

Establishing A Procedure of Using Commercial Protease to Collect Fish Protein Hydrolysate (FPH) from Non-Toxic Pufferfish (*Lagocephalus Wheeleri*) as a Functional Food Ingredient

Bui Thi Thu Hien^{1*}, Pham Thi Diem¹, Vu Xuan Son¹, Nguyen Viet Nghia¹, Tran Nhat Anh¹, Dang Minh Nhat², Bui Xuan Dong², Hoang-Dung Tran³, Le Danh Tuyen⁴, Vu Thi Thu Hien⁴ and Nguyen Khac Bat^{1*}

¹Research Institute For Marine Fisheries, Ministry of Agriculture and Rural Development, 224 Le Lai Street, May Chai Ward, Ngo Quyen District, Hai Phong City 04218, Viet Nam.

²The University of Da Nang - University of Science and Technology, 54 Nguyen Luong Bang Street, Hoa Khanh Bac Ward, Lien Chieu District, Da Nang City 50608, Viet Nam.

³Nguyen Tat Thanh University, 298A-300A Nguyen Tat Thanh Street, Ward 13, District 4, Ho Chi Minh 72820, Viet Nam.

⁴National Institute of Nutrition, 48B Tang Bat Ho Street, Pham Dinh Ho Ward, Hai Ba Trung District, Hanoi 11611, Viet Nam.

<http://dx.doi.org/10.13005/bbra/2840>

(Received: 05 May 2020; accepted: 13 June 2020)

In this study, we aim at establishing the first standard procedure of using commercial protease to collect fish protein hydrolysate (FPH) from the non-toxic pufferfish *Lagocephalus wheeleri* and set general specification of FPH as a functional food ingredient. Pufferfish *Lagocephalus wheeleri* was sorted and collected as by-caught from Vietnamese Northern and Central seas. General standard analyzing methods were used for sensory, nutritional, and chemical properties. The hydrolysis conditions were evaluated as a 1.2% mixture of Flavourzyme and Protamex proteases (1:1) at 55°C in 6 hours. Qualified FPH was a bright yellowish color clear liquid with specialty fish smell and sweetness of amino acid with an aftertaste, total nitrogen 20.08 ± 0.25 g/l, Na.a/Nts ratio over 50%, with no TTXs detected. The hydrolysis conditions and processes were discussed and shown to be similar to other studies on different fish species. The benefit of making FPH using the materials from non-toxic pufferfish was also analyzed. Organization and control of accurate non-toxic pufferfish collection is the essential step to ensure a safe and highly commercialized product. The process established in this study can be upgraded into large-scale production of pufferfish FPH and allows further research for value-added products that are benefiting the Vietnamese fisheries industry.

Keywords: Fish Protein Hydrolysate (FPH); Non-Toxic Puffer-Fish; *Lagocephalus Wheeleri*; Commercial Protease.

The puffer fishes are commonly known for all types of fish poisoning and have been recognized from ancient times. There are as many

as 120 species of puffer fish that live in tropical seas. In Viet Nam, there are an estimated 46 puffer species, distributed along the coast from

*Corresponding author E-mail: nkbat2005@gmail.com

Northern to Southern of the country with a reserve of approximately 37,387 tons. A majority of the puffers yield is the subfamily Tetraodontinae, including *Lagocephalus lunaris*, *Lagocephalus wheeleri*, and *Lagocephalus spadiceus*, which accounts for 84.7% of all puffers. *Lagocephalus wheeleri* (*L. wheeleri*) is considered less or very mild toxic.¹

Although puffers are a valuable source of high nutritional food with extensive reserves, the research, development, and consumption of products from pufferfish are inadequate to the potential. Pufferfish are mostly abandoned right at sea by fishermen or used as animal feeds. There is only a small amount of pufferfish exported to Korean and Chinese at a low price.¹

There were few studies in Viet Nam on pufferfish, mostly focused on evaluating the toxicity of the pufferfish to specify the highly toxic, mildly toxic and non-toxic pufferfish in Vietnam coasts¹. An attempt to utilize pufferfish was made in using the poisonous pufferfish *Torquigener gloerfelti* to make fish sauce following the traditional procedure. However, there was still a prominent amount of Tetrodotoxin (TTX) remaining after 12 months of processing (accounted for 6.07 – 13.57% of total raw fish toxicity), making the product unusable by human.²

Despite the potential of puffer fish, there was no study on utilizing non-toxic pufferfish in the food industry. Finding value-added products from the *Lagocephalus wheeleri* non-toxic pufferfish is necessary to utilize resources and increase fishermen's incomes.

Fish protein hydrolysate (FPH) has been widely considered the most important source of proteins and bioactive peptides.³ As a product of the hydrolysis process, FPHs contain native fish proteins that maintain their nutritional values and have functional, physicochemical, and sensory characteristics improved⁴. Producing FPH from non-toxic pufferfish *L. wheeleri* as by-catch from fishing activities is a practical approach as it helps utilize the use of this species and increase economic effectiveness.

Commercial proteases such as Alcalase, Neutrase, Protamex, and Kojizyme were commonly used in FPH research.³ Among the physicochemical properties of a hydrolysis procedure, the degree

of hydrolysis and the amount of nitrogen amino acids are the most critical index as they are directly related to the peptide length, nutritional value, and sensory property of the hydrolysate.^{4,5} Therefore, the choice of hydrolysis method and its conditions, which highly reliant on the type of fish and ways of handling and processing, are essential to the quality of FPH product and its related value-added products such as capsules, nutritional powders, or syrup.

In this study, we aimed at establishing a small-scale standard procedure of using commercial protease to produce FPH from non-toxic pufferfish *Lagocephalus wheeleri* and forming a general specification for pufferfish protein hydrolysate as a functional food ingredient. This study will also create a foundation for further research on further high-quality products from this valuable resource.

MATERIALS AND METHODS

Materials

Raw Pufferfish Meat

Specimens of the pufferfish *Lagocephalus wheeleri* were collected as by-catch from fishing ports in Northern Gulf provinces such as Hai Phong, Nam Dinh, Quang Ninh, and in the Central, such as Thanh Hoa, Da Nang. The collecting personnel were trained to recognize the non-toxic pufferfish (*L. wheeleri*) by its main characteristics and appearances. The collected bright eye, fresh, and unbroken fish were preserved with two layers of ice in iceboxes, and then transported to the Research Institute for Marine Fisheries (RIMF). In the laboratory, the pufferfish were sorted again to exclude underqualified and off-species fish (if any). Pufferfish that confirmed to be *L. wheeleri* were gutted, skinned, and cut off heads and fins then frozen at -18°C.

When used in experiments, the frozen fish meat was thawed, cleaned, minced, weighed in a 500ml Erlenmeyer flask, and heated up in the water bath or the incubator. The fish meat samples were analyzed for basic chemical properties and Tetrodotoxins.

Protease

The commercial proteases for food production by Novozyme (Denmark) were Alcalase 2.5L with enzyme activity 2.5 AU/g

(Aminopeptidase Units per gram); Flavourzyme 500 LAPU/g (Leucine Aminopeptidase Units per gram); and Protamex 1.5 AU/g.

Setting up the Experiment

The experiment was set to evaluate the indexes in consecutive order. In each test, one index was assessed by varying its value, while other factors in the analysis were fixed at preset values. The chosen value of the previous experiment will be used in later experiments.

The criteria to choose the optimal value for each experiment were the value of amino acid nitrogen and the ratio of amino acid nitrogen to total nitrogen (Na.a/Nts).

Experiment 1

Determining the Proteases

The experiment analyzed four proteases, Alcalase, Neutrase, Protamex, Flavourzyme,

and three mixtures of proteases, Alcalase + Flavourzyme, Neutrase + Flavourzyme, Protamex + Flavourzyme. The ratio of the individual proteases in the mixtures was 1:1. The hydrolysis procedure was done by adding 40% water and 0.5% protease (individual or combination) to the prepared fish meat at 50°C for 6 hours. Then, the protease was inactivated at 95°C in 15 minutes. The sensory properties, the value of nitrogen amino acid, and the Na.a/Nts ratio of the hydrolysates were assessed.

The optimum individual protease or mixture of proteases was chosen based on the best combination of all three criteria: the best sensory score, the highest nitrogen amino acid, and the highest Na.a/Nts ratio.

Table 1. The sensory scoring scale

Parameter	Scales					Weighing factors
	5	4	3	2	1	
Color	Creamy white	Milky white	White	Yellowish	Yellow	0.6
Smell	Special flavors smell of fish meat	Special smell	Mild fishy	Heavy fishy	Sour fishy smell	0.8
Taste	Specialty sweet with after taste	Mild sweet	Mildly sweet, a little acrid, no after taste	No taste, a little acrid	Bitter and acrid taste	1.6
Texture	Homogenous liquid	Homogenous	Less Homogenous	Foaming, not homogenous	Foaming and clotted	1

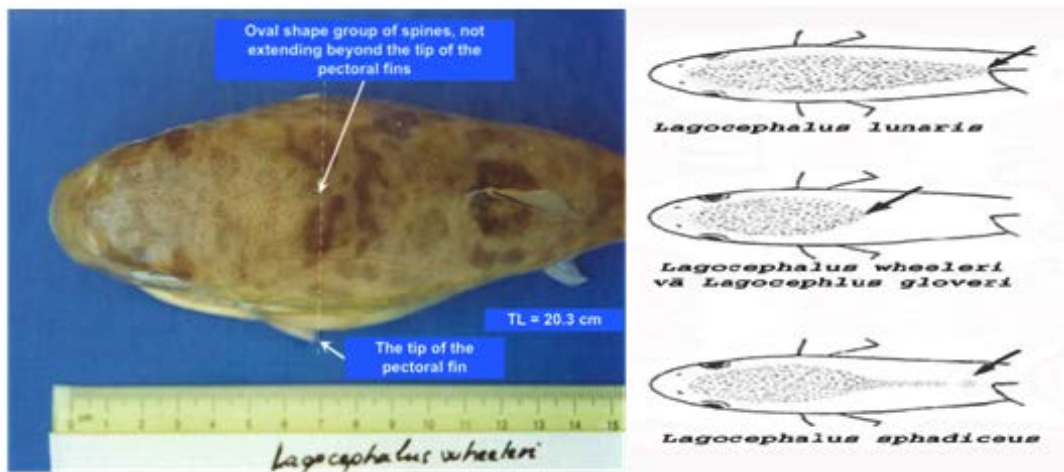


Fig. 1. Images to recognize the dorsal spines of non-toxic *Lagocephalus wheeleri*; A – Dorsal surface and the endpoint location of the dorsal spines. B – The arrows showed the endpoints of the dorsal spines on some different puffer species

Experiment 2

Determining the Ratio of Protease to Fish Meat

The optimum result chosen in experiment 1 was added to fish meat at 0.3, 0.5, 0.8, 1.0, 1.2, 1.4, and 1.6 percent together with 40% water and maintained at 50°C for 6 hours. Then, the protease was inactivated at 95°C in 15 minutes.

The optimum protease/fish meat ratio was chosen by analyzing the resulting hydrolysates.

Experiment 3

Determining the Ratio of Each Protease in the Mixture

The chosen mixture of protease (if any) was tested at the ratio of (1:1), (1:2), (2:1), (1:3), (3:1) added to the fish meat at the chosen ratio from the previous experiment with similar reaction conditions as the previous experiment. The optimal ratio of individual proteases that provided the best hydrolysis result was determined.



Fig. 2. The uniformly collected fish from fishing ports

Experiment 4

Determining the Time of Hydrolysis

Time of hydrolysis of 4, 5, 6, 7, 8 hours was tested with experimenting conditions using the best results from experiments 1, 2, and 3 at 50°C. After the hydrolysis time, the enzyme was inactivated at 95°C in 15 minutes. The optimal time was chosen based on the analysis of resulting hydrolysates.

Experiment 5

Determining the Temperature of Hydrolysis

The experiment was performed with the optimal parameters from previous experiments 1-4 at the temperature of 40°C, 45°C, 50°C, 55°C and 60°C. Then the hydrolysates were inactivated at 95°C in 15 minutes. The optimal reaction temperature was chosen based on the analysis result comparison.

Methods of Analysis

The sampling method was performed according to TCVN 5276-90. The value of amino acid nitrogen (Na.a) was analyzed according to TCVN 8764:2012 and total nitrogen (Nts) according to the internal method EHC-TP2-047 (Ref FAO Food 14/7-1986). TTX was detected according to NAF 04/10 (2010). Lipid was analyzed by the internal method EHC-TP2-050 (Ref FAO Food 14/7-1986). Other chemical and nutritional indexes were analyzed using appropriate methods.

The sensory assessment was performed using a scoring method based on TCVN 3215-79, consisting of a sensory committee of 5 people with a scoring scale as presented in table 1.



Fig. 3. Raw fish material processing procedure

Table 2. Percentage of usable pufferfish material after processing

Raw fish (%)	Gut (%)	Skin and fins (%)	Head and bone (%)	Processed meat (%)
100	6.82 ± 1.75	11.5 ± 1.33	38.74 ± 1.85	42.94 ± 2.00

Data Processing Method

Each experiment was performed three times, with three samples per time. The results were an average of all results. Experimenting data processing and Figuring was done using MS Excel 2010 software. Data analysis was done using Design Expert (version 10) software.

RESULTS AND DISCUSSION

Training of Material Choice

The experts from the RIMF came to the fish trading centers and trained the purchasing people how to recognize the non-toxic *L. wheeleri* based on the fish's appearance as following (see figure 1):

Table 3. The chemical, nutritional và toxicity properties of pufferfish (*L. wheeleri*)

No.	Testing parameters	unit	Results
1	Tetrodotoxin (TTX)	MU	Not detected (LOD = 5MU)
2	Lipid	%	0,68
3	Protein	%	19.9
4	Saturated fat	g/100 g	0.265
5	Unsaturated fat	g/100 g	0.415
6	Polyunsaturated fat	g/100 g	0.305
7	Monounsaturated fat	g/100 g	0.109
8	Myristic Acid (C14:0)	g/100 g	0.017
9	Palmitic Acid (C16:0)	g/100 g	0.15
10	Stearic Acid (C18:0)	g/100 g	0.085
11	Cis-oleic Acid (C18:1 n9)	g/100 g	0.090
12	Cis-linoleic Acid (C18:2 n6)	g/100 g	0.052
13	Arachidonic Acid (C20:4) (ARA)	g/100 g	0.053
14	cis-4,7,10,11,13,16,19-Docosahe xaenoic acid/DHA (C22:6)	g/100 g	0.188
15	Omega-3	g/100 g	0.194
16	Omega-6	g/100 g	0.108
17	Omega-9	g/100 g	0.090
18	(d) Alanic (Total)	g/100 g	0.96
19	(d) Acid aspartic (Total)	g/100 g	1.91
20	(d) Cystine/Cysteine (Total)	g/100 g	0.45
21	(d) Acid glutamic (Total)	g/100 g	4.24
22	(d) Glycin (Total)	g/100 g	0.80
23	(d) Histidin (Total)	g/100 g	0.56
24	(d) 4-Hydroxyprolin (Total)	g/100 g	0.25
25	(d) Isoleucin (Total)	g/100 g	0.82
26	(d) Leucin (Total)	g/100 g	1.42
27	(d) Lysin (Total)	g/100 g	2.04
28	(d) Methionine (Total)	g/100 g	0.47
29	(d) Phenylalanin (Total)	g/100 g	0.56
30	(d) Prolin (Total)	g/100 g	1.01
31	(d) Serin (Total)	g/100 g	0.56
32	(d) Threonin (Total)	g/100 g	0.86
33	(d) Tyrosin (Total)	g/100 g	0.67
34	(d) Valin (Total)	g/100 g	0.86
35	(d) Amino acid (Total)	g/100 g	18.5
36	(a)(f) Moisture	%	78.8
37	(a)(f) Total ash	%	1.45

1. There are small spines on the back and the belly. The needles on the dorsal surface are distributed in an oval shape, starting from behind the nostrils, not extending beyond the tip of the pectoral fins (quite a large distance to the base of the dorsal fin).
2. The dorsal and head have a greenish color. The sides of the head and the sides along the body are iridescent yellows.
3. The nostril is white.
4. Several greyish spots are on the top of the head, in the middle of the dorsal surface, and near the base of the dorsal fin and tail fin.
5. Caudal fin concaves in the midpoint.

All of the purchasing centers were able to recognize and choose the by-catch non-toxic *L.wheeleri* pufferfish correctly to collect and send to the RIMF (see Figure 2). To be safe, the expert in the RIMF checked 100% of the received fish. The accuracy of the collection was 99%.

Processing for Preservation

The processing procedure was established, including cleaning, gutting, skinning, and freezing at -18°C (see Figure3).

The percentage of usable material pufferfish after processing was determined in Table 2.

Determining the Nutritional Value of the Material Fish Meat

The main chemical, nutritional properties, and Tetrodotoxin (TTX) of the material pufferfish meat were shown in Table 3.

The non-toxic *L.wheeleri* pufferfish meat showed high nutritional value with 19.9% protein, 0.68% lipid, and other health enhancement components such as Vitamin E 143.8 mg/100g, Vitamin A 7.6 mg/100g, Omega-3 194 mg/100g and DHA 188mg/100g, suitable to be used as a food supplement.

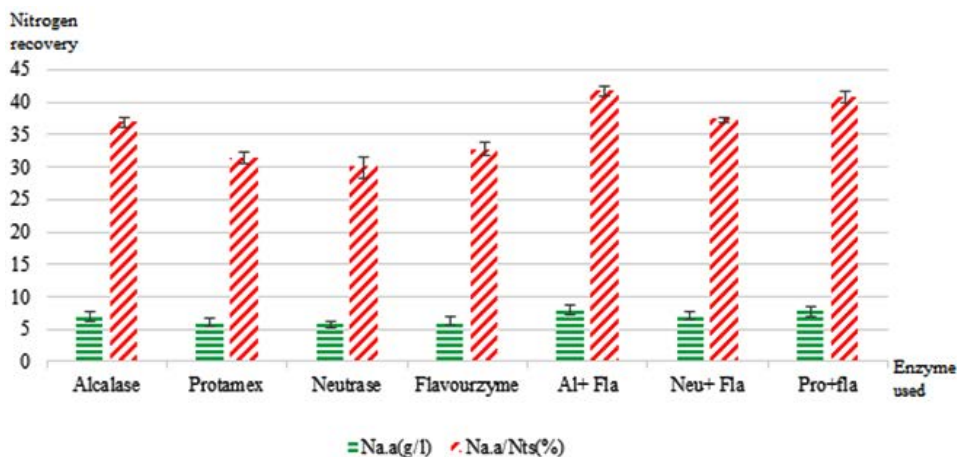


Fig. 4. The value of Nitrogen from amino acid and the ratio of Nitrogen from amino acid to total Nitrogen by types of protease used

Table 4. The sensory assessment results of FPHs

Protease	The average sensory score				Average score	Quality level (According to TCVN 3215-79)
	Color	Smell	Taste	Texture		
Alcalase®	3.1	3	1.5	3	10.6±0.67	Bad
Flavourzyme®	4	3	2.8	2.1	11.9±0.68	Average
Neutrase®	3.2	3.1	2.2	2.4	10.9±0.43	Bad
Promatex®	3.3	3	3	4	13.3±0.41	Average
FlaAlc	3.8	3.5	3	4	14.3±0.38	Average
FlaNeu	4	4	2.8	3	13.8±0.55	Average
FlaPro	4	3.2	4.1	4	15.3±0.36	Good

**Determining the Optimal Hydrolysis Parameters
Select the Optimal Protease for the Hydrolysis**

Four commercial proteases were used in this experiment, three of which, Alcalase®, Neutrase®, and Promatex®, were endo-protease and one of which, Flavourzyme®, was a premix of endo and exo-protease. Flavourzyme® was described to be able to decrease the typical bitterness of the hydrolysate when used in a small dose together with other endo-protease.⁶ Therefore, we evaluated three more mixtures of the endo-proteases with Flavourzyme®, Flavourzyme®+Alcalase® (FlaAlc), Flavourzyme®+Neutrase® (FlaNeu), and Flavourzyme®+Promatex® (FlaPro). The analysis results of the hydrolysates were presented in Figure 4.

It was clear from the data shown in Figure 2 that the type of protease profoundly

affected the value of amino acid and nitrogen recovery performance. Among the four types of proteases, Alcalase had the best performance with 36.8% nitrogen recovery, while the other three performed similarly at a lower level. In the mixture group, FlaAlc and FlaPro provided high performance at 41.71% and 40.73% nitrogen recovery, respectively. The sensory assessment was done on all the proteases tested, and the results were shown in Table 4.

Among the three proteases giving the best performance, Alcalase had the lowest sensory score, with only 10.6 points due to its strong bitterness. FlaPro got the highest sensory score 15.3 points for creating FPH with creamy white color, specialty sweetness of amino acids, and the definite aftertaste. The result agreed to other previous studies that the combination of

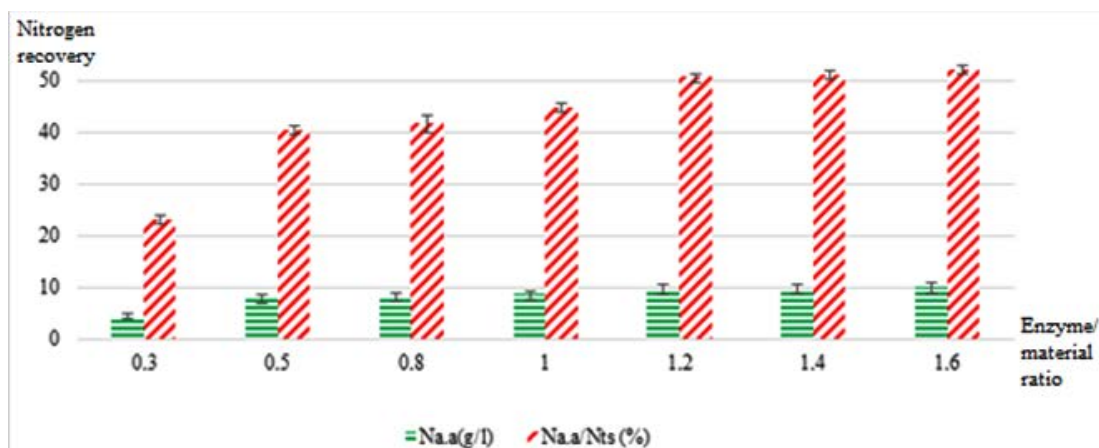


Fig. 5. The effect of the protease to material ratios to the hydrolysis performance

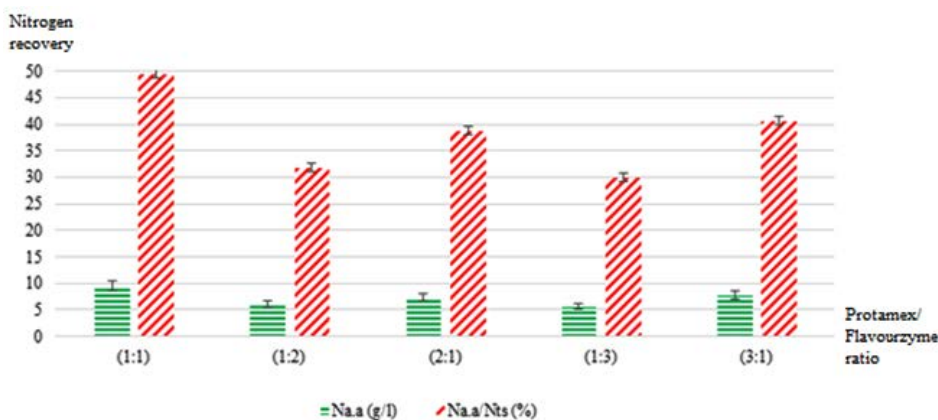


Fig. 6. The effect of the enzyme ratio in the mix to the hydrolysis performance

endo-protease and exo-protease helped increase the hydrolysis performance compared to using individual protease.^{7,8}

Evaluating the two parameters, the hydrolysis performance, and sensory scores, we decided that the protease mixture FlaPro was the most appropriate choice. Therefore, we chose FlaPro to use in our next experiments.

Select the Optimal Protease to Material Ratio

The result of the amino acids and Na.a/Nts ratio by a protease to material ratios was presented in Figure 5.

The enzyme ratio has a high impact on the hydrolysis reaction. When the enzyme ratio increased, Na.a/Nts ratio increased accordingly. However, when enzyme ratio reaches a certain point, the concentration of endo and exo-peptidase are high enough to cut almost all protein chains to peptide, stopping the hydrolysis reaction,

preventing the Na.a/Nts ratio from increasing further.

The results showed that when increasing the protease percentage from 0.3 to 1.2 percent, Na.a/Nts ratio increased quickly accordingly from 23.06% to 50.54%. When continuing the increment of protease percentage to 1.4% and 1.6%, the Na.a/Nts ratio stopped increasing at 51.03% and 52.01%, respectively. Therefore, the optimal proportion of protease was chosen at 1.2% to material fish meat.

Select the Ratio of the Individual Proteases in the Mixture

The ratio of each enzyme in the mix profoundly affected the quality of the FPHs. The results were shown in Figure 6. The proportion of the protease mixture to the material fish meat was 1.2%.

The ratio of each enzyme in the enzyme mixture profoundly influenced the hydrolysis

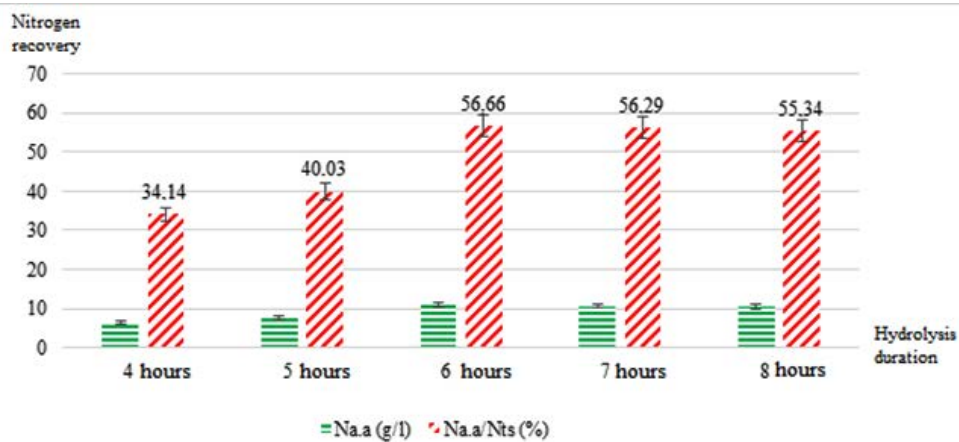


Fig. 7. The influence of time to amino acid value and Na.a/Nts ratio of the FPHs

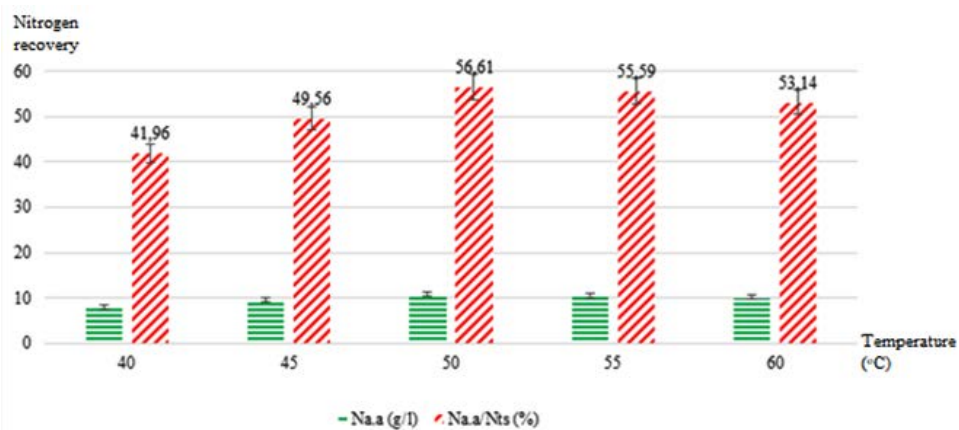


Fig. 8. The effect of the temperature to amino acid value and Na.a/Nts ratio in FPHs

reaction. At the ratio 1:1 of the two proteases Protamex and Flavourzyme, the amino acid nitrogen obtained to reach the highest at 9.43g/l and the Na.a/Nts ratio was 49.56%. Other ratios, such as (1:2), (1:3), (2:1), and (3:1), resulted in less effective hydrolysis with received Na of 6.07, 5.69, 7.37, and 7.75 g/l, respectively. Therefore, the best Protamex/Flavourzyme ratio was chosen to be 1:1.

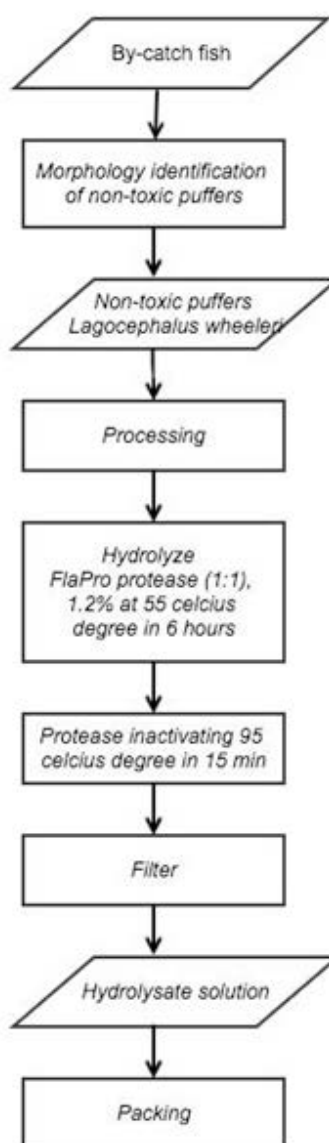


Fig. 9. Initial procedure for hydrolyzing non-toxic puffers *Lagocephalus wheeleri* for functional food ingredients

Select the Optimal Reaction Time

The time of hydrolysis significantly affected the ratio of Na.a and Na.a/Nts as seen in Figure 5. The Na.a value of the resulting FPHs rose from 6.49 g/l in the 4h to 10.78 g/l in the 6h reaction. Na.a /Nts ratio also increased proportionally from 34.14% to 56.66%. However, when the hydrolysis time was longer than 6h, the hydrolysis performance did not noticeably increase, instead even decreased slightly at 8h. The time of 4h generated the lowest yield of Na.a and Na.a/Nts. Therefore, 6h was chosen to be the optimum time for the hydrolysis (see Figure 7).

Select the Temperature of Hydrolysis

The Na.a/Nts ratio increased from 41.96% to 56.61% when the temperature was raised from 40°C to 55°C then decreased slightly to 55.59% and 53.14% at 55°C and 60°C, respectively (see Figure 8). Therefore, to save the operation cost and get the maximum performance, 50°C was chosen for our hydrolysis procedure.

Establishing the Process to use Protease to Produce FPH from Non-Toxic Pufferfish

We suggested the procedure to use commercial protease to provide FPH from non-toxic pufferfish (*L. wheeleri*) to use as a functional food ingredient, as presented in the Flow Figure 9.

The clean material fish meat was minced, then added 40-45% water and 1.2% FlaPro (1:1) protease (weight/volume). The hydrolysis reaction took place at 53±2oC in 6-7h. After that, the enzymes were inactivated by increasing the temperature to 95°C in 15 minutes.

The resulting FPH was a bright yellowish color clear liquid with specialty fish smell and sweetness of amino acid with an aftertaste. The product had total nitrogen of 20.08 ± 0.25 g/l, the ratio of Na.a to Nts over 50.3%, and no TTXs detected.

DISCUSSION

Utilizing non-toxic pufferfish in the food industry is a practical and necessary approach in Vietnam for some reason.

Firstly, as our initial analysis revealed, pufferfish *L. wheeleri* has outstanding nutritional value with ideal protein percentage, essential amino acids, and various other dietary components such as DHA, Omega-3, and Vitamin E.

Secondly, despite having extensive reserves in Vietnamese oceans, the pufferfish is abandoned as a human consumption resource. In the domestic market, all fresh pufferfish trading is banned as human food. The pufferfish, as by-caught is, therefore, only used as animal feed and fertilizers, which is a waste of resources and an economic loss for the fishing industry.

Finally, the demand for functional foods in the Vietnamese market is high and expanding. Within the last five years, it is considered a booming functional food industry in Vietnam. Besides functional food for health enhancement, anti-aging, disease prevention, supplements for individual nutritional needs are a niche product that is mostly imported. The RIMF has been successful in applying biotechnology in producing dietary supplements from various subjects such as amino acid-rich hydrolysates and powder from prawns and fish, and oyster powders. Therefore, developing a new product for individual nutritional needs from non-toxic pufferfish is both scientifically and economically feasible.

Making fish protein hydrolysate (FPH) using commercial microbial enzyme was researched and applied extensively by many authors thanks to its several benefits, for example, easily controllable and less depended to seasons, species comparing to hydrolyzing using autolytic enzymes⁴; more stable hydrolysis conditions than animal or plant-based proteases⁵; producing higher quality FPHs than chemical hydrolysis⁶; and allowing enzyme selection to optimize the hydrolysis reaction for a specified protein and easily ended by inactivating the enzyme using high temperature.¹⁰ Ananey-Obiri *et al.* (2019)¹¹ suggested a hydrolysis procedure for the fish by-product to obtain FPH, and similar processes were applied in several other hydrolysis condition evaluation studies. Nguyen *et al.* (2011)¹² assessed the hydrolysis of Yellowfin Tuna by-products using Protamex. Wisuthiphaet *et al.* (2016)¹³ compared and reported that Alcalase was more effective than acid in hydrolyzing by-caught fish for FPH with 6% (w/w) at a temperature of 61.23°C and a reaction time of 27.36 minutes. Muzaiifa *et al.* (2012)¹⁴ evaluated the effectiveness and conditions of the hydrolysis of fish by-products using Alcalase and Flavourzyme, showing that Alcalase provided better hydrolysis performance than Flavourzyme

(82.66% and 73.51% respectively). However, in the sensory assessment part in our study, Alcalase was not chosen for its standout bitterness preventing its application in human consumption products. The disadvantage of the bitterness in products also mentioned in other works such as He *et al.* (2013)⁵ and Fu *et al.* (2018)¹⁵ The results of our study were shown to be similar to findings from other authors on many different subjects.

CONCLUSION

Our study suggested the first process of hydrolyzing non-toxic pufferfish (*Lagocephalus wheeleri*) caught in Vietnamese seas using commercial proteases to produce FPH. Various extensive researches on the pufferfish family (poisonous and non-poisonous) in Vietnamese seas were the foundation for us to conduct this study on non-toxic pufferfish. In this study, we put particular concern on the sorting phase to accurately recognize and collect the non-toxic pufferfish and the post-production quality check to verify the amount of TTXs in the products. With our initial success in this study, we concluded that the organizing and strictly controlling the recognition and collection of the non-toxic pufferfish could be the essential step in the whole process to ensure a safe and highly commercialized product.

The process established in this study can be upgraded to a large scale to utilize the pufferfish resource domestically to produce high-quality FPH for human consumption. The finding also allows further research for value-added products that are benefiting the Vietnamese fisheries industry.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Industry and Trade, The Socialist Republic of Viet Nam for the project “Processing of functional-food products from the meat of Vietnam nontoxic puffer-fish”, No 02/SXTN02.18. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

1. Nguyen V. L., Nguyen X. T., *et al.* Toxicity of puffer species and solutions for safe and

- hygienic processing methods from harvest to consumption. Research Institute for Marine Fisheries. August 2006. Accessed from <http://www.rimf.org.vn/baibaocn/chitiet/tinid-147> (in Vietnamese)
2. Sato S., Dao V. H. Tetrodoxin toxicity in fish sauce made from *Torquigener gloerfelti*. *Vietnam J. Mar. Sci. Technol.* 2013; **13**(3):261-5. (in Vietnamese)
 3. Chalamaiah M., Dinesh Kumar B., Hemalatha R., Jyothirmayi T. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities, and applications: a review. *Food Chem.* 2012; **135**(4):3020-38.
 4. Kristinsson H. G., Rasco B. A. Fish Protein Hydrolysates: production, Biochemical, and Functional Properties. *Rev. Food. Sci.* 2000; **40**(1):43–81.
 5. He S., Franco C., Zhang W. Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). *Res. Int.* 2013; **50**(1):289–97.
 6. Enzymes at work. Accessed from: https://www.novozymes.com/-/media/Project/Novozymes/Website/website/document-library/Brochures_Downloads/General-materials/Enzymes_at_work.pdf
 7. Kamnerdpetch C., Weiss M., Kasper C., Scheper T. An improvement of the potato pulp protein hydrolyzation process by the combination of protease enzyme systems. *Microb. Technol.* 2007; **40**:508–14.
 8. Nchiencia H. A., Morawicki R. O., Gadang V. P. Enzymatic hydrolysis of poultry meal with endo- and exopeptidases. *Poultry Sci.* 2010; **89**: 2273-80.
 9. González-Tello P., Camacho F., Jurado E., Páez M. P., Guadix E. M. Enzymatic hydrolysis of whey proteins: I. Kinetic models. *Biotechnol Bioeng.* 1994; **44**(4):523-8.
 10. Pasupuleti V. K., Braun S. (2008) State of the art manufacturing of protein hydrolysates. In: *Protein hydrolysates in biotechnology* (Pasupuleti VK, Demain AL, eds). Amsterdam: Springer Netherlands. 2008; pp 11–32.
 11. Ananey-Obiri D., Matthews L. G., Tahergorabi R. Proteins From Fish Processing By-Products. In: *Proteins: Sustainable Source, Processing and Applications* (Galanakis CM, ed). Amsterdam: Elsevier. 2019; pp 163-191.
 12. Nguyen H. T. M., Sylla K. S. B., Randriamahatody Z., Donnay-Moreno C., Moreau J., Tran L. T., Bergé J. P. Enzymatic Hydrolysis of Yellowfin Tuna (*Thunnus Albacares*) By-Products Using Protamex Protease. *Food Sci.* 2011; **49**: 48-55.
 13. Wisuthiphaet N., Klinchan S., Kongruang S. Fish Protein Hydrolysate Production by Acid and Enzymatic Hydrolysis. *King Mongkut Int. J. Appl. Sci. Technol.* 2016; **9**(4):261-70.
 14. Muzaifa M., Safriani N., Zakaria F. Production of protein hydrolysates from fish by product prepared by enzymatic hydrolysis. *Aquar. Conserv. Legis.* 2012; **5**(1):36-9.
 15. Fu Y., Chen J., Bak K. H., Lametsch R. Valorisation of protein hydrolysates from animal by-products: perspectives on bitter taste and debittering methods: a review. *J. Food Sci. Technol.* 2018; **54**(4):978-86.