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Changes in tra catfish flesh meat (*Pangasianodon hypophthalmus*) during refrigerator storage and the lipid properties of flesh meat

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Abstract

In this study, the lipid properties of tra catfish flesh meat (*Pangasianodon hypophthalmus*) during refrigerator storage as well as the properties of oil extracted from tra catfish flesh meat were investigated. The quality of the samples was maintained, with lipid content and peroxide value falling within the acceptable range after 15 days of storage. The yield of oil extracted using isopropanol:hexane at a ratio of 2:3 was higher (56.2%) than that extracted using ethanol:hexane at a different ratio. Isopropanol:hexane (2:3) also showed the highest L^* (lightness/brightness) value (33.35) and the lowest b^* (yellowness/blueness) value (7.35). The polyunsaturated fatty acid levels as well as the omega 3 and 6 fatty acid levels of oil were highest in this solvent at a ratio of 2:3. Thus, tra catfish flesh meat could be used as a material for lipid extraction.

Keywords: tra catfish flesh meat; oil; fatty acid; solvents; refrigerator storage.

Practical Application: Tra catfish flesh meat oil with extracting in isopropanol:hexane showed higher yield and PUFA level than that extracted in ethanol:hexane.

1. Introduction

Tra catfish are the most important freshwater fish in the Mekong Delta Region, Vietnam, and their processed products are very important foodstuffs for domestic consumers and exportation. Frozen tra catfish fillets are the main export product in Vietnam. According to the Vietnam Association of Seafood Exporters and Producers, by November 2022, the total value of tra catfish exports had reached $ 2.3 billion (VASEP, 2022). However, the filleting process discards a large amount of byproducts (fish heads, skin, scales, bones, flesh, and viscera). Fish flesh and viscera are potential sources for lipid extraction. Furthermore, the fatty acid (FA) composition of the lipids extracted from the viscera of tra catfish includes high levels of saturated FAs (SFAs; palmitic and stearic acids) and monounsaturated FAs (MUFAs; oleic acid), but low levels of long-chain polyunsaturated FAs (PUFAs; docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)) (Ho & Paul, 2009; Men et al., 2005). However, the above results refer only to lipids from the viscera oil of tra catfish, and there is limited publication regarding lipid extraction from tra catfish flesh meat. Therefore, the purpose of this study was to investigate the effect of refrigeration storage time on the quality of tra catfish flesh meat, the properties of the lipids, and the FA composition by using extraction solvents.

2. Materials and methods

2.1. Preparation of tra catfish flesh meat

Flesh meat of tra catfish was collected from a frozen seafood processing company located in Can Tho City. The samples were washed with chilled water, stored on ice, and immediately sent to a laboratory. Finally, the samples were kept in polyethylene bags, stored in a refrigerator (4°C), and then collected to check lipid content changes (lipid content and peroxide value (PV)) at 0, 3, 6, 9, 12, and 15 days.

2.2. Lipid extraction

2.2.1. Isopropanol:hexane extraction

Raw flesh meat from tra catfish was ground for homogenization. A sample (10 g) was mixed with 100 mL of isopropanol:hexane at different ratios (3:2, 1:1, and 2:3) and gently stirred at room temperature for 30 min. The filtrate was collected from the extraction sample. Subsequently, anhydrous sodium sulfate (1,000 mg) was added to the filtrate, and the mixture was vortexed for 30 s. The solvent was removed by rotary evaporation at 50°C for 2 h. The residue was collected, weighed, and stored at 20°C in a dark, inert atmosphere.

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2.2.2. Ethanol:hexane extraction

Raw flesh meat from tra catfish was ground for homogenization. The sample (10 g) was mixed with 100 mL of ethanol:hexane at various ratios (3:2, 1:1, and 2:3) and gently stirred at room temperature for 30 min. The lipid collection procedure was similar to that described above.

2.3. Lipid content in the tra catfish flesh meat

Lipids of fish flesh meat were extracted from a sample with methanol/chloroform/0.88% KCl (1/1/0.5, v/v/v) according to the method of Bligh and Dyer (1959). Then, the solvent was evaporated at 35°C using a rotary evaporator. Finally, the lipid content of samples was determined gravimetrically, and the collected data were expressed as a percentage of the wet-weight samples.

2.4. Peroxide value

The PV of the lipid sample was analyzed according to the method described by Takagi et al. (1978) with minor modifications. Briefly, ground meat or lipid extraction solution (50 mg) was mixed with 5 mL of chloroform and 10 mL of acetic acid. Then, the mixture was blended with 1 mL of 50% potassium iodine, placed in the dark for 5 min, and then added to 9 mL of 2% cadmium acetate and continuously stored in the dark until the two phases were clearly separated. The absorbance of the upper aqueous phase was measured at 410 nm using a visible spectrophotometer (Genesys 30, USA). The PV (mEq O_2/kg) was calculated using the Equation 1 (Weng et al., 2009):

$$PV = [(a - b) \times 60.14 + 0.69](8w)^{-1} \times 1{,}000 \quad (1)$$

Where:

a and b: the absorbances of the sample and blank, respectively;

w: the sample weight (g).

2.5. Lipid recovery yields

In all extraction solvents, the recovery yield of oil extraction was determined based on the procedure reported by Sahena et al. (2010) and expressed as the proportion of extracted oil compared to the total oil content in the sample.

2.6. Determination of the acid value

First, 2 mL of 1% phenolphthalein indicator and 125 mL of 1:1 toluene:isopropyl alcohol were added to a 250-mL Erlenmeyer flask. The sample was neutralized with 0.1 N KOH until a faint but permanent pink color appeared. An oil sample (0.25 g) and a neutralized solvent mixture (125 mL) were added and mixed in another 250-mL Erlenmeyer flask. The sample was then titrated with 0.1 N KOH until a permanent pink color appeared. The acid value of the oil (mg KOH/g of sample) was calculated according to the method described by Iberahim and Tan (2020) as follows (Equation 2):

R:

$$\text{Acid value} = \frac{(A - B) \times N \times 56.1}{W} \quad (2)$$

Where:

A: the volume of standard alkali used in the titrating sample (mL);

B: the volume of the standard alkali used in titrating the blank (mL);

N: the normality of the standard alkali;

W: the mass of the sample (g);

56.1: the molecular weight of KOH (g).

2.7. Analysis of oil color

The color of the oil sample was determined according to the AOCS Official Method Cc13e92 (AOCS, 2009). For this purpose, a colorimeter (PCE–CSM 2, China) was used, and the results are expressed based on L^* (lightness/brightness) and b^* (yellowness/blueness).

2.8. FA analysis

The FA composition was determined using an Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent 7683 B autoinjector and a flame ionization detector. Helium was used as the carrier gas, with a flow rate of 1.6 mL/min. The individual FAs were separated according to their different migration rates using a Varian CP7419 capillary column (50 m × 250 µm × 0.25 µm; Agilent Technologies, Santa Clara, CA, USA). After maintaining the temperature at 60°C for 1 min, the column was temperature-programmed at 30°C/min to 130°C, at 1.3°C/min to 195°C, and at 30°C/min to 240°C, where it was maintained for 10 min. The temperature of the injector was 240°C and the temperature of the detector was 250°C.

2.9. Statistical analysis

All experiments were performed four or more times, and data analysis was conducted using the SPSS software package (SPSS 16.0 for Windows). Statistical differences were analyzed using one-way ANOVA and Duncan's multiple range test at 95% probability.

3. Results and discussion

3.1. The lipid changing of flesh meat from tra catfish during 15 days in refrigerator

The lipid contents and PVs of fresh tra catfish flesh meat after 15 days of storage in a refrigerator increased with increasing storage time at 4°C (Table 1). The lipid content in the tra catfish meat increased from 31.5 to 45.09% after 15 days of cold storage. In a similar study, the lipid content in mussel meat decreased from 43.5 to 33.6% after 15 days of cold storage (Bejaoui et al., 2021). Storage conditions (temperature and time) had a greater

effect on the final lipid content than the packaging method (Nguyen et al., 2023).

The PV is the most commonly used assay for the oxidation of fats and oils. It measures the rancidity or degree of oxidation but not the stability of the fat. Oxidation of lipids is an important problem in seafood products, particularly fish, because it can lead to high amounts of PUFAs (Goulas & Kontominas, 2007), which cause quality deterioration and the formation of unpleasant odors. In the present study, during refrigerator storage, the PV of flesh meat increased significantly after 15 days (from 1 to 3 mEq/kg). In a previous study, an increase in the PV was found in oily Monterey sardine stored at 0°C for 15 days (Pacheco-Aguilar et al., 2000), but these findings disagree with those of Li et al. (2016) and Tenyang et al. (2017) who observed a decrease in the PV of commercial catfish in Cameroon and surimi products during cold storage. Thus, the differences in the PV might be due to storage temperature, concentration of pro-oxidants and enzymes, ionic strength, and oxygen consumption (Bustabad, 1999). For quality fish oil, the PV must be ≤5 mEq/kg, as stipulated by the Global Organization for EPA and DHA (GOED) and the Food and Agricultural Organization of the United Nations (FAO, 2005). In this study, the PV of the fish oil obtained was 3 mEq/kg after 15 days of storage in a refrigerator, which was within the acceptable range. Furthermore, the total aerobic microorganisms (CFU/g) of flesh tra catfish meat after 15 days of storage at a cold temperature were 5.45×10^3 CFU/g, which was within the safety range.

3.2. The effect of extraction solvents on the quality of oil from flesh meat of tra catfish

3.2.1. Lipid recovery yield

The selection of the solvent and ratio is very important for extracting oil from fish with a high oil content. As shown in Table 2, the yield of oil extracted from the flesh meat of tra catfish using isopropanol:hexane was higher than that extracted using ethanol:hexane at a similar ratio. Isopropanol acts as a co-extractant for the oil. Since the cell membrane is mostly composed of protein molecules that enclose polar lipids, such as phospholipids, glycolipids, and cholesterol, isopropanol, which acts as a polar solvent, destroys the complex lipid-protein interactions before the lipids are extracted with hexane (Halim et al., 2011). Hence, an isopropanol:hexane ratio of 2:3 is most suitable for maintaining the nonpolar characteristics for oil extraction and the polar characteristics for disruption of the protein matrix.

Table 1. The lipid changing of tra catfish flesh during 15 days of cold storage (on wet basis)*.

Storage time (days)	Peroxide value (mEq/kg)	Lipid content (%)
3	1.00±0.990^{a}	31.50±1.466^{a}
6	1.10±0.240^{a}	34.51±0.752^{ab}
9	1.17±0.141^{a}	37.32±1.554^{bc}
12	2.20±0.990^{b}	40.65±1.691^{c}
15	3.00±0.833^{c}	45.09±1.133^{d}

*Results are means±standard deviation (n=3). Different superscripts (a–d) within a column are significantly different among samples ($p<0.05$).

Table 2. The recovery yield of lipid*.

Ratio	Isopropanol:hexane	Ethanol:hexane
3:2	34.7±4.52^{a}	34.2±6.58^{a}
1:1	44.4±4.67^{b}	38.4±4.78^{b}
2:3	56.2±9.98^{c}	41.6±7.34^{c}

*Results are means±standard deviation (n=3). Different superscripts (a–c) within a column are significantly different among samples ($p<0.05$).

3.2.2. Acid value

The acid value was used to determine the free FA content of the oil. The acid value indicated the amount of KOH (mg) required to neutralize 1 g of oil. The acid values of the fish oil extracted from the flesh meat of the tra catfish using isopropanol:hexane and ethanol:hexane at various ratios are presented in Table 3. The acceptable limit of the acid value is 7–8 mgKOH/g (Deepika et al., 2014). In the present study, the acid values of the fish oil extracted with isopropanol:hexane at three ratios (3:2, 1:1, and 2:3) were 4.94, 3.65, and 2.62 mgKOH/g, respectively, whereas those extracted with ethanol:hexane were 4.26, 3.22, and 2.35 mgKOH/g, respectively. The acid values of oil from the flesh meat of the tra catfish were within the acceptable limit and lower than those of oil from the meat of the Atlantic salmon (7.48 mgKOH/g) according to Iberahim and Tan (2020). The acid value of fish oil is significantly affected by the oil composition, extraction method, and freshness of the raw material (Deepika et al., 2014).

3.2.3. The color changes of oil

Oil color is one of the most important parameters affecting the manufacturing costs of fish products. To obtain oil with an acceptable color, high costs are typically incurred to increase the efficiency of the production process (Noriega-Rodríguez et al., 2009).

In this study, the lightness values of oil samples from tra catfish flesh meat extracted using isopropanol:hexane and ethanol:hexane at various ratios were in the range of 21.54–33.35 and 23.38–28.53, respectively, while the yellowness varied from 7.34–10.97 and 8.9–11.24 at different ratios, respectively (Table 4). In terms of yellowness, the oil extracted with an isopropanol:hexane ratio of 2:3 had the lowest value (7.34). Based on color measurements, oil extracted from tra catfish meat with isopropanol:hexane at a ratio of 2:3 had a bright yellow color, which was indicated by the highest L^* value and lowest b^* value compared to those of other samples.

Table 3. The acid value of oil from flesh of tra catfish (mgKOH/g)*.

Ratio	Isopropanol:hexane	Ethanol:hexane
3:2	4.94±0.431^{c}	4.26±0.376^{c}
1:1	3.65±0.285^{b}	3.22±0.223^{b}
2:3	2.62±0.433^{a}	2.35±0.314^{a}

*Results are means±standard deviation (n=3). Different superscripts (a–c) within a column are significantly different among samples ($p<0.05$).

3.2.3. The FA profile of oil from tra catfish flesh meat

The average FA contents, expressed as proportions of SFAs, MUFAs, and PUFAs in the tra catfish flesh meat oil, are shown in Table 5. The tra catfish flesh meat oil was relatively enriched with SFAs. The SFA group in the tra catfish meat oil extracted with isopropanol:hexane and ethanol:hexane had the lowest content at a ratio of 2:3 (40.63% using isopropanol:hexane and 39.18% using ethanol:hexane). The high proportion of SFAs in the tra catfish flesh meat oil in the current study was similar to the mean value for oil from a fillet of this fish (42.63%) (Ho & Paul, 2009). Palmitic acid was found to be predominant in tra catfish flesh meat oil. Palmitic acid was predominant in the SFA group, as found in freshwater channel catfish (*Ictalurus punctatus*) (19.2%) (Sathivel et al., 2002) and tra catfish fillet (*Pangasianodon hypophthalmus*) (29.33%) (Ho & Paul, 2009).

Table 4. The color changing of oil extracted from flesh tra catfish meat*.

Ratio	Color of oil			
	Isopropanol:hexane		Ethanol:hexane	
	*L**	*b**	*L**	*b**
3:2	21.54±0.391^{a}	8.73±0.332^{ab}	27.51±1.690^{b}	9.97±0.472^{b}
1:1	26.86±0.023^{b}	10.97±1.564^{b}	28.53±0.536^{b}	11.24±0.351^{c}
2:3	33.35±0.523^{c}	7.34±1.653^{a}	23.38±1.863^{a}	8.90±0.299^{a}

*Results are means±standard deviation (n=3). Different superscripts (a–c) within a column are significantly different among samples ($p<0.05$).

The proportion of MUFAs ranged from 42.5 to 43.81%, depending on the ratio of solvents. Oleic acid (C18:1n-9) was the most prevalent MUFA, and it was higher in tra catfish flesh meat oil extracted by isopropanol:hexane than that extracted by ethanol:hexane at a similar ratio. Steiner-Asiedu et al. (1991) reported a correlation between oleic acid content and water temperature in normal fish habitats. Furthermore, Ho and Paul (2009) reported that fish species living in freshwater had higher oleic acid content than those living in seawater. The PUFA proportions in oil from tra catfish flesh meat showed the highest content (11.634%) when oil was extracted using isopropanol:hexane at a ratio of 2:3. Linoleic acid (C18:2n-6) was the major PUFA in tra catfish flesh meat oil (9.51–9.81%).

Of the various ratios of solvents, tra catfish had the highest proportion of EPA (10.8%) and DHA (1.604%) with an extracted solvent ratio of 2:3 for ethanol:hexane and isopropanol:hexane, respectively. The low contents of EPA and DHA in tra catfish flesh meat oil were similar to those in tra catfish fillet oil (EPA and DHA were 0.31 and 4.74%, respectively) (Ho & Paul, 2009). Lower levels of DHA and EPA were found by Haard (1992) in fish species farmed in freshwater than in their seawater counterparts. This can be explained by seawater fish obtaining omega-3 FAs from oceanic plankton (Steffens, 1997). Haliloglu et al. (2004) reported that seawater rainbow trout meat (*Oncorhynchus mykiss*) had a remarkably higher proportion of EPA than its freshwater counterparts. Furthermore, Ho and Paul (2009) also reported high EPA and DHA content in Atlantic salmon from seawater when compared to that in fillets from fish farmed in freshwater.

Table 5. The fatty acid profile (%) of oil from tra catfish flesh.

Fatty acid	Isopropanol:hexane			Ethanol:hexane		
	3:2	1:1	2:3	3:2	1:1	2:3
Saturated fatty acids (SFA)						
C14:0	3.49	3.33	3.28	3.67	3.78	3.46
C16:0 (palmitic acid)	29.51	28.66	28.76	29.89	29.76	27.67
C18:0 (stearic acid)	8.55	8.49	8.59	8.56	8.10	8.05
Σ SFA	41.55	40.48	40.63	42.12	41.64	39.18
Monounsaturated fatty acids (MUFA)						
C16:1n7	1.01	1.03	1.00	1.04	1.02	0.97
C18:1n9 (oleic acid)	40.64	41.52	41.29	40.79	40.42	40.84
C20:1n9	1.23	1.26	1.27	1.12	1.06	1.17
Σ MUFA	42.88	43.81	43.56	42.95	42.5	42.98
Polyunsaturated fatty acids (PUFA)						
C18:2n6 (linoleic acid)	9.71	9.51	9.81	9.74	9.66	9.63
C20:4n6 (arachidonic acid)	0.21	0.2	0.22	0	0.21	0.2
C18:3n3 (linolenic acid)	0.6	0.6	0.65	0.57	0.59	0.61
C20:4n3	0.47	0.51	0.57	0.46	0.46	0.43
C20:5n3 (EPA)	0.084	0.086	0.094	0.089	0.094	0.086
C22:6n3 (DHA)	0.24	0.23	0.29	0.23	0.24	0.21
Σ PUFA	11.314	11.136	11.634	11.089	11.254	11.116
Omega-3 fatty acid	1.394	1.426	1.604	1.03	1.26	1.24
Omega-6 fatty acid	9.92	9.71	10.03	10.86	10.72	10.8

DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid.

4. Conclusion

Changes in the quality of tra catfish meat during refrigerator storage were identified. Tra catfish meat was maintained in quality for lipid extraction after 15 days of storage. The oil from tra catfish flesh meat showed the highest recovery yield, brightness, and PUFA levels when extracted with isopropanol:hexane at a ratio of 2:3. Furthermore, the DHA content of the oil sample was higher than the EPA content, as indicated by the FA profile analysis. Therefore, oil can be extracted from the fresh meat of tra catfish.

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