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Original Research Article

UTILIZATION OF *PANGASIUS HYPOPHTHALMUS* BY-PRODUCT TO PRODUCE PROTEIN HYDROLYSATE USING ALCALASE ENZYME

Nguyen Phuoc Minh

TraVinh University, Vietnam

Abstract: *Pangasius hypophthalmus* is one of the major fish species in the Mekong River fishery, one of the largest and most important inland fisheries in the world. Unfortunately increasing production of catfish also resulted in increasing its by-product. This source is rich in protein so we can utilize it to produce value-added product. Protein hydrolysate is a case in point. Protein hydrolysate can be used to improve or modify the physicochemical, functional properties such as solubility, fat absorption, waterholding capacity, foaming properties, emulsifying properties and or sensory properties of proteins without losing its nutritional value. That is the aim of our research in utilizing *Pangasiushypophthalmus*by-product to produce protein hydrolysate. Optimal conditions for protetin hydrolysis are presented at temperature 60^{o}C and pH 5 in 180 minutes with 40% water supplementation. We suggest using 10% hot water to remove fat. The protein hydrolysate concentrating process is conducted at pressure 0.33 atm, temperature 80^{o}C in 137 minutes. Alcalase enzyme is positively used for hydrolysis.

***Keywords: Pangasius hypophthalmus*, by-product, alcalase, hydrolysis, protein hydrolysate**

1. Introduction

Pangasius hypophthalmus, so-called Tra catfish, is one of the major fish species in the Mekong Delta fishery, and is the key fish species in Vietnamese aquaculture.Tra catfish is considered the major aquaculture success story of the country and is a significant source of socio-economic development, which is essential for earning foreign currency through the export of valuable fish, providing employment opportunities and encouraging local and foreign investors (Phuong &Oanh, 2010).

For Correspondence:
dr.nguyenphuocminhATgmail.com
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The *Pangasius hypophthalmus* fillet accounts for 33-38% and the left-over is the by-product. The large amount of waste was head, bones, skins and fat while fish oil take over 15.3% of fish weight. There was over 200 thousand tons of pangasius fish oil that were not enhanced the value and utilized effectively every year (Luc et al., 2013). Traditionally, all by-products are used for fishmeal production (Thuy et al., 2010). *Pangasius hypophthalmus* by-products have been used as raw materials for production of gelatin and collagen. These products have been proven to have nutraceutical and functional properties and have been widely used in food, cosmetics and medicine.

There are several researches utilized *Pangasius hypophthalmus* by-product to produce protein concentrate, protein isolate, and protein hydrolyaste. E. Bárzana, M. García-Garibay (1994) used fish by-product for the production of fish meal, fish protein concentrate or fish protein hydrolyzates.Production of fish protein hydrolysate via enzymatic hydrolysis is one way to add value to proteinaceous fish waste (Aspmo et al., 2005).Amiza et al., (2013) examined physicochemical properties of silver catfish (Pangasius sp.) frame hydrolysate.Thy et al., (2014) studied chemical composition and foaming, emulsifying abilities of fish protein isolate (FPI) which obtained in the hydrolysis of *Pangasius hypophthalmus* by-products were proceeded; and compared to the ones of commercial soy protein isolate (SPI), commercial whey protein isolate (WPI). Cao Xuan Thuy et al., (2014) conducted enzymatic hydrolyis optimization of *pangasius hypophthalmus* by-products to obtain fish protein isolate (FPI) with foaming function.

By careful control of the hydrolysate process, it is possible to produce hydrolysate with different
degrees of hydrolysis and different functional properties. The choice of substrate, protease enzyme employed and degree of hydrolysis can greatly affect the physicochemical properties of hydrolysate. Commercial enzyme, Alcalase has been strongly recommended for fish hydrolysis (Shahidi et al., 1995).

Purpose of our research is to investigate the utilization of *Pangasius hypophthalmus* by-product to produce protein hydrolysate using alcalase enzyme. This is an approach not only to enhance added value from *Pangasius hypophthalmus* by-product but also reduce pollution. We focus on examining raw material *Pangasius hypophthalmus* by-product characteristics; hydrolysis by bromelin as well as acomparision of hydrolysis efficiency between bromelin and alcalase.

2. Material & Method

2.1 Material

Pangasius hypophthalmus fishes areoriginated from processing factories in Mekong river delta, Vietnam. Bromelin, deposit enzyme and alcalase 2,4L are supplied from Dong Nam Co. Ltd, HCM City, Vietnam.

2.2 Research method

2.2.1 Examing composition percentage of Pangasiushypophthalmus fillet, chemical composition in Pangasius hypophthalmus by-product

Composition percentage of *Pangasius hypophthalmus* fillet is verified to know percentage of edible part and by-product so that we can estimate the importance of this source for utilization. Chemical composition in *Pangasius hypophthalmus* by-product is also examined such as protein, lipid, moisture, ash.

2.2.2 Effect of different factors influencing to hydrolysis

2.2.2.1 Effect of hydrolysis time

Five *Pangasius hypophthalmus* by-productsamples (without enzyme supplementation) are hydrolized in different intervals: 30, 60, 90, 120, 150, 180 and 210 minutes. Testing parameters include soluble protein and nitrogen acid amin.

2.2.3 Effect of enzyme ratio influencing to hydrolysis

Five *Pangasius hypophthalmus* by-productsamples (with bromelin enzyme supplementation) are hydrolized in different enzyme ratios (sample #1: control; sample #2: 0.07% bromelin; sample #3: 0.1% bromelin; sample#4: 0.15% bromelin; sample#5: 0.2% bromelin). All of them are hydrolized at temperature 60^{o}C, pH 5.0 with 40% water. Each sample has 100 gram. Testing parameters include soluble protein and nitrogen acid amin.

2.2.4 Comparision of hydrolysis efficiency to protein hydrolysate by bromelin and alcalase enzyme

We compare the hydrolysis efficiencyof protein hydrolysateby bromelin(in pineapple and extract) and alcalase enzyme. Testing parameters include enzyme activity, soluble protein and nitrogen acid amin

2.3 Statistical analysis

All data are processeed by Excel 2003.

3. Result & Discussion

3.1 Edible percentage of *Pangasius hypophthalmus*

Table 1.Edible percentage of *Pangasius hypophthalmus*

Composition	Percentage (%)
Fillet	38.6
Skin	4.9
Fat	3.5
Meat in abdomen	10.5
Viscera	5.8
Head and bone	37

From table 1, fish fillet accounts for 38.6%, the other part 61.4% is by-product. This by-product is very enormous so we utilize this source to produce value added product.

Table 2. Chemical composition in *Pangasius hypophthalmus* fillet

Composition	Percentage (%)
Crude protein	16.1
Lipid	8.2
Moisture	73.6
Ash	1.6

Table 3. Chemical composition in *Pangasius hypophthalmus* by-product

Composition	Percentage
Crude protein	16.21
Nitrogen acid amin	0.73
Lipid	29.82
Dry matter	50.87
Ash	2.37

3.2 Effect of different factors influencing to hydrolysis

3.2.1 Effect of hydrolysis time

Table 4. Effect of hydrolysis time

Hydrolysis time (minutes)	30	60	90	120	150	180	210
Soluble protein (g/100g)	1.425	1.455	1.475	1.615	1.645	1.790	1.790
Nitrogen acid amin (g/l)	4.60	5.11	6.07	6.62	6.85	6.92	6.90

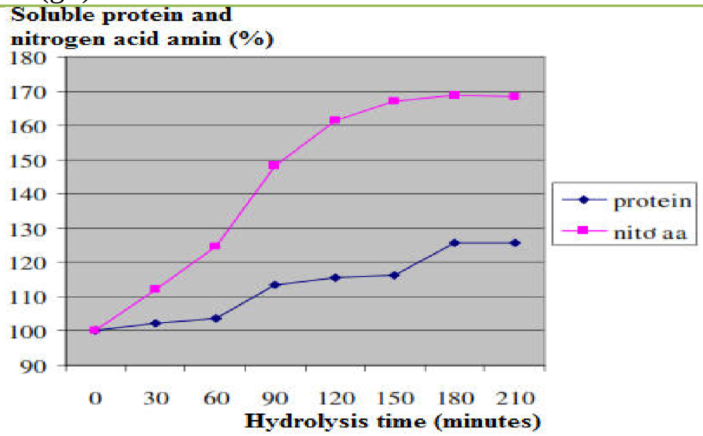

Figure 1. Effect of hydrolysis time to soluble protein and nitrogen acid amin

The longer hydrolysis time is, the more soluble dry matter is extracted. After 180 minutes of hydrolysis, we get the highest soluble protein. So we choose this value for further experiments.

3.2.2 Effect of enzyme ratio to soluble protein and nitrogen acid amin

Table 5.Effect of enzyme ratio to soluble protein

Hydrolysis time (minutes)	Soluble protein (g/100g)				
	Sample #1 (control)	Sample #2 (0.07% bromelin)	Sample #3 (0.1% bromelin)	Sample #4 (0.15% bromelin)	Sample #5 (0.2% bromelin)
0	1.425	1.425	1.425	1.425	1.425
30	1.455	1.555	1.600	1.645	1.670
60	1.475	1.600	1.655	1.705	1.760
90	1.615	1.645	1.675	1.795	1.840
120	1.645	1.675	1.740	1.865	1.885
150	1.655	1.680	1.750	1.960	1.970
180	1.790	1.980	2.030	2.520	2.560
210	1.790	1.850	1.980	2.260	2.470

From table 5, when we increase enzyme ratio, we get more soluble protein. At 01.5% enzyme bromelin and hydrolysis time 180 minutes, soluble protein is 2.520 g/100g. Meanwhile at 0.2% enzyme bromelin and hydrolysis time 180 minutes, soluble protein is noticed at 2.560 g/100g.

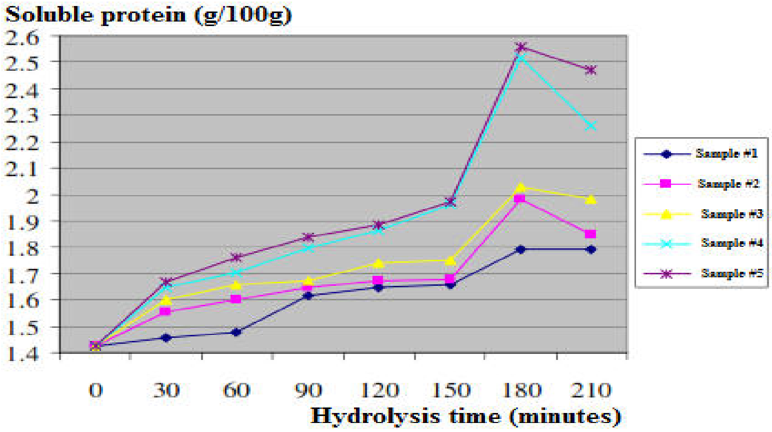

Figure 2.Effect of enzyme ratio to soluble protein

Table 6. Effect of enzyme ratio to nitrogen acid amin

Hydrolysis time (minutes)	Soluble protein (g/100g)				
	Sample #1 (control)	Sample #2 (0.07% bromelin)	Sample #3 (0.1% bromelin)	Sample #4 (0.15% bromelin)	Sample #5 (0.2% bromelin)
0	4.10	4.10	4.10	4.10	4.10
30	4.60	5.41	5.52	7.09	7.09
60	5.11	5.64	5.82	7.21	7.23
90	6.07	6.57	6.31	7.54	7.61
120	6.62	6.98	7.03	7.69	7.82
150	6.85	7.13	7.18	8.03	8.14
180	6.92	7.41	7.43	8.40	8.49
210	6.90	7.29	7.34	8.02	8.17

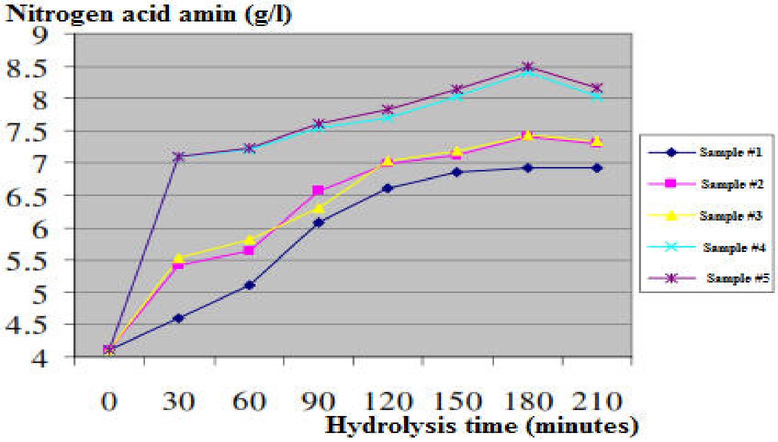

Figure 3. Effect of enzyme ratio to nitrogen acid amin

With 0.2% enzyme bromelin, we get maximum nitrogen acid amin. So we choose this value for further experiments.

3.3 Comparision of hydrolysis efficiency of protein hydrolysate by bromelin and alcalase enzymeto the soluble protein and nitrogen acid amin

Table 7. Hydrolysis efficiency by different enzymes to the soluble protein and nitrogen acid amin

Enzyme	Ratio (%)	Activity (UI/ml)	%soluble protein	%nitrogen acid amin
Bromelin	0.15	6.08	137.54	195.85
Deposit enzyme	0.15	2.88	110.04	156.68
Alcalase -2,4L	0.15	2.4AU	316.38	375.65

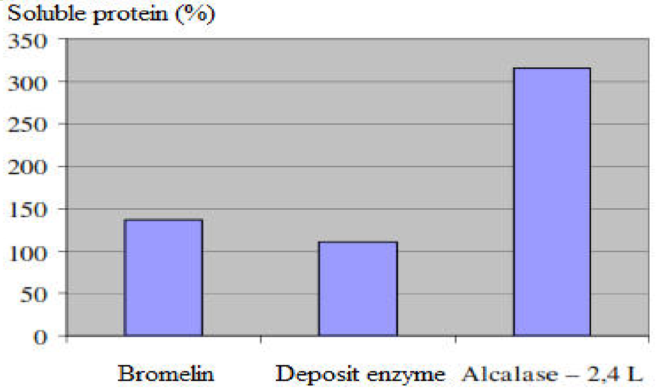

Figure 4.Hydrolysis efficiency by different enzymes to the soluble protein

From figure 4, we see alcalase enzyme show the best solube protein, so we choose this enzyme for protein hydrolysis.

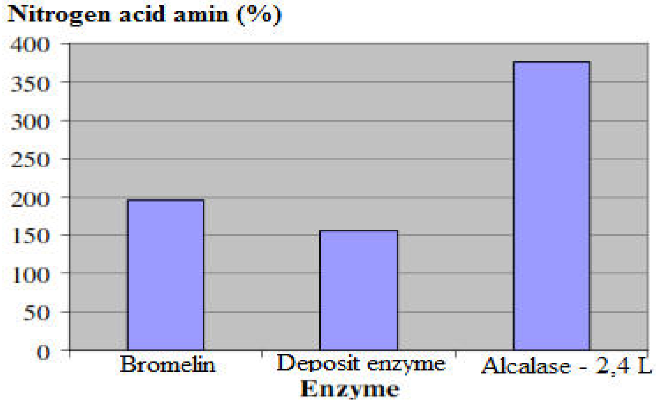

Figure 5.Hydrolysis efficiency by different enzymes to nitrogen acid amin

From figure 5, we see alcalase enzyme hydrolizes effectively to get maximum nitrogen acid amin. So we choose this enzyme for protein hydrolysis

4. Conclusion

High protein content in fishery waste make them more perishable, may bring undesirable effects to environment pollution, as well as high cost in managing waste disposal. Production of fish protein concentrate is one way to add value to proteinaceous fish waste.

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