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Deodorizing pangasius oil from by-products using Absorbent and enzymatic methods

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Vietnam is the number one Striped Pangasius (*Pangasianodon hypophthalmus*) producer and exporter and the Catfish industries by-products implied a potential economic value when appropriately utilized. Catfish oil was not widely applied to human consuming products due to its fishy smell. In this study, we investigated two low-temperature Pangasius oil deodorizing: Absorption over activated charcoal and fermentation using lactic acid bacteria (LAB). The optimum ratio of activated charcoal used was 0.2%. The optimal fermentation conditions were 5% LAB, 3.4% sugar in 8 hours. The physicochemical characteristics of the raw Catfish oil and Catfish oils after deodorization were analyzed and the sensory assessment was done. The Acid value (AV) was decreased from 3.363 mg NaOH/l in raw oil to 2.172 and 2.186 in charcoal treating and fermentation oils, respectively. Similarly, Peroxide value (Pv) decreased from 0.9103 meqO_2/kg to 0.830 with the absorbent method and 0.776 with the LAB fermentation method. Trimethylamine (TMA) was 19.37 mgN/100g in crude Catfish oil and lowered to 2.079 in the absorbent and 1.516 in the fermentation method. There was a dramatic improvement in the sensory scores after treatment with both methods (70 in crude oil to 111 in activated charcoal and 105 in fermentation treated oils). Both methods suggested useful application in Catfish oil deodorization in the Vietnam condition.

Keywords: catfish oil, deodorize, absorbent, enzyme, *Pangasianodon hypophthalmus*

INTRODUCTION

Pangasius fish (*Pangasianodon hypophthalmus*) is one of the critical aquaculture commodities and contributes a massive portion of export value in Vietnam. Vietnam is the top producer and accounted for more than half of the striped Pangasius production in the world. In 2018, the total Pangasius production in Vietnam was 1.42 million Metric Tons making an export value of 2.26 billion USD to 150 countries worldwide. The top four export markets for Vietnamese Pangasius are the United States, the European Union, China, and Southeast Asia (Nguyen and Jolly, 2017).

The Vietnamese Pangasius are sold mainly in frozen fillets, frozen steaks, and wholes. Vietnam has developed a modern production system for these sectors with certified manufacturers, to comply with the highest quality demanding market, such as USA and EU. However, Pangasius by-products, accounted for 50% to 75% of the whole

production (Guérard *et al*., 2005) in the forms of heads, viscera, bones, fat, and trimmed meats are environmental concerns and low market value. Thammapat *et al*. (2010) reported that the viscera contained the highest lipid content in Pangasius, up to 93.32%. The most predominant fatty acids were monounsaturated fatty acid (MUFA), ranging from 32.7% - 39.9%, followed by saturated fatty acids (SFA) and polyunsaturated fatty acid (PUFA). There were attempts to utilize Pangasius oil, such as making biodiesel (Santya *et al*., 2019) or cooking oil. In Vietnam, some manufacturers, such as the Asia Fish Oil Corporation (AFO), have produced premium fish cooking oil from Pangasius by-products for local and abroad markets. The high percentage in SFA in Pangasius oil also suggests another application in making shortening and margarine.

A disadvantage of using Pangasius oil for human consumption application was the unfavorable sensory features, especially the smell and color. In the process of fish oil refining, deodorizing is one crucial step. A typical deodorization process was steam distillation when high temperature under low temperature (180-220oC in 1-10mbar) was used to remove the volatile compounds (the secondary oxidation products) but posed the risk of degradation (Carvajal and Mozuraityte, 2016). Song *et al*. (2018) studied several deodorizing methods such as liquid-liquid extraction, green tea polyphenol treatment, and solid phased absorption to compare to the industrial scale distillation. The author suggested that the latter's high temperature risked inducing peroxidation, degradation, and isomerization of lipids. Low-temperature deodorization was considered more efficient in removing unfavorable smells yet maintaining the best quality fish oil refining.

In this study, we investigated the two low-temperature deodorizing methods for Pangasius oil, the absorbent using the activated charcoal and the lactic acid fermentation, then evaluated their effectiveness by physicochemical characteristics and sensory assessment.

MATERIALS AND METHODS

Obtaining Pangasius oil

Steamed Pangasius by-products, including mainly viscera, head, bone, skin, and trimmed meat… were then pressed to extract the lipid-rich liquid. The liquid was centrifuged using a three-phased decanter to obtain yellowish homogenous oil and used as materials for experiments in this study.

Pangasius oil quality evaluation

Methods of sensory quality assessment: The sensory quality of the Pangasius oil, including taste, smell, color, and transparency, was assessed according to TCVN 2627:1993. Scoring was done according to TCVN 3215-1979. A trained committee of 6 members evaluated the quality of each sensory parameter based on a scale from 0-5 points, in which 0 indicated spoilage and 5 indicated the best quality. The total score for each parameter is 30 points. The total overall score is 120 points.

The lipid concentration was determined according to TCVN 3703-2009, using a Soxhlet extractor (Soxhlet R106S - Behr, Germany) to extract lipid from a solution, then dried and weighed. The lipid concentration was calculated as a percentage (%) of fat obtained to total sample weight.

The Iodine index was determined according to TCVN 6122:2010. The Acid value (AV), expressed by mg of potassium hydroxide to neutralize the organic acids in 1 gram of fat, and the free fatty acids (FFA) were determined according to TCVN 6127:2010. The saponification value, defined as mg of potassium hydroxide to saponify 1 g of fat, was detected according to TCVN 6126:2015. The peroxide value representing the degree of oxidative of oils was found using the method standardized in TCVN 6121:2010. The method of determining trimethylamine (TMA) in seafood and seafood products was done according to Woyewodal et al., 1986.

Deodorizing Pangasius oil with the absorbent method (with powder activated charcoal).

Activated charcoal powder, after being washed in clean water and dried, was added into 100g Pangasius oil in 0% (control), 0.05%, 0.1%, 0.15%, and 0.2%. The mixtures were then heated to 45°C in 60 minutes and filtered. The experiment was repeated three times. The amount of activated carbon that produced the final product with the lower acid value (AV) and free fatty acid value (FFA) and higher sensory evaluation score was chosen as optimum.

Deodorizing Pangasius oil by Lactic acid fermentation

Choosing lactic acid fermentation bacteria. Two bacteria strains, *Lactobacillus brevis* and *Lactobacillus plantarum*, were cultured in liquid

MRS medium at 37°C in 24 hours. Each strain was then added to 100g Pangasius oil at a concentration of 3%, with 5% sugar and fermented at 37°C in 8 hours. The final lactic acid product, determined according to TCVN 3702-90, was used to evaluate each strain's fermentation ability, the higher lactic acid production, the better. The bacterial strain that can produce more lactic acid would be chosen for later experiments.

Choosing the optimum fermentation conditions.

The optimum fermentation conditions for the bacteria strain of choice in the previous experiment to obtain the highest lactic acid production were assessed and selected in the following operations:

Experiment 1: Choosing the optimum amount of bacteria added

Lactic fermentation bacteria were added in each of 100g Pangasius oils at 0% (control), 1%, 2%, 3%, 4%, 5%, 6%, 7%, and 8%(repeated 3 times) and fermented at 37°C in 8 hours in the natural pH value of Pangasius oil (6.5). Five percent of sugar was also added in all trials. The optimal amount of bacteria that produced the highest lactic acid was chosen. This value would be used in the next experiments.

Experiment 2: Choosing the optimal amount of sugar added

Adding the optimal amount of bacteria found in the previous experiment into each of 100g Pangasius oil. In each mixture, 0.5%, 1%, 2%, 3%, 4%, 5%, 7%, and 9% were added and fermented at 37°C in 8 hours in the natural pH value of Pangasius oil (6.5). The experiment was repeated three times. The sugar content in the formula that produced the highest lactic acid was chosen to use in the next experiments.

Experiment 3: Choosing the optimal fermentation time

The same fermentation of 100g Pangasius oil with the optimum amount of bacteria and sugar from previous experiments were done in 6, 7, 8, 9, and 10 hours. The test was repeated three times. The fermentation time that produced the highest lactic acid product was chosen as optimum.

Experiment 4: Calculating the optimum fermentation condition formula

Based on the results of the previous three experiments, a regression formula was built based on the Box-Behnken design method with three factors: the number of bacteria (%), amount of sugar (%), and fermentation time (hour). Testing the accuracy of the formula was performed by laboratory fermentation experiment with the optimum conditions calculated by the equation.

Data analysis

Data were analyzed using Microsoft Office Excel 2016 and Design-Expert 11.

RESULTS

Analyzing the quality parameters of the material Pangasius oil

The quality parameters of raw Pangasius oil were presented in table 1 and the sensory quality assessment in table 2.

Deodorizing using the absorbent method

The acid value (AV) and free fatty acid (FFA) of the deodorizing tests were shown in Figure 1, and the sensory assessment results in Figure 2. It was clear that when the amount of activated charcoal increased, the AV and FFA decreased while the sensory score increased. In test 5, there was a sharp increase in sensory scores, from 68 in the control sample to 111 in the deodorized sample. The change in color was easily observed (see figure 3).

The optimum activated carbon for deodorizing was 0.2%.

Deodorizing Pangasius oil by Lactic fermentation

Choosing the lactic acid bacteria (LAB) strain. Both LAB strains, *Lactobacillus plantarum* and *Lactobacillus brevis*, were able to perform fermentation in fish oil medium with 5% added sugar. *Lactobacillus brevis* produced higher lactic acid (1.68 ± 0.049 g/l compared to 1.1 ± 0.075 g/l) and was chosen for the next experiments.

Choosing the optimum lactic acid fermentation conditions.

The effect of the ratio of LAB was presented in Figure 4. The obtained lactic acid increased when the ratio of LAB increased, reached the maximum at the ratio of 5% LAB, and decreased from the ratio of 6%. The optimum ratio of LAB was suggested to be 5%. A higher concentration of bacteria could create a nutritional competition in the growth stage, bringing the fermentation process to the stable and equilibrium phases, inhibiting lactic acid production. The range from 4% - 6% was chosen to use as a factor in the later

optimal condition regression model design.

The effect of the ratio of added sugar was presented in Figure 5. The lactic acid amount increased proportionally with the percentage of sugar added and reached a maximum at 3% (2.55g/l). Then, it reduced as higher sugar concentration inhibited LAB growth. The optimum sugar ratio was proposed to be 3%, and the range from 2%-4% was chosen for later regression model construction.

With 5% of LAB and 3% sugar added, a series of fermentation tests were done to determine the optimum fermentation time. It was clear from Figure 6 that the optimal time for the lactic acid fermentation could be 8 hours, as there was the highest lactic acid produced (2.32 g/l). When the fermentation took longer than 8 hours, the substrates decreased, and LAB will use lactic acid as substrates to grow. The range from 7-9 hours was chosen for optimal condition regression model construction, suggesting that the optimal time was 8 hours

A regression model that showed the relationship between lactic acid content and the factors: LAB ratio, percentage of added sugars, and fermentation time was constructed using Design-Expert 11 and shown in equation (1) as follows:

$$Y= -57.6 +9.76A + 0.52B + 8.59C -0.02AB + 0.05AC + 0.12BC – 1.00A^2 – 0.20B^2 – 0.57C^2 \quad (1)$$

(In which Y: Amount of Lactic acid; A: LAB ratio; B: added sugar ratio; C: time)

The p-value was 0.0001 (<0.005), $R^2 = 0.9988$, Adj $R^2 = 0.9966$ and the difference of Adj R^2 and Pred R^2 (0.9821) was 0.015 < 0.2 demonstrated that the selected model can be used to predict other conditions of an experiment (see Figure 7-10).

The optimum fermentation conditions were predicted, and a laboratory experiment was done to test the predicted conditions with results shown in Table 3. There was a trivial difference between the predicted and actual lactic acid obtained. The optimal fermentation conditions, therefore, were 5% LAB, 3.4% sugar in 8 hours.

Analyzing and comparing the quality parameters of Pangasius oils after *deodorized*

Two samples of Pangasius oils were deodorized with the absorbent and lactic acid fermentation methods' optimal conditions. The quality parameters were analyzed and compared to that of raw Pangasius oil, as shown in Table 4.

Table 1: Initial quality parameters of Pangasius oil

No.	Parameters	Unit	Methods	Results
1	Lipid value	%	TCVN 3703:2009	99.76±0.049
2	Acid value	mg NaOH/g	TCVN 6127:2010	3.363±0.029
3	Free fatty acid	%	TCVN 6127:2010	1.692±0.008
4	Saponification value	mgKOH/g	TCVN 6126:2015	192.81±0.325
5	Peroxide value	meqO_2/kg	TCVN 6121:2010	0.9103±0.024
6	Iodine index	gI_2/100g	TCVN 6122:2010	64.87±0.216
7	Trimethylamine(TMA)	mgN/100g	According to Woyewodal et al., 1986	19.37±0.155

Table 2: Initial Pangasius oil sensory evaluation

Sample	Parameters	Score	Total score	Average score
Initial Pangasius oil	Taste	18	70	11.67
	Transparency	20		
	Color	22		
	Odor	10		

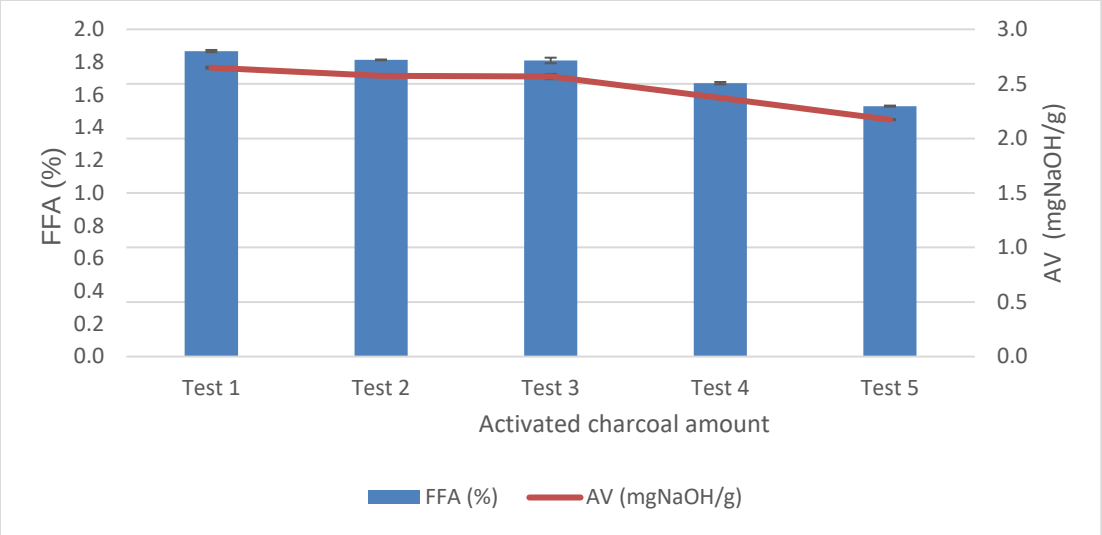

Figure 1: Acid Value and Free Fatty acid value in absorbent deodorizing tests.

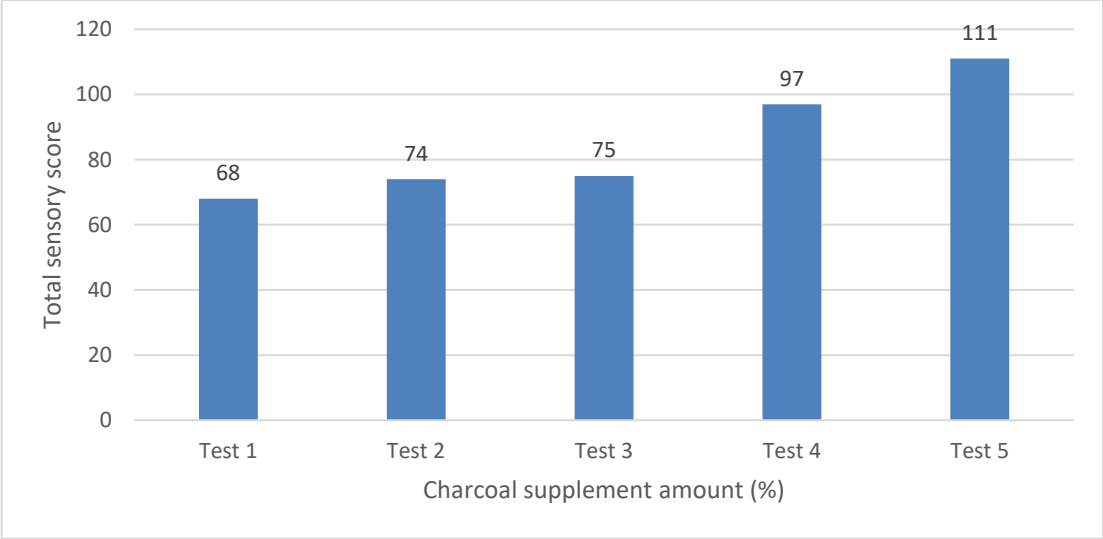

Figure 2: The sensory assessment results of absorbent deodorizing tests

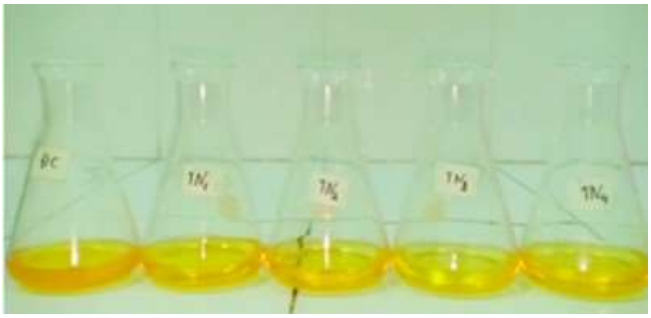

Figure 3: The color of samples after deodorizing with activated carbon

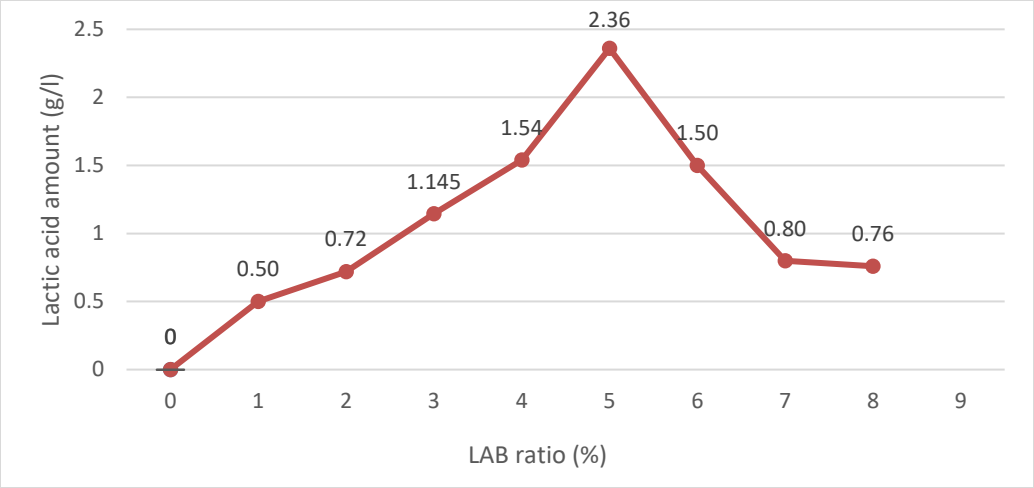

Figure 4: The effect of the ratio of LAB on the fermentation process.

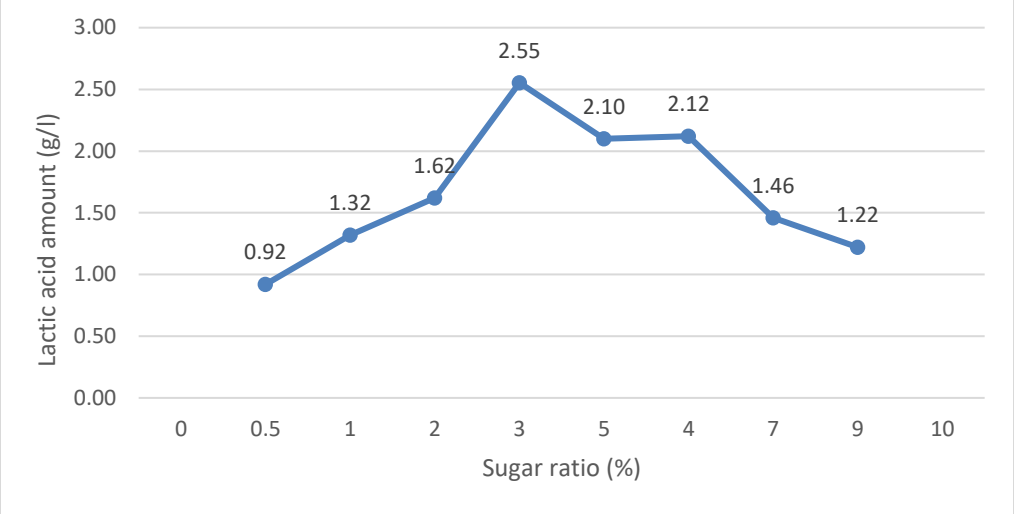

Figure 5: The effect of the ratio of added sugar on the fermentation process

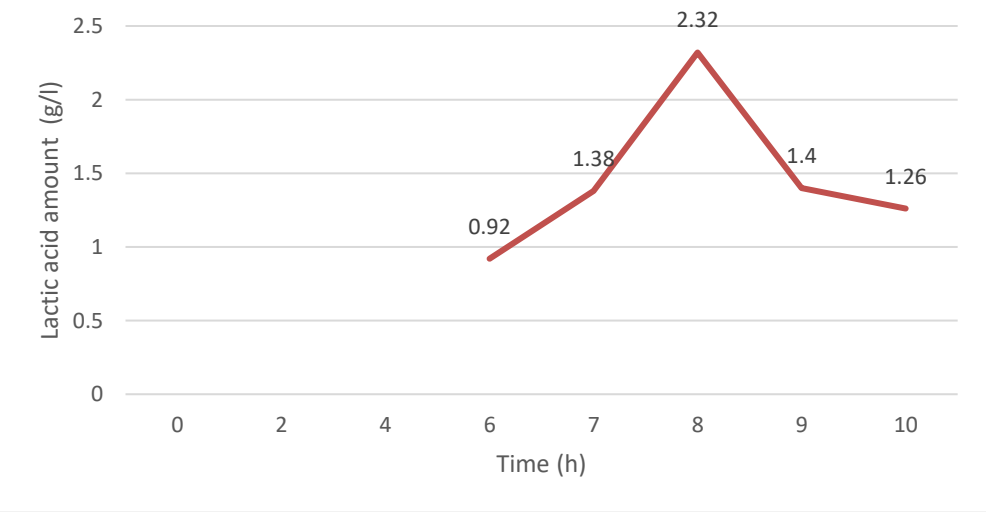

Figure 6: The effect of fermentation time

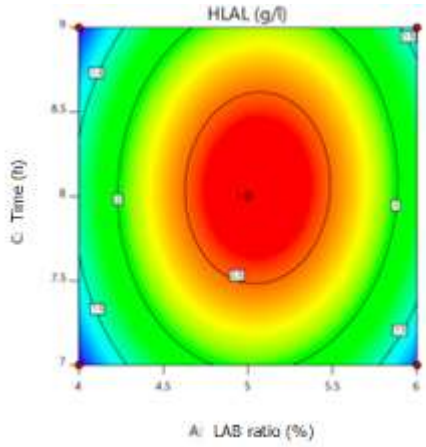

Figure 7:Contour line graph of the effect of LAB ratio and time on HLAL.

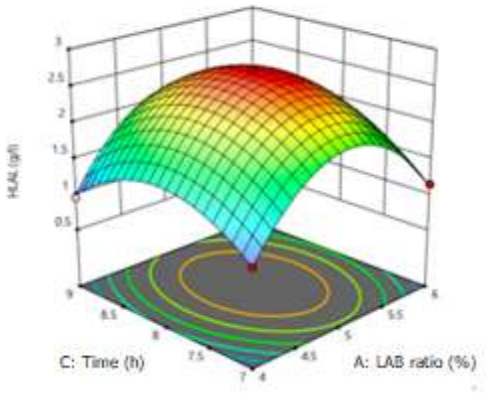

Figure 8: The 3D graph of the effect of LAB ratio and time on HLAL.

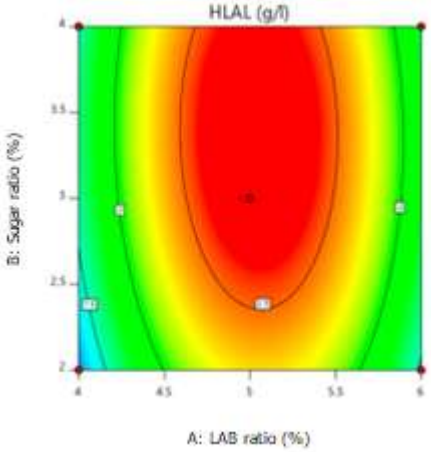

Figure 9: Contour line graph of the effect of LAB ratio and sugar ratio on HLAL.

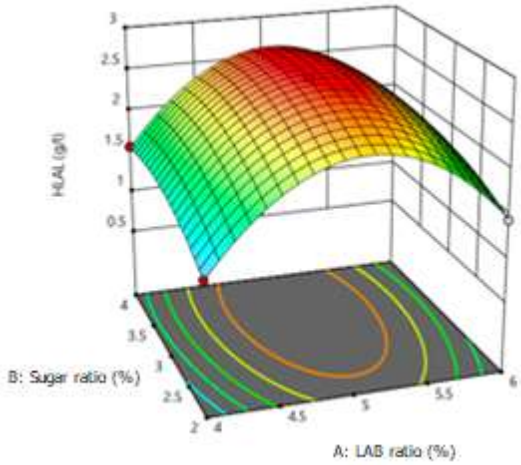

Figure 10: The 3D graph of the effect of LAB ratio and sugar ratio on HLAL.

Table 3: Predicted and actual optimum fermentation conditions

	LAB (%)	Sugar (%)	Time (hour)	Lactic Acid (g/l)
Predicted	4.98	3.41	7.97	2.70
Practical	5	3.4	8	2.68 ± 0.057

Table 4: Quality parameters of Pangasius oil before and after deodorizing

No.	Parameters	Units	Raw oil	Absorbent method	Fermentation method
1	Lipid value	%	99.76±0.050	98.73±0.811	94.32±0.629
2	Acid value	mg NaOH/g	3.363±0.010	2.172±0.005	2.186±0.008
3	Free fatty acid	%	1.692±0.008	1.531±0.003	1.541±0.006
4	Saponification value	mgKOH/g	192.81±0.325	185.15±0.651	179.93±0.420
5	Peroxide value	meqO_2/kg	0.9103±0.024	0.830±0.047	0.776±0.063
6	Iodine index	gI_2/100g	64.87±0.021	60.29±0.109	62.98±0.258
7	Trimethylamine (TMA)	mgN/100g	19.37±0.155	2.079±0.011	1.516±0.163

Table 5: Comparing the sensory assessment results

Methods	Parameters	Score	Total score	Average
Absorbent	Taste	26	111	18.5
	Transparency	30		
	Color	27		
	Odor	28		
Lactic fermentation	Taste	26	105	17.5
	Transparency	30		
	Color	23		
	Odor	26		

DISCUSSION

The oxidation parameters, such as acid value (AV) and peroxide value (PV), are routinely used worldwide as indicators of the rancidity of oils (Robards *et al*., 1988). Many regulators have set limits to both parameters as quality standards of fish oils.

In this study, the crude Pangasius oil was not conformed to CODEX/FAO in AV (3.363 mg NaOH/g against <3) (Food and Agriculture Organization, 2010). The parameter lowered after both activated carbon and LAB treatments and came below the limit (2.172 and 2.178, respectively).

The PV of crude Pangasius oil was 0.9103 meqO_2/kg, relatively good compared to the CODEX/FAO limit of 5 meqO_2/kg. The PV after treatments was improved slightly to 0.830 with the absorbent method and 0.776 with the LAB fermentation method. LAB fermentation showed a better effect in improving the PV parameter. The result indicated the potential of using Pangasius oil from by-product for human consumption as it conformed to most international standards for fish oils, while as high as 13.9% of the fish oil products in the database exceeded the limit (De Boer *et al*., 2018).

Trimethylamine (TMA) is a product of decomposition and generally associated with the odor of fouling fish, infections for bad breath. TMA is produced from Trimethylamine oxide (TMAO) in fish cells. The smell of fish oils is considered strongly related to TMA from the fish itself and bacteria growth in the oil. In this study, the TMA value was 19.37 mgN/100g in crude Pangasius oil, suggesting its unfavorable smell showing through the low sensory assessment score. TMA value was greatly improved through deodorizing treatments, to 2.079 with the absorbent method and 1.516 in fermentation method. Mohri and Kanauchi (2019) reported that using LAB *Lactobacillus plantarum* H78 could significantly improve the smell of fish meat by eliminating TMA to 30-38% compared to control samples. Chung and Lee (2009) also effectively deodorized fish oil by removing TMA by absorption over zeolite catalysts.

The sensory results showed an agreement with physicochemical results in evaluating the effectiveness of the deodorization. After treatment using both methods, the sensory scores had been dramatically improved. The extent of improvement was not much distinction between both methods.

This study's results suggested that both absorption over activated charcoal method and LAB fermentation method were relatively effective in deodorizing Pangasius crude oil from by-products. The results agreed with studies with other objects. Yang (2007) deodorized the

hydrolyzed clam meat protein by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* fermentation. Liu (2011) studied to produce shellfish protein beverage, in which he used *Lactobacillus bulgaricus* and *Streptococcus thermophilus* to create a better smell and taste for the product. Chakraborty and Deepu (2014) used activated charcoal as a step in the four-step refining procedure for Indian Oil Sardine.

CONCLUSION

Absorption over activated charcoal and fermentation using LAB was examined and suggested effective uses to deodorize Pangasius oil from Pangasius by-products. This study could be an essential step in the refining procedure of Pangasius oil to use for human consumption. With its high content of saturated fat, refined Pangasius oil could be further applied in value-added products such as shortening, potentially utilizing the prominent products of Vietnam.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

Conceptualization, N.T.K.H., P.T.V., and B.T.T.H.; methodology, N.T.K.H. and B.T.T.H.; software, N.T.K.H.and N.T.B; validation, L.C.L, T.V.T., N.K.B., and B.T.T.H..; formal analysis, N.T.K.H., P.T.D., and N.T.H.; investigation, N.T.K.H., P.T.V.,N.T.H., N.T.A., D.T.T. and N.K.B.; resources, N.T.A. , D.T.T. and N.K.B.; data curation, N.T.K.H. and B.T.T.H; writing—original draft preparation, N.T.K.H., P.T.V., and B.T.T.H..; writing—review and editing, N.K.B and B.T.T.H..; visualization, N.T.K.H., P.T.D. and N.T.B.; supervision, B.T.T.H.; funding acquisition, B.T.T.H. All authors have read and agreed to the published version of the manuscript.

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