PARTIAL BIOCHEMICAL CHARACTERIZATION OF EGG MASSES OF THE WEDGE SEAHARE

Dolabella auricularia (Lightfoot, 1786)

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ABSTRACT

Egg masses of the wedge seahare *Dolabella auricularia* are gathered as human food in the Visayas region and other areas in the Philippines, but there have been no studies examining their appropriateness for human consumption. This study was conducted to characterize the biochemical components of the egg masses. Biochemical characterization was assessed through proximate composition analysis and an amino acid profile. The egg masses comprised 91.10 percent moisture, 3.43 percent ash, 2.85 percent crude protein, 1.77 percent carbohydrates, and 0.85 percent fats. The 23 amino acid included six (6) essential components, nine (9) non-essential components, and eight (8) others. The essential amino acids included leucine, valine, isoleucine, phenylalanine, threonine and histidine, while the non-essential amino acids included taurine, tyrosine, serine, glycine, aspartic acid, glutamic acid, alanine, glutamine and proline. The other components included phospho-serine, urea, aromatic amino acid, citrulline, beta-alanine, gamma-aminobutyric acid, ammonia and ornithine. The results show that egg masses contain amino acids that are important for human health, thus making it another food commodity from the ocean.

Keywords: Opisthobranch - mollusks, proximate composition, amino acids

INTRODUCTION

Seafoods are important to the diet of Filipinos and can be served either fresh or cooked. They are of high demand at present because of their considerable health benefits and for being nutritious (Skonberg and Perkins, 2002). Seafood products are considered potential and cheap source of animal protein due to their amino acid composition and digestibility (Louka *et al*, 2004). Also, the vitamins and minerals in seafood products are sufficient to warrant healthy living (Murray and Burt, 2001). On the other hand, measurement of fish constituents is sometimes necessary to meet the requirements of food regulation or commercial purposes (Osibona, 2011).

The proximate composition of fish serves as an index for fish quality (Hernandez *et al.,* 2001) and provides information in terms of the nutritive value (Steffens, 2006). It can also ensure food regulation requirements and commercial specifications (Waterman, 2000). At present, fish magnetize the attention of the diet conscious consumers as a potential source of animal protein and essential nutrients (Fawole *et al,* 2007). Protein is essential for the sustenance of life. Fish can be an excellent source of protein because of their amino acid components and the degree of their digestibility (Louka *et al,* 2004). Fishery products have been found to be rich in protein needed by

human in terms of nutritional nourishment. Fish have been proven to be an excellent food source due to its amino acid composition and its degree of digestibility (Louka *et al.*,2004). Osibona (2011) categorized amino acids into three categories (major, medium and minor amino acids) while there are some literature that categorize amino acids into essential and non-essential.

The island province of Cebu has a total coastal area of 18,000 km (CRMP, 1996) making it a very good source of marine resources. It is popularly known for its abundant seafood products, which are sold at the public market and even in a small stall or "*talipapa*" near and within beaches and resorts (personal observation). Some products are common and identical to those in other provinces, while others are available only in selected places. One example is egg masses of the wedge sea hare *Dolabella auricularia* which are known locally in Cebu and other areas as "*lukot*". It attracts the attention of seafood lovers due to its tangled form that resembles noodles and its distinct taste.

The wedge sea hare, *D. auricularia* belongs to the *Opisthobranch* mollusks (*Aplysiidae*). It is mostly found on shallow tropical shorelines and has been observed to feed on algae or seagrasses. It is a cross fertilizing hermaphrodite in which fertilization is internal, with one individual transferring sperm via an eversible penis located on the right side of the head to the dorsally located gonophores of a second individual (Van Horn, 2005). The species lays gelatinous string-like egg masses usually cemented to eel grass or seaweed. Inside the string are the segregated egg capsules arranged spirally with four capsules to each spiral turn. Moran and Woods (2007) reported that every capsule contains 50 to 150 embryos loosely packed inside the sleeve. An individual sea hare can lay a string of eggs at the average rate of 5.9 cm or 230 capsules or 41,000 eggs per minute with individual eggs measuring 77 microns in diameter (Mac Ginite, 1934). The wedge sea hare is essentially nocturnal; its activities can be observed to feed, mate, spawn and move about (Calumpong, 1979). Spawning and recruitment occur throughout the year, with peaks in May and July and September to October (Calumpong and Pauly, 1984).

In the Philippines, there are limited published articles or studies on *D. auricularia*. The studies of Calumpong, 1979; Calumpong and Pauly, 1984; Lozada *et al.*, 2005 and Iballe *et al.*, 2008 focused only on biology and culture, and not on the utilization of egg masses as human food. Egg masses are popular food not only in the Philippines but in other parts of the world like Samoa, Kiribati and Fiji Islands (Singh and Vuki, 2009). In the aforementioned places both the organism and the egg masses are collected for food, and are eaten either raw or half cooked, and marinated with lemon juice (Fay-Sauna, 2001).

Egg masses of *D. auricularia* in the Philippines are either eaten raw in a form of a salad or added as one of the ingredients in cooking "Tinolang Isda" (a staple soup in the Visayas region) and shellfishes. The egg masses have been traditionally consumed as human food for quite a long time, and there have been no reported incidents/cases of food poisoning due to ingestion of the egg masses. Anecdotal information tells us that eating an egg mass can bring many health benefits to its consumer. However, these claims have not been scientifically or empirically proven. This scenario provides a venue for a call to characterize egg masses in terms of their biochemical composition to warrant their appropriateness for human consumption. To our knowledge, there have been no studies conducted on the biochemical characterization of egg masses to determine if they are safe for human consumption. Thus, this study was conceptualized and directed towards this purpose.

MATERIALS AND METHODS

Sample Collection and Preparation

Egg masses of D. auricularia were collected from the coastal areas of Danao City and Carmen, Cebu. They were harvested fresh from the sources prior to the analyses. Prior to packing, the egg masses were cleaned of debris and placed into a tray lined with a paper cloth to remove the surplus moisture before being placed in a ziplock cellophane bag. Samples were placed in a styropack box lined with crushed ice and transported to the laboratory for analyses.

Proximate Composition

Egg mass samples were analyzed for moisture and ash content following the procedures of AOAC (1995), total fat was measured using the Bligh and Dyer Method (1959), and crude protein was measured using the modified Kjeldahl Method (AOAC, 1995). Carbohydrates were calculated by the difference between 100 percent and the sum of the other components. Triplicate determinations were carried in each analysis.

Amino Acid Profile

The amino acid composition of the egg masses was analyzed using an amino acid analyzer. This analysis was done in triplicate.

RESULTS AND DISCUSSION

Proximate Composition

The chemical characteristics of the egg masses of D. auricularia were determined through proximate analysis. Percentage composition of moisture, protein, total fat, ash and carbohydrates are shown in Table 1.

Proximate Composition	Percentage			
Moisture	91.10			
Total Fat	0.85			
Protein	2.85			
Ash	3.43			
Carbohydrates	1.77*			

Table 1. Proximate Composition of *Dolabella auricularia* Egg Masses

-*calculated by difference

- Values are mean of 3 replicates

The egg masses had a high moisture product; the percent composition (Table 1) was even higher than that of sea urchin roe, which is 78.97 percent (Mol, et al, 2008). Results show that since the egg masses are high in moisture content, they will have better digestibility and help supplement the water requirements of consumers. On the other hand, lipids have been found to be the highest and most efficient source of energy of which it contains more than twice from that of the carbohydrates and protein (Soundarapandian *et al*, 2008). The total fat composition of the egg masses, (0.85%) was much lower than that of sea urchin roe (3.05%) (Mol, et al, 2008). Stansby (1982) classified a foodstuff to be low in oil if its composition is less than five (5) percent. The low percent composition of the crude fats of the egg mass could be attributed to its high moisture content. Other possible reasons could be possibly attributed to the fact that most mollusks utilize either fat or protein during embryogenesis to meet their energy requirements (Shakuntala and Pandian, 1972). The low percentage of lipids in the egg masses could benefit people trying to control their lipid intake.

The percent composition of protein (Table 1) in the egg masses was high compared to the other components because it forms the largest quantity in fish (Steffens, 2006). High protein supply in the body aids in the regulations of blood sugar and facilitates in losing weight (Sutharshiny and Sivashanthini, 2011). The high percentage of protein and low fat content in seafood is advantageous (Pigott and Tucker, 1990). Based on these results, the egg masses of the wedge sea hare are a potent source of high protein and low fat food from the sea.

High levels of ash content (Table 1) were found in the egg masses. That level of ash content is oftentimes associated with a relatively high mineral content (Ratana-Arpon, 2006). Bolawa *et al.*(2011) reported that the high ash content of the products can be a good source for minerals such as calcium, potassium, zinc, iron and magnesium. These minerals are important for human nutrition (Sikorski *et al*, 1990).

The carbohydrate content of the egg masses was 1.77 percent. This was expected since in proximate composition, carbohydrates constitute only a minor percentage (Soundarapandian *et al.*, 2008). Results suggest that the egg masses can be recommended as part of the diet for those who are observing their sugar levels since most of the carbohydrates found in fishery products contains glucides (Sudhakar *et al*, 2011) with some traces of glucose, fructose, sucrose and other mono and disaccharides (Okuzumi and Fujii, 2000). Glucides have been known to regulate the sugar level of the body.

Amino Acid Profile

The biochemical composition of the egg masses was determined to provide comprehensive information to the consuming public. Results show that the egg masses contained 15 amino acids, of which six (6) are essential and seven (7) other components (Table 2). More amino acids were found in the egg masses than in the roe of salmon which contains only 10 amino acids (Seagran *et al*, 1953).

Amino acids are the building blocks of proteins and their nutritional importance can be

seen in their indispensable/essential amino acid concentration. Because of this, they are essential for the synthesis of enzymes and some hormones and neurotransmitters. Mc Ardle *et al* (2000) recommend that the daily protein intake for men and women should be at least 0.9g of protein/kg body weight for adolescents and 0.8 g of protein/kg body weight for adults. While the Recommended Dietary Intake for amino acids is 184 mg/kg body weight (WHO/FAO/UNU Expert Consultation, 2002).

The essential amino acids valine, leucine and isoleucine perform the important functions of increasing the protein which serves as a source of energy during exercise. According to Kreider (1999) protein and amino acids are among the most common nutritional supplements taken by athletes. Phenylalanine, threonine and histidine produce various useful amines,

Table 2. Amino Acid Profile of the *D. auricularia* Egg Masses

		Standard				
Amino Acid	Egg Mass	µg/ml	nmol/ml	μg/ml	nmol/ml	
Phospho-serine	P-ser ³	2.574	13.909	9.253	50.000	
Taurine	Tau ["]	19.373	154.834	6.256	50.000	
Urea	Urea 3	7.6555	127.451	120.120	2000.000	
Aspartic acid	Asp ²	4.299	32.300	13.310	100.000	
Threonine	Thr -	2.664	22.362	11.912	100.000	
Serine	Ser 2	2.707	25.758	10.509	100.000	
Glutamic acid	Glu	23.842	162.045	14.713	100.000	
Glutamine	Gln	0.501	3.427	14.615	100.000	
Aromatic amino acid	AAA ³	0.595	3.694	8.058	100.000	
Glycine	Gly ²	7.670	102.165	7.507	50.000	
Alanine	Ala	7.562	84.866	8.910	100.000	
Citrulline	Cit [,]	0.254	1.452	17.519	100.000	
Valine	Val	5.643	48.186	11.715	100.000	
Isoleucine	lle	5.253	40.044	13.117	100.000	
Leucine	Leu	0.761	5.801	13.117	100.000	
Tyrosine	Tyr ²	3.367	18.583	18.119	100.000	
Beta-alanine	b-ala ³	1.214	7.349	16.519	100.000	
Phenylalanine	Phe	0.535	6.007	8.910	100.000	
Gamma-aminobutyric acid	GABA ³	0.239	2.321	10.312	100.000	
Ammonia	NH,	3.874	227.911	1.700	100.000	
Ornithine	Orn ³	0.240	1.814	13.217	100.000	
Histidine	His	0.230	1.484	15.516	100.000	
Proline	Pro ²	4.525	39.300	11.513	100.000	
TOTAL	105.575	1133.040				

¹Essential Amino Acid;² Non Essential Amino Acid; ³other components

form active sites of enzymes and produce histamine and others, respectively. Based on the unique functions of these essential amino acids in our bodies and the fact that they cannot be synthesized, it is imperative that they be included in the diet of every individual to compensate the amino acid requirements. The Essential amino acids in an appropriate amount are required for the maintenance of life, growth, synthesis of vitamins and reproduction (Sudhakar *et al*, 2011). Balance nutritional components in the body system must be of paramount importance to every individual to warrant healthy living. The lowest level in any of the essential amino acids in a protein source limits the utilization of the protein, thus making it the first limiting amino acid (Paulraj and Sridhar, 2001) while high levels of amino acids can trigger the occurrence of inflammatory diseases (Shoda *et al*,1996). Chukwuemeka (2008) reported that for an adult to remain healthy, essential amino acids as lysine. methionine, phenyalanine, threonine, tryptophan and valine must be incorporated in the diet. Results show (Table 2) that three (3) of the previously mentioned amino acids were present in the egg masses, making them a good source of essential amino acids and are highly recommended to be always incorporated in the diet to help supplement the amino acid requirements of human.

In terms of non-essential amino acids, tyrosine is used by our body in the production of various amines, while serine plays an essential role in the functioning of the central nervous system (CNS) by providing precursors for amino acids, protein and nucleotide synthesis, neurotransmitter synthesis and L-serine derived lipids (Tabatabaie et al, 2010). Glycine and alanine are used for the production of glutathione and porhyrin, a component of hemoglobin, respectively. Aspartic acid and glutamic acid are both fast-acting energy sources to the body. Glutamine and proline are used to maintain the normal functioning of the gastrointestinal tract and muscles and serve as the main components of collagen that constitutes the skin and other tissues, respectively. Taurine, an intracellular sulphur-containing amino acid (Boucknooghe et al, 2006), is not part of the human body's structural proteins, which is why it remains free in the tissues and bloodstream and is abundant in the heart, the skeletal muscles and nervous system. Taurine is recognized for its diverse biological function into our body. It has been shown to be a potent antioxidant and effective against diabetes mellitus by decreasing the concentration of glucose and fructosamine, and increasing the contents of insulin, c-peptide and glycogen in the liver (Gavrovskaya et al, 2008). Another study revealed that supplementing taurine in the diet of an infant can help aid in bile acid secretion, fat absorption and proper liver function (Hayes et al, 1989). Taurine has been proven to be more effective than a placebo in preventing alcohol relapse, however, its effectiveness appeared to be dose dependent (Wilde, et al, 1997). Another study revealed that taurine is very useful in treating alcohol dependency in a dosage of one (1) gram three times daily, and fewer psychotic episodes were observed compared to people who took a placebo (Ikeda, 1977). Taurine has been shown by Matsuyama et al (1983) to decrease the level of bilirubin and bile acids in a double-blind, randomized study of an acute hepatitis patients in the amount of four (4) grams three times daily after meals. The egg masses which contained nine (9) non essential amino acids can be a very good food source that would aid/supplement for proper nourishment.

In terms of the other components of the egg masses, it was found that aside from essential and non-essential amino acids, they also had eight other components (Table 2). Gamma-aminobutyric acid as dietary supplement works as a primary inhibitory neurotransmitter in the

central nervous system in adults, has been claimed to either increase or decrease human growth hormone levels and may increase the level of the hormones insulin and glucagon, however, these claims have not yet been proven empirically and scientifically. Citrulline, an amino acid which serves as an important constituent of the liver, acts as a detoxifying agent by removing toxic compounds such as ammonia in the system. Orthinine an amino acid derived from the breakdown of arginine during the citric cycle, helps build muscles and reduce body fat. Urea, a waste product at the end of protein metabolism, is used by the body as a raw material to synthesize amino acids and proteins. Ammonia is essential in the body as a building block for making proteins and other complex molecules. Beta-alanine, is a naturally occurring beta-amino acid, classified as nonproteinogenic amino acid, is thought to be used by the body in the building of proteins.

This study showed that, egg masses of the wedge sea hare contain amino acids that can help supplement the amino acid requirements for healthy living. As shown in Table 2, eating an egg mass, introduces into the body several essential and non-essential amino acids that can help balance nutritional requirements. Eating an egg mass is far better than taking a synthetic dietary supplement because supplements contain only target amino acids while egg masses contain several amino acids that will be assimilated into the body as a potent source of amino acids and another ideal food commodity from the marine environment.

CONCLUSION

The results of this study show that the egg masses of *D. auricularia* contain a number of essential amino acids that are needed by man in his diet. Thus, egg masses can be ideal as food for human.

RECOMMENDATION

Further studies are suggested to determine the profile of the fatty acid component, its vitamins and minerals content and to screen the egg masses for any possible biologically active compounds to maximize its utilization and consumption.

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