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Original Research Article

HYDROLIZED FISH OILQUALITY FROM *PANGASIUS HYPOPHTHALMUS* BY-PRODUCT AND ITS STABILITY IN PRESERVATION

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Abstract
Pangasius hypophthalmus, also known as freshwater Tra catfish, is the main commercial farmed fish in the Mekong Delta of Vietnam. The rapid development of fishery products processing in Vietnam has had a significant impact on the socio-economic development of the country. This development also generates a large amount of fishery by-product. We come to investigate the hydrolysis of *Pangasius hypophthalmus* by-product to produce fish oil and its stability during preservation. The chemical compositions in *Pangasius hypophthalmus* by-product include 18.31% protein; 7.34% lipid; 63.09% moisture; 8.13% ash. Lipid recovery is 67.83%. Hydrolized fish oil contains 99.58% lipid; 0.35% moisture; 3.6 mg KOH/g acidity index; 216.28 mg KOH/g saponification; 212.68 mg KOH/g esterification; 195 gI2/100g iodine; 1 meq/kg peroxide index. *Pangasius hypophthalmus* fish oil contains essential fatty acids, especially DHA and EPA. So we also investigate the effect of temperature and vitamin ratio to fish oil stability during preservation.Our data demonstrate that low temperature preservation shall limit the lipid hydrolization and oxidation. Vitamin E plays a key role in lipid oxidation prevention.

Keywords: *Pangasius hypophthalmus* by-product, hydrolysis, fish oil stability, preservation.

1. Introduction

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. The traditional development of capture-based aquaculture for this species, particularly in Viet Nam, probably began because it is a prolific spawner, producing relatively large numbers of larvae that are easily harvested from the flowing river (Hung et al., 2004). *Pangasius* species have a low to moderate fat content with high levels of protein. The amount and composition of the fat content will be influenced by the feed used in aquaculture operations. A nutrition label for a

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four ounce raw portion of *Pangasius* is provided. The actual nutrient content of products that are consumed will be affected by added ingredients and the cooking method that is used.

Pangasius hypophthalmus plays an important role in aquaculture production. Unfortunately increasing production of catfish also resulted in increasing its by-product. The yield of *Pangasius hypophthalmus* by-product is very high (50%). Therefore *Pangasius hypophthalmus* by-products are potential to be a source of rich omega-3 oils. Saturated fatty acids (SFA) were most abundant in *Pangasius hypophthalmus* (42.6%) while salmon (37.2%) and seabass (39.0%) were rich in polyunsaturated fatty acids (PUFA). Tra *Pangasius hypophthalmus*catfish contains Docosahexaenoic acid (DHA), but its percentage was lower (4.7%) than salmon (20.2%) and seabass (18.7%) (Ho, B. T. and Paul, D. R., 2009).

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.

Fatty acids in *Pangasiushypophthalmus*oil that contained bioactive substances such as DHA and EPA are put into processing of functional food. These acids are major components of brain and retina which prevented the eyes diseases and the memory impairment in the old (Shahidi and Wanasundara, 1998). Besides, DHA is essential for the development of brain and retina in children, especially for third trimester foetus and infant (Shahidi and Wanasundara, 1998; Eduardo, 2010). In addition, these fatty acids reduce cholesterol and triglyceride in blood which prevent cardiovascular diseases (Simopoulos, 1997). The demand for food such as fish oil capsules, high level content of DHA milk, supplementary the fatty acids (DHA, EPA) food oil was increasing while the raw materials to enrich these fatty acids were deficient.

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Nguyen Tien Luc and Nguyen Anh Minh (2014) isolate the fatty acid from pangasius fish oil by hydrolysis method and determine the factors that affect the enrichment of the high bioactive chemical elements such as DocosahexaenoicAcid (DHA) and Eicosapentaenoic Acid (EPA) which are two polyunsaturated fatty acids in Omega-3 group by urea complexation process. At the same time, the optimization determined the relationship between mathematics and technology to choose the best technological solution. The recovery efficiency of fish oil reached maximum value at the urea-to-fatty acid ratio of 3.2, complexation time of 16 hours and complexation temperature of -10.6°C. Under the optimal conditions, the enrichment ratio of DHA and EPA also reached maximum value of 75.5 and 78.9% in order to enhance the value of pangasius oil raw material and significantly improve the quality of final product.

So it's essential to utilize *Pangasius hypophthalmus* by-product to produce value-added productsincluding fish oil, not only enhancing the value of *Pangasius hypophthalmus* but also limit of pollution from fish processing factories. Our research focuses on determining the quality of fish oil hydrolized from *Pangasius hypophthalmus* by-product as well as its stability in preservation.

2. Material & Method

2.1 Material

Pangasius hypophthalmus by-product is collected from processing factories in Tra Vinh province, Vietnam.

Figure 1. ***Pangasius hypophthalmus*** **by-product**

2.2 Research method

2.2.1 Determine chemical composition in Pangasius hypophthalmus by-product

Pangasius hypophthalmus by-product → grind finely → determine moisture content, protein, lipid and ash.

2.2.2 Fish oil extraction by hydrolysis

Raw material is finely grinded, stored at -18^{o}C ± 2 and defrosted at 0-4^{o}C before hydrolysis. The hydrolysis is conducted as follows: water/material (1:2), mix thoroughly, heat to 50^{o}C and then add enzyme 0.03%. Make sure the temperature fluctuation not over 0.5^{o}C. After hydrolysis, take sample out of water batch and dip into hot water 95^{o}C in 15 minutes to inactivate enzyme. Use sieve to remove particles and collect filtrate. Centrifuge this filtrate at 5000 rpm in 30 minutes to separate into three parts: lipid, hydrolized fluid and centrifuged deposit. Fish oil is secondly centrifuged to eliminate foreign matter utmost, kept at -18^{o}C.

2.2.3 Lipid recovery

Pangasius hypophthalmus by-product hydrolysis → Fish oil → Lipid determination in fish oil → Lipid recovery.

2.2.4Quality of fish oil

- Sensory characteristics include color, flavour, and clearance.
- Chemical composition in fish oil:lipid, moisture, acidity, saponification, esterification, iodine, peroxide, fatty acids.

2.2.5 Effect of preservation temperature and time to fish oil quality

Hydrolized fish oil is separated into several portions, each portion 50 gram. Experiment is arranged as follows:

Sample #1: Normal temperature, vitamin E 0%
Sample #2: Temperature (0-4^{o}C), vitamin E 0%
Sample #3: Temperature (0–4^{o}C), vitamin E 0.01%
Sample #4: Temperature (0-4^{o}C),vitamin E 0.03%
Sample #5: Temperature (0-4^{o}C),vitamin E 0.05%
Sample #6: Temperature (-18^{o}C), vitamin E 0%
Sample #7: Temperature (-18^{o}C),vitamin E 0.01%
Sample #8: Temperature (-18^{o}C),vitamin E 0.03%
Sample #9: Temperature (-18^{o}C),vitamin E 0.05%
After 15 days, we check fish oil quality such as acidity and peroxide index.

2.3 Testing method

Moisture content: drying to basic weight at 105^{o}C by TCVN 3700-1990.
Lipid content: by Folch method
Saponification index: by TCVN 6126:2007
Acidity index: titration by TCVN 6127: 2010
Esterification index: Saponification index - Acidity index
.
Peroxide index: titration by TCVN 6121:2010
Iodine index: titration by TCVN 6122:2010
Fatty acids: by gas chromatography

2.4Statistical analysis

All data are processed by Excel 2003

3. Result & Discussion

3.1Chemical compostion in *Pangasius hypophthalmus* by-product

Table 1. Chemical composition in *Pangasius hypophthalmus* by-product

Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
18.31	7.34	63.09	8.13

Lipid content in *Pangasius hypophthalmus* by-product is quite high so it's ideal to extract fish oil. Moreover, the protein content is very high 18.31% so this source can also be utilized to produce protein concentrate for fish sauce fermentation or fish meal. By that, the fish oil extraction and hydrolysis shall be enhanced also
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3.2 Lipid recovery

After hydrolysis *Pangasius hypophthalmus* by-product, we get fish oil. Take this fish oil to determine lipid recovery.
Fish oil receiving from 3kg *Pangasius hypophthalmus* by-product:150g
Lipid content in fish oil: 99.58%
Lipid receiving from 3 kg of *Pangasius hypophthalmus* by-product: 150 * 99.58/100 = 149.37g
Lipid content in raw material: 7.34%
Lipid content in 3kg raw material: 3000 * 7.34/100 = 220.2g
Lipid recovery: 149.37/220.2 * 100 = 67.83%
We can see the high lipid recovery 67.83% proved that the fish oil extraction is efficient.

3.3 Quality of hydrolized fish oil

3.3.1 Sensory characteristics

Table 2. Sensory characteristics of fish oil

Criteria	Fish oil
Color	Yellow
Clearance	Quite clear
Flavor	Specific flavour

3.3.2 Chemical compositions in fish oil

Table 3. Chemical compositions in fish oil

Lipid (%)	Moisture (%)	Acidity (mgKOH/g)	Saponification (KOH/g)	Esterification (mgKOH/g)	Iodine (g I_2/100g)	Peroxide (meq/kg)
99.58	0.35	3.6	216.28	212.68	195	1

From table 3, we can see the high percentage of lipid in fish oil, low moisture content so it's ideal for preservation.

3.3.3 Fatty acids in fish oil

Saturated fatty acid (SFA) contains very low (2.88%). Meanwhile the mono-unsaturated fatty acids are quite high (20.31%), the poly-unsaturated fatty acids (25.56%) and high-unsaturated fatty acids (23.36%). In general, *Pangasius hypophthalmus* by-product has important fatty acid such as linoleic acid, linolenic acid, DHA, EPA. Linoleic acid (C18:2ω6) accounts for 6.23% and linolenic acid (C18:3ω3) account for 5.25%. These fatty acids are essential for human body because they are not synthesized; only supplied through food consumption. Moreover, fish oil also contains DHA (C22:6ω3) and EPA (C20:5ω3) belongs to omega 3 group. EPA contributes to cholesterol decrement and cardiovascular disease prevention. DHA is good for human brain. These fatty acids are easily decomposed by oxidation so it's necessary to have appropriate preservation.

Table 4. Fatty acids in fish oil

Compositions in fatty acids	Fatty acid in fish oil (%)
Saturated fatty acids	
C14:0	0.88
C16:0	0.58
C18:0	1.42
∑ Saturated fatty acids	2.88
Mono-unsaturated fatty acids	
C14:1ω5	3.14
C16:1ω7	3.58
C18:1ω9	4.67
C20:1ω9	6.27
C24:1ω9	2.65
∑Mono-unsaturated fatty acids	25.56
Poly-unsaturated fatty acids	
C18:2ω6	6.23
C18:3ω3	5.25
C20:2ω6	4.52
C20:3ω3	5.78
C20:3ω6	3.78
∑ Poly-unsaturated fatty acids	25.56
High-unsaturated fatty acids	
C20:4ω6	6.27
C20:5ω3	7.47
C22:63ω3	9.62
∑ High-unsaturated fatty acids	23.36
W3	28.12
W6	20.8

3.4 Fish oil stability during preservation

During preservation, fish oil is under acidity and rancidity, The acidity is owing to hydrolysis to produce free fatty acids and glycerin; rancidity is owing to oxidation by oxygen present. To evaluate the hydrolysis and oxidation of fish oil, we need to determine acidity index and peroxide index. Through these values, we can estimate fish oil quality during preservation.

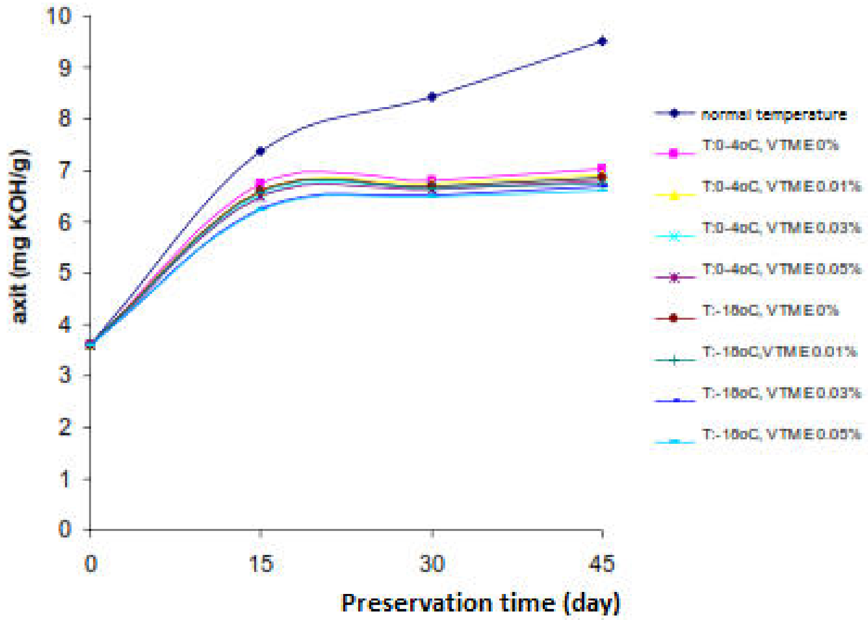

Figure 2. Acidity index in fish oil by preservation time

From figure 2, acidity index is increasing during preservation, quickly in the first 15 days and slightly after that. The acidity indices of all samples after 15 days are over permitted level. After 30 days, acidity index in sample preserved at normal temperature is 8.44 (mgKOH/g). After 45 days, this index in sample preserved at normal temperature is 9.51. Meanwhile, acidity index in sample preserved at-18^{o}C is very low (3.6 mgKOH/g) and over permitted level after 15 days. So it's necessary to perform quickly in extraction to avoid fish oil hydrolysis.

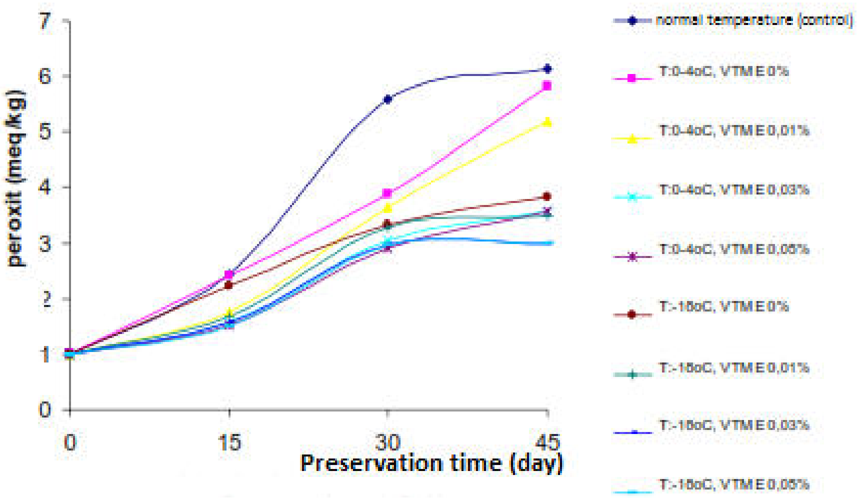

Figure 3. Peroxide index in fish oil by preservation time

From figure 3, we can see the increasing trend of peroxide index in 45 days. In the first 15 days, the increment is slowly. After 30 days, peroxide value of sample preserved at normal temperature is 5.58 meq/kg (permitted level 5 meq/kg). Peroxide values of other samples still increase but in allowance limit. Preservation in low temperature (-18^{o}C) shows the low peroxide index compared to preservation at 4 ^{o}C and normal temperature.After 45 days, peroxide index of sample preved at normal temperature still slightly increases. Sample preserved at T:-18^{o}C, vitamin E 0.05% has the lowest peroxide index, which is similar to sample preserved at T:-18 ^{o}C, vitamin E 0.03%. After 45 days, peroxide indice of samples at normal temperature; T: 0-4 ^{o}C, vitamin E 0% and T:0-4^{o}C, vitamin E 0.01% are all over permitted level. Other samples have peroxide indices below permitted level. Sample preserved by vitamin E has the peroxide index lower than sample without vitamin E in the same temperature. So preservation low temperature and vitamin E supplementation have an important role in oxidation prevention.

4. Conclusion

The major by-products of seafood processing in Vietnam are from catfish processing are very valuable if they can be used as raw materials for processing value-added products. Previously, these by-products were considered as waste and therefore were usually transported for landfill and/or were used in fishmeal production with low economic value. Nowadays, the value of these by-products has been more realized. In this research, we have sucessfully evaluate the hydrolized fish oil from *Pangasius hypophthalmus* by-product as well as its stability in preservation.

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