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RESEARCH ON LIPID CHEMISTRY OF SEA URCHIN *TRIPNEUSTES GRATILLA* AND SEA URCHIN *DIADEMA SAVIGNYI* COLLECTED IN NHA TRANG, KHANH HOA AND APPLICATION ORIENTATION IN FOOD TECHNOLOGY

SUMMARY OF DISSERTATION ON CHEMISTRY OF NATURAL PRODUCTS

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LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Hoa Dinh Thi Kim**, Long Pham Quoc, Phi Hung Nguyen, Phuong Doan Lan and Thang Tran Dinh, "Research on the component of lipid classes, fatty acid from egg and body of sea urchin *Diadema savignyi* (Audouin, 1809)", *Journal of Pharmacognosy and Phytochemistry 2018; 7(1): 836-840*
2. **Dinh Thi Kim Hoa**, Luu Hong Son, Nguyen Thi Tinh, Ta Thi Luong, Nguyen Xuan Vu, Doan Lan Phuong, "Optimization of enzymatic protein hydrolysis conditions of sea urchin *Tripneustes gratilla* by using Alcalase#, 2021 Viet Nam National Conference of Biotechnology, Thai Nguyen University Publishing House, (2021) 969 - 975.
3. **Dinh Thi Kim Hoa**, Pham Quoc Long, Doan Lan Phuong, Research on the composition of lipids, fatty acids, and amino acids from egg and body of sea urchin *Tripneuster gratilla*, *Vietnam Journal of Science and Technology* ***56*** *(4A) (2018) 30-38,*
4. **Dinh Thi Kim Hoa,** Hoang Thi Bich, Pham Quoc Long, Tran Quoc Toan, Doan Lan Phuong, Protein hydrolysis of eggs from the sea urchin *Tripneuster gratilla* by the industrial enzyme Alcalase$^{\#}$, *Vietnam Journal of Science and Technology* ***57*** *(2) (2019) 133-138,* doi:10,15625/2525-2518/57/2/12894
5. **Dinh Thi Kim Hoa**, Luu Hong Son, Nguyen Lan Nhi, Doan Lan Phuong, The research on the optimization of protein hydrolysis from egg of sea urchin *Diadema savignyi* by Alcalase$^{\#}$ enzyme", **Viet Nam Journal of Agriculture and Rural Developmetn**, *volume 2 No.5 2023, 91-100.*
6. **Thi-Kim-Hoa Dinh**, Phi-Hung Nguyen, Doan Lan Phuong, Thi-Phuong-Ly Dang, Pham Minh Quan, Thi-Kim-Dung Dao, Valeria P, Grigorchuk, Pham Quoc Long, Component and content of Lipid classes and Phospholipid molecular species of egg and body of the Vietnamese sea urchin *Tripneustes gratilla*, *Molecules* **2023**, *28*, 3721. https://doi.org/10.3390/molecules28093721
7. **Dinh Thi Kim Hoa**, Doan Lan Phuong, Patent Patent (application accepted): Process for producing hydrolyzed sea urchin egg powder with Alcalase enzyme, Valid application accepted, application number 1-2003-02315.

I. PREFACE

1. The urgency of the thesis

Sea urchins are a large group of marine invertebrates in the Echinodermata phylum (spiny-skinned animals), with medicinal and nutritional value. Many studies on sea urchins have discovered biologically active compounds that can be isolated, purified, and converted into pharmaceuticals or functional foods. Extracts and hydrolysates from sea urchin eggs have different biological activities, with notable compounds including glycosides, polysaccharides, glycolipids, sulfate-polysaccharides, and phospholipids.

Despite their high medicinal and nutritional value, the beneficial chemical components of sea urchins have not been thoroughly researched to the extent that isolation and extraction techniques can produce high-tech food or pharmaceutical products.

Applying hydrolysis technology to sea urchin eggs is a new and promising research direction. Biological hydrolysis is highly efficient, with gentle reaction conditions, safety for workers, and high-quality products. Hydrolyzed protein powder from sea urchin eggs can have various applications in medicine or be added to food products to enhance absorption in children and the elderly. Additionally, sea urchin eggs are rich in valuable phospholipids (PL) and fatty acids. Evaluating the molecular forms of PL and their concentrations in sea urchin eggs is a very new and important research direction.

Therefore, the content of this dissertation focuses on studying the phospholipid class in the eggs and body of the sea urchin *Tripneustes gratilla* and the sea urchin *Diadema savignyi* collected from Nha Trang, Khanh Hoa, aiming to guide the source of raw materials and create low molecular weight protein and phospholipid powders, thereby increasing the economic value of sea urchins.

2. Research aims of the thesis

- Study the basic chemical composition of the eggs and body of the sea urchin (*Tripneustes gratilla*) and the sea urchin (*Diadema savignyi*) collected from Nha Trang, Khanh Hoa.
- Research the composition of lipid classes and fatty acid content in the eggs and body of the sea urchin (*Tripneustes gratilla*) and the sea urchin (*Diadema savignyi*).
- Determine the molecular species of phospholipids and their ratios in each phospholipid subclass of total lipid from egg and body samples of the sea urchin (*Tripneustes gratilla*) and the sea urchin (*Diadema savignyi*).

- Develop the technology for hydrolyzing the eggs of the sea urchin (*Tripneustes gratilla*) and the sea urchin (*Diadema savignyi*) using Alcalase enzyme to create health supplement product with low molecular weight protein as the main component, thus creating the first food products from sea urchins in Vietnam.

3. Research contents of the thesis

- Determine the chemical composition, total lipid content, lipid classes, and the composition and quantity of fatty acids present in four study samples from the body and eggs of the sea urchin (*Tripneustes gratilla*) and the sea urchin (*Diadema savignyi*).

- Identify the molecular species of phospholipids in four study samples from the body and eggs of the sea urchin (*Tripneustes gratilla*) and the sea urchin (*Diadema savignyi*).

- Develop a process for hydrolyzing the eggs of the sea urchin (*Tripneustes gratilla*) and the sea urchin (*Diadema savignyi*) using Alcalase enzyme, and optimize the key process parameters affecting the hydrolysis process.

- Complete the technology for producing health supplement products from sea urchin egg and evaluate the quality of the achieved products.

4. New contributions of the thesis

- This is the first work to study in detail the composition and content of lipid classes, fatty acid, and phospholipid of sea urchin (*Tripneustes gratilla*) and sea urchin (*Diadema savignyi*) collected in Nha Trang, Khanh Hoa.

- Phospholipid molecular species such as phosphatidylchloline (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidyl-acid (PA), lyso phospholipids (LPC, LPE, LPS) of two species of Sea urchin *Tripneustes gratilla* and *Diadema savignyi* have been identified for the first time. The results of the research reveals that phospholipid of two investigated sea urchins consists of 7 classes such as PI, PS, PE, PA, PC, LPC, LPE. There are 24 molecular species of PE, 76 molecular species of PC, 16 molecular species of PS, 11 molecular species of PA, 24 molecular species of PI, 19 molecular species of LPC, 10 molecular species of LPE. In addition, 23 molecular species of Sulfoquinovosyl diacylglycerols (SQDG), sulfolipids, were also detected.

- A hydrolyzed product from sea urchin egg by using enzyme technology combined with membrane filtration was created for the fist time. The product was rich in amino acids, oligopeptides and low molecular weight proteins. The product meets the standards of a functional food product.

5. Thesis structure

The dissertation consists of 139 pages, including 38 figures, 65 tables, and 2 diagrams. The structure of the dissertation is as follows: Introduction (3 pages); Chapter 1: Overview (28 pages); Chapter 2: Materials and Research Methods (4 pages); Chapter 3: Experiments (15 pages); Chapter 4: Results and Discussion (76 pages); Conclusion and Recommendations (3 page); List of Published Works from the Dissertation (1 page); References (9 pages).

THESIS CONTENT

PREFACE

The introduction section addresses the scientific significance, practical relevance, research objectives, and tasks of the dissertation, as well as the novel aspects discovered through the research.

CHAPTER 1. OVERVIEW

The overview consists of three major parts: the first part provides an overview of the research subject (lipids and phospholipids); the second part provides an overview of the research materials (chemical composition and biological activity of sea urchins in general and the two sea urchin species studied); the third part provides an overview of the enzymatic hydrolysis technology for proteins.

CHAPTER 2. RESEARCH MATERIALS AND METHODS

2.1. Research materials

Samples of the sea urchin *Tripneustes gratilla* (Linnaeus, 1758) and the sea urchin *Diadema savignyi* (Audouin, 1809) were collected from Hon Tam, Nha Trang, Khanh Hoa, Vietnam, and were classified by Dr. Nguyen An Khang from the Nha Trang Institute of Oceanography – Vietnam Academy of Science and Technology.

2.2. Research methods

2.2.1. The methods for determining the basic chemical components of the materials

The Kjeldahl method is used to determine total protein content; the Lowry method is used to determine soluble protein content; the ashing method is used to determine ash content; the drying method at 150°C is used to determine moisture content; and chromatography is used to determine amino acid composition.

2.2.2. The total lipid extraction method

The total lipid extraction method is Blight - Dyer method.

2.2.3. The methods for isolating lipid classes and lipid subclasses

Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) are two methods used to isolate lipid classes and lipid subclasses.

2.3.4. The methods for determining the composition and content of lipid classes

The composition and content of lipid classes are determined based on the thin-layer chromatography (TLC) method combined with the Sorbfil TLC Videodensitometer imaging analysis program from Krasnodar, Russia.

2.3.5. Determination of fatty acid composition and content

A mixture of fatty acid methyl esters is analyzed using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), with the use of the NIST standard spectral library for comparison.

2.2.6. Methods for determining the molecular form and structure of compounds and phospholipid subclasses

The molecular forms of phospholipids are analyzed using high-resolution mass spectrometry (HRMS), recorded on a Shimadzu LCMS-IT-TOF device provided by Shimadzu (Kyoto, Japan).

2.2.7. Multifactorial optimization method for the protein hydrolysis process

The experiment to optimize the protein hydrolysis process from sea urchin eggs is designed using the Box-Behnken model, with three variables at three levels and 17 experimental units, each repeated three times with the selected variables.Phương pháp đánh giá độc tính cấp và độc tính bán trường diễn của sản phẩm

2.2.8. Methodology for assessing acute toxicity and subchronic toxicity of the product

CHAPTER 3. EXPERIMENTS

The experimental section provides a detailed description of the steps involved in the experiments and the research methods outlined in Chapter 2. These include experiments to analyze total protein content, soluble protein content, ash content, total lipid content, lipid classes, fatty acid composition, and more.

Additionally, Chapter 3 discusses the process flow diagram of the technology for hydrolyzing sea urchin eggs. It explores the influence of certain technological factors such as the water-to-material ratio, the amount of enzyme added, hydrolysis time, pH, and hydrolysis temperature. This allows the identification of the three factors that most significantly affect the amount of soluble protein obtained, which is then used to optimize the technology.

CHAPTER 4. RESULTS AND DISCUSSION

4.1. The chemical composition of the body and egg of the sea urchin (*Tripneustes gratilla*) and the sea urchin (*Diadema savignyi*)

Table 4.1. Results of the chemical composition of the body and egg of sea urchins

4.2. Lipid classes and their content in the body and egg of the sea urchin (*Tripneustes gratilla*) and the sea urchin (*Diadema savignyi*)

No.	Components	*Tripneustes gratilla egg*	*Tripneustes gratilla body*	*Diadema savignyi* egg	*Diadema savignyi* body
1	Water (%)	75.28 ± 0.83	68.12 ± 0.24	80.13 ± 0.61	71.51 ± 0.52
2	Total ash (% wet base)	2.05 ± 0.03	5.47 ± 0.22	1.93± 0.01	4.12 ± 0.02
3	Total lipid (% wet base)	4.41 ± 0.03	1.32 ± 0.03	3.18 ± 0.02	1.33 ± 0.04
4	Total Protein (% wet base)	12.45 ± 0.14	4.17 ± 0.11	11.74 ± 0.15	3.81 ± 0.08
5	Soluble Protein (mg/g)	141.48 ± 0.62	38.71 ± 0.22	82.43 ± 0.17	26.91 ± 0.09

Table 4.2. Results of the composition and content of lipid classes in the eggs and body of the sea urchin Tripneustes gratilla

No.	Lipid class	Research sample	
		Egg (%)	Body (%)
1	**PoL**	4.41 ± 0.05	6.36 ± 0.04
2	**MDAG và DAG**	1.11 ± 0.01	1.43 ± 0.01
3	**ST**	5.69 ± 0.05	6.63 ± 0.04
4	**FFA**	4.76 ± 0.03	4.49 ± 0.03
5	**TAG**	78.37 ± 0.64	76.10 ± 0.57
6	**MADAG**	3.31 ± 0.05	3.24 ± 0.05
7	**HW**	2.35± 0.04	1.75 ± 0.02

Table 4.3. Results of the composition and content of lipid classes in the egg and body of the sea urchin Diadema savignyi

No.	Lipid class	Egg (%)	Body (%)
1	**PoL**	20.47 ± 0.05	33.94 ± 0.60
2	**MDAG và DAG**	12.45 ± 0.01	19.57 ± 0.26
3	**ST**	4.14 ± 0.05	9.28 ± 0.16
4	**FFA**	55.27 ± 0.03	32.39 ± 0.30
5	**TAG**	4.43 ± 0.04	2.50 ± 0.08
6	**MADAG**	3.24 ± 0.05	2.32 ± 0.07

PoL: polar lipid; ST: sterol; FFA: free fatty acid; TAG: triacylaglycerol; MADAG: monoalkyldiacylglycerol; HW: wax; MDAG và DAG: Mono and Diacyl Glycerol

The results from Table 4.2 show that for the sea urchin *Tripneustes gratilla*, triacylglycerol is the largest and most prominent type within the total lipids of both the egg and body samples, accounting for 78.37% and 76.10%, respectively. This study also indicates that the percentage of sterol is low as a polar lipid class, with 5.69% in the egg sample and 6.63% in the body sample. The remaining three groups (fatty acids, monoalkyl diacylglycerol, and hydrocarbon + wax) have low contents, below 5% for both samples.

For the sea urchin *Diadema savignyi*, triacylglycerol constitutes the largest proportion within the total lipids of the egg sample (55.27%) and the second-highest after polar lipids in the body sample (32.39%). Polar lipids are also the main component of the total lipids of both the body and egg samples, with contents of 33.94% and 20.47%, respectively.

4.3. Fatty acid components and content in the body and egg of the sea urchin (*Tripneustes gratilla*) and the sea urchin (*Diadema savignyi*)

4.3.1. Fatty acid components and content in the body and egg of the sea urchin (*Tripneustes gratilla*)

In this study, 25 and 24 fatty acids were identified in the egg and body samples, with 12 to 22 carbon atoms respectively. Fatty acids 14:0, 16:1n-9, 16:0, 18:1n-9, 20:4n-6, and 20:5n-3 were found to have relatively high concentrations. Palmitic acid (C16:0) and myristic acid (14:0) were observed to be the dominant saturated fatty acids (SFA) in all samples, ranging from 3.59% to 14.50% (C14:0) and from 11.74% to 25.10% (C16:0), respectively, in the egg and body samples.

Table 4.4. Fatty acid components and content in the body and egg of the sea urchin (Tripneustes gratilla)

No.	Fatty acid	Egg	Body	No.	Fatty acid	Egg	Body
1	12:0	0.08	-	15	18:0	1.39	1.57
2	14:0	14.50	3.59	16	20:0	0.23	0.12
3	14:1n-7	2.03	0.33	17	20:3n-3	0.32	0.68
4	15:0	0.44	0.20	18	20:2n-6	0.67	-
5	16:1n-9	8.66	3.59	19	20:1n-9	2.50	6.25
6	16:2n-4	0.32	-	20	20:4n-6	10.95	30.96
7	16:1n-7	3.08	2.04	21	20:5n-3	6.42	13.39
8	16:0	25.10	11.74	22	20:3n-6	2.46	5.55
9	18:4n-3	3.67	2.64	23	20:4n-3	1.15	1.46

No.	Fatty acid	Egg	Body	No.	Fatty acid	Egg	Body
10	18:2n-6	1.86	1.81	24	22:6n-3	0.22	0.31
11	18:1n-9	8.87	4.62	25	22:1n-9	0.52	0.27
12	18:1n-7	0.87	0.91	26	22:6n-6	-	0.30
13	18:3n-3	2.19	2.19	27	22:4n-6	-	0.57
14	18:3n-6	0.85	0.64	28	Other	0.65	4.27
SFA		41.74	17.22	**Omega-6**		16.79	39.83
MUFA		26.53	18.01	**Omega-9**		20.55	14.73
PUFA		31.08	60.50	**PUFA/SFA**		74.46	3.51
Omega-3		13.97	20.67	**n3/n6**		0.83	0.52

The PUFA/SFA ratio and n3/n6 ratio found in the total lipids from the eggs and body of *Tripneustes gratilla* are (74.46 and 3.51) and (0.83 and 0.52), respectively, which are high ratios. According to the World Health Organization (WHO), the eggs and lipid extracts from *Tripneustes gratilla* used in the study are classified as top-quality food products and are very beneficial for human health.

4.3.2. Fatty acid components and content in the body and egg of the sea urchin *Diadema savignyi*

The fatty acid composition in the total lipids of the eggs and body of *Diadema savignyi* is very diverse, with the presence of 27 fatty acids in the sea urchin egg lipid sample and 30 fatty acids in the sea urchin body sample with carbon chains ranging from 14 to 24. The composition mainly includes fatty acids such as 14:0, 16:0, 20:4n-6, 20:1n-9, and 18:0 in relatively high amounts, while other fatty acids have lower contents, with up to 24 fatty acids present at levels below 1%.

Table 4.5. Fatty acid components and content in the body and egg of the sea urchin Diadema savignyi

No.	Fatty acid	Egg	Body	No.	Fatty acid	Egg	Body
1	**14:0**	**17.35**	**9.38**	17	**18:0**	**5.74**	**9.71**
2	a-15:0	0.59	0.48	18	19:0	0.41	0.69
3	15:0	1.35	1.11	19	19:1n-9	0.96	1.77
4	16:1n-9	4.41	2.72	20	a-19:0	-	0.18
5	16:2n-6	0.37	0.15	21	20:0	0.69	0.46
6	a-16:0	0.15	-	22	20:1n-7	0.50	0.63
7	**16:0**	**31.40**	**27.49**	23	**20:1n-9**	**9.90**	**12.90**
8	i-17:0	-	0.29	24	**20:4n-6**	**8.58**	**11.34**
9	a-17:0	0.57	-	25	20:5n-3	1.76	1.75

No.	Fatty acid	Egg	Body	No.	Fatty acid	Egg	Body
10	17:0	1.20	1.40	26	20:2n-6	2.07	4.48
11	18:4n-3	0.24	0.29	27	21:1n-9	0.64	1.23
12	18:2n-6	1.29	1.01	28	21:0	0.18	0.26
13	18:1n-9	3.42	2.93	29	22:4n-6	0.18	0.20
14	18:1n-7	2.78	2.75	30	22:4n-3	0.13	0.30
15	18:3n-6	0.51	0.35	31	24:1n-7	0.17	0.17
16	i-19:0	-	0.27	32	24:1n-9	0.20	0.35
				33	Other	0.13	0.51
SFA		60.08	52.26	**Omega-6**		13.28	17.67
MUFA		23.61	26.27	**Omega-9**		20.46	22.86
PUFA		15.96	20.94	**PUFA/SFA**		0.27	0.40
Omega-3		2.31	2.47	**n3/n6**		0.17	0.14

For both the egg and body samples of *Diadema savignyi*, saturated fatty acids (SFA) accounted for a dominant proportion, at 60.08% and 52.26% in the eggs and body, respectively. Meanwhile, the content of monounsaturated fatty acids (MUFA) was 23.61% and 26.27%, and polyunsaturated fatty acids (PUFA) were 15.96% and 20.94%, respectively.

4.4. Phospholipid molecular species of the total lipid from the sea urchin (*Tripneustes gratilla*) and the sea urchin (*Diadema savignyi*)

The results indicate the presence of five classes of glycerolipids (GLP) in the phospholipids (PL) of both egg and body samples of both studied sea urchins, including phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidic acid (PA), and phosphatidylcholine (PC), as well as one glycosyldiacylglycerol known as sulfoquinovosyl diacylglycerol (SQDG). The identification of PL showed that 20 molecular species were found in the phosphatidylinositol (PI) class from the phospholipids of both body and egg samples of *Tripneustes gratilla* sea urchin and 23 molecular species of PI in the body sample and 24 molecular forms of PI in the egg sample of *Diadema savignyi* sea urchin. For yellow sea urchin samples, among the PI molecular species, PI 18:0/20:4 (m/z $[M-H]^-$ 885.5562) was the main component, with concentrations of 38.65% (in the egg sample) and 48.19% (in the body sample). Next were PI 20:0/20:5 (m/z $[M-H]^-$ 911.5645, with molecular formula $C_{49}H_{85}O_{13}P$), PI 20:0/20:4 (m/z $[M-H]^-$ 913.5814, with molecular formula $C_{49}H_{87}O_{13}P$), and PI 18:0/20:5 (m/z $[M-H]^-$ 883.5401, with molecular formula $C_{47}H_{81}O_{13}P$), accounting for 13.37%,

11.49%, and 9.36% in the egg samples and 12.64%, 9.89%, and 6.96% in the body samples, respectively.

In the sea urchin samples, 14 molecular species of PS were identified in the egg samples and 15 molecular species of PS in the body samples, while in both body and egg samples of *Diadema savignyi* sea urchins, 16 molecular species of PS were found.

Among the PS molecular species in *Tripneustes gratilla* sea urchin samples, PS 20:1/20:1 with negative ion $[M-H]^-$ at m/z 842.5962 (corresponding to molecular formula $C_{46}H_{86}NO_{10}P$) had the highest concentrations of 42.48% in the eggs and 44.41% in the body samples. For the egg samples of *Diadema savignyi* sea urchins, the molecular species PS 18:0/20:4 and PS 20:1/20:1 were the highest at 21.781% and 20.067%, respectively. However, in the body samples, the molecular specie PS 20:1/20:1 was significantly higher, accounting for 34.591%, while PS 18:0/20:4 accounted for only 9.052%. PS 20:1/20:4 and PS 20:1/21:1 were the second highest molecular specie in both egg and body samples of sea urchins *Diadema savignyi*. For phosphatidylethanolamine (PE), 22 molecular species were found in *Tripneustes gratilla* sea urchin samples. In the body and egg samples of *Diadema savignyi* sea urchins, 23 molecular species of PE were recorded; one additional PE molecular specie was discovered compared to the two *Tripneustes gratilla* sea urchin samples, namely PE 16:0/20:4, but it was present in very low concentrations, all below 1% in both body and egg samples.

For the phosphatidic acid (PA) class, 11 molecular species were identified in the egg and body samples of both sea urchins. For the two egg and body samples of *Tripneustes gratilla* sea urchins, the phosphatidylcholine class contained 73 species, corresponding to 73 molecular species, while *Diadema savignyi* sea urchins showed 77 molecular species of PC.

Lyso phosphatidylcholine (LPC) molecules are formed when PC loses one fatty acid group. In the body and egg samples of *Tripneustes gratilla* sea urchins, 21 molecular species of LPC were observed, and *Diadema savignyi* sea urchins recorded 20 molecular species of LPC.

Lyso-phosphatidyl-ethanolamines (lysocephalins, LysoPtdEtn, LysoPE, or LPE) belong to the ester phospholipid group within phospholipids. Ten molecular species of LPE were found in both sea urchins.

4.5. The result of research on the hydrolysis of sea urchin egg by using Alcalase enzyme

Table 4.22. Influence of added water ratio on the amount of soluble protein obtained

Ratio of water/material (v/w)	***Tripneustes gratilla* Soluble protein (mg/g)**	***Diadema savignyi* Soluble protein (mg/g)**
0.5/1.0	$140.24^{e} \pm 0.17$	$138.24^{e} \pm 0.16$
1.0/1.0	$171.29^{a} \pm 0.21$	$150.24^{a} \pm 0.13$
1.5/1.0	$165.71^{b} \pm 0.30$	$146.69^{b} \pm 0.21$
2.0/1.0	$163.81^{c} \pm 0.09$	$153.81^{c} \pm 0.09$
2.5/1.0	$159.87^{d} \pm 0.15$	$143.27^{d} \pm 0.11$

Table 4.23. The result of research on suitable Alcalase ratio

Enzyme ratio (%) (v/v)	***Tripneustes gratilla* Soluble protein (mg/g)**	***Diadema savignyi* Soluble protein (mg/g)**
0.5	$171.30^{b} \pm 0.34$	$150.26^{b} \pm 0.21$
1.0	$199.48^{a} \pm 0.21$	$190.24^{a} \pm 0.13$
1.5	$198.50^{a} \pm 0.22$	$190.30^{a} \pm 0.12$
2.0	$197.18^{a} \pm 0.31$	$189.12^{a} \pm 0.16$
2.5	$196.04^{a} \pm 0.17$	$189.14^{a} \pm 0.15$

Table 4.24. Result of research on the effect of pH to sea urchin egg hydrolysis

pH	***Tripneustes gratilla* Soluble protein (mg/g)**	***Diadema savignyi* Soluble protein (mg/g)**
6,0	$146.11^{d} \pm 0.34$	$141.26^{e} \pm 0.21$
6,5	$183.65^{b} \pm 0.21$	$175.41^{b} \pm 0.13$
7,0	$198.40^{a} \pm 0.24$	$190.34^{a} \pm 0.13$
7,5	$183.42^{b} \pm 0.13$	$173.13^{c} \pm 0.13$
8,0	$172.84^{c} \pm 0.17$	$169.14^{d} \pm 0.15$

Table 4.25. Result of research on the effect of temperature to sea urchin egg hydrolysis

Temperature (^oC)	***Tripneustes gratilla*** **Soluble protein (mg/g)**	***Diadema savignyi*** **Soluble protein (mg/g)**
35	$141.13^e \pm 0.12$	$129.26^d \pm 0.17$
40	$186.51^c \pm 0.19$	$153.45^c \pm 0.13$
45	$203.34^a \pm 0.18$	$193.34^a \pm 0.13$
50	$189.13^b \pm 0.13$	$176.34^b \pm 0.13$
55	$171.31^d \pm 0.16$	$153.42^c \pm 0.13$

Table 4.26. Result of research on the effect of hydrolysis time to sea urchin egg hydrolysis

Time of hydrolysis (hour)	***Tripneustes gratilla*** **Soluble protein (mg/g)**	***Diadema savignyi*** **Soluble protein (mg/g)**
5.0	$159.28^d \pm 0.15$	$148.19^d \pm 0.13$
5.5	$203.48^c \pm 0.24$	$193.25^c \pm 0.16$
6.0	$219.51^a \pm 0.17$	$210.13^a \pm 0.12$
6.5	$218.24^a \pm 0.33$	$210.15^a \pm 0.14$
7.0	$201.41^b \pm 0.27$	$197.35^b \pm 0.17$

Applying regression analysis methods to the experimental data, a quadratic polynomial model representing the total soluble protein content in the solution after hydrolysis is obtained as follows:

The result of sea urchin *Diadema savignyi*:

$Y = 203.14 + 38.25*A - 3.8*B + 41.13*C + 17.61*A*B + 33.90*A*C - 10.01*B*C - 47.27*A^2 - 53.55*B^2 - 78.60*C^2$

The result of sea urchin *Tripneustes gratilla:*

$Y = + 217.73 + 46.41*A + 0.95*B + 44.49*C + 8.04*A*B + 36.33*A*C - 11.53*B*C - 56.48*A^2 - 62.41*B^2 - 78.43*C^2$

Y is the soluble protein content obtained, and the values A, B, and C represent the values of the factors A: Enzyme ratio (%); B: Water/material ratio (v/w); C: Hydrolysis time (hours), respectively. Considering the impact of each factor (while keeping other factors at their average levels within their running ranges), the obtained regression equation shows that the factor impacting the soluble protein content is temperature, followed by the water/material ratio and hydrolysis time. ANOVA analysis is used to evaluate the model. The ANOVA analysis results are presented in Tables 4.28 and 4.29.

Table 4.28. ANOVA (Analysis of Variance) analysis of optimization of hydrolysis from sea urchin Diadema savignyi egg

Source	SS	DF	MS	F standard	P value
Model	84291.84	9	9365.76	373.46	< 0.0001
A - enzyme ratio	11706.03	1	11706.03	466.78	< 0.0001
B - Ratio of water/material	115.29	1	115.29	4.60	0.0692
C - Time of hydrolysis	13535.88	1	13535.88	539.75	< 0.0001
AB	1240.10	1	1240.10	49.45	0.0002
AC	4596.16	1	4596.16	183.27	< 0.0001
BC	400.80	1	400.80	15.98	0.0052
A^2	9408.82	1	9408.82	375.18	< 0.0001
B^2	12073.66	1	12073.66	481.44	< 0.0001
C^2	26015.11	1	26015.11	1037.36	< 0.0001
Residual	175.55	7	25.08		
Lack of Fit	88.43	3	29.48	1.35	0.3763
Error	87.11	4	21.78		
Total SS	84467.39	16			

Table 4.29. ANOVA (Analysis of Variance) analysis of optimization of hydrolysis from sea urchin Tripneustes gratilla egg

Nguồn	SS	DF	MS	F standard	P value
Model	101300	9	11250.80	270.87	< 0.0001
A - enzyme ratio	17233.89	1	17233.89	414.92	< 0.0001
B- Ratio of water/material	7.16	1	7.16	0.17	0.0690
C - Time of hydrolysis	15834.88	1	15834.88	381.24	< 0.0001
AB	258.73	1	258.73	6.23	0.0412
AC	5279.48	1	5279.48	127.11	< 0.0001
BC	531.76	1	531.76	12.80	0.0090
A^2	13434.04	1	13434.04	323.43	< 0.0001
B^2	16400.17	1	16400.17	394.85	< 0.0001
C^2	25901.88	1	25901.88	623.61	< 0.0001
Residual	290.75	7	41.54		
Lack of Fit	190.74	3	63.58	2.54	0.1945
Error	100.01	4	25.00		
Total SS	101500	16			

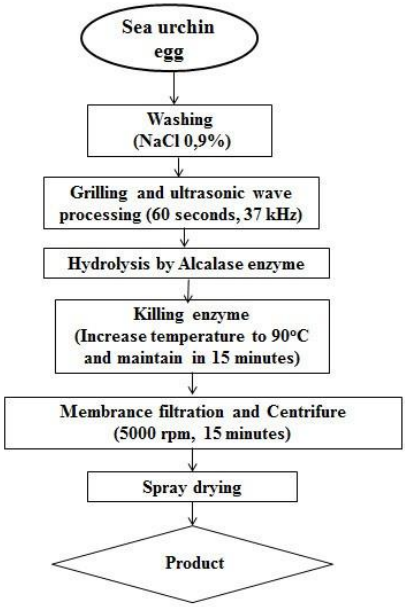
The significance and suitability of the model were assessed by analyzing table 4.28 and table 4.29, ANOVA analysis. The probability value of the model (P-value) is less than 0.0001, which is less than 0.05, indicating that the chosen model is significant. The regression coefficient (R^2) is 0.9954, showing that 99.54% of the experimental data is consistent with the model's predicted data. The Design-Expert software was used to optimize the total soluble protein content obtained from the hydrolysis of sea urchin eggs using the expected value function method. The interaction between enzyme ratio and hydrolysis time (keeping the water/material ratio at an average level) is the largest, followed by the model interaction between enzyme ratio and water/material ratio (keeping the hydrolysis time at an average level). The least interaction occurs in the model interaction between water/material ratio and hydrolysis time (keeping the enzyme ratio at an average level). The optimal conditions for maximizing the objective function are: enzyme ratio at 1.32%, water/material ratio at 0.88/1.0 mL/g of material, and hydrolysis time at 6.13 hours. Under these conditions, the total soluble protein content calculated to be achieved is 212.63 mg/g of sea urchin eggs. This result shows high consistency with experimental verification.

General process of protein hydrolysis from sea urchin eggs

Raw material handling: 1 kg of sea urchin egg frozen at -20^{o}C are thawed at normal temperature conditions. Then the entire egg mass is washed 3 times with clean water to remove impurities on the surface of sea urchin eggs. All sea urchin eggs are then submerged in a basin of water containing 0.9% NaCl salt solution and gently washed while removing all tendons and muscles in the body cavity of sea urchin eggs. The thorns are placed on a tray with drainage holes and left to drain.

After being drained, the sea urchin eggs are put into the screw mill, and the mill is turned on to grind the ingredients. After being crushed, the raw materials are put into a device that emits ultrasonic waves at a frequency of 37KHz for 60 seconds.

Hydrolysis of sea urchin egg: After that, the ground sea urchin egg is added with water at a ratio of 1/1.1 (v/w) to sea urchin *Tripneustes gratilla* eggs and 0.88/1. (v/w) with sea urchin *Diadema savignyi*; Add Alcalase enzyme at a rate of 1.1% for sea urchin *Tripneustes gratilla* and 1.32% for sea urchin *Diadema savignyi* and put it into an automatic hydrolysis device with a stirrer. Set the parameters for the hydrolysis device including temperature 45^{o}C, pH = 7.0 and time of 6.27 hours with sea urchin *Tripneustes gratilla* egg and 6.13 hours with black sea urchin *Diadema savignyi* egg, stirring speed only maintain 1200 rpm.



Kill enzymes and kill microorganisms: Finish the hydrolysis process, turn off the stirrer and set the device temperature to 90^{o}C. Monitor when the machine reports that the fluid temperature reaches 90^{o}C, then start counting the 15-minute timer. After 15 minutes, turn off the device completely and let it cool at room temperature to obtain a semi-finished product.

Rough filtration of semi-finished products: After 60 minutes, the hydrolysis - semi-finished product mixture has reached a temperature of 25^{o}C, the entire liquid mass is passed through a protein filter membrane with a pore size of 10 kDa and put into a centrifuge. Centrifuge at a speed of 5000 rpm for 15 minutes, obtaining the filtrate and solid residue, in which the filtrate contains free amino acids, oligopeptides and low molecular weight soluble proteins (less than 10 kDa) and mixed with oil, while the residue contains non-hydrolysable sea urchin egg components. This solid residue is dried, packaged and used as an attractant added to animal feed.

Drying and recovery of free amino acids, oligopeptides and low molecular weight proteins: Free amino acids, oligopeptides and soluble low molecular weight proteins passing through the membrane are recovered, spray dried at 180^{o}C until reaching a moisture content of less

than 10%, a hydrolyzed sea urchin egg powder product is obtained rich in amino acids, oligopeptides and low molecular weight proteins (<10 kDa). The resulting hydrolyzed sea urchin egg powder can be supplemented with sorbic acid at a rate of 0.2% of the dried powder weight for better preservation. The hydrolyzed sea urchin egg powder obtained can be used as a nutritional supplement to increase vitality and nourish the body for debilitated people.

Completing the production process and trial production with sea urchin egg raw materials. Project on developing standards for food products that protect health from sea urchin eggs. Acute and chronic toxicity, evaluation of antioxidant activity, analysis of total protein content, amino acids, total lipids, fatty acid composition and mineral composition of the product were all analyzed.

Table 4.30. Results of experimental production batches of sea urchin egg hydrolysis

No.	Material mass (kg)	Achieved product mass (kg)	Efficiency (%)
1	20.3	1.95	9.60
2	20.1	2.01	10.02
3	19.8	2.07	10.45
	Σ = 60.2	**Σ = 6.08**	**TB = 10.02**

Table 4.31. Number of dead mice and external manifestations observed in mice after consuming sea urchin egg health supplement product (SUEHSP)

Group	SUEHSP (g/kg)	Number of dead mice in 72 hours	External manifestations within 0 - 72 hours
1	5	0	After ingesting the sample, the mice moved and ate normally, with good light and sound reflexes.
2	6	0	24 hours after ingesting the sample, the mice moved and ate normally, with good light and sound reflexes
3	7	0	After 24 hours of ingesting the sample, the mice exhibited normal movement and feeding behavior, with intact light and sound reflexes.
4	8	0	After consuming the sea urchin health supplement, some mice exhibited reduced movement and decreased intake of food and water

5	9	0	After consuming the sea urchin health supplement, some mice showed reduced movement and decreased food and water intake
6	10	0	After consuming the sea urchin health supplement, some mice exhibited reduced movement and decreased intake of food and water

Table 4.32. Results of monitoring the weight of mice in different groups

Group (g/kg)	The average weight of the experimental mice			
	Before using product	**Day 1**	**Day 4**	**Day 7**
5	21.09± 0.27	21.17 ± 0.31	22.08 ± 0.28	23.13 ± 0.25
6	21.33 ± 0.30	21.37 ± 0.35	22.32 ± 0.31	23.42 ± 0.32
7	21.28 ± 0.33	21.32 ± 0.32	22.23 ± 0.26	23.36 ± 0.30
8	21.55 ± 0.31	21.60 ± 0.26	22.65 ± 0.33	23.68 ± 0.29
9	21.47 ± 0.28	21.55 ± 0.31	22.51 ± 0.34	23.65 ± 0.30
10	21.34 ± 0.34	21.31 ± 0.29	22.23 ± 0.31	23.39 ± 0.26
P	>0.05	>0.05	>0.05	>0.05

Table 4.33. The effects of sea urchin egg health supplements on the body weight of rabbits

Group	Body weight (kg/mouse)		
	Before experiment	**After 2 weeks**	**After 4 weeks**
Control sample	1.93 ± 0.27	2.41 ± 0.34	2.58 ± 0.41
SUEHSP (100 mg/kg/day)	1.91 ± 0.24	2.37 ± 0.35	2.63 ± 0.38
SUEHSP (300 mg/kg/day)	1.88 ± 0.25	2.17 ± 0.29	2.54 ± 0.36
SUEHSP (500 mg/kg/day)	1.83 ± 0.31	2.13 ± 0.32	2.49 ± 0.42

Table 4.34. The effects of sea urchin egg health supplements on the red blood cell count of rabbits

Index	**Control sample**	**SUEHSP (100 mg/kg/day)**	**SUEHSP (300 mg/kg/day)**	**SUEHSP (500 mg/kg/day)**

White blood cells ($\times 10^9$/L)	5.90 ± 0.82	5.95 ± 0.75	5.93 ± 1.03	5.90 ± 0.82
Red blood cells ($\times 10^{12}$/L)	5.21 ± 0.38	5.26 ± 0.47	5.21 ± 0.43	5.25 ± 0.53
Hemoglobin (g/L)	9.75 ± 1.05	9.88 ± 1.23	9.62 ± 1.03	9.86 ± 1.38
HCT (fl)	62.38 ± 1.42	63.98 ± 1.59	61.86 ± 1.92	61.78 ± 1.68
MCV (g/L)	5.96 ± 0.96	5.93 ± 0.78	5.93 ± 1.03	5.90 ± 0.98
Platelets ($\times 10^9$/L)	326.12 ± 12.39	328.90 ± 15.53	334.68 ± 19.23	331.12 ± 12.39

Table 4.35: Effects of SUEHSP on AST, ALT, and Creatinine Activity in Rabbit Blood

Index	**Control sample**	***SUEHSP* (100 mg/kg/day)**	***SUEHSP* (300 mg/kg/day)**	***SUEHSP* (500 mg/kg/day)**
AST (UI/L)	42.73 ± 3.62	41.90 ± 2.82	41.88 ± 3.17	42.16 ± 3.54
ALT (UI/L)	55.26 ± 4.71	56.63 ± 4.33	57.01 ± 3.53	61.20* ± 4.42
Creatinin (UI/L)	1.18 ± 0.18	1.17 ± 0.20	1.21 ± 0.16	1.20 ± 0.15

Table 4.36. Results of dissecting some internal organs after consuming SUEHSP

Organ	**Control sample**	**SUEHSP (100 mg/kg/day)**	**SUEHSP (300 mg/kg/day)**	**SUEHSP (500 mg/kg/day)**
Liver	Brown color, homogeneous liver tissue with no damage or necrosis.	Brown color, homogeneous liver tissue, no damage or necrosis.	Brown color, homogeneous liver tissue, no damage or necrosis.	Brown color, homogeneous liver tissue, no damage or necrosis.
Kidney	Light brown color, both kidneys are symmetrical, no signs of water	Light brown color, both kidneys are symmetrical, no signs of water	Light brown color, both kidneys are symmetrical, no signs of water	Light brown color, both kidneys are symmetrical, no signs of water

	retention	retention	retention	retention
Spleen	Dark brown color, homogeneous tissue, no swelling.	Dark brown color, homogeneous tissue, no swelling.	Dark brown color, homogeneous tissue, no swelling.	Dark brown color, homogeneous tissue, no swelling.

Table 4.37. Changes in the weight of mice consumed SUEHSP

Group	**The average weight of mice when consuming SUEHSP (g/mouse)**				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control sample	21.20 ± 0.46	24.88 ± 0.44	26.35 ± 0.82	28.29 ± 1.22	30.31 ± 1.47
***SUEHSP* (100 mg/kg/day)**	21.50 ± 0.41	24.87 ± 0.42	26.48 ± 0.40	28.02 ± 0.42	30.38 ± 0.56
***SUEHSP* (300 mg/kg/day)**	21.35 ± 0.90	24.98 ± 1.05	27.10 ± 0.93	28.09 ± 0.87	29.80 ± 0.69
***SUEHSP* (500 mg/kg/day)**	21.62 ± 0.34	24.47 ± 0.42	26.73 ± 0.39	28.73 ± 0.71	30.60 ± 0.81

Table 4.38. Hematological characteristics of mice consumed SUEHSP

Index	**Control sample**	**SUEHSP (100 mg/kg/day)**	**SUEHSP (300 mg/kg/day)**	**SUEHSP (500 mg/kg/day)**
White blood cell ($\times 10^9$/L)	6.21 ± 0.42	6.34 ± 0.07	6.20 ± 0.34	3.73 ± 0.92
Red blood cell ($\times 10^{12}$/L)	8.16 ± 0.25	8.54 ± 0.17	8.41 ± 0.16	8.47 ± 0.22
Hemoglobin (g/L)	121.67 ± 2.67	127.33 ± 2.03	127.67 ± 0.88	126.67 ± 2.85
HCT (fl)	0.42 ± 0.01	0.43 ± 0.00	0.43 ± 0.01	0.44 ± 0.01
MCV (g/L)	51.27 ± 0.19	51.47 ± 0.48	51.03 ± 0.62	51.50 ± 0.65
MCH (pg)	14.90 ± 0.15	14.83 ± 0.33	14.80 ± 0.40	14.93 ± 0.12
MCHC	290.33	288.00 ± 1.53	290.00 ± 2.08	289.67 ± 1.76

(g/L)	± 1.76			
RDW (%)	11.90 ± 0.35	12.10 ± 0.21	12.10 ± 0.25	12.10 ± 0.35
HDW (g/L)	15.43 ± 0.18	16.63 ± 0.58	16.07 ± 0.23	16.40 ± 0.70
CHCM (g/L)	275.67 ± 1.33	277.00 ± 2.65	278.00 ± 4.62	277.67 ± 3.48
CH (pg)	14.13 ± 0.12	14.27 ± 0.12	14.67 ± 0.32	14.30 ± 0.06
MPV (fL)	6.23 ± 0.07	6.57 ± 0.07	6.60 ± 0.06	6.60 ± 0.15
% NEUT	10.23 ± 3.22	11.87 ± 0.35	11.70 ± 0.59	11.80 ± 1.30
% LYMPH	51.80 ± 2.47	46.60 ± 2.00	45.77 ± 2.74	46.23 ± 6.35
% MONO	26.80 ± 2.51	33.97 ± 1.51	34.80 ± 1.54	34.30 ± 3.46
% EOS	0.30 ± 0.06	0.43 ± 0.03	0.47 ± 0.03	0.50 ± 0.12
% BASO	0.27 ± 0.03	0.33 ± 0.07	0.33 ± 0.07	0.30 ± 0.10
% LUC	7.27 ± 1.23	6.77 ± 0.18	6.90 ± 0.72	6.83 ± 2.60
Platelets ($\times 10^9$/L)	677.67 ± 2.60	639.67 ± 8.57	666.00 ± 7.23	656.00 ± 16.74

Table 4.39. Biochemical characteristics of mice consumed SUEHSP

Index	**Control sample**	**SUEHSP (100 mg/kg/day)**	**SUEHSP (300 mg/kg/day)**	**SUEHSP (500 mg/kg/day)**
AST (UI/L)	118.80 ± 1.88	118.70 ± 1.46	117.00 ± 2.25	118.53 ± 2.09
ALT (UI/L)	34.17 ± 0.79	35.57 ± 0.69	37.33 ± 0.75	39.73* ± 0.67
Creatinin (UI/L)	25.67 ± 0.66	26.00 ± 1.01	26.10 ± 0.38	26.20 ± 0.64

Table 4.50. Dissection results of experimental mice internal organs when consuming SUEHSP

Group	Dissection results of experimental mice internal organs when consuming SUEHSP		
	Liver	**Kidney**	**Spleen**
Control sample	Liver tissue is homogenous, with no damage or necrosis.	Both kidneys are relatively symmetrical, with no signs of water retention	No swelling
SUEHSP (100 mg/kg/day)	Liver tissue is homogenous, with no damage or necrosis.	Both kidneys are relatively symmetrical, with no signs of water retention	No swelling
SUEHSP (300 mg/kg/day)	Liver tissue is homogenous, with no damage or necrosis.	Both kidneys are relatively symmetrical, with no signs of water retention	No swelling
SUEHSP (500 mg/kg/day)	Liver tissue is homogenous, with no damage or necrosis.	Both kidneys are relatively symmetrical, with no signs of water retention	No swelling

Table 4.51: Weight of some internal organs (grams per 10 grams of body weight)

Group	Weight of some internal organs (grams per 10 grams of body weight)		
	Liver	**Kidney**	**Spleen**
Control sample	0.388 ± 0.017	0.105 ± 0.004	0.038 ± 0.001
SUEHSP (100 mg/kg/day)	0.394 ± 0.034	0.103 ± 0.005	0.039 ± 0.002
SUEHSP (300 mg/kg/day)	0.397 ± 0.025	0.104 ± 0.003	0.039 ± 0.001
SUEHSP (500 mg/kg/day)	0.396 ± 0.011	0.102 ± 0.003	0.038 ± 0.001

Table 4.52: Effect of SUEHSP on superoxide anion inhibition activity in mouse serum

Group	n	Inhibition activity	% control	P value
Control sample	11	27.44 ± 0.62	100	

Experiment 1	11	36.25 ± 1.54	132.11	< 0.05
Experiment 2	11	39.70 ± 2.43	144.67	< 0.05

Table 4.53: Total protein and amino acid composition of SUEHSP

Composition	**Content** (% MK)	**Composition**	**Content** (% MK)
Soluble protein	74.67	Tyrosine	2.038
Aspartic acid	4.956	Cysteine	4.385
Aspartic acid	4.970	Valine*	2.365
Serine	2.834	Methionine*	1.548
Histidine*	1.820	Phenynalanine*	2.558
Glycine	4.965	Isoleucine*	2.987
Threonine*	2.653	Leucine*	2.582
Alanine	2.475	Lysine*	4.965
Agrinine	4.296	Proline	2.120

Table 4.54: Lipid and fatty acid composition of SUEHSP

Composition	**Content** (% DM)	**Composition**	**Content** (% DM)
Total Lipid	21.56	C18:3 (n-6)	0.93
C12:0	0.07	C20:0	0.45
C14:0	13.67	C20:3 (n-3)	0.65
C14:1 (n-7)	2.14	C20:4 (n-6) (AA)	9.85
C15:0	0.60	C20:5 (n-3) (EPA)	7.18
C16:1 (n-9)	8.64	C20:2 (n-6)	0.83
C16:0	19.82	C20:1 (n-9)	2.76
C16:2 (n-4)	1.01	C20:4 (n-3)	1.42
C16:1 (n-7)	2.73	C22:6 (n-3) (DHA)	2.51
C18:0	2.07	C22:1 (n-9)	0.67
C18:2 (n-6)	2.76	C22:6 (n-6)	0.12
C18:1 (n-9)	11.73	C22:4 (n-6)	0.31
C18:4 (n-3)	3.13	Khác	1.51
C18:1 (n-7)	0.78	Tổng axit béo no	36.68
C18:3 (n-3)	1.66	Tổng axit béo không no	61.81

Table 4.55: Hormonal composition of SUEHSP

Sample	**Carotenoid (ng/g)**	**Testosteron (ng/g)**	**Estradiol (ng/g)**	**Progesteron (ng/g)**
SUEHSP	331.54	0.32	30.81	0.16

*Table 4.56: Composition of macrominerals and microminerals in **SUEHSP***

Sample	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	As (mg/kg)	Se (mg/kg)
SUEHSP	158.35	17.03	9.98	50.25	7.39	8.16

Sea urchin egg health supplement product

CONCLUSION

1. Research results on chemistry, component of lipid classes, and phospholipids

The main chemical composition of the eggs and bodies of the sea urchin (*Tripneustes gratilla*) and sea urchin (*Diadema savignyi*) collected from Nha Trang, Khanh Hoa, has been studied. The water content accounts for 75.28% in eggs and 68.12% in the bodies of the *Tripneustes gratilla* sea urchin, while it's 80.13% and 71.51% respectively in eggs and bodies of the *Diadema savignyi* sea urchin. The total protein content in the eggs of both sea urchin species is about 3.5 times higher than in the bodies, with total protein content in the eggs being 12.45% for *Tripneustes gratilla* sea urchins and 11.74% for *Diadema savignyi* sea urchins. The soluble protein content in *Tripneustes gratilla* sea urchin eggs is 141.48 mg/g, whereas it's 82.43 mg/g in *Diadema savignyi* sea urchin eggs, significantly lower in the bodies at 38.71 mg/g and 26.91 mg/g for *Tripneustes gratilla* and *Diadema savignyi* sea urchins respectively.

This is the first study to delve deep into the chemical composition of lipids, lipid classes, fatty acid components, and phospholipid molecular species found in the bodies and eggs of the *Tripneustes gratilla* sea urchin *Tripneuster gratilla* and sea urchin *Diadema savignyi*.

The total lipid content in the eggs of both sea urchin species is about three times higher than in the bodies. The total lipid content in *Tripneustes gratilla* sea urchin eggs accounts for 4.41%, and for *Diadema savignyi* sea urchin eggs, it's 3.18%, whereas in the bodies, the proportion is 1.32% and 1.33% respectively.

It was discovered that both the eggs and bodies of the two sea urchin species consist of 7 lipid classes in total, including polar lipid, monoacyl and diacyl glycerol, sterol, fatty acids, triglyceride, monoalkyl diacylglycerol, hydrocarbon, and wax. However, lipid samples from the bodies and eggs of the *Diadema savignyi* sea urchin only revealed 6 lipid classes and lacked the monoacyl and diacyl glycerol components.

In both studied species, triglyceride content in total lipid is the highest, followed by sterol, fatty acids, and polar lipids. Triglyceride content in *Tripneustes gratilla* sea urchin egg accounts for 78.37% (egg) and 76.10% (body), while in *Diadema savignyi* sea urchins, it's 55.27% (egg) and 32.39% (body) - significantly lower compared to *Tripneustes gratilla* sea urchins. Conversely, sterol and polar lipid content in *Diadema savignyi* sea urchins is much higher compared to *Tripneustes gratilla* sea urchins. Specifically, sterol content in *Diadema savignyi* sea urchins is 12.45% (egg) and 19.57% (body), whereas in *Tripneustes gratilla* sea urchins, it's 5.69% (egg) and 6.63% (body). Polar lipid content in *Diadema savignyi* sea urchin egg is 20.47% and in body is 33.94%, while in *Tripneustes gratilla* sea urchins, it's 4.41% in egg and 6.36% in body.

The fatty acid composition of the total lipid of the 4 egg and body samples of sea urchins has been studied, identifying 25 fatty acids in *Tripneustes gratilla* sea urchin egg and 29 fatty acids in *Diadema savignyi* sea urchin egg. Typical fatty acids found in both *Tripneustes gratilla* and *Diadema savignyi* sea urchin egg include: 14:0; 16:1 (n-9); 16:0; 18:1 (n-9); and 20:4 (n-6).

The study also reported on the molecular species of phospholipids in the bodies and eggs of *Diadema savignyi* and *Tripneustes gratilla* sea urchins. The results revealed that phospholipids of sea urchin bodies and eggs consist of 7 lipid classes including PI; PS; PE; PA; PC; LPC; and LPE. Specifically, there are 24 molecular forms of PE; 76 molecular forms of PC; 16 molecular forms of PS; 11 molecular forms of PA; 24 molecular forms of PI; 19 molecular species of LPC; and 10 molecular species of LPE. Additionally, 23 molecular species of SQDG were detected in the total lipid of the study samples. These are the first findings of such data in lipid samples of the bodies and eggs of *Tripneustes gratilla* sea urchins and sea urchins *Diadema savignyi*.

2. Research results in developing product for application in food technology

The research project has developed a hydrolysis process for both sea urchin *Tripneustes gratilla* and *Diadema savignyi*, to produce low molecular weight proteins (under 10 kDa) using the enzyme Alcalase. A regression equation has been derived to describe the soluble protein content of both *Diadema savignyi* and *Tripneustes gratilla* sea urchin eggs under the optimal conditions of three key technological parameters influencing the hydrolysis process: enzyme ratio (%);water/material ratio (mL/g); and hydrolysis time (hours).

For *Diadema savignyi* sea urchin eggs, the technological parameters include water/material ratio of 0.88/1 (w/v); Alcalase enzyme ratio of 1.32%; hydrolysis temperature of 45°C; hydrolysis time of 6.13 hours; pH maintained at 7; and soluble protein content achieved is 212.63 mg/g.

For *Tripneustes gratilla* sea urchin eggs the technological parameters include water/material ratio of 1/1 (v/w); Alcalase enzyme ratio of 1.1%; hydrolysis temperature of 45°C; hydrolysis time of 6.27 hours; pH maintained at 7; and soluble protein content achieved is 230.045 mg/g. The production technology exhibits high stability and achieves a recovery rate of 10.02%.

The quality and biological activities of the studied product have been evaluated. The results show that the soluble protein content of the product reaches 74.67% (dry sample), which consists of low molecular weight proteins under 10 kDa that have been proven to be highly biologically active peptides. The amino acid and fatty acid components of the product have been analyzed. The product contains 17 amino acids, including all 8 essential amino acids, particularly with a high lysine content of 4.965%. The fatty acid composition is diverse and includes all three essential fatty acids: AA (9.85%); EPA (7.18%); and DHA (2.51%).

The product has been evaluated for acute *in vivo* toxicity (not considered toxic via oral administration) and subchronic *in vivo* toxicity (not considered toxic) in mice.

The product contains carotenoids (331.54 ng/g dry weight) and important steroids for the body such as testosterone (0.32 ng/g dry weight), progesterone (0.16 ng/g dry weight), estradiol (30.81 ng/g dry weight).

The hydrolyzed protein product from *Tripneustes gratilla* sea urchin eggs has been registered and declared as a health supplement product under the name "Sea Urchin Egg Health Supplement."

RECOMMENDATION

- It is necessary to conduct in-depth research on the biological activity and nutrient absorption capabilities of different groups such as the elderly, children, and adults for the sea urchin egg health supplement. Therefore, further specialized trials should be carried out before the process is put into industrial production.

- The hydrolyzed protein product needs to be fractionated into different segments based on sizes that are smaller than 10 kDa. From there, evaluate the beneficial biological activities of each obtained fraction and direct the creation of different products with various activities and applications for different groups of users.

- The identified phospholipid molecules, particularly those make up a large proportion, need to be separated and tested for beneficial biological activity. To further verify the chemical structure of phospholipid molecules in each phospholipid class, it is necessary to analyze the fatty acid composition of each phospholipid subclass instead of just analyzing the fatty acid composition of the total lipid sample.

- It is necessary to analyze the sterol content in lipids from the eggs and bodies of *Diadema savignyi* sea urchins, as it makes up a very large proportion of the total lipids in this species.