.229 to 98.99 mg/g fat and linolenic acid content of 0.041 to 13.53 mg/g fat. From 10 species of deep sea fishes that were tested by HPLC (High Performance Liquid Chromatography), there were 4 types of fishes which had positive taurine content. The 4 types of species were *Antigonia capros*, *Diretmoides pauciradiatus*, *Neoscopelus microchir* and *Zenopsis conchifer*.

Based on the results of previous studies, deep sea fishes are very potential to be developed as a natural supplement in the form of natural taurine and aphrodisiac. The purpose of this study was to determine the characteristics of the raw material that will be developed as a source of taurine, to conduct taurine extraction with combination treatment of temperature and time and to determine the best extraction treatment that will produce crude taurine powder containing quite high taurine levels.

Corresponding Author: Sugeng Heri Suseno, Department of Aquatic Products Technology, Bogor Agricultural University, Jl. Agatis, Dramaga, Bogor 16680, West Java-Indonesia, Tel.: (+62 251) 8622909-8622906; Fax: (+62 251) 8622907

MATERIALS AND METHODS

Materials and equipments: Raw materials that used in this study were escolar fish (*Lepidocybium flavobrunneum*) and deep sea lobster, which were caught in South Sea of Java (Pelabuhan Ratu, West Java-Indonesia). Other materials that used were materials for taurine analysis, proximate analysis and amino acid analysis.

Equipments that used in this study were erlenmeyer, pipette, spatula, measuring glass, beaker glass, filter paper, plastic, aluminum foil, calico cloth, bowl, tray, electric stove, pan, water-bath shaker, some containers, paper label, funnel, separating funnel, ladle, digital scales, analytical scales, freezers, freeze dryer, Kjehdal tube, the Shimadzu GC 2010 and HPLC instruments.

Methods: The research was conducted in two stages, namely preliminary research and primary research. Preliminary research includes characterization of raw materials in the form of escolar (*Lepidocybium flavobrunneum*) and deep sea lobster. Characterization of raw materials included proximate analysis, taurine levels analysis and amino acid composition analysis. The primary research included extraction of taurine from flesh and viscera of selected sample and characterization of the extracted product which included taurine levels analysis, proximate analysis and sensory test of products.

Taurine extraction: Extraction method that used in this study was the solvent extraction method which distilled water was used as solvent. The ratio of solvent with the sample was 1:1 with a measured pH of 6.5. Taurine extraction was conducted as a combination of different time and temperature. Each treatment as combination of different time and temperature performed in three replicates. Temperatures that used were 30, 45, 60, 75 and 100°C, respectively. Length times of extraction that used were 30, 45, 60 and 75 min, respectively. Water bath shaker was used for extraction in this study. Furthermore, the filtrate that obtained from extraction was filtered using mesh sieve, then it was filtered again using filter paper. A filtered filtrate was then dried using freeze dryer to produce a product in the form of powder.

Analysis: Analysis which were conducted in the characterization of raw materials and extraction products included analysis of water content (AOAC, 2005), protein content (AOAC, 2005), fat content (AOAC, 2005), ash content (AOAC, 2005), carbohydrate content (Winarno, 2008), analysis of the amino acids composition (AOAC, 2005) and analysis of taurine levels (AACC, 1994).

RESULTS AND DISCUSSION

Characteristics of raw materials:

Morphology and morphometrics characteristics gindara fish and lobster: Raw materials which used in this study were escolar (*Lepidocybium flavobrunneum*) and lobster, originating from the sea of southern Java (Pelabuhan Ratu). Morphology of escolar and lobster can be seen in Fig. 1. Escolar and lobster were biota of the result of bycatch which were caught by the local fishermen, using fishing equipments and lobster traps.

The results of measurement of total length, width, height and weight of escolar and lobster can be seen in Table 1. Characteristics of escolars in this study have big eyes, black skin, white flesh, their flesh were very soft and rich in fiber. Lobsters have characteristics that the color of carapace has dominant orange, the body consists of two main parts, namely the cephalothorax is covered by a hard spiny carapace, where five periopod attached to the structure and a body consists of meat, the back covered by the carapace, there are pleopod and tail (telson) were attached to the body structure.

Schwartz (1997) observed escolars were caught in the waters of southeastern Beaufort and eastern Gulf Stream using longline tuna, the results of his study, they caught eight of escolars which have total length of 74.9 to 157 cm. The results also indicated that the average weight of escolar 1060 grams. Shchwartz (1997) showed that escolars's weights between 450 to 6300 g. Escolars used in this study have a different length and weight of escolar were observed in the study of Schwartz (1997), it can be caused by the differences habitat which have different seasons, temperature, salinity and water pressure. In addition, the age also affects the escolar's difference. This is consistent with the statement Nurjanah and Abdullah (2011) which states that the characteristics of the aquatic biota is influenced by age, sex, habitat and food.



Fig. 1: Morphology of (a) escolar and (b) lobster

Table 1: Morphometric of escolar and lobster

Sample	Total length (cm)	Width (cm)	Height (cm)	Total weight (g)
Escolar	52	11	8	1060
Lobster	55	8	4	640

Table 2: Yield of escolar and lobster

Sample	Flesh yield (%)	Viscera yield (%)
Escolar	59.81	11.41
Lobster	20.47	4.62

Table 3: Chemical composition of escolar flesh and tuna flesh as comparison

Parameter	Escolar (%)	Tuna (%)*
Water	65.56	70.58
Ash	0.97	1.30
Fat	3.86	1.01
Protein	18.14	22.00
Carbohydrate	11.98	5.11 (by different)

*: Ikhsan (2007)

Table 4: Chemical composition of lobster flesh and viscera

Parameter	Lobster flesh (%)	Lobster flesh (%)
Water	83.45	75.37
Ash	1.05	1.42
Fat	0.51	7.05
Protein	12.29	13.89
Carbohydrate	2.70 (by different)	2.27 (by different)
Total asam lemak	31.31	52.65

Yield: Yield is the percentage ratio between the weight of the material parts that can be used with a total weight of material. Rendemen value is used to determine the economic value of a product or material (Nurjanah *et al.*, 1996). Yield of flesh and viscera of escolar and lobster can be seen in Table 2.

Table 2 showed that the highest yield founded in escolar's flesh 59.81%, while the lowest yield founded in the lobster viscera 4.62%. Jayanti's (2008) study showed that escolars have a yield greater than the other deep sea fish *Antigonia capros*, *Antigonia rubicund*, *Caelorinchussmithi*, *Coryphaenoides sp.*, *Diretmoides pauciradiatus*, *Lamprogrammus niger*, *Neoscopelus microchir*, *Setarchesguenteheri*, *Zenopsis conzifer*, but *Diretmoides veriginiae* species have a higher yield than escolar. Effendi (1997) stated that weight and total length of fish are influenced by internal and external factors. Internal factors meant sex, age, level of maturity of gonads. External factors meant temperature, salinity and food. Kusumo (1997) stated also that the greater edible portion, the greater the nutritional value of fish. It caused by amount of minerals, proteins and fats that can be utilized from the fish.

Chemical composition gindara fish and lobster: The chemical composition of escolar and lobster can be determined by proximate analysis. Proximate analyzes were done on samples of escolar's flesh, lobster's flesh, lobster's viscera. The result of proximate analysis from escolar and lobster can be seen in Table 3 and 4.

Moisture content of escolar flesh is 65.56%, moisture content of lobster flesh is 83.45% and moisture content of lobster viscera 75.37%. It is not much different from the results of the study of Asty (2007), Ikhsan (2007), Damayanti (2005) and Fanany (2005), that study showed that the moisture content in the deep-sea fish have values ranging from 70.00 to 86.26%. Escolar have a lower water content compared with tuna. This is due to the characteristics of the habitat. The deep sea have high salinity more than 34.2%. The water conditions with high solubility of

inorganic salts which cause hypotonic processes in fish. This course will be related to the value of water holding capacity of fish muscle, water holding capacity value will affect the moisture content in the body of the fish.

Ash content of escolar flesh was 0.97%, while the ash content of lobster flesh was 1.05% and lobster viscera was 1.42%. This result is not much different when compared to research Asty (2007) and Ikhsan (2007) which stated that the ash content in the fish generally range between 1-2.5%. Escolar contain high levels of ash which is not much different as well with the tuna.

Ash content related to mineral of a material, the minerals in a food are divided into two kinds of salts, the organic salts and inorganic salts (Budianto, 2009). Ash content and its constituent components will depend on the type of material being observed and how to ashing (Sudarmadji *et al.*, 2007).

Fat content of escolar was 3.86%. Fat content of escolar was greater than fat content of tuna (1.01%). Fat content of lobster flesh was lower than fat content of lobster viscera. Fat content will be influenced by several factors such as fishing season, geographic location, species, gonad maturity and size (Kusumo, 1997).

Protein content of escolar flesh was 18.14%, while the protein content in the lobster flesh 12.29 and 13.89% in the lobster viscera. The study of Chasanah and Barus (1994) and Fanany (2005) showed that the protein content in the fish ranged from 11-18%. Protein content of escolar flesh is lower than protein content of tuna fish. It can be caused by differences in the availability of different foods and the pelagic zone and zone mesopelagis (Davis, 1991). According Nybakken (1992), the higher the depth of the water, so food availability will decrease. Least amount of food availability cause a decreasing food intake of fish in the sea so that the nutrients especially proteins become lower.

Carbohydrate content of escolar was 11.98%, while the carbohydrate content of lobster was 2.7% in the flesh and 2.27% in the viscera. Carbohydrate content of escolar greater than the lobster and tuna fish.

Taurine levels: Taurine (IUPAC name: 2-aminoethanesulfonik acid) is a non-essential amino acid because it can be synthesized from cysteine and methionine (Welborn and Manahan, 1995). Humans have limited capabilities in the biosynthesis of taurine, especially infants (Gaull, 1986; Schuller-Levis and Park, 2003), taurine are added to the formula milk as supplement taurine intake for infants. Taurine has important role in several important processes in the body system such as membrane stabilization, osmoregulation, antioxidation and development of the central nervous system and retina (O'Flaherty *et al.*, 1997; Schaffer *et al.*, 2000).

Table 5: Taurine levels of fresh sample

Sample		Taurine level (mg/100 g)
Escolar	Flesh	44.201
	Viscera	43.915
Tuna	Flesh	30.855
	Viscera	29.759
Lobster	Flesh	19.672
	Viscera	12.736

Table 6: Amino acid composition of fresh escolar flesh

Amino acid	w/w (%)
Aspartic	1.44
Glutamic	2.04
Serine	0.58
Histidine*	0.89
Glycine	0.57
Threonine*	0.66
Arginine*	0.83
Alanine	0.78
Tyrosine	0.50
Methionine*	0.43
Valine*	0.74
Phenylalanine*	0.54
Isoleucine*	0.70
Leucine*	1.08
Lysine*	1.21
Total asam amino	13.01

*: Essential amino acid

Results of analysis taurine of fresh samples using HPLC (High Performance Liquid Chromatography) are presented in Table 5. Taurine content of tuna fish was used as a comparison for the taurine content of two samples.

Results showed that the taurine content of fresh escolar greater than taurine content of fresh tuna and fresh lobster. Taurine content of fresh escolar's flesh was 44.201 mg/100 g, fresh escolar's viscera was 43.915 mg/100 g. Jayanti (2008) studied on the taurine content of some deep-sea fish, such as fresh *Zenopsis conchifer* 34.54 mg/100 g, *Diretmoides pauciradiatus* of 31.51 mg/100 g, *Antigonia capros* of 29.7 mg/100 g and *Neoscopelus microchir* of 25.4 mg/100 g. Taurine content of escolar is not much different from the taurine content of other fish in the sea.

Some deep sea fishes species which had been studied had higher taurine levels than pelagic fish, such as Skipjack tuna which had taurine content at 3 mg/100 g. However, taurine content in deep sea fish was lower than the Japanese oyster (1178 mg/100 g) (Okuzumi and Fujii, 2000). Gormley *et al.* (2007) showed the taurine content of some fish commonly sold in supermarkets, such as plaice (146 mg/100 g fresh weight), cod (108 mg/100 g fresh weight), mackerel (78 mg/100 g wet weight) and salmon aquaculture (60 mg/100 g fresh weight). In the general, seafood products, especially invertebrates such as mollusks and crustaceans contain high concentrations of taurine (Spitze *et al.*, 2003).

Amino acid composition of escolar: Amino acid composition was analyzed in this study is the

composition of amino acids in escolar's flesh, this is related to the analysis result of taurine content of escolar. It showed a high value of taurine content. High levels of taurine in escolar will be a reference for the development of crude concentrate products taurine on primary research, so that the amino acid composition of escolar also needs to be analyzed. Amino acid content of fresh escolar flesh presented in Table 6.

Amino acid composition analysis was done in this study is based on acid hydrolysis method, so that only fifteen kinds of amino acids consisting of nine essential amino acids are histidine, threonine, arginine, methionine, valine, phenylalanine, isoleucine, leucine and lysine and six non-essential amino acids are aspartic acid, glutamic acid, serine, glycine, alanine and tyrosine that can be analyzed. Essential amino acids for adults consisting of lysine, leucine, isoleucine, threonine, methionine, valine, phenylalanine and tryptophan, while the amino acids essential for children are arginine and histidine. Non-essential amino acids consisting of aspartic acid, glutamic acid, alanine, asparigin, cysteine, glycine, proline, tyrosine, serine and glutamine (Winarno, 2008).

The highest content of non essential amino acid which can be found in escolar was glutamate and aspartate, which respectively 2.04 and 1.44%. Glutamic acid and aspartic acid are a non essential amino acid that is important because it can produce the characteristic aroma and taste of the food. Glutamic acid can be produced by the human body. Glutamic acid ions in which there are several types of glutamate can stimulate nerves that exist in the human tongue. These properties can be exploited by the flavoring industry where salt derived from glutamic acid (also known as monosodium glutamate) is well known as a food flavoring.

The highest content of essential amino acids in the escolar flesh fish was lysine and leucine, which respectively 1.21 and 1.08%. Lysine serves as the basis of blood antibodies, strengthens circulatory system, maintaining the growth of normal cells, with proline and vitamin C to form collagen and reduce excessive blood triglyceride levels. Lysine deficiency can cause fatigue, difficulty concentrating, hair loss, anemia, stunted growth and reproductive disorders. Arginine, lysine and leucine is an essential amino acid important in aquatic animals (Meade *et al.*, 2005).

Escolar crude taurine powder characteristics:

Sensory characteristics of escolar crude taurine powder: Data on preliminary research give the conclusion that escolar has good potential for further extraction to produce crude taurine extract in general, crude taurine powder which extracted from escolar's flesh has appearance in brownish yellow to brown, fishy smell, dry and crisp texture but some products have a wet texture because of its hygroscopic

Table 7: Taurine levels of escolar crude taurine powder

Treatment	Taurine level (mg/100 g)	
Flesh	30°C, 30'	69.28
	30°C, 60'	83.71
	45°C, 30'	75.45
	45°C, 60'	63.28
	60°C, 30'	72.04
	60°C, 60'	71.13
	75°C, 30'	76.82
	75°C, 60'	80.29
	75°C, 75'	72.52
Viscera	75°C, 30'	75.19
	75°C, 75'	83.55

Table 8: Chemical composition of escolar crude taurine powder

Parameter	wb (%)	db (%)
Water	10.16	
Ash	9.44	10.51
Protein	76.91	85.61
Fat	3.48	3.87

characteristic which related to freeze drying process. Freeze drying process contributes to the characteristic of final product. Crude taurine powder which extracted from escolar's has a brown color, fishy smell and tough texture.

Taurine levels in escolar crude taurine powder:

Extraction is the separation of a component using a solvent (Kustiariyah, 2006). Extraction method used in the study was macerated by water bath shaker. The filtrat which the result of extraction was dried using a freeze-drying method (freeze drying) for 48 h, so it produce crude taurine powder. The results of taurine analysis in crude taurine powder from escolar are presented in Table 7.

Results showed that the highest taurine content was produced from the escolar flesh which extracted by a treatment of 30°C for 60 min, and the taurine content was 83.71 mg/100 g. While the lowest levels of taurine produced from extraction at 45°C for 60 min at flesh escolar was 63.28 mg/100 g. If compared with the results of taurine content from fresh escolar flesh, the extraction produces a higher taurine content.

Results of extracts obtained will depend on several factors, including the condition of the natural compounds, the extraction method used, sample particle size, condition and storage time, long extraction times, as well as a comparison between the amount of solvent by the number of samples (Kustiariyah, 2006).

Heat treatment on extraction gave the positive and negative impacts on the products to be produced and the rate of change will depend on the heat treatment applied. The advantage of hot process is to kill pathogens, anti-nutrient enzyme inactivation, increasing digestibility and bioavailability of the product. Warming also can provide the desired taste. Destruction of components, browning reactions, racemization and the loss of water-soluble compounds are some of the adverse effects of heat treatment (Meade *et al.*, 2005).

Dragnes *et al.* (2009) reported the concentration of taurine in the eye and fillet of salmon was respectively 0.8 and 0.7 mg/g, whereas high concentrations of taurine which can be found in cod liver and salmon was 8.3 and 5.6 mg/g. Spitze *et al.* (2003) stated that the concentrations of taurine are most numerous in muscles, viscera and brain. Larsen *et al.* (2007) reported that concentrations of taurine and creatine compounds in a material can be decreased during salting and ripening.

Chemical composition of escolar crude taurine powder:

The results of taurine content analysis showed that the best treatment to produce products with high taurine content is treated at a temperature of 30°C for 60 min. Crude taurine product of the best treatment was then analyzed to determine its chemical composition. The results of the proximate analysis of crude taurine powder of escolar are presented in Table 8.

Based on Table 8, the protein content of the crude taurine powder increased from 18.14 to 76.91% (wb) moisture content of the crude taurine powder decreased from the fresh samples from 65.56 to 10.16%. This is due to the freeze-drying process, whereas most of the water is drawn out of the sample or material through sublimation mechanism. So the crude taurine powder including products that classified high protein and have a low water content.

Eshtiaghi *et al.* (1994) stated that one of the drying method for drying the product is frozen (freeze drying). High vacuum pressure treatment and freezing temperatures can result in texture, color, rehydration capacity and other parameters are good. Freeze drying can produce products with higher quality than the other way because of freeze drying can produce a product that has a rigid structure due to the sublimation process. With a rigid structure, the product becomes porous and does not shrink in the dry state. When water is added to the products, rehydration process will happen quickly and back to the form which almost fresh. In addition, during this process there is almost no liquid in the material so as to prevent the transport of substances that are soluble in water and minimize degradation reactions.

CONCLUSION

Results of this study indicated that escolar fish can be used as raw material whose high in taurine content. It was supported by the yield of escolar flesh and viscera which were higher than the lobster yield. The highest level of taurine in escolar crude taurine powder was resulting from the extraction treatment at a temperature of 30°C for 60 min and its value was 83.71 mg/100 g.

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