

## ROLE OF PHOSPHORIC ACID AND ACTIVATED CARBON IN DEGUMMING AND BLEACHING STAGES OF TRA CATFISH OIL REFINING PROCESS

Pham Thi Hoan<sup>1</sup>, Ngo Thanh Binh<sup>2</sup>

<sup>1</sup>Ho Chi Minh City University of Technology and Education, Vietnam

<sup>2</sup>Tran Dai Nghia University, Vietnam

Received 08/01/2020, Peer reviewed 18/01/2020, Accepted for publication 10/02/2020

### ABSTRACT

*The purpose of the present study was to investigate the efficiency of phospholipid removal using phosphoric acid and tra catfish (*Pangasianodon hypophthalmus*) oil bleaching using activated carbon. Crude oil was extracted from tra catfish fat, a by-product, by hot water. Crude oil had phosphorus content of 19.63 mg/kg, Abs<sub>228nm</sub> of 0.91, melting and solidification points of 33.8°C and 24°C, respectively. After the refining process, chemical and physical properties of fish oil had many advantaged changes. Phosphorus content was reduced to 4.48 mg/kg, Abs<sub>228nm</sub>, melting and solidification points were decreased to 0.32, 10.2°C and -2.0°C, respectively. Besides, quality parameters such as peroxide value (4.22 meq O<sub>2</sub>/kg), iodine value (67.88 gI/100g), and saponification value (162 mg KOH/g) were analyzed. Moreover, total content of unsaturated fatty acids was 60.6% including palmitoleic, oleic, linoleic,  $\gamma$ -linolenic, paullinic, eicosadienoic, dihomo- $\gamma$ -linolenic, erucic acids. Refined tra catfish oil is a pale yellow, liquid state at ambient temperature without strange odor.*

**Keywords:** Fish oil refining; activated-carbon; phosphoric acid; fatty acids; *Pangasianodon hypophthalmus*.

### 1. INTRODUCTION

Crude edible oil contains undesirable substances such as free-fatty acids, gummy materials and colouring matters. Gummy materials and colouring matters are due to the presence of phospholipids (phosphatides) and pigments passing to the oil with the crushing extraction or pressing treatments. Phospholipids are phosphoric acid diesters of glycerol where the phosphoric acid part is esterified by alcohol (choline, ethanolamine) or a polyol (inositol). If the phosphoric acid is not linked to any alcohol it is named phosphatidyl acid. Pigments consist of carotenoids, chlorophyll, gossypol and related compounds [1]. These substances should be removed from the oil not only for the improvement of the nutritional value and sensory properties (color, odor) but also for enhancement of the product shelf- life.

Theoretically, there are many refining processes used to remove oil impurities [2, 3]. Many species of marine fish have been studied for fish oil production; however, little attention has been paid to produce catfish oil from processing waste. There are many researches on the process of recovery and quality definition of edible oil from channel catfish (*Ictalurus punctatus*) viscera [4], herring (*Clupea harengus*) by-products [5] or carp (*Cyprinus carpio*) viscera [6]. In this study, crude oil, recovered from tra catfish fat, was processed through the following steps: degumming (using 85% phosphoric acid), neutralization, winterization, wax separation, bleaching (using activated carbon) and deodorizing.

Degumming was carried out with the primary purpose of eliminating the phospholipid compounds including phosphatidyl acid (PA), phosphatidyl choline

(PCh), phosphatidyl inositol (PI), phosphatidyl ethanolamine (PE). Both PCh and PI completely soluble in water and they are called hydratable phospholipids (HP). PE is partly water-soluble. PA is completely insoluble in water in simple form or in the combination of metal ( $Mg^{++}$ ,  $Ca^{++}$ ). PE and PA are called non-hydratable phospholipids (NHP) [1].

The effect of degumming was evaluated primarily on the reduction of phosphorus content in the oil. The acid degumming was found more effective for phospholipid removal than for the conversion from NHP to HP [1, 7]. This behavior can be explained by the change in electric charge on the functional groups of the phospholipid molecules under defined pH condition [8]. Theoretically, Phosphoric and citric acids are used because they are food grade, sufficiently strong, and they bind divalent metal ions ( $Mg^{++}$ ,  $Ca^{++}$ ) to form an insoluble salt complex in oil [2]. Therefore, we propose to study the effect of phospholipid removal in tra catfish crude oil using phosphoric acid.

## 2. METHODS

*Sample preparation.* Tra catfish fat was collected from Go Dang seafood factory, Ben Tre, Vietnam. The fat was divided into small bags, vacuumed and preserved at minus 20°C for further steps. The thawed fat was finely ground with water at a ratio of 1:5 (w/v). The mixture was placed in a sealed container and heated at 70°C for 15 min [4]. The mixture was then filtered through a sieve ( $\phi = 2$  mm) in order to recover the liquid-phase. Catfish crude oil was separated from the supernatant phase by centrifugation (2500×g, 30 min). The crude oil was degassed under vacuum pressure and then stored in a zip-lock bag at -20°C for the next steps.

The oil refining process (degumming, neutralization, bleaching, winterization and dewaxing, and deodorization) was carried out according to Sathivel S. et al. (2003) with slight modification.

*Degumming.* A sample of crude oil (100 g) was heated (60, 70, 80 and 95°C) in a water bath. At each treated temperature, phosphoric acid solution (85%, v/v) from 0.1 to 0.4 (% v/w) was added to the oil. The mixture was thoroughly stirred for 5 min. The oil was then cooled to room temperature and centrifuged (2500×g, 10 min) for impurity removing.

*Neutralization.* The degummed oil was neutralized. NaOH solution was added to degummed oil (100 g) to reach pH 7.0 and the mixture was heated (65°C, 30 min) with constant stirring. The sample was then cooled to room temperature and stood for 6 hours. After centrifugation (2500×g, 10 min), the oil was decanted. Fifty milliliters of demineralized water were added to the centrifuged sample to wash out any remaining soap. This washing was repeated three times. The remaining water and impurities were removed by centrifugation (2500×g, 10 min).

*Bleaching.* The neutralized oil (100 g) was bleached using an activated carbon (6-12 mesh size coconut shell charcoal, 500 kg/m<sup>3</sup>, pH 7.0, insoluble in water and other solvents) at the ratio of 1-5 (% w/w). The mix was heated up to 70°C for 20 min with constant stirring with a magnetic stirrer bar [5]. Then, activated carbon was removed from the oil by centrifugation (2500×g, 30 min). The oil was filtered by polyester filter paper ( $\phi = 25$   $\mu$ m).

*Winterization and dewaxing.* The bleached oil (100 g) was chilled to reach 25°C. Then, n-butanol (40 ml) was added and cooled at first to reach 0°C for 12 hours and then was subsequently cooled to reach -18°C for removal of the residue (oil wax). The separation of the liquid-olein and solid stearin fractions was carried out by centrifugation (4500×g, 20 min).

*Deodorization.* The dewaxed oil (100g) was added to the flask and heated up to 150°C for 30 min under vacuum (32 mmHg). Volatile products were condensed in a cooling system installed on the vacuum line, and the distillate was collected from the column.

The fatty acid composition was determined by chromatography according to AOAC Standard 2012 (969.33). The phosphorus content was determined according to AOCS Ca 12-55 (2009). The absorbance of the oil was measured by UV/VIS spectrophotometer at 228 nm AOCS Method Cc13c-50. The free fatty acid content (FFA), peroxide value (PV), iodine value (IV), melting point of the oil and iron content were determined according to AOAC (949.28), AOAC (1999), AOCS (Method Cd 1-25), AOCS (Cc 3025, 2009), and AOAC 2012 (999.11), respectively.

All experiments were performed at least triplicate. The mean value and standard deviation were reported. Analysis of variance (ANOVA) was conducted, and the mean separations were analyzed by Fisher's least significant difference ( $p < 0.05$ ). All statistical analyses were conducted using Minitab 16 software.

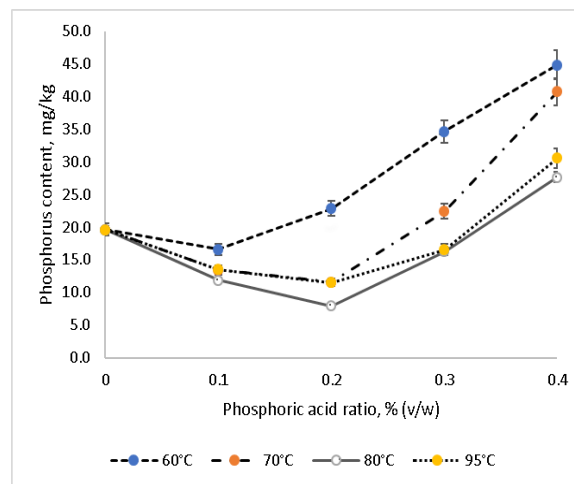
### 3. RESULTS AND DISCUSSION

Tra catfish crude oil recovered from extraction has phosphorus content of 19.63 mg/kg which is higher than quality requirement in refined fish oil ( $< 5$  mg/kg) [3]. Furthermore, the melting point of the oil is  $33.8^{\circ}\text{C}$ , which causes the oil to be in the semi-solid state. The oil is yellow and has a fishy smell. Thus, the refining of fish oil is necessary to remove the impurities in the oil, improve the nutritional quality as well as the sensory quality. As a result, the storage time of fish oil products can also be increased.

#### 3.1 Effects of phosphoric acid ratio on the efficiency of the phospholipid removal

The results (Figure 1) showed that, at the same treated temperature, the increase of phosphoric acid ratio (from 0 to 0.2% to crude oil weight) resulted in the decrease of phosphorus content. However, the higher acid content ( $> 0.2\%$ ) resulted in the fast increase of phosphorus content in oil sample. These changes may be due to the excess phosphoric acid used in oil degumming

process. Phosphoric acid itself contains phosphorus. When the excess acid is not completely removed, it will increase the phosphorus content of the refined oil.



**Fig.1.** Influence of phosphoric acid ratio and temperature on the phosphorus content in tra catfish oil

These results are consistent with the results of Deffense's study in 2017 on the use of phosphoric acid in vegetable oil degumming. In this study [9], the author suggested that only 85% of phosphoric acid should be used in the oil degumming in small amounts (from 0.1% to 0.3% of crude oil volume), because of the excess phosphoric acid may cause problems with the increase in phosphorus content in refined oil.

In another study by Dijkstra et al. 1986, the authors showed the influence of phosphoric acid ratio used in the degumming process to phosphorus content of vegetable oil. The results showed that when the phosphoric acid ratio increased from 0.2% to 0.4% of the crude oil volume, the phosphorus content gradually decreased. The lowest phosphorus content in oil (3.9 mg/kg) was recorded when degumming with 0.4% phosphoric acid. However, when increasing the phosphoric acid ratio to 0.5%, the phosphorus content in the oil increased.

Our results (Figure 1) also showed that, when increasing the temperature from  $60^{\circ}\text{C}$  to  $80^{\circ}\text{C}$ , the efficiency of degumming with phosphoric acid increased. However, the

increase in temperature to 95°C resulted in the reduction of degumming efficiency. Treated temperature at 80°C was found to be more efficiency than the others. The lowest phosphorus content (7.96 mg/kg) was reach at the treatment of 0.2% phosphoric acid at 80°C in 5 minutes. The best phospholipid removal efficiency using phosphoric acid was approximately 60%.

### 3.2 Effects of activated carbon on the bleaching of fish oil

The temperature and time of bleaching process using activated carbon are important factors. Actually, the temperature affects the quality of the edible oil, the lower temperature is, the less damage of oil sample does. However, the temperature should be high enough for the absorption of impurities and color compounds take place. The temperature of bleaching should be 70-110°C during 15-20 min [3]. Therefore, the condition of 70°C for 20 min was used for our treatment. The color of the oil can be determined using a spectrophotometer [10, 11]. Results of oil absorption at wavelength of 228 nm, phosphorus content and peroxide value (PV) are presented in Table 1.

The more adding activated carbon (0-3 %, w/w) resulted in the decrease of Abs<sub>228nm</sub> value (from 0.85 to 0.32), phosphorus content (6.54 to 4.13 mg/kg) and PV (from 2.8 to 0.73 meq.O<sub>2</sub>/kg) (Table 1). However, the higher content of activated carbon (>3 %, w/w) these quality properties were unchanged. These results are similar to that of previous studies [11, 12]. In a limited extent, the increase of activated carbon content causes the increase in absorbing surface area. However, the higher content of activated carbon did not cause further absorption. Furthermore, too much activated carbon might cause problems in filtering and increase in oil loss.

The activated carbon ratio of 3% (w/w) led to the phosphorus content decrease (approximately 37%) and the PV reduction (73.93%) comparing to the neutralized fish oil. This can be explained by the fact that, in addition to the coloring compounds, activated carbon can absorb some other impurities if their size is smaller than the voids on the surface of the coal [3].

The proportion of activated carbon used in the bleaching process is carefully considered because this is related to the oil recovery efficiency after treatment. Activated carbon has a very high oil holding capacity, usually the oil loss is in the range of 20-40% depending on the amount of coal used. Therefore, to minimize oil loss, the lowest activated carbon ratio (3%) was chosen and the quality properties (phosphorus content of 4.13 mg / kg, Abs<sub>228nm</sub> of 0.32, PV of 0.73 meq.O<sub>2</sub>/kg) reached the Codex 2017 for edible oil [13].

### 3.3 Chemical and physical properties of tra catfish oil after each stage of refining process

The chemical and physical properties of crude tra catfish oil after each stage of treatment were presented in Table 2. Obviously, quality indexes (FFA, PV, phosphorus content, Abs<sub>228nm</sub> and IV) had markedly changed through each processing step.

The phosphorus content decreased during two treatment stages: degumming (59.45%) and bleaching (36.85%). In the other stages, the efficiency of phospholipid removal was almost negligible. Refined tra catfish oil has phosphorus content of 4.48 mg/kg. This result is consistent with reference standard of O'Brien (2009) [3] and Codex stan (2017) [13].

**Table 1.** Effect of activated carbon ratio on oil quality properties

No	Activated carbon (%, w/w)	Abs <sub>228nm</sub>	Phosphorus content (mg/kg)	PV (meq.O <sub>2</sub> /kg)
1	Neutralized oil	0.85 ± 0.01 <sup>a</sup>	6.54 ± 0.19 <sup>a</sup>	2.80 ± 0.14 <sup>a</sup>

N <sup>o</sup>	Activated carbon (%, w/w)	Abs <sub>228nm</sub>	Phosphorus content (mg/kg)	PV (meq.O <sub>2</sub> /kg)
2	1	0.63 ± 0.00 <sup>b</sup>	6.00 ± 0.04 <sup>b</sup>	1.47 ± 0.04 <sup>b</sup>
3	2	0.45 ± 0.02 <sup>c</sup>	4.14 ± 0.10 <sup>c</sup>	1.07 ± 0.14 <sup>c</sup>
4	<b>3</b>	<b>0.32 ± 0.01<sup>d</sup></b>	<b>4.13 ± 0.08<sup>cd</sup></b>	<b>0.73 ± 0.02<sup>d</sup></b>
5	4	0.33 ± 0.01 <sup>d</sup>	4.36 ± 0.17 <sup>d</sup>	0.74 ± 0.00 <sup>d</sup>
6	5	0.33 ± 0.00 <sup>d</sup>	4.46 ± 0.08 <sup>d</sup>	0.72 ± 0.02 <sup>d</sup>

In the same column, different letters show statistically significant differences (p < 0.05).

**Table 2.** Physical and chemical properties of tra catfish oil after treatment stages

N <sup>o</sup>	Stage	Phosphorus content (mg/kg)	Abs <sub>228nm</sub>	PV (meqO <sub>2</sub> /kg)	FFA (% Oleic)	IV (gI/100g)
1	Crude oil	19.63 ± 0.28 <sup>a</sup>	0.91±0.01 <sup>b</sup>	3.55±0.14 <sup>a</sup>	0.68±0.03 <sup>a</sup>	62.48±0.31 <sup>a</sup>
2	Degumming	7.96 ± 0.15 <sup>b</sup>	1.26±0.05 <sup>a</sup>	3.40±0.13 <sup>a</sup>	0.62±0.01 <sup>b</sup>	62.40±0.41 <sup>a</sup>
3	Neutralization	6.54 ± 0.19 <sup>b</sup>	0.84±0.01 <sup>bc</sup>	2.80±0.14 <sup>b</sup>	0.26±0.02 <sup>c</sup>	65.43±0.32 <sup>b</sup>
4	Bleaching	4.13± 0.08 <sup>c</sup>	0.26±0.02 <sup>d</sup>	0.73±0.02 <sup>c</sup>	0.12±0.00 <sup>d</sup>	65.52±0.26 <sup>b</sup>
5	Winterization and dewaxing	4.44±0.20 <sup>c</sup>	0.25±0.01 <sup>d</sup>	0.70±0.02 <sup>c</sup>	0.12±0.00 <sup>d</sup>	68.39±0.32 <sup>c</sup>
6	Deodorization	4.48 ± 0.16 <sup>c</sup>	0.77±0.05 <sup>c</sup>	4.22±0.15 <sup>d</sup>	0.06±0.01 <sup>e</sup>	67.88±0.12 <sup>c</sup>

In the same column, different letters show statistically significant differences (p < 0.05)

The absorption of oil (Abs<sub>228nm</sub>) increased under degumming treatment (from 0.91 to 1.26). This can be explained by the separation of NHP (PE, PA) compounds under the action of citric acid which results in the formation of HP (PCh, PI). HP dissolves in water to form suspended particles that increase the turbidity of the oil. Next, Abs<sub>228nm</sub> decreased through the neutralization, bleaching, winterization and dewaxing. In fact, during these stages, the oil was continuously washed and removed impurities such as pigments, metals, phospholipids and oxidation products resulting in the increase of turbidity level of the oil. However, at the deodorization stage, the absorption of the oil increased to 0.77. It might be the result of oxidation of some components in the oil at high temperature (150°C). Color enhancement may also be due to the accumulation of non-volatile decomposition products such as

triacylglycerols or products of the Maillard reaction [14].

The peroxide value did not change during degumming process, but it was decreased under neutralization, bleaching, winterization and dewaxing processes. However, after deodorization stage the PV was reached to 4.22 meq.O<sub>2</sub>/kg. These are similar to the variability of PV in the study of Crexi et al. (2010) [6]. Clearly, the PV was less than <5.0 meq. O<sub>2</sub>/kg, which is required for edible oil [13].

The results (Table 2) also showed that, the FFA content of the oil decreased through processing stages (from 0.68% to 0.06%). Free fatty acid was mainly removed during neutralization process (58%). During other stages, the amount of removed FFA was unnoticed. This can be explained by the neutral reaction between the alkaline solution (NaOH) and residual phosphoric acid and free fatty acids found in the oil [4].

In addition, the IV increased after each stage of treatment. This is due to the effective removal of the impurities which already presented in the fish oil.

In summary, under refining process, fish oil quality has been markedly improved by the reduction of free fatty acid content, PV, phosphorus content, absorption and increase of iodine value (IV).

### 3.4 Quality properties of fish oil before and after refining process

The analytical results (Table 3) showed that, refined fish oil has the quality that met

the criteria for refined edible oil: the PV of 4.22 meq.O<sub>2</sub>/kg; the free fatty acid content of 0.06 % oleic; the phosphorus content reached 4.48 mg/kg; the moisture content of 0.04%; melting point and solidification point decreased from 33.8°C and 24°C to 10.2°C and -2.0°C, respectively; iron content was 0.32 mg/kg. These qualities are in line with Codex stan 2017 [13] for fish oil and reference standards of Bimbo (1998) [15] or O'Brien (2009) [3].

*Table 3. Quality properties of crude fish oil and refined fish oil*

№	Name of property	Unit	Content		Reference standards	
			Crude fish oil	Refined fish oil	Crude fish oil	Refined fish oil
1	Peroxide value	meq. O <sub>2</sub> /kg	3.55	4.22	≤20*	≤ 5**
2	Free fatty acid content	% oleic	0.68	0.06	2 – 5*	≤ 0.6*
3	Saponification value	mg KOH/g	158	162	-	-
4	Iodine value	gI/100g	62.48	67.88	-	-
5	Melting point	°C	33.8	10.2	10 – 15*	-
6	Solidification point	°C	24	-2.0	-	-
7	Phosphorus content	mg/kg	19.63	4.48	5 – 100	<5*
8	Moisture content	%	0.07	0.04	0.5 - 1%*	≤ 0.2*
9	Iron content	mg/kg	0.40	0.32	0.5 – 7	≤ 1.5*
10	Refractive index	nD/28.2°C	1.4582	1.4900	-	-
11	Viscosity	C <sub>p</sub> . 28°C	57	48	-	-

- No specified; \*Standards are referenced by Bimbo 1998 or O'Brien 2009;

\*\* The Codex 2017 standards for fish oil.

The fatty acid composition of refined tra catfish oil was compared with the fatty acid composition of refined salmon oil and tuna oil (Table 4). The results showed that refined tra catfish oil was rich in unsaturated fatty acids (60.6%), including palmioleic, oleic, linoleic,  $\gamma$ -linolenic, paullinic, eicosadienoic,

dihomo- $\gamma$ -linolenic, erucic acids. In which, oleic acid and linoleic acid percentages were 43.9 and 12.2%, respectively. Omega-6 fatty acids (Linoleic acid (C18:2),  $\gamma$ -Linolenic acid (C18: 3), eicosadienoic acid (C20: 2) and dihomo- $\gamma$ -linolenic (C20:3)) are human interesting because organism cannot synthesize them. They are mainly provided

through food [6]. Content of omega-6 fatty acids in tra catfish oil (13.8%) were higher than that of salmon oil and tuna oil. Thus, refined tra catfish oil can be used as a daily edible oil.

Table 4. Fatty acid composition in crude fish oil and refined fish oil

№	Name of fatty acid	Content (% of total fatty acids)			
		Crude tra catfish oil	Refined oil		
			Tra catfish oil	Salmon oil*	Tuna oil*
1	C14:0 myristic acid	3.8	3.6	1.5-5.5	ND
2	C16:0 palmitic acid	32.1	29.0	6.5-12.0	14.0-24.0
3	C16:1 (n-7) palmitoleic acid	0.7	0.9	2.0-5.0	ND
4	C18:0 stearic acid	7.0	6.5	2.0-5.0	ND
5	C18:1 (n-9) oleic acid	39.9	43.9	30.0-47.0	10.0-25.0
6	C18:2 (n-6) linoleic acid	12.8	12.2	8.0-15.0	ND
7	C18:3 (n-6) $\gamma$ -Linolenic acid	0.6	0.6	ND	ND
8	C20:0 arachidic acid	0.2	0.2	0.1-0.5	ND
9	C20:1 (n-7) paullinic acid	1.6	1.7	NA	NA
10	C20:2 (n-6) eicosadienoic acid	0.4	0.5	NA	NA
11	C20:3 dihomogamma-linolenic acid	0.5	0.5	NA	NA
12	C22:0 behenic acid	0.1	0.1	0.1-0.5	ND
13	C22:1 (n-9) erucic acid	0.3	0.3	3.0-7.0	ND
14	Total saturated fatty acids	43.2	39.4	NA	NA
15	Total <b>unsaturated</b> fatty acids	56.8	60.6	NA	NA

\* Based on Codex 2017; ND = non-detected, defined as  $\leq 0.05\%$ ; NA = not applicable or available

#### 4. CONCLUSION

The lowest phosphorus content (7.96 mg/kg) was reached at the treatment of 0.2% phosphoric acid at 80°C. The best phospholipid removal efficiency using phosphoric acid was approximately 60%. Bleaching tra catfish oil with 3% (w/w) activated carbon led to the most appropriate efficiency. After refining process, phosphorus content in oil was reduced to 4.48

mg/kg, Abs<sub>228nm</sub>, melting and solidification points were decreased to 0.32, 10.2°C and -2.0°C, respectively. Besides, quality properties such as peroxide value (4.22 meq. O<sub>2</sub>/kg), iodine value (67.88 gI/100g), and saponification value (162 mg KOH/g) reached the standards for edible oil. Refined tra catfish oil is pale yellow, liquid state at ambient temperature without strange odor.

#### REFERENCES

- [1] Kovári K. "Recent developments, new trends in seed crushing and oil refining", OCL, Vol. 11(6), 381-387, 2004.
- [2] Akoh C.C. and Min D.B. *Food lipids: Chemistry, Nutrition, and Biotechnology*. Edited by Casimir C. Akoh and David B. Min. 3rd ed. Taylor & Francis Group. LLC, 2008.
- [3] O'Brien R.D. *Fat and Oils: formulating and processing for applications*. Richard D. O'Brien. 3rd ed. Taylor & Francis Group. LLC, 2009.
- [4] Sathivel S. et al., "Oil production from catfish viscera", JAOCS, Vol. 80, 377-382, 2003.

- [5] Aidos I., Van Der Padt A., Boom R.M. and Luten J.B., “*Quality of Crude Fish Oil Extracted from Herring Byproducts of Varying States of Freshness*”, Food science, Vol. 8(2), 458-465, 2003.
- [6] Crexi V.T. et al., “*Production and refinement of oil from carp (Cyprinus carpio) viscera*”, Food Chemistry. Vol. 119, 945–950, 2010.
- [7] Dijkstra A.J. et al. “*Process for producing degummed vegetable oils and gums of high Phosphatidic acid content (Patent style)*”, Patent Number: 4698185, 1987.
- [8] Dijkstra A.J. and Opstal M.V., “*The total degumming process*” JAOCS, Vol. 66(7), 1002-1009, 1989.
- [9] Deffense E. 2017. *Chemical degumming*. AOCS library
- [10] Dáša Kružlicová et al. “*Classification and characterization of olive oils by UV-Vis absorption spectrometry and sensorial analysis*”, Journal of Food and Nutrition Research, Vol. 47(4), 181–188, 2008.
- [11] Budi H. S. “*Bleaching of crude palm oil (cpo) using adsorbent prepared from pyrolyzed coffee residues*”, in Regional Symposium on Chemical Engineering (RSCE), 2004.
- [12] Nandini.N and Sivasakthivel. S. “*Bleaching of sunflower waste oil by absorption on activated carbon and improved by ozonisation*”, American International Journal of Research in Science, Technology, Engineering & Mathematics, Vol. 7(1), 35-39, 2014.
- [13] Codex stan 329-2017. Standard for fish oils.
- [14] Antoniassi R., Esteves W., and Antonio José de Almeida Meirelles. “*Pretreatment of Corn Oil for Physical Refining*”, JAOCS, Vol. 75(10), 1411-1415, 1998.
- [15] Bimbo A.P. “*Guiderlines for characterising food grade fish oil*”, Inform, Vol. 9(5), 473-483, 1998.

**Corresponding author:**

PhD. Pham Thi Hoan

Ho Chi Minh City University of Technology and Education

E-mail: hoanpt@hcmute.edu.vn