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PROTEIN HYDROLYSIS OF EGGS FROM THE SEA URCHIN *TRIPNEUSTES GRATILLA* BY THE INDUSTRIAL ENZYME ALCALASE[#]

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Abstract. The sea urchin *Tripneustes gratilla* (Linnaeus, 1758) is an important economic sea product of Viet Nam because of the culinary, nutritional, and medicinal values of its eggs. In order to develop a value-added product from the sea urchin eggs, we investigated in this study their partial hydrolysis using the industrial enzyme alcalase to produce a protein hydrolysate containing free amino acids and oligopeptides, which are valuable dietary supplements.

Keywords: Sea urchin eggs, partial protein hydrolysate, enzyme alcalase.

Classification numbers: 1.3.1; 1.3.2; 1.4.3.

1. INTRODUCTION

The sea urchin *Tripneustes gratilla* (Linnaeus, 1758) is a shellfish belonging to the Echinoidea family that lives in groups between rocks, stones and algae at a depth of 20 m in the sea. This shellfish is bowl-shaped, usually dark or brown in colour, 7-8 cm in diameter and has a shell covered with spines. Some recent studies show that the egg of sea urchin has high nutritional and pharmacological value. These results also demonstrate the presence of aminoacids, vitamin A, vitamin E, trace elements (Fe, Mg and Zn), which are highly nutritive [1]. Generally, fish and shellfish meat is considered to be highly nutritious owing to its content of essential aminoacids and proteins. In addition to their dietary importance, proteins also influence food organoleptic properties: proteins affect food texture and small peptides and aminoacids contribute to food flavor [2]. Sea urchins, and especially its eggs are important economic sea products of Viet Nam. The results of many reseaches on the chemical composition

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of sea urchin eggs found in the world have discovered that the gonade of sea urchin has antibacterial effect. Moreover, eggs of sea urchin also contain essential aminoacids, β-carotene and docosahexaenoic acid (DHA) [3]. Chen and his colleagues pointed out that the composition of the sea urchin egg contains 80 % of unsaturated fatty acids (PUFA), in addition to eicosapentaenoic acids - Omega-3 (EPA), arachidonicacid - Omega-6 (AA) and natural carotene containing antioxidants such as echinenone, carotene and fucoxathine [4].

Enzymatic hydrolysis is a good way to protein recovery from by products and produce value added products from wastes [5]. Alcalase, a popular industrial enzyme in Viet Nam, is separated and purified from microbial sources. By using alcalase enzyme it is easy to adjust hydrolysis, calculate the required amount of base to maintain constant pH during hydrolysis. Selection of this enzyme is also based on its characteristics for the non-suckingability of aminoacids, resulting in a non-bitter hydrolysis product at the end of the hydrolysis. These features are essential [6]. The enzymatic treatment helps to create well defined peptide profiles and there is an extensive review on the application of enzymatic protein hydrolysates in human nutrition.

In order to develop a value-added product from the sea urchin eggs, we investigate in this study the partial hydrolysis of these eggs using the industrial enzyme alcalase to produce a protein hydrolysate containing free amino acids and oligopeptides, which are valuable dietary supplements.

2. MATERIALS AND METHODS

2.1. Materials

The sea urchins *Tripneustes gratilla* (Linnaeus, 1758) were collected in Hon Tam, NhaTrang, Khanh Hoa, Viet Nam and classified by Dr. Nguyen An Khang, NhaTrang Institute of Oceanography at the Vietnam Academy of Science and Technology. All samples were cleaned and stored at 4 ^{o}C under standard condition.

The industrial enzyme alcalase which was produced from *Bacillus licheniformis* was supplied by Novozyme (Kobenhavn, Denmark) and had declared activity of 2.4 AU/g and density of 1.18 g/ml.

2.2. Methods

To create amino acid standard samples, 95 μL of the 250 pmol/μL amino acid standard mixture was mixed with 5 μL of 10 mM norvaline and these standard samples were analyzed directly by RP-HPLC, within 24 h after preparation. The linear measured solutions were prepared in duplicate by diluting the 1 nmol/μL amino acid standard solution with the concentrations of 20, 50, 130, 250, or 500 pmol/μL of amino acid standard mixture together with 0.5 mM norvaline.

Protein samples: Glass test tubes (50 × 6 mm) were marked with incisions and soaked in a detergent solution for at least 12 h. Firstly, they were rinsed completely in Milli-Q water and after that they were put in an oven at 100 °C for drying. Protein samples (7–75 μg) were transferred into the glass test tubes and spiked with 0.5 mM norvaline. They were quickly spun in a low-velocity centrifuge, then frozen and dried in a lyophilizer. Samples were then transferred into the reaction vial which contained 0.5 mL of constant-boiling HCl on the bottom. Up to 12 test tubes could be accommodated in one reaction vial. These reaction vials were

precisely closed and transferred into a pre-heated oven at 110 °C for 18 h. All reaction vials were cooled at room temperature, then opened under an aspirated hood carefully. The test tubes were centrifuged and removed remaining liquid again in the lyophilizer to remove any liquid traces (condensed vapors). The dried residues were dissolved in 100 µL of 0.1 N HCl and transferred into the HPLC glass insert vials.

Instrument: An Agilent 1100 Liquid Chromatography system which was equipped with a binary pump delivery system (G1312A), robotic autosampler (G1313A), column thermostat (G1316A) and multi-wavelength detector (G1365A) was used to carry out these analyses.

Analytical procedure: Chromatographic conditions were followed by the Agilent methods. Shortly, the partial protein hydrolysate samples and the norvaline-spiked amino acid standard solutions were automatically derivatized with OPA by programming the robotic autosampler. After that, an amount equivalent to 2.5 µL of each sample was injected on a Zorbax Eclipse-AAA column, 5 µm, 150 × 4.6 mm (Agilent), at 40 °C and detection was conducted at λ = 338 nm. Mobile phase A: 40 mMNaH_2PO_4, pH 7.8 (this pH was kept stable by NaOH). Mobile phase B: acetonitrile/methanol/ water (45/45/10 v/v/v). The separation was obtained at a flow rate of 2 mL/min with a gradient program that allowed for 1.9 min at 0 % B followed by a 16.3-min step that raised eluent B to 53 %. Then washing at 100 % B and equilibration at 0 % B was performed in a total analysis time of 26 min. The technology parameters were determined based on single factorial experiments and the amount of total dissolved protein was used to choose the optimum parameter.

3. RESULTS AND DISCUSSION

3.1. The hydrolysis process

Sample treatment

After washing with distilled water and with a solution of 0.1 % NaCl, 100 g egg samples were minced to reduce the size of the materials and make them easier link with the active centre of the enzyme.

Pasteurization

1 ml of distilled water was added to 1 g of the clean egg samples and then heated at 90 ^oC for 15 min. Under this condition, almost all bacteria were killed and some protein, lipid components were denatured gradually, which facilitate the hydrolysis process.

Hydrolysis with alcalase

The hydrolytic liquid was maintained at the room temperature for 5 minutes and then alcalase was added at a rate of 1 % of the weight of the material. The whole liquid samples were stirred at 300 rpm at 45 – 50 ^oC for 6 h.

Stopping enzymatic activity

To keep the product from adverse changes, the enzyme alcalase remained need to be inactivated. For this purpose, the hydrolytic liquid was heated at 90 ^oC for 15 min.

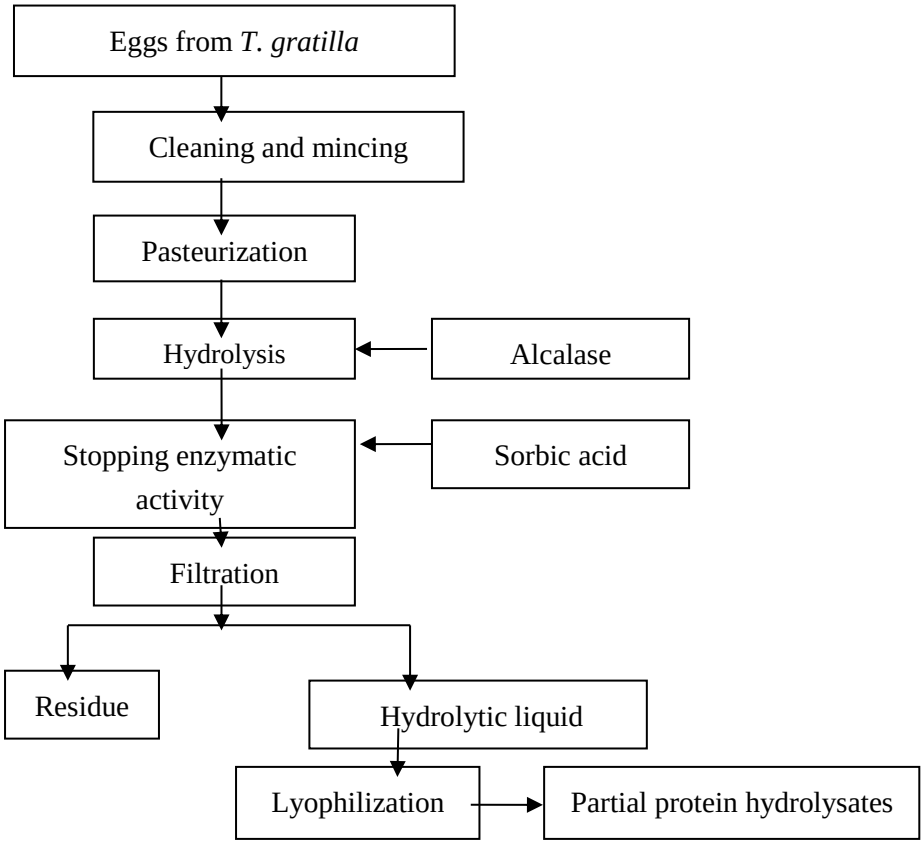

Figure 1. The flow chart of the hydrolytic process of eggs from sea urchin *T. gratilla* by the enzyme alcalase.

Filtration

The hydrolytic liquid was then centrifuged at 8000 rpm for 10 min to remove pellets, and then filtered (filter 200 µm pore size).

Freeze dry

The hydrolytic liquid was freeze-dried until a moisture content of 12% was achieved. The protein hydrolysate powder was packed in plastic bags under vacuum.

3.2. Quantitative determination of free amino acids

From the analytical result acquired, we found that the total protein contents in sea urchin eggs and their protein hydrolysate with alcalase enzyme are 181.29 mg/g and 219.51 mg/g**.** A total protein content of 181.29 and 219.51 mg/g were found for the sea urchin eggs and the hydrolysate powder, respectively. The amount of free amino acids in fresh egg samples from sea urchin *T. gratilla* and hydrolysate powder that was produced by the process mentioned above were shown in Table 1.

Table 1. Content of free amino acids of eggs and the hydrolysate powder.

No	Amino acid	Free amino acids (mg/g)		No	Amino acid	Free amino acids (mg/g)	
		Before hydrolysis	After hydrolysis			Before hydrolysis	After hydrolysis
1	Aspartate	-	-	10	Cysteine	-	-
2	Glutamate	-	-	11	Valine*	-	-
3	Serine	-	-	12	Methionine*	-	-
4	Histidine*	-	-	13	Phenylalanine*	-	-
5	Glycine	31.76	-	14	Isoleucine*	-	-
6	Threonine*	-	101.09	15	Leucine*	-	1.94
7	Arginine	4.45	5.22	16	Lysine*	-	-
8	Alanine	-	-	17	Proline	226.65	254.99
9	Tyrosine	-	-				
Free amino acids		**262.86**	**363.24**				
Essential amino acids		**0**	**103.03**				
Common amino acids		**262.86**	**260.21**				

(-): trace; * : essential amino acid.

As illustrated in Table 1, by HPLC, 17 amino acids were found in the egg samples. This result is similar to what previous research found in other species of sea urchin [8, 9]. Among these amino acids there were 8 essential amino acids (histidine, threonine, valine, methionine, phenylalanine, isoleucine, leucine, and lysine) with a total content of 103.03 mg/g. The others were 7 common amino acids (glutamic acid, serine, glycine, alanine, tyrosine, cysteine, and proline) with a total content of 262.86 mg/g. In addition, Table 1 also showed that the free amino acid content was increased in the hydrolysate powder. Especially, the contents of the essential amino acids were considerably improved. Thus, the content of threonine increased from 0 to 101.09 mg/g and that of leucine from 0 to 1.94 mg/g. The amino acid composition, in general, is very important in term of nutrition and food flavor [10]. The content of total amino acids (TAA) affects the nutritional value of the food, while free amino acids (FAA) affect its flavor. Moreover, amino acids are precursors of many biological compounds, notably proteins, and play an important role for energy production. Deficiency or excess of one or more of the amino acids is known to limit protein synthesis, growth, or both [11]. From many previous studies, the taste components of some seafood products have been discovered. Especially, glycine, alanine, valine, glutamine, and methionine were found as components of the taste of the sea urchin, with glycine and alanine responsible for its sweetness and valine responsible for its bitterness. In addition, glutamine contributes to the umami taste of sea urchin [12].

4. CONCLUSIONS

The partial hydrolysis of the eggs from the sea urchin *Tripneustes gratilla* using the industrial alcalase produced a hydrolysate powder containing more free amino acids, especially more essential free amino acids, than the eggs. This suggests that the obtained hydrolysate powder has better functional properties than the initial eggs.

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