Food Research 8 (6) : 1 - 6 (December 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Characterization and anti-aging activities of Haruan (*Channa striata*) fish oil

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Article history:
Received: 4 December 2022
Received in revised form: 25 February 2023
Accepted: 3 December 2023
Available Online: 3 November 2024

Keywords:
Haruan fish oil,
Antioxidant activity,
Anti-collagenase,
Extraction method,
Yield

DOI:
https://doi.org/10.26656/fr.2017.8(6).593

Abstract

Haruan fish or snakehead fish (*Channa striata*) is a freshwater fish widely cultivated in Indonesia. Fish oils are believed to have some biological activities which are beneficial to human health, including antioxidant and antiaging activities. The objectives of this study are to determine the characteristics and yield of Haruan fish oil (HFO) using three extraction methods (steaming, maceration, and pressing), to determine radical scavenging activity (RSA) of fish oils using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radicals and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals and to determine anti-collagenase activity of HFO. The extraction of HFO from the flesh part using the steaming method resulted in the highest RSA values either using DPPH or ABTS radicals. HFO could inhibit the activity of collagenase by 26.87±0.391%. The highest yield of HFO was obtained using the steaming method, with extraction yields of flesh and offal of 2.02% and 2.05%, respectively. The acid values (AV) of HFOs obtained from different parts using the steaming method were 1.87±0.04 (flesh part), 1.88±0.07 (head) and 1.93±0.05 (offal). The saponification values (SV) of HFO using the same extraction method were 6.30±0.26, 6.17±0.07, and 6.30±0.23 for flesh, head and offal, respectively. The peroxide (PV) and iodine (IV) values were 85.37±2.86 and 198.51±7.68, 103.70±2.21 and 206.44±5.24, as well as 96.05±2.93 and 193.68±1.70, for flesh, head and offal, respectively. These parameters (AV, SV, PV and IV) met the requirements. Haruan fish oil has the potential to be developed as a source of the human diet.

1. Introduction

Skin aging is a physiological process that happens to every human being. Skin aging is divided into intrinsic or chronological aging associated with increasing age and extrinsic aging associated with external factors. Photoaging is a term used for sun exposure that contains ultraviolet (UV) light, this is one of the most important extrinsic factors as a cause of accelerating the skin aging process (Syamsul *et al.*, 2021). Various methods are available to prevent and treat skin aging, ranging from using photoprotector materials to topical medicines, both natural and synthetic. Sources of antiaging and antioxidants in fish oil are found in polyunsaturated fatty acid (PUFA), Omega 3, and others (Ikhsan *et al*., 2021).

Haruan fish or snakehead fish is one of the characteristics of swampy waters in Kalimantan (especially South Kalimantan and East Kalimantan), which is very popular with the community as a complete snack of "ketupat kandangan and nasi kuning". This fish contains albumin, which the human body needs to overcome diseases caused by reduced blood protein. Haruan fish is a freshwater carnivore because it likes to prey on small fish for food. Haruan fish oil contains PUFA. The saturated fatty acids in HFO are palmitic, stearic, and myristic, while the unsaturated fatty acids in HFO are oleic, linoleic and linolenic acid (Syifa *et al*., 2022).

Haruan fish or snakehead fish with the scientific name of *Channa striata* belong to the family of Chanidae and is one of the popular carnivorous freshwater fishes having high commodity values in the fish market (Bich *et al.*, 2020). Haruan fish are widely found in tropical and subtropical countries such as Indonesia and

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

Malaysia. Haruan fish is a freshwater, air-breathing, and carnivorous fish, a valuable source of protein in the Asia Pacific region (Ikasari *et al.*, 2020). The cultured Haruan fish has become an economically important freshwater fish in developing countries due to its beneficial effects on human health. High protein in this fish potentially prevents stunting (Pasaribu *et al.*, 2020). Haruan fish consisting of the highest albumin and omega-3 could accelerate the healing progress of a scratch wound as it is helpful to form new tissue during the growth period (Ikasari *et al.*, 2020). Haruan fish enhances the synthesis of different glycosaminoglycans in healing wounds and increases the rate of wound contraction, leading to a quicker healing process (Baie and Sheikh, 2000). Omega -3 polyunsaturated fatty acid contained in HFO can regulate prostaglandin synthesis and also influence the immune system (Durkin *et al.*, 2021).

Several techniques have been used to assess the antioxidant activities of fish oils *in vitro*, including lipid peroxidation inhibition, radical scavenging methods using 2,2'- diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), and metal-chelating (Ilza and Sukmiwati, 2020; Putri *et al*., 2021). Antioxidant test methods have been widely used for fish oil tests and these methods can provide fast and reliable results (Bag and Chattopadhyay, 2018). Therefore, this study intended to determine the characteristics and yield of HFO using three extraction methods (steaming, maceration, and pressing), to evaluate the antioxidant test using DPPH radical scavenging assay and ABTS radical scavenging assay and to evaluate anti-collagenase tests for HFO.

2. Materials and methods

2.1 Materials

Haruan fish were collected from Samarinda, East Borneo, Indonesia. DPPH (Sigma Aldrich), ABTS (Sigma Aldrich), and collagenase kit (Sigma Aldrich). The other reagents and solvents used for analysis were of analytical grade (Merck).

2.2 Extraction method

Extraction of oil components in haruan fish uses three different methods: steaming method, maceration method, and pressing method. Haruan fish sample preparation in the steaming method consists of offal, flesh, and fish head after being washed, cut into small pieces, and then mashed using a blender, including offal, flesh, and fish head, and mashed using a blender. The sample of 300 g and 1 L of water were put into the steamer and steamed at 800ºC for 20 mins. The crude oil obtained was then refined to separate impurities from the pure fish oil. The maceration method extracted wet fish samples using chloroform:water (1:1) for 6 hrs. The solvent was then removed using a rotary evaporator at 40ºC. The pressing method is slightly modified (Bako *et al*., 2017). The haruan fish sample was wrapped in a filter cloth, placed in a press machine column, and pressed with a pressure of 100 kN for 2 mins. The oil obtained was collected in a 15 mL flacon. To remove the water content and other impurities, 10 g of haruan fish oil was mixed with 0.3 g of bentonite and 1 g of anhydrous sodium sulfate in a flacon as the purification method. If there are still suspended particles in the fish oil obtained, they are separated by centrifugation with a force of 5000 rpm for 10 mins. The upper phase is pure oil and was put into a new container, and pure oil from the upper phase was placed in a fresh container and given a calculated weight (Putri *et al.*, 2021). All fish oils obtained were then subjected to antioxidant evaluation using DPPH radical scavenging assay, ABTS radical scavenging assay, and collagenase test.

2.3 Free fatty acid value determination

Free fatty acid value (FAV) is the number of milligrams of KOH required to neutralize the free fatty acids in 1 g of oil or fat. FAV was measured according to the AOAC official method. The oil samples were placed in an Erlenmeyer flask, and 10 mL of ethanol was added and mixed with the indicator of 1 mL phenolphthalein. The mixture was titrated using 0.1 N KOH in ethanol with consistent shaking until the colour changed from white to pink, which is described as the endpoint of the titration (Heidari *et al*, 2020).

2.4 Saponification value determination

The saponification value (SV) is defined as the number of milligrams of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of sample (Yusuf *et al*., 2015). A weight of 1 g of samples was placed in an Erlenmeyer flask then 30 mL of KOH in ethanol was added. The Erlenmeyer was connected to an air condenser and boiled for 30 mins to complete the saponification. A few drops of phenolphthalein indicator were added to the warm solution and titrated with 0.5 N HCl until the colour changed from pink to colourless.

2.6 Peroxide value determination

The peroxide value (PV) is expressed as milliequivalents (meq) of radicals per kilogram of fat or oil. PV was determined using the AOAC official method (Ikhsan *et al*., 2021). One gram of oil sample was dissolved in 30 mL chloroform: acetic acid (2:3) solution. A 0.5 mL saturated KI solution was added to the mixture and left for 1 min in a dark place. Starch 1.5% as an indicator was added and titrated with 0.01 N

https://doi.org/10.26656/fr.2017.8(6).593

sodium thiosulphate.

2.7 Iodine value determination

The iodine value (IV) measures the total number of double bonds in fats and oils. A 0.3 g sample was weighed in an Erlenmeyer flask with a stopper. The samples were dissolved in 10 mL chloroform and 25.0 mL Wijs reagent (iodine chloride 1% in acetic acid glacial). The mixture was then placed in the dark for 30 mins. Ten millilitres of KI 15% solution and 15 mL of water were added, then titrated with sodium thiosulphate 0.1 N until the yellow colour almost disappeared. A few drops of 0.5% starch indicator were added, and the titration continued until the blue colouration disappeared. The same procedure was used for the blank test (Ikhsan *et al*., 2021).

2.8 DPPH free radical scavenging assay

A small adjustment was made to the DPPH radical scavenging test (Rohman *et al.*, 2020). Fifty microlitres of each fish oil sample was added to 1 mL 2,2-diphenyl-1-picrylhydrazyl (DPPH) 0.4 mM and diluted with ethanol to 5.0 mL. The solution mixture was allowed to stand at room temperature in the dark for 30 mins. The absorbance of evaluated solutions was measured using a spectrophotometer (Hitachi, U-2900, Japan) at 515 nm and corrected with blank solutions containing solvent and the studied samples (Akanbi and Barrow, 2018; Syamsul, Umar, Wahyuni *et al.*, 2022). The absorbance of control solutions containing DPPH solution was also measured. DPPH radical scavenging activity was calculated as:

$$\%\text{RSA} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs control})} \times 100\%$$

2.9 ABTS radical scavenging assay

The solution of 7.4 mM ABTS was prepared by weighing 203 mg of ABTS powder and then dissolved with distilled water to 50 mL. Afterwards, a potassium persulfate solution was made by weighing 64.8 mg of potassium persulfate powder dissolved in 50 mL of distilled water. The dilution was carried out 50 times. Approximately 200 µL of ABTS stock solution and potassium persulfate were taken and then diluted with ethanol to 10 mL. This solution is called the ABTS control. ABTS control was read the absorbance, while the ABTS control blank was ethanol. Prepared a sample solution and blank, dissolved 25 µL of sample into a test tube, and added 4975 µL of ABTS control. Homogenization was carried out with a vortex for 1 min, then incubated for 17 mins (Syamsul, Supomo, Jubaidah *et al.*, 2022). The blank sample 25 µL was added with 4975 µL of ethanol, then homogenized with a vortex for one minute and let stand for 30 mins, recorded the absorption at each concentration, then calculated the RSA of the HFO (Rohman *et al*., 2020).

2.10 Collagenase test

HFO from different extraction techniques was input into the desired well(s) in 96 well plates and collagenase Assay Buffer ad 100 µL. The collagenase (0.35 U/mL) amount of 10 µL was added for the control. Furthermore, for the inhibitor control, added to the desired well amount of 10 µL of supplied collagenase (0.35 U/mL) and 2 µL of inhibitor (1.10 Phenanthroline). The collagenase buffer assay was carried out by adding collagenase buffer to the control wells and inhibitor control wells up to a volume of 100 µL and for background control reagent was added to 100 µL collagenase buffer.

The collagenase inhibitor assay was carried out dissolved the collagenase inhibitor in up to 100× the final test concentration, and in the well of the inhibitor test was added 2 µL of the collagenase inhibitor and 10 µL of the collagenase (0.35 U/mL). An enzyme control (EZ) parallel well was prepared by adding 10 µL of the supplied collagenase. Collagenase assay buffer was added until 100 µL for the volume of test inhibitor and Enzyme control. Then incubated at room temperature for 10 mins. For each reaction, 100 µL of the reaction mixture was prepared, containing 40 µL of collagenase substrate (FALGPA) and 60 µL of collagenase assay buffer. Measured in kinetic mode, absorbance (A) at 345 nm in a microplate reader at 37°C for 5-15 mins. Samples with low activity were measured for 1-3 hrs.

$$\%\text{anti collagenase} = \frac{(\text{Abs sample} - \text{Abs control})}{(\text{Abs sample})} \times 100\%$$

2.11 Statistical analysis

Values are presented as the mean ± standard deviation (SD) of triplicate determination. Statistical analysis for physicochemical values was carried out by independent t-test using SPSS software version 21.0 (SPSS Inc., USA) and the significance was defined at $p<0.05$.

3. Results and discussion

In this study, HFO was obtained from three different extraction methods, the steaming method on wet samples, the maceration method using chloroform-water on wet samples, and the pressing method using dried samples. Table 1 shows the extraction yield of HFO (%), acid value (AV), saponification value (SV), peroxide number (PV), and iodine number of (IV) HFO. The results of the ANOVA test analysis followed by LSD showed that the extraction using the steaming method offered the highest yield than the other methods, the

https://doi.org/10.26656/fr.2017.8(6).593

Table 1. Characterization of HFO along with the extraction methods.

Sample	Code	Part of Fish	Extraction Methods	Yield (%)	Free Fatty Acid Value (%)	Peroxide Value (meq/kg sample)	Iodine Value (g/100 g sample)	Saponification Value (mg KOH/g)
1	HFlO	Flesh	Steaming	2.02	1.87±0.04	6.30±0.26	85.37±2.86	198.51±7.68
2	HHO	Head	Steaming	1.02	1.88±0.07	6.17±0.07	103.70±2.21*	206.44±5.24
3	HOO	Offal	Steaming	2.05	1.93±0.05	6.30±0.23	96.05±2.93*	193.68±1.70
4	HFlO	Flesh	Maceration	1.70	1.89±0.03	4.72±0.26*	93.39±4.02*	189.13±4.70
5	HHO	Head	Maceration	1.03	1.84±0.01	4.27±0.06*	114.28±2.71*	181.15±10.43*
6	HOO	Offal	Maceration	1.50	1.94±0.02	5.56±0.25*	85.99±1.25	193.16±4.64
7	HFlO	Flesh	Pressing	1.92	1.88±0.03	7.19±0.25*	113.91±1.59*	189.67±1.53
8	HHO	Head	Pressing	1.07	1.84±0.09	7.03±0.06*	119.57±0.29*	199.29±5.57
9	HOO	Offal	Pressing	1.88	1.85±0.08	7.19±0.25*	101.22±1.84*	190.88±2.65

HFlO: haruan flesh oil, HHO: haruan head oil, HOO: haruan offal oil.
*Representative data are statistically significantly different (P<0.05) when compared with steaming HFlO.

pressing method and the maceration method.

The pressing method provided HFO with the highest yield at 2.02% flesh and 2.05% offal. Based on the Indonesian National Standard (Badan Standardisasi Nasional, SNI) for cooking oil and the International Fishmeal, the standard value for AV is 7 mg KOH/g sample, PV is 10 meq O_2/kg, IV is <140, and SV is 196–206. The category of PV is determined based on the level of oxidation. If the oxidation level is lower than 1, this indicates fresh oil, an oxidation level of 1-5 indicates low category, an oxidation level of 6-10 indicates medium category, an oxidation level of higher than 10 indicates high category and an oxidation level >20 indicates very high category (Gotoh *et al*., 2011). PV with an oxidation level >20 has a rancid odour due to the presence of aldehydic compounds in the evaluated samples. In terms of quality, the values of AV, SV, PV and IV were in accordance with the requirements.

Radical scavenging activities (RSA) values in DPPH and ABTS to HFO extracted from haruan fish (flesh, heads and offal) using three different extraction methods were shown in Table 2. The highest RSA values in DPPH using the steaming method for the flesh was 23.15±0.32% the maceration method (chloroform-water) for the offal was 21.65±0.98%, and the pressing method for the flesh was 21.44±0.98%. The different extraction methods HFO show that the RSA values in DPPH using the steaming method are higher than the RSA values of the maceration method, and the RSA values of the pressing method are smaller than the maceration method and steaming method.

The extraction method of steaming on HFO using antioxidant DPPH has higher antiradical activity compared to the maceration method and the pressing method. The highest RSA values in ABTS using the steaming method for the flesh was 17.73±1.97%, the maceration method (chloroform-water) for the flesh was 15.76±2.15%, and the pressing method for the flesh was 15.93±1.24%. The different extraction methods HFO show that the RSA values in ABTS using the steaming method are higher than the RSA values of the pressing method, and the RSA values of the maceration method are smaller than the pressing method and steaming method. The extraction method of steaming on HFO using antioxidant ABTS has higher antiradical activity compared to the maceration method and the pressing method. Based on the results of the antioxidant test showed that the values of RSA in DPPH and ABTS in HFO with the steaming method obtained the highest

Table 2. Radical scavenging activity of fish oil from *Channa striata* and extraction methods.

Sample	Code	Part of Fish	Extraction Method	RSA DPPH (%)	RSA ABTS (%)
1	HFlO	Flesh	Steaming	23.15±0.32	17.73±1.97
2	HHO	Head	Steaming	22.08±0.49	16.25±0.49
3	HOO	Offal	Steaming	22.40±1.13	14.78±1.78
4	HFlO	Flesh	Maceration	20.26±0.96*	15.76±2.15
5	HHO	Head	Maceration	21.44±0.81*	14.45±2.53
6	HOO	Offal	Maceration	21.65±0.98*	15.60±1.50*
7	HFlO	Flesh	Pressing	21.44±0.98*	15.93±1.24*
8	HHO	Head	Pressing	20.26±0.96*	14.29±1.78
9	HOO	Offal	Pressing	21.22±0.32*	14.12±2.48*

HFlO: haruan flesh oil, HHO: haruan head oil, HOO: haruan offal oil.
*Representative data are statistically significantly different (P<0.05) when compared with steaming HFlO.

https://doi.org/10.26656/fr.2017.8(6).593

RSA values when compared to the pressing method and the maceration method. Aging is a natural process that is closely related to degenerative processes. The occurrence of free radicals due to oxidative processes is the basis of the most commonly adopted theory. Antioxidants are one of the efforts that are often carried out to prevent aging. Extrinsic skin aging is dominated by exposure to solar radiation (Photoaging) (Lephart *et al*., 2018). Free radicals are formed because of oxidative stress, a major cause of intrinsic and extrinsic aging. The use of antioxidants is often one effort to prevent aging or at least aging healthily (Wong *et al*., 2010). Figure 1 shows that the test extracted inhibited collagenase in a dose-dependent manner, that dose 2.5 µL shows 17.53±1.477%, dose 5 µL shows 23.91±0.878%, and dose 10 µL shows 26.87±0.391%. HFO (10 µL) inhibited collagenase activity by 26.87±0.391%. Independent T-Test showed that HFO significantly different ($p<0.05$) with control.

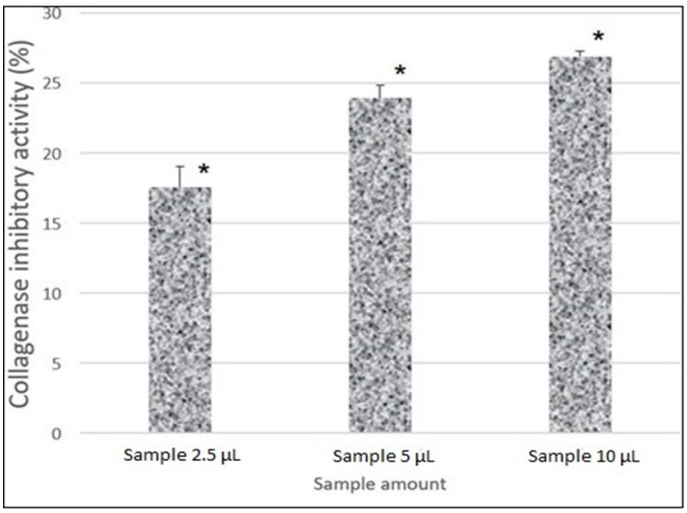

Figure 1. Collagenase inhibitory effects were performed using the test kits, fluorescence was measured in kinetic mode, and the absorbance (A) at 345 nm in a microplate reader. The data represent three sample amounts, *P<0.05, compared to the control.

Regarding anti-elastase activity, the aging process is natural over time, but free radicals can also accelerate the aging process. Both physical and physiological changes can identify the aging process. The physical change can be characterized by wrinkles, lessening skin elasticity, uneven pigmentation, brown spot, laxity, and a leathery appearance (Widowati *et al*., 2017). Collagenases are essential components of the matrix metalloproteinases family of proteins, and their main function is breaking the triple-helix collagen (Nagase *et al*., 2006).

4. Conclusion

The highest yield of HFO was obtained by using the steaming technique, with extraction yields of flesh and offal of 2.02% and 2.05%, respectively. HFO (10 µL) inhibited collagenase activity by 26.87±0.391%. The highest yield of HFO was obtained by using the steaming technique, with extraction yields of flesh and offal of 2.02% and 2.05%, respectively. Regarding the oil quality. In addition to the steaming method, the free fatty acid value (FAV) of flesh: was 1.87±0.04, the head: was 1.88±0.07, and the offal: 1.93±0.05, saponification value (SV) of flesh: was 6.30±0.26, head: 6.17±0.07, and offal: 6.30±0.23, peroxide value (PV) of flesh: 85.37±2.86, head: 103.70±2.21, and offal: 96.05±2.93, and iodine value (IV) of flesh: 198.51±7.68, head: 206.44±5.24, and offal: 193.68±1.70, met the requirements. This research could be an alternative method for predicting RSA and inhibited collagenase activity with high accuracy.

Conflict of interest

The authors declare no conflict of interest.

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