

The process for the production of high phospholipid containing eicosanoids and soluble oligopeptides from the *Oyster*. sp.

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Abstract

Oysters could be found in seashore and estuarine areas and is regarded as a valuable export product with high economic value. In addition, the oysters could serve as an efficient assimilator of nutrients and potential source of polyunsaturated fatty acids (PUFA), mainly omega-3 fatty acids, which have been found to be responsible for a wide array of health benefits. In this paper, we report a process for the production of high phospholipid containing eicosanoids and soluble oligopeptides from the *Oyster*. sp. This result shows phospholipid layer containing high eicosanoids with 34.4% and soluble oligopeptides containing 8 essential acid amides. 19.53 g histidine per 100 g oligopeptides pointed out that hydrolyzed oysters are highly nutritional and valuable pharmacological products.

Keywords: Oyster, hydrolyzed, eicosanoid, phospholipid.

INTRODUCTION

Oysters have been recognized as one of the valuable and nutritious aquatic foods. The significance of oysters lies in the abundance of important minerals such as calcium, phosphorus and iron that simply a small number of oysters could provide to the daily average diet of humans [1]. It has also been found that the iodine content of oysters far exceeds that of other foods such as milk, eggs, or beefsteak. The importance of dietary iodine is substantiated by the association between increased incidence of goiter and cretinism and the shortage of iodine in foods and drinking water [2]. In addition, oysters are a potential source of omega-3 polyunsaturated fatty acids (PUFA), in particular, eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and docosapentaenoic acid (DPA, 22:5n-3) have been found to be responsible for a wide array of health benefits [3].

Phospholipids (PL) play multiple roles in cells such as forming the semi-permeability barrier of the cell membrane and intracellular organelles and actively participate in signal transduction in response to both external and internal stimuli to the brain. PUFA in the PL form, due to their better bioavailability, higher tissue-delivery capacity and enhanced health-promoting effects, have been attracting research interest from scientists worldwide, for example, DHA abundantly existing in membrane PL significantly influences cell survival through modulation of signaling pathway, carries important medicinal implications in treatment of brain disorders [4, 5].

Omega-3 and Omega-6 PUFA are important precursors to the synthesis of eicosanoids because eicosanoid is a class of molecules derived from 20-carbon ("eicosa" is Greek definition for 20) polyunsaturated fatty acids, most frequently arachidonic acid (AA). The eicosanoids include the prostaglandins, thromboxanes, leukotrienes, and lipoxins. These molecules almost always act on the cells that produce them or on neighboring cells, i.e., over short distances and time periods, and therefore they can be classified as autocrine/paracrine hormones. They are widely distributed in the cells and tissues of the body and possess wide

array of biological activities. The eicosanoids play important roles in endocrine systems. Virtually every other endocrinological/physiological system discussed in the other chapters of the book [6] also involves the local production and action of one or more of the eicosanoids.

Oligopeptide is used to refer to a short peptide with fewer members of amino acids as opposed to polypeptide, which is a peptide comprised of two to twenty amino acids. Many studies have shown that oligopeptides eventually degrade into non-toxic or low-toxic metabolites *in vivo*. Additionally, compared with recombinant proteins and antibodies, oligopeptides possess lower molecule weight and immunogenicity, which enable them to penetrate deeply into the organs. With low molecular weight, these peptides will be absorbed in the intestinal tract more effectively than intact protein and free amino acids of equivalent amounts. Therefore, oligopeptides are used as potential drugs for cancer, diabetes, high blood pressure, highly strengthening fitness and immunity,... [7]. It has been proved that marine protein is cut to oligopeptide by protease enzymes (pepsin, alcalase, trypsin, α -chymotrypsin, papain,...).

The studies of oysters are mostly oriented into fast food or functional foods. The method of processing is still simple, mainly manual or hydrolyzed technic, then they are dried into protein powder. On the other hand, a dual procedure that both isolates phospholipids and hydrolyzes to oligopeptides has not been studied. Due to these reasons, the purpose of our study is to establish a process to isolate high phospholipid containing eicosanoids and soluble oligopeptides with high nutritional and pharmacological values from oysters.

THE PROCESS FOR THE PRODUCTION OF HIGH PHOSPHOLIPID CONTENT AND SOLUBLE OLIGOPEPTIDES

Material

The oysters were collected in January 2019 in Hai Phong city, Vietnam and transferred to Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology

shortly afterwards. Soft tissues of oysters were then separated.

Equipment, tools and chemicals

Equipment: high performance liquid chromatography - high resolution mass spectrometry (HPLC-HRMS) and gas chromatography (GC).

Tools: centrifuge, ultrasound, vacuum evaporator, specialized grinder.

Chemicals: acetone, hexane, ethyl acetate, alcalase enzyme, NaCl, (NH₄)₂SO₄.

Technological scheme

The isolation process was presented in figure 1.

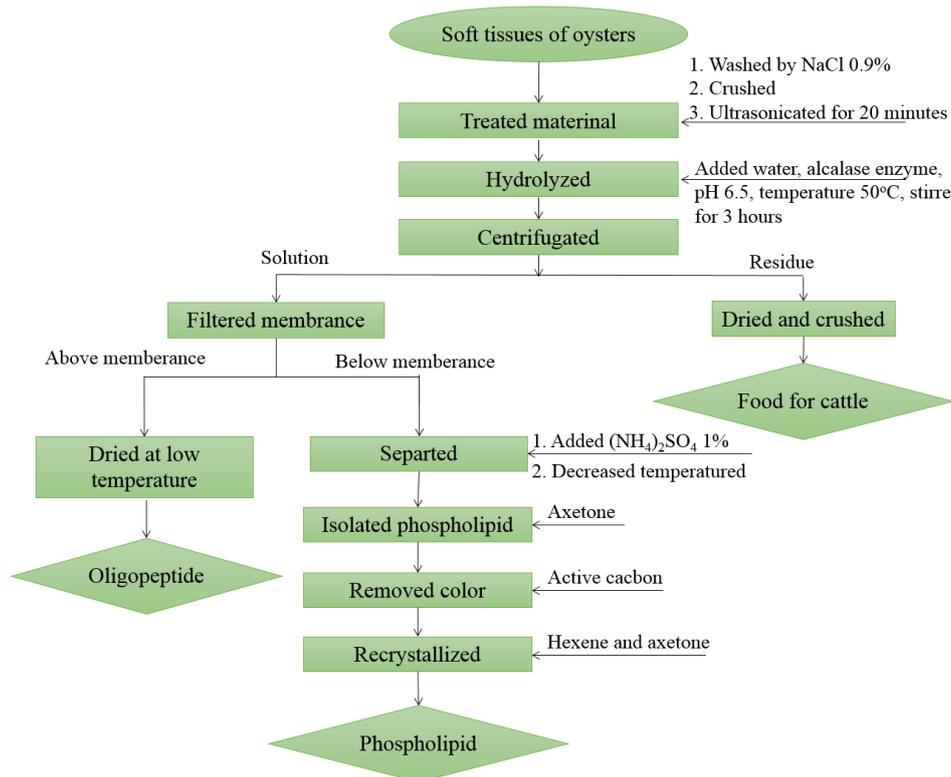


Figure 1. Technological scheme of isolation of high phospholipid containing eicosanoids and soluble oligopeptides

Demonstration of technology diagram in 5 steps:

Step 1 - Material treatment: Soft tissues of oysters were washed by NaCl 0.9% to remove dirt and crushed by specialized grinder. After that, they were ultrasonicated at 20–30 kHz for about 20 minutes.

Step 2 - Hydrolyzing oysters under optimal conditions: The water/substrate ratio is 60 % (w:w), the enzyme/substrate ratio is 0.5% (v:w), pH 6.5, temperature 50°C, stirring at 200 rpm for 3 hours.

Step 3 - Producing food for cattle: the hydrolyzed mixtures were centrifuged at

4,000–6,000 rpm to separate solution and residue. This residue was dried, then crushed and packaged for use as animal feed.

Step 4 - Isolating soluble oligopeptides: In the solution obtained in step 3, the dissolved oligopeptides was isolated from the oil-water mixture in the high-pressure filter and super membrane filter. The filtrate was compressed at a pressure of 3 atm in a pressure vessel and then injected through successively placed cellulose acetate membranes with pore sizes of 100 kDa, 30 kDa, 10 kDa, 5 kDa and 1 kDa respectively to obtain oligopeptides. The solubility was trapped on the membrane and

recovered. Oligopeptides were dried at temperatures below 100°C, and used as functional foods that have the effects of nourishing the body, preventing depression and fatigue.

Step 5 - Isolating phospholipid: Lipid was separated from the solution in step 4 by the addition of (NH₄)₂SO₄ 1% at the ratio of 100/1 (v:w) at 5°C, stirring gently for 30 minutes. The mixture was left to dissociate completely in 12 hours, and the lipid was collected.



Figure 2. Phospholipid layer separated from the oyster

Lipids were supplemented with acetone, at the ratio of 1:4 (m:v). The mixture was shaken for 32 s. After shaking for the first time, the acetone suspension was filtered out and a layer of lipid residue insoluble in acetone remained in the flask. Acetone continued to be added and the process was repeated 3 times. After fractionation, two parts are obtained: lipid (non-polar) soluble in acetone and lipid (non-polar rich in PL) insoluble.

The obtained PL rich residue was bleached and impurities were removed by dissolving in ethyl acetate at the ratio of 1:50 (w:v). The activated carbon powder was added with the ratio of activated carbon:phospholipid-rich residue of 1:5 (w:w), shaken for 5 minutes and then the activated carbon was filtered out. The process was repeated one time. The filtrate after shaking with activated carbon 2 times was exhausted by evaporation, a layer of oil was obtained with orange color, plasticity and consistency.

The obtained oil was fractionally crystallized with n-hexane. The n-hexane solution was stirred continuously at 100 rpm, maintained at 0°C. Cold acetone (-15°C) was slowly added to a triangle flask containing n-hexane until crystallization was terminated. A layer of light brown glue settled on the bottom of the flask. The above turbidity was removed, the brown colloidal layer at the bottom of the flask was washed with cold acetone, obtaining the phospholipid layer from the oyster (fig. 2).

PROPERTY OF PRODUCTIONS

Acid amides

The acid amide composition of soluble oligopeptides was 30.32 g/100 g including 16 acid amides. Among these, 8 essential acid amides were found (table 1). Histidine accounted for the highest percentage at 19.53%. The contents of threonine and isoleucine were lower, at 0.85% and 0.74%, respectively. Five acid amides: lysine, methionine, leucine and tryptosine accounted for equal content, approximately 0.4%.

This result shows that soluble oligopeptides of hydrolyzed oysters have high histidine content which is an essential amino acid that is not synthesized de novo in humans, thus, humans and other animals must ingest histidine or histidine-containing proteins. The histidine amino acid is a precursor for histamine, an amine produced in the body necessary for inflammation and is an important neurotransmitter, such as immune response capacity, sexual and reproductive health, the wake-up cycle - biological sleep and function of the digestive system. Deficiency of histidine risks anemia, especially in people with arthritis and kidney diseases [8].

Table 1. Acid amide composition of phospholipid layer

Acid amides	Composition (g/100 g)	Acid amides	Composition (g/100 g)	Acid amides	Composition (g/100 g)
Aspartic	0.54	Arginine	2.42	Methionine	0.34
Glutamic	0.43	Threonine	0.85	Lysine	0.48
Serine	0.28	Proline	2.19	Leucine	0.47
Histidine	19.53	Cystine	0.01	Isoleucine	0.74
Glycine	0.14	Tyrosine	0.36	Total acid amides	30.32
Alanine	1.09	Valine	0.47		

Fatty acids

Phospholipid layer was first treated with 2% H₂SO₄ in methanol commenced in 2 hours at 80°C in a screw top vial, followed by purification by TLC development in hexane - diethyl ether (95:5, v:v). GC analysis was employed to analyze fatty acid methyl esters (FAME) with column temperature of 210°C. Identification of FA was carried out by comparing obtained results with authentic standards and reporting equivalent chain lengths [9]. Injector and detector temperatures were 240°C.

The fatty acid composition of phospholipid layer comprised a total of 28 fatty acids and aldehyde dimethyl acetals (DMA) whose carbon atom number ranges from 14 to 22

(table 2). Abundant FA were 14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 16:3n-3, 20:1n-11, 20:4n-6 (AA), 22:2nmi, 20:5n-3 (EPA) and 22:6n-3 (DHA). Saturated fatty acids occupied 31.2% of PL layer content. 68.6% was proportion of unsaturated fatty acids (USFA) that have got 20.6% monounsaturated fatty acids (MUFA). A major MUFA in the composition was n-7 MUFA with the content of about 9%. Polyunsaturated fatty acids (PUFA) take up to 79.4% of USFA in PL layer content. Among PUFA, EPA and DHA accounted for high composition, at 10.9 and 16.8% respectively. Specifically, eicosanoid accounted for 34.4% in PL layer and 43.3% in PUFA. This result shows that the extracted PL layer contains high eicosanoid content.

Table 2. Fatty acid composition of phospholipid layer

Rt	Fatty acid	Content (%)	Rt	Fatty acid	Content (%)
3.619	14:0	2.8	13.757	20:0	0.2
4.292	15:0	0.9	14.508	20:1n-11	2.9
5.275	16:0	18.5	14.653	20:1n-9	0.4
5.597	16:1n-7	2.1	15.027	20:1n-7	2.4
5.819	17:0	0.4	15.584	20:2-nmi	0.3
6.496	17:0	1.8	18.109	20:3n-6	0.2
7.06	16:3n-3	9.7	19.431	20:4n-6	2.9
7.919	DMA 18:1	1.7	23.554	20:5n-3	8.7
8.279	18:0	6.6	26.994	21:3n-3	1.4
8.749	18:1n-9	2.1	27.621	22:2nmi	5.1
8.927	18:1n-7	4.3	31.521	21:5n-3	0.4
9.871	18:2n-6	1.4	37.167	22:5n-6	0.8
11.481	18:3n-6	0.3	41.55	22:5n-3	1.5
11.706	18:3n-3	0.9	45.729	22:6n-3	13.4
12.767	18:4n-3	0.8		Other	5.3

CONCLUSIONS

This study contributed an advanced process for the production of high phospholipid containing eicosanoids and soluble oligopeptides from the *Oyster*. sp. Obtained results show phospholipid layer containing high

eicosanoids with 34.4% and soluble oligopeptides containing 8 necessary acid amides. 19.53 g histidine per 100 g oligopeptides pointed out that hydrolyzed oysters are highly nutritional and valuable pharmacological products.

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REFERENCES

- [1] Mitchell, P. H., 1917. Nutrition of oysters: glycogen formation and storage (Vol. 35). *US Government Printing Office*.
- [2] Jones, D. B., 1926. The nutritional value of oysters and other sea food. *American Journal of Public Health*, 16(12), 1177–1182. Doi : 10.2105/AJPH.16.12.1177-a.
- [3] Liu, Z. Y., Zhou, D. Y., Wu, Z. X., Yin, F. W., Zhao, Q., Xie, H. K., Zhang, J. R., Qin, L., and Shahidi, F., 2018. Extraction and detailed characterization of phospholipid-enriched oils from six species of edible clams. *Food Chemistry*, 239, 1175–1181. <https://doi.org/10.1016/j.foodchem.2017.07.035>.
- [4] Liu, C., Li, Y., Li, J., and Chen, S., 2012. Lipid composition of Chinese shellfish and molecular species of phospholipids in triangle pearl mussel. *Lipidomics: Sea food, marine based dietary supplement, fruit and seed*, 41–50.
- [5] Ghasemifard, S., Turchini, G. M., and Sinclair, A. J., 2014. Omega-3 long chain fatty acid “bioavailability”: A review of evidence and methodological considerations. *Progress in Lipid Research*, 56, 92–108. <https://doi.org/10.1016/j.plipres.2014.09.001>.
- [6] Laneuville, O., 2003. Eicosanoids.
- [7] Ji, Y., Qiao, H., He, J., Li, W., Chen, R., Wang, J., Wu, L., Hu, R., Duan, J., and Chen, Z., 2017. Functional oligopeptide as a novel strategy for drug delivery. *Journal of Drug Targeting*, 25(7), 597–607. <https://doi.org/10.1080/1061186X.2017.1309044>.
- [8] Kopple, J. D., and Swendseid, M. E., 1975. Evidence that histidine is an essential amino acid in normal and chronically uremic man. *The Journal of Clinical Investigation*, 55(5), 881–891. <https://doi.org/10.1172/JCI108016>.
- [9] Christie, W. W., 1988. Equivalent chain-lengths of methyl ester derivatives of fatty acids on gas chromatography A reappraisal. *Journal of Chromatography A*, 447, 305–314. [https://doi.org/10.1016/0021-9673\(88\)90040-4](https://doi.org/10.1016/0021-9673(88)90040-4).