JIPK. Volume 16 No 1 April 2024
Sinta 1 (Decree No: 158/E/KPT/2021)
e-ISSN:2528-0759; p-ISSN:2085-5842
Available online at https://e-journal.unair.ac.id/JIPK

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Short Communication

Effect of Ultrasonic Assisted Extraction with Ethanol for Removing Lipid on Catfish (*Pangasius* sp.) Skin as a Collagen Sources and Its Characteristic

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OPEN ACCESS

ARTICLE INFO

Received: June 05, 2023
Accepted: July 03, 2023
Published: December 29, 2023
Available online: Jan 27, 2024

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Keywords:
Fisheries
Fish Skin
Pangasius sp.
Ultrasonic
Lipid Reduction

Abstract

Fish skin is in collagen (80-90%). However, catfish (*Pangasius* sp.) skin has a high lipid content which can reduce the quality of collagen. Therefore, treatment is needed to remove the lipid using ultrasonic assisted extraction (UAE) with ethanol. Hence, the aim of present study was to remove lipid content from catfish skin as a raw material of collagen by using UAE with different concentrations of ethanol (25, 50, 75%). The research was conducted in three stages: removing impurities, collagen extraction and characterization of collagen. The result exhibited that UAE-ethanol treatment was capable to remove up to 85.6% of lipid content and the produced collagen was potential to be utilized as an alternative source of collagen based on its properties

Cite this as: Agustina, M., Patmawati, Mubarok, S., Sulmartiwi, L., Wulandari, D. A., Zai, K., Raseetha, S., Pujiastuti, D. Y., Nirmala, D., Werdani, M. C. K., Moechthar, O., & Husein, M. A. A. (2024). Effect of Ultrasonic Assisted Extraction with Ethanol for Removing Lipid on Catfish (*Pangasius* sp.) Skin as a Collagen Sources and Its Characteristic. *Jurnal Ilmiah Perikanan dan Kelautan*, 16(1):274–284. http://doi.org/10.20473/jipk.v16i1.46061

1. Introduction

The fish processing industries, especially the fillet industry, produce around 40% of either solid or liquid waste such as skin, fins, bones, scales, and viscera (Nurilmala *et al*., 2022). Fish skin is regarded as an essential by-product of the fish processing industry, which is rich in fibrillar collagen, which accounts for approximately 80-90% of dry weight (Espinales *et al*., 2023). Catfish (*Pangasius sp.*) is prominently suited as a collagen source, whose skin allegedly contains a high collagenous protein content of 93.39% (Kartika *et al*., 2016). Collagen from fish by-products is generally found as type 1 collagen with distinct properties, predominantly composed of two α1-chains and one α2-chain coil around in a triple helix structure. Both the α1 chain and the α2 chain consist of a long helical domain preceded by a short N-terminal peptide and followed by a short C-terminal peptide. Type I collagen is abundantly found in the connective tissue of vertebrates (Gómez-Guillén *et al*., 2011). Collagen has good functional properties of water absorbability and gel formation capacity (Schmidt *et al*., 2016), which makes it widely utilized in various industries such as medicine, pharmaceuticals, cosmetics, biofilm, and as a source of biomaterials or tissue engineering (Chen *et al*., 2016; Arumugam *et al*., 2018; Ghomi *et al*., 2021).

Collagen extraction requires a suitable solvent and method to fully diminish non-collagenous components like lipids, which are apt to affect end product quality (Karim *et al*., 2020). Several studies on various catfish skins have shown high lipid content, e.g., *Pangasius sp.* (15%) (Veeruraj *et al*., 2015), *P. hypophthalmus* (7.90±1.03%) (Suptijah *et al*., 2018), and *P. djambal* (6.61±0.84%) (Hastarini *et al*., 2012). Thus, ethanol solvent is chosen for removing lipid content to be less than 1%, according to the National Standard Indonesia of SNI 8076:2014. According to Karim *et al.* (2020), ethanol solvents, which has a higher partition coefficient than lipid, may detach the lipid matrix from the cell wall structure, as the solvent partition coefficient is imposed to be the parameter of solute migration convenience. In addition to further assisting lipid reduction on catfish (*Pangasius sp.)* collagen, ultrasonic assisted extraction (UAE) may accelerate ethanol works by forming cavitation bubbles, which leads to high shear force and causes cell wall disruption. These additional forces also significantly shorten extractability duration (Chemat et al., 2017; Lu et al., 2023).

The UAE is one of the extraction techniques out of possible modifications such as alkali, acid, enzymatic hydrolysis, salt solubilization, microwave assisted, and mechanical agitation (Lin *et al*., 2010; Kim *et al*., 2012; Yang and Shu, 2014; Pal and Suresh, 2016; Schmidt *et al*., 2016; Jin *et al*., 2019; Matinong *et al*., 2022). The UAE has been customized by combining traditional solvent extraction and novel ultrasonic technology to improve overall extraction efficiency (Lu *et al*., 2023). Kim *et al.* (2012) reported that collagen extraction using ultrasound with a frequency of ≥20 kHz can significantly improve yield and reduce extraction time. In addition, ultrasound can generate turbulence, affecting the enhanced rates of chemical reactions and mass transfer energy. The amplitude and duration of the ultrasound treatment can markedly increase extraction yield and optimally remove non-collagenous components. Generally, the ultrasound method is a simple technique to reduce non-collagenous components from the skin samples while reducing chemical utilization for environmental purposes (Kadam and Tilve, 2015; Schmidt *et al*., 2016; Senadheera *et al*., 2020). The UAE has several advantages, such as low cost, high yield, simple operation (Xie *et al*., 2014a), and a reduction in time extraction (Khan *et al*., 2010) due to the mass transfer rate increment, which may promote solvents to enter the cell wall and destroy the skin tissues as a consequence of producing cavitation energy, which may alter the compound state from the solid phase into the liquid phase (Xie *et al*., 2014b; Agarwal, 2018). The UAE has been widely used for extracting bioactive compounds, especially from marine commodities (Belwal *et al*., 2018). In order to evaluate the quality of fish skin from catfish (*Pangasius* sp.) as raw material for collagen production, the collagen was extracted by using UAE combined with ethanol solvent. This study aimed to examine the effect of a combination of UAE and different concentrations of ethanol on removing lipid content from catfish skin (*Pangasius* sp.) as a raw material for collagen and its characterization.

2. Materials and Methods

2.1 Material

Ten kilograms of catfish (*Pangasius* sp.) were taken from a fish fillet company in Surabaya, East Java, Indonesia. Other materials used in this research included absolute ethanol (SmartLab, Indonesia), sodium hydroxide (NaOH) (Merck, 1.06498.1000 1), acetic acid (CH_3COOH) (Merck), TRIS buffer (Vivatis), distilled water, and materials for analysis.

2.2 Method

2.2.1 Sample fish skin of catfish (Pangasius sp.*) preparation*

Catfish skin samples were cleaned from the remaining meat and dirt using a knife. Then, the catfish

skin samples were washed using running tap water five times and distilled water three times. After washing, the samples were cut into a size of 1x1 cm^2. All processes were carried out at a chilling temperature (±4°C) to maintain the quality of the catfish skin. Then, the samples were stored in the freezer (-18°C) until used in further processing (around two weeks). Proximate analysis was conducted to evaluate the quality of the raw skin, especially the lipid content.

2.2.2 Removing lipids using ultrasonic-assisted extraction with different concentrations of ethanol

Based on the previous step, the proximate analysis exhibited a total lipid content on catfish skin of 9.10%. The lipids of catfish skin were removed using ultrasonic-assisted extraction (UAE) (GT Sonic (bath cleaner): ultrasonic power (100W) and ultrasonic frequency (40KHz)) with different concentrations of ethanol (25, 50, and 75%) at a cold temperature for 10 minutes. The UAE samples were compared to the control treatment, which was carried out using a maceration process with different ethanol concentrations (25, 50, and 75%) for 24 hours at 4°C. The ratio of skin to ethanol was 1 to 10 (w/v) with three times replication. The mixed samples were centrifuged at 7319 rpm for 10 minutes, and then the supernatant was removed. The collected skin was washed using distilled water. Then, the treated samples were evaporated using an electric fan at room temperature for two hours to fully remove the remaining ethanol. The treated samples were checked for lipid content reduction by the Soxhlet technique. The best treatment was used for collagen extraction from catfish skin.

2.2.3 Bleaching process to remove the color of the skin of catfish

The skin samples from the previous step of removing lipid contents were placed in the beaker glass for the bleaching process. This process was carried out to remove non-collagenous proteins by immersing samples in NaOH 0.1 M for 24 hours and replacing the solution every eight hours. Then, the samples were washed with distilled water three times until they reached a neutral pH of 7. The treated samples were dried using tissue paper (PASEO) and stored in the freezer until utilized for further experimentation.

2.2.4. Extraction of collagen from catfish skin

Collagen extraction was carried out using an ultrasonic assisted extraction (UAE) method with acetic acid 0.2 M to reduce time consumption during the extraction process. The catfish skin samples from the previous step were extracted with acetic acid in an ultrasonic bath for 150 minutes. This process was carried out at a chilling temperature of ≤ 10°C. Then, the sample was filtered using Whatman No. 42, and the liquid was taken. The liquid sample was treated by adding NaCl until it reached a concentration of 2.5 M. The TRIS buffer was added to neutralize the treated solution. The liquid samples were centrifuged at 6.000 rpm for 15 minutes, and the supernatant was taken separately. Furthermore, the supernatant liquid was dried using a freeze dryer at -54°C for 24 hours. The dried collagen was stored at a cold temperature for further analysis.

2.2.5 Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis

Collagen samples were suspended in a 5% SDS solution and incubated at 85°C for 60 minutes in a water bath (Aminudin *et al*., 2015). The SDS-PAGE analysis was conducted in accordance with the previous method illustrated by Li *et al*. (2013), which initially began with preparing the suspended sample for centrifugation at 6.000 rpm for five minutes at room temperature to remove the insoluble component. Secondly, the dissolved sample was analyzed by using SDS-PAGE with 7.5% running gel and 4% stacking gel in a ratio of 20 μl sample in 5 μl loading buffer (4 mg bromophenol blue, 0.9 ml distilled water, 1.6 ml Tris-Cl pH 6.8, 4 ml SDS 10%, and 2.5 ml glycerol 80%). Thirdly, the sample was added to the well and electrophoresized at a voltage of 100 V for four hours. After electrophoresis, the gel was stained with Coomassie blue R-250 0.1% (w/v) in methanol 45% (v/v) and acetic acid 10% (v/v) for 12 hours (Li *et al*. 2013).

2.2.6. Yield analysis

Collagen yield is the proportion of the raw material weight to the total amount of collagen produced. The calculation aims to determine the economic value and effectiveness of material production (Girsang *et al*., 2020). The yield of collagen was calculated based on the total used sample divided by the total wet basis of collagen times 100% (Normah and Afiqah, 2018) as the following equation:

$$Collagen\ (g/100g) = \frac{Total\ wet\ basis\ of\ collagen\ (g)}{Total\ weight\ of\ initial\ fish\ skin\ used\ (g)}\ X\ 100\%$$

…Eq 1

2.3 Data Analysis

This study used descriptive analysis to check the effectiveness of lipid removal from catfish *(Pangasius sp*.) skin using ultrasonic-assisted extraction (UAE) with different concentrations of ethanol (20, 50,

and 75%). The results were represented as the mean of three repetitions with standard deviations. The collagen quality was analyzed by comparing the results of an experiment with the National Standard Indonesia (SNI 8076:2014) for collagen products and evaluating the potential of fish skin as a raw material to produce collagen.

3. Results and Discussion

3.1 Catfish (Pangasius sp.) skin characteristics

The proximate analysis was important properties to determine characteristics of raw materials such as moisture, ash, lipid, and protein content. The result showed the proximate analysis result of catfish (*Pangasius* sp.) skin including moisture content (62.69 ± 0.02%), ash content (0.12 ± 0.02%), lipid content (9.17 ± 0.03%), and protein (24.66 ± 0.04%) (Table 1). These proximate results were slightly different with the previous research conducted by Suptijah *et al.* (2018), which reported that the proximate analysis of catfish (*Pangasius* sp.) skin was moisture content (65.59%), ash content (0.195), lipid content (2.69%), and protein (30.28%). This characteristic discrepancy might happen due to different sources of raw material, feed used while rearing, aged, and fish habitat.

The moisture content in the catfish skin was 62.69% (Table 1) which was coincide with the finding result of Suptijah *et al.* (2018) who reported moisture content of catfish was 65.59%. Nowsad (2007) stated that moisture content in fish might broadly vary between 60 to 80% which depended on the fish species, sex, age, season and environmental condition (Chan *et al*., 2021). However, this study result was markedly lower than other species of *P. hypophthalmus* (78.20%) (Rao *et al*., 2013) and Vietnamese Pangasius (83.83-85.59%) (Guimaraes *et al*., 2016). Hence, the moisture content of the catfish skin used in this study was still within the range of moisture content of general fish skin and considered as fresh condition.

The ash content of catfish (*Pangasius* sp.) skin in this study was 0.12%, which was smaller than 0.19% of ash content reported by Suptijah *et al.* (2018) (Table 1). The ash content in this study was lower compared to the other catfish species ash content of *P. hypopthalmus* (0.25%) (Thitipramote and Rawkdkuen, 2011) and *P. sutchi* (0.73%) (See *et al*., 2010). Low ash content of less than 0.5% indicate a good quality raw material for collagen and gelatin manufacture. See *et al.* (2010) stated that ash content in raw material may be removed by carrying out a demineralization process to ensure resulting collagen quality. Matinong *et al.* (2022) reported that collagen extraction is dominantly relied to raw material treatment on reducing all non-collagenous components such as ash, lipid, and other impurities. The removal of non-collagenous matter may be carried out during the overall extracting process started with pre-treatment of collagen sources, extraction of collagen, and purification process.

The lipid content of catfish skin was 9.17% (Table 1), which was higher than previous research on catfish (*Pangasius* sp.) by Suptijah *et al.* (2018) (2.69%) but exhibited lower lipid content compared to *P. sutchi* (10.65%) (See *et al*., 2010) and *P. hypopthalmus* (20.24%) (Thitipramote and Rawkdkuen, 2011). The high lipid content in catfish skin indicates that the removal of lipids is highly needed to improve the quality of the skin, which can affect the quality of collagen. Combination treatment of ultrasonication with solvent modification may be opted as the fittest treatment in optimally removing lipid content in fish skin. Shon *et al.* (2011) stated that the presence of lipids will interfere with the effectiveness of collagen in its application in various products. Therefore, the treatment of the skin catfish by using ethanol and ultrasonics was carried out to reduce or eliminate high levels of lipid in fish skin. By using this step, the obtained collagen could achieve the best quality befitted to national standards (SNI 8076:2014) or international standard such as Food Drug Administration (FDA) and Food Chemical Codex (FCC).

The protein content of catfish skin was 24.62%, which was higher than *P. sutchi* (18.96%) (See *et al*., 2010) but lower than *Pangasius* sp. (30.28%) (Suptijah *et al*., 2018) and *P. hypopthalmus* (27.26%) (Thitipramote and Rawkdkuen 2011). This data illustrated high protein properties of the used catfish skin in this experiment which met the criteria to be used as collagen raw material. Higher protein content means that there are sufficient amounts of amino acid to be converted into collagen (Suptijah *et al*., 2018).

The species variation of catfish seemingly affects the overall chemical composition (Table 1), which was predominantly caused by differences in species, age, habitat, types of feed, and material preparation (Songchotikunpan, 2008). Based on the proximate test results, catfish (*Pangasius* sp.) used in this research process has met the criteria to be utilized as raw material for the manufacture of collagen, but it has a little drawback with having lipid content of 9.10%, which was higher than previous similar research by Suptijah *et al.* (2018) with lipid content of 2.69%. Therefore, the treatment

of catfish (*Pangasius* sp.) skin using ultrasonic with ethanol was carried out in this subsequent experiment process to thoroughly reduce lipid content until of less than 1% as regulated by the requirement standard for ameliorating its overall quality (SNI 8076:2014) (BSN, 2014; Gadi *et al*., 2017).

3.2 Effect of UAE and ethanol solvent for removing lipid on catfish (Pangasius sp.) skin

The analysis results of lipid content reduction on catfish (*Pangasius sp.*) skin between two different treatments of UAE-ethanol and maceration-ethanol could be seen in Table 2. The results showed that both treatments were significantly capable of reducing lipid up to 85.6% from previous lipid content of 9.10±0.20%. However, UAE treatment for 10 minutes was proved to have better removal capability compared to maceration treatment for 24 hours. UAE treatment exhibited the ability to reduce lipid content of 53-85.6%, while maceration might reduce lipid content of 35.93-49.89%. The UAE treatment profoundly able in optimally reducing lipid because of the use of ultrasonic waves which may generate a large amount of energy from cavitation caused by vibrations and provides sufficient energy to

Table 1. Proximate skin of catfish (*Pangasius* sp.) And various types of catfish (wet weight basis)

No	Component	Value (%)[1]	Value (%)[2]	Value (%)[3]	Value (%)[4]
1	Ash content	0.12 ± 0.02	0.19	0.73	0.23
2	Moisture content	62.69 ± 0.02	65.59	39.42	51.85
3	Lipid content	9.17 ± 0.03	2.69	10.65	20.48
4	Protein content	24.66 ± 0.04	30.28	18.96	27.26
5	Carbohydrate content	3.48 ± 0.03	1.25	3.42	0.42

Research Data [1](*Pangasius* sp.); [2] (*Pangasius* sp.) (Suptijah *et al*., 2018); [3] (*P. Sutchi*) (See *et al*., 2010); [4] (*P. hypopthalmus*) (Thitipramote and Rawkdkuen, 2011).

Table 2. Lipid content of catfish (*Pangasius* sp.) Skin treatment by ultrasonic and maceration in ethanol at 4°C

No	Treatment	Initial Lipid content before treatment (%)	Lipid content after treatment (%)
	Ultrasonics treatment for 10 minutes		
1	Distillate water	9.10±0.20	3.40±0.05
2	Ethanol 25%		1.31±0.04
3	Ethanol 50%		2.95±0.06
4	Ethanol 75%		4.25±0.05
	Maceration treatment for 24 hours		
1	Distillate water	9.10±0.20	4.35±0.10
2	Ethanol 25%		4.56±0.03
3	Ethanol 50%		5.57±0.05
4	Ethanol 75%		5.83±0.08

enhance the effectivity of extraction process due to the shock effect of ultrasonic energy (Kim *et al*., 2012). The use of high ultrasonic frequency of 40 kHz in this research induces the occurrence of myriad cavitation bubbles which may accelerate the extraction of catfish (*Pangasius* sp.) skin (Kim *et al*., 2012; Zou *et al*., 2017). In additions, UAE requires shorter extraction time which is important for scale-up industry because the increase of time efficiency can heavily save energy and costs. It is an important thing that needs to be considered to generate profits for the industry, besides maintaining the final product quality.

The effect of ethanol solvent for reducing lipid with UAE and maceration process was gradually decreasing along with the concentration increment (25, 50, 75%) (Table 2). Ethanol portrayed best ability in reducing lipid on both treatment methods at the lowest concentration of 25%, from previous lipid content of 9.10%±0.20% reduced to 1.31±0.04% (UAE) and 4.56±0.03% (maceration). The lipid reduction occurs as interaction between ethanol and the phospholipid layer of the cell membrane, leading to a disruption of the membrane physical structure (Patra *et al*., 2006). Gurtovenko *et al*. (2009) evaluated the effect of ethanol concentration (2.5-30%) on phospholipid membranes and reported that concentrations lower than 30% was apt to promote membrane structure disruption, as well as a decrease of the membrane thickness which stimulates lipid detachment. Previous study comparing two different ethanol concentrations of 25 and 75% in extracting lipid by Jaeschke *et al*. (2016), who found that up to 83% of the total lipid content of the biomass was able to be extracted with the assistance of novel extraction technologies. This finding was aligned with the present result which showed the ability of ethanol with assistance of UAE method in reducing up to 85.6% of previous lipid content in catfish (*Pangasius* sp.) skin.

3.3 Collagen Characteristics

The results of proximate analysis showed good properties of collagen such as moisture content (11.98±0.03), lipid content (0.95%), protein content (83.15%), and ash content (0.66%). Based on these results, it depicted that all parameters were already befitted to the criteria of National Standard Indonesia SNI 8076: 2014 (Table 3). These results proved that catfish (*Pangasius* sp.) skin by-product has the potential to be utilized as collagen sources and advanced applied to related fields such as medicine, pharmaceutical, cosmetic, and biomaterial sources (Chen *et al*., 2016; Arumugam *et al*., 2018; Ghomi *et al*., 2021). According to Hadinoto *et al*., (2019), moisture content depletion is heavily influenced by ultrasonication duration as it has longer exposure with solvent during the extraction process. The moisture content is also closely related to the ash content, as low hydrogen ions or moisture existence in collagen fiber caused by ethanol treatment slightly helps in reducing the ash content on collagen by diminishing impurities and minerals found in fish skin (Desmelati *et al*., 2020). Hence, the high protein content in catfish (*Pangasius* sp.) is caused by the usage of low acetic acid concentration which is likely to disrupt the collagenous protein detachment (Schmidt *et al*., 2016). UAE may accelerate collagen structure disruption by acetic acid reaction, because the ultrasound frequency is possible to swiftly break down the skin cell wall and induces collagenous protein to be extracted from fish tissue (Zou *et al*., 2017). Moreover, low lipid content in this study indicates the effectiveness of ethanol pre-treatment which is prone to dissolve fat and reduce other collagen impurities (Shaik *et al*., 2021).

The yield of collagen produced by UAE from catfish (*Pangasius* sp.) skin was 7.23±0.15% (wet weight basis) (Table 3). This yield result was considered higher compared to other previous studies on similar

Table 3. Proximate analysis of collagen and yield collagen extract

No	Parameter Test	Value (%)	National Standard Indonesia SNI 8076: 2014
1	Moisture content	11.98±0.03	≤12%
2	Ash content	0.66±0.05	≤1%
3	Lipid content	0.95±0.029	≤1%
4	Protein content	83.15±0.56	≥75%
5	Yield (wet weight basis)	7.23±0.15	

catfish species *P. hypopthalmus* (5.1%) (Singh *et al*., 2011) and *P. pangasius* (6.44%) (Fabella *et al*., 2018). The higher yield on this study predominantly caused by the use of UAE technique which is able to loosen fish skin tissues. As a consequence, the solvent of acetic acid is more easily and effective to penetrate the skin which results in more high yield. Some researchers also have reported extracting some active compounds such as polyphenolic compounds, oil, natural color, and some organic acids from natural resources (Vilkhu *et al*., 2008; Shirsath *et al*., 2012; Pingret *et al*., 2013). The UAE method has profoundly advantage as stated by Pezeshk *et al*. (2022), that ultrasound treatments might significantly increase collagen extraction yield from the tuna skin up to 2.7 times, compared to the conventional extraction with acetic acid immersion.

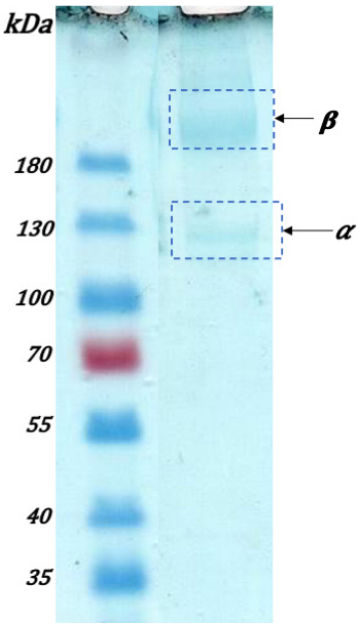

Figure 1. SDS-PAGE analysis of collagen produces by ultrasonic assisted extraction with acetic acid

Regarding to SDS-PAGE analysis results, collagen was composed of α and β chains as major components. The molecular weight of collagen produced by ultrasonic from the catfish (*Pangasius* sp.) was β (≥180 kDa) and α (130 kDa) as shown in Figure 1. These results were not much different from Abbas *et al*. (2022) reported that molecular weight collagen from catfish skin consisted of two distinct α chains (slightly larger than 116 KDa) and their dimer a β chain (200 kDa), which is typical of type 1 collagen. In addition, the molecular weight of collagen from Nile perch (*Lates niloticus*) was 120 and 115 kDa for α chains (Muyonga *et al*., 2004). Pezeshk *et al*., (2022) also reported that the molecular weight of collagen from the skin of tuna was around 122 and 118 kDa for α1 and α2 chain. The ultrasonication duration collagen extraction was reported to possess a strong correlation to the collagen structure regardless of the solvent concentration. Along with the ultrasonication time increase, a more compact and rigorous structure will be formed as indicated by the presence of triple helix structure (Silvipriya *et al*., 2015). Based on this result, ultrasonic process was proved to be less likely cause the molecular weight change of SDS-PAGE band in term of polypeptide pattern marks by having insignificantly different molecular weight.

4. Conclusion

The UAE treatment with various concentrations of ethanol has shown better ability in reducing lipids from catfish (*Pangasius* sp.) skin compared to maceration treatment. The ethanol ability on lipid reduction gradually decreased along with the concentration increment. UAE with ethanol 25% for 10 minutes was capable of decreasing lipid content in the fish skin up to 85.6%, while the maceration for 24 hours was only able to reduce lipid for 49.89%. This result demonstrated that the utilization of UAE method with low concentration of ethanol (25%) exhibited an effective result in reducing lipid compared to the conventional method. The proximate analysis on collagen from catfish (*Pangasius* sp.) skin has been befitted the criteria of the Indonesian national standard with moisture (11.98%), protein (83.15%), lipid (0.95%), ash (0.66%), and wet weight basis yield (7.23%). Therefore, catfish (*Pangasius* sp.) could be utilized as alternative sources of collagen, even though further research is highly needed to find an optimized conditions for the extraction process such as extraction time, solvent concentration, solvent ratio, raw material properties, and temperature of extraction.

Acknowledgement

This research was supported by Universitas Airlangga with No. 1111/UN.3.1.12 /PT/2020 and Universitas Gadjah Mada, with grant (63.31.01/UN1/FFA/UP/SK/2023).

Authors' Contributions

The contribution of each author is as follows, Maulida Agustina, Patmawati, and Money Carattri Kusuma Werdani, Mohamad Akmal Alwi Husein; collected data, analyzed, and wrote the article. Patmawati, Shofy Mubarok, Laksmi Sulmartiwi, Raseetha Siva, Diah Anggraini Wulandari, Khadijah, Dwi Yuli Pujiastuti, Dwita Nirmala, Oemar Moechthar; supervised the research, devised the main conceptual ideas, wrote the article, did a critical revision of the article. All authors discussed

the results and contributed to the final manuscript.

Conflict of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding Information

The research was funded by Universitas Airlangga, with grant (1111/UN3.1.12/PT/2020).

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