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Extraction of healthy oils from fish viscera by conventional and advanced technologies

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SUMMARY: Fish viscera is a by-product of fish processing that has limited use as added-value products. The current issue of circular economy to produce recycled and zero waste products, therefore, the utilization of fish viscera for valuable products is important to explore. One of the valuable components of fish viscera is fish oil, which is characterized by being high in polyunsaturated fatty acids (PUFA). The limited exploration of fish viscera for potential utilization requires a comprehensive review of the visceral characteristics of various fish species. This article reviews the nutritional characteristics of various fish viscera, lipid class composition, fish viscera oil characteristics, and various methods of fish viscera oil extraction, both by conventional and advanced technologies. The main contribution of this review is to provide information about fish viscera, their potential as a source of fish oil, and extraction methods which are suitable for various industrial applications and purposes, including health applications for ω-3 fatty acids.

KEYWORDS: *By-product; Extraction; Fatty acid; Fish oil; Non-Conventional Extraction Method; Viscera.*

RESUMEN: ***Extracción de aceites saludables de vísceras de pescado mediante tecnologías convencionales y avanzadas.*** Las vísceras de pescado son un subproducto del procesamiento del pescado que se utiliza de forma limitada para productos de valor añadido. Es muy importante actualmente el tema de la economía circular, para producir productos reciclados y sin desperdicio. Así, con la utilización de vísceras de pescado podemos obtener productos con alto valor añadido. Uno de los componentes valiosos de las vísceras de pescado es su aceite, caracterizado por un alto contenido en ácidos grasos poliinsaturados (PUFA). La exploración de las vísceras de los peces, para una utilización potencial amplia, requiere una revisión exhaustiva de las características viscerales de varias especies de peces. Este artículo revisa las características nutricionales de vísceras de varios pescados, la composición de las diferentes clases de lípidos, las características del aceite de vísceras y varios métodos de extracción del aceite de vísceras de pescado, tanto con tecnologías convencionales como avanzadas. La principal contribución de esta revisión es proporcionar información sobre las vísceras de pescado, su potencial como fuente de aceite de pescado y los métodos de extracción adecuados para diversas aplicaciones y propósitos industriales, incluidos los usos para la salud de los ácidos grasos ω-3.

PALABRAS CLAVE: *Aceite de pescado; Ácido graso; Extracción; Extracción no convencional; Subproducto; Vísceras.*

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1. INTRODUCTION

Fish oil is one of the important oils for the food and pharmaceutical industries since this oil contains health-beneficial fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The main advantage of consuming fish oil as a supplement or fish oil-containing foods from fortified products is the health benefits from long-chain omega-3 fatty acids. Omega-3 fatty acids have been widely reported to be beneficial for the treatment of cardiovascular disease. Nutritional supplementation of ω-3 fatty acids, mainly EPA and DHA proved its anti-inflammatory, hypocholesterolemic, anticancer, stroke therapeutics, and immunomodulatory effects. EPA and DHA when oxidized by endogenous compounds possess anti-inflammatory effects that lead to the recovery of patients infected with COVID-19 (Rogero *et al.,* 2020).

Traditionally, the sources of fish oil are whole fish body, fish flesh, and by-products of fish canning and meal processing. The increasing demand for fish oil driven by population growth, necessitates the search for new sources of fish oil. The fish processing industry produces by-products in large quantities. The by-product of evisceration consists of the head, viscera, carcass, scales, skin, and fins (Villamil *et al.,* 2017). Fish viscera is generated in a considerable portion approximately 12-18% of the total weight of processed whole fish (Estiasih *et al.*, 2021). The global fish production volume amounted to 184.6 million metric tons in 2022 (Shahbandeh, 2023). Therefore, the estimated fish viscera production is 22.15–33.23 million metric tons. The viscera comprise the mixture of several organs including the stomach, intestines, liver, spleen, and pancreas. These by-products are usually discarded from all kinds of fish processing such as whole cooking, skin-on, or skin-off fillets, and canning.

Fish viscera provides a resource of rich lipids and proteins. Lipid comprises 5-36% and protein of 5-22% depending on the fish species and habitat. Therefore, one of the aims of fish viscera oil extraction is to have fish oil containing EPA and DHA for health purposes. Some studies reported the use of fish viscera oils for biodiesel production (El-Rahman *et al.,* 2018) and feeds.

Fish viscera is one of the alternative sources of fish oil containing omega-3 fatty acids. The oil content in fish viscera is affected by the fish species but generally contains omega-3 fatty acids as valuable fatty acids for health. The portion of oil in the viscera is not a predominant component so fish viscera oil extraction is suggested to be an integrated process with the separation of other components, mainly proteins and their derivatives.

In line with the current issue of circular economy to produce recycled and zero waste products, the utilizations of fish viscera for valuable products is very important to explore. A sustainable extraction and refinement of fish viscera oil from various fish waste materials should be encouraged since the process will produce valuable fish oil. The extraction of oil from fish viscera could be integrated with other valuable material production from fish viscera such as protein and its derivatives, and some digestive enzymes such as proteases, lipases, and amylases (Estiasih *et al.,* 2021).

Fish cell membranes are arranged into a double layer of phospholipids which acts as an extraction barrier. Reduction of thickness, formation of pores, and finally damage to cell membrane permeability by certain energies can facilitate the release of fish oils. Accordingly, tightly attached oils in the cell membrane structure can be extracted (Guo *et al.,* 2018). The extraction of fish oils by conventional methods (such as soxhlet, Randall, rendering, and solvent extraction) has several drawbacks such as low yield, long time, high-energy consumption, and less eco-friendliness. In addition, they can reduce the oxidative stability and quality of oils due to prolonged processes and enhanced heating. Non-conventional and advanced methods such as infrared-assisted extraction (IR), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), electric field (EF), and supercritical fluid extraction (SFE) are able to extract oil quickly, with high oil recovery, as well as prolonged oxidative stability of the extracted oils. The non-conventional and advanced methods significantly disintegrate the cell structure of the materials (Afolabi *et al.,* 2018; Guo *et al.,* 2018), thereby oil extraction is easier. The comparison of fish viscera oil extraction by conventional and non-conventional and advanced methods is interesting to discuss. The studies on fish viscera oil extraction, characterization, and utilization are still limited. This article aims to review the composition of fish viscera, their lipid composition and fatty acid profile, various methods

of fish viscera oil extraction, and their characteristics based on various limitedly published research works.

2. PROXIMATE COMPOSITION OF FISH VISCERA

Viscera comprises 12-18% of the whole fish's body weight, and the rest are heads 9--12%, bones 9–15%, 9–12%, skin 1–3%, and scales 5% (Villamil *et al.,* 2017). The viscera occupy the highest portion of by-products from fish processing (Estiasih *et al.,* 2021). The composition of fish viscera is very important for determining the type of suitable process for obtaining added-value products.

The composition of fish viscera may vary depending on the fish species (Table 1). Fish habitat diversity does not seem to affect the composition of viscera. Moisture content is the highest portion of the composition of fish viscera, followed by protein or lipids. Removing the water from the viscera results in significant amounts of fat and protein. Therefore, fish viscera are the source of fat and protein for further use in a suitable product. Ash is also present but in the lowest quantity. Some fish such as tilapia, Persian sturgeon, and yellowfin tuna have high ash contents at more than 4% (Table 1).

Horn *et al.* (2007) reported a seasonal variation in whole viscera and four fractions (roe, stomachs, milt, and liver), obtained at different times during the spawning season. In general, the body composition of the fish showed seasonal variations as reported by Baghtasingh *et al.* (2016) for sardines (*Sardinella gibbose*). The variation was greater for lipid content, while proteins, ash, and moisture contents did not vary significantly. The fish body composition is affected by spawning period and seawater temperature.

Kacem *et al.* (2011) reported seasonal variations in the proximate composition of fish viscera from *Sardinella aurita, Sarpa salpa,* and *Sepia officinalis*.

Table 1. Some fish viscera composition (% w.b)

Fish Species	Protein	Lipid	Moisture	Ash	References
Freshwater fish					
Common carp (*Cyprinus carpio* var. *communis*)	12.03 ± 0.10	22.98 ± 0.09	63.96 ± 0.25	1.03 ± 0.05	Al-Hilphy *et al.* (2020)
Red tilapia (*Oreochromis* sp.)	4.57 ± 0.23	33.06 ± 1.60	62.69 ± 1.90	0.73 ± 0.04	Arias *et al.* (2022)
Channel catfish (*Ictalurus punctatus*)					
Whole viscera	14.7	33.6	50.1	-	Sathivel *et al.* (2022)
Digestive tract	13.4	5.8	79.5	-	Sathivel *et al.* (2022)
Liver	11.4	8.8	74.9	-	Sathivel *et al.* (2022)
Gallbladder	2.6	0.3	88.9	-	Sathivel *et al.* (2022)
Visceral storage fat	1.3	90.7	8	-	Sathivel *et al.* (2022)
Brackish water fish					
Persian sturgeon (*Acipenser persicus*)	15.48 ± 0.25	15.68 ± 1.34	39.00 ± 0.00	5.76 ± 0.05	Ovissipour *et al.* (2009)
Seawater fish					
Rainbow smelt (*Osmerus mordax*)	14.68 ± 0.21	5.60 ± 1.31	78.63 ± 1.60	0.59 ± 0.08	Yin *et al.* (2022)
Atlantic salmon (*Salmo salar*)	10.73 ± 1.54	27.19 ± 1.55	61.24 ± 0.75	1.30 ± 0.06	He *et al.* (2011)
Yellowtail kingfish (*Seriola lalandi*)	11.12 ± 0.54	23.75 ± 1.94	63.90 ± 3.54	1.17 ± 0.09	He *et al.* (2011)
Yellowfin tuna (*Thunnus albacares*)	21.50 ± 0.50	5.08 ± 1.53	69.66 ± 2.32	4.46 ± 1.21	Ovissipour *et al.* (2012)
Gilthead sea bream (*Sparus aurata*)					
Guts	12.89	34.11	47.70	1.09	Pateiro *et al.* (2020)
Liver	10.11	25.76	55.50	1.08	
Round sardinella (*Sardinella aurita*)	1.36 ± 0.15 to 15.88 ± 0.88	3.90 ± 0.28 to 25.40 ± 0.16	49.06 ± 0.06 to 78.15 ± 0.91	1.33 ± 0.05 to 2.78 ± 0.04	Pateiro *et al.* (2020)
Salema porgy (*Sarpa salpa*)	7.71 ± 0.10 to 13.49 ± 0.41	0.58 ± 0.30 to 4.02 ± 0.14	83.37 ± 0.09 to 89 ± 0.07	1.31 ± 0.07 to 3.48 ± 0.05	Kacem *et al.* (2011)
Common cuttlefish (*Sepia officinalis*)	3.83 ± 0.27 to 6.65 ± 0.11	0.36 ± 0.16 to 3.63 ± 0.16	75 ± 0.67 to 85.04 ± 0.14	2.32 ± 0.00 to 6.17 ± 0.05	Kacem *et al.* (2011)

The highest fluctuation was found in protein content, which differed from the edible parts of the whole body of many marine species. A significant difference was also found in ash content. The variation in lipid content was affected conversely by moisture content. The seasonal variation in small abalone viscera was also reported not only in proximate composition but also total free amino acids (FAA), adenosine 5′-triphosphate (ATP)-related compounds (ARC), and glycogen (Chiou *et al.,* 2001).

Many factors affect fish composition such as sex, age, season, temperature, diet, life cycle, size, and location. The feed of the fish affects the viscera composition. Therefore, the compositional variation and their affecting factors should be taken into account in fish viscera utilization for added-value products, including fish viscera oil. Fluctuations in oil composition potentially make quality standardization difficult to obtain (Jacobsen *et al.,* 2022). However, season variations in viscera composition have not been reported for fish from tropical seawaters, since this region does not have high differences between seasons.

Table 1 shows that the different fractions of fish viscera have different compositions. Fat is the highest portion of the storage fat in fish viscera, and it is suitable as a source of fish viscera oil. The data on the composition of the viscera fraction remains limited and it could not be concluded based on the two data in Table 1 alone. Different fractions of viscera from channel catfish (*Ictalurus punctatus*) and gilthead sea bream (*Sparus aurata*), such as liver and guts, reveal different compositions. In gilthead seabream fish, the fat content is higher in the gut compared to the liver. Meanwhile, in channel catfish, the liver has a higher fat content than the digestive tract. More studies are needed to obtain comprehensive data on the composition of the fish viscera fraction.

For a large-size fish, the separation of viscera fractions is easily obtained and important for appropriate viscera processing. Codfish (*Gadus morhua*) liver is high in fat and has been used commercially to produce cod liver oil (Meidell *et al.,* 2023). Jacobsen *et al.* (2022) studied five species of coastal fish (cod, ling, hake, monkfish, and coalfish) liver composition and found seasonal variations in lipid content, peroxide value, free fatty acid content, and fatty acid composition. The lipid content and composition among species varied similarly with higher values in fall (October, November) and lower values in spring (March, April). Not all the liver from fish species is suitable to produce fish oil due to the low lipid and omega-3 fatty acids content, and high free fatty acids. Other fractions of fish viscera have not been explored intensively for fish oil production.

Interestingly, Table 1 shows that visceral storage fat contains a considerable amount of fat which shows potential for fish oil production. The abdominal fat of catfish (*Pangasius hypothalamus*) was easily separated and extracted by rendering (Ayu *et al.,* 2019). The belly fat of some fish species is obviously visible and easily separated. Catfish lipid and channel catfish (*Ictalurus punctatus*) belly fat contains lipids of 88 and 90%, respectively.

However, fish viscera contain a considerable amount of lipids with highly polyunsaturated fatty acids. These fatty acids are susceptible to oxidation and cause an undesirable fishy flavor. Some microbiomes also live in the gut of viscera and make them deteriorate easily. The digestive tract is part of fish viscera which is responsible for producing several enzymes such as lipases and proteases (Estiasih *et al.,* 2021). Quality degradation could occur rapidly in fish viscera due to oxidation, enzymatic, and microbial degradation which starts shortly after catching the fish, Optimal handling and processing strategies are required to produce high-quality ingredients from fish viscera, including fish oil. The study of Meidell *et al.* (2023) showed that the maximum for both cod viscera and liver storage at 4 °C before oil extraction is 2 days to meet the standard quality for foods.

3. LIPID CLASSES COMPOSITION OF FISH VISCERA

Lipids are acknowledged as important macro-nutrients and exert some biological activities. Fish lipid is considered the most complex compound and has several thousand species of lipids (He *et al.,* 2021). The study of Meidell *et al.* (2023) showed that Atlantic cod (*Gadus morhua*) contained triglycerides as the major lipid class, diglyceride, monoglyceride, free fatty acids, cholesterols, and phospholipids. Sinanoglou *et al.* (2017) reported that lipid class profiles consisted of neutral and polar lipids, with neutral lipids in higher quantity than polar lipids. Neutral lipids were dominated by triglycerides and free fatty acids, sterols, monoglycerides, and 1,2 diglycerides were also found. Meanwhile, polar lipids consisted of l-lyso-phos-

phatidylcholine; phosphatidylcholine; phosphatidylethanolamine; phosphatidylinositol; phosphatidylserine; and sphingomyelin.

He *et al.* (2021) reported five lipid classes in tilapia viscera consisting of glycerolipids (GLs, 50.54–60.75% of total lipid species), glycerophospholipids (GPs, 19.25–26.53%), sphingolipids (SPs, 10.94–12.86%), saccharolipids (SLs, 4.72–5.85%) and fatty acyls (FAs, 4.34–6.04%). The amount of free fatty acids in fish viscera is affected by fish handling after catching and storage time. Meidell *et al.* (2023) reported that increasing storage time resulted in increasing free fatty acid and diglyceride, decreasing triglyceride, and fluctuating the level of cholesterol. The hydrolysis of triglycerides during viscera handling resulted in diglycerides, monoglycerides, and free fatty acids. Moreover, viscera also consisted of guts which are rich in digestive enzymes including lipase as the lipid hydrolyzing enzyme. Therefore, viscera handling is the critical factor in utilizing viscera to increase their added values.

Fish viscera was reported to contain phospholipids at higher levels than whole-body fish oil. Tilapia (*Oreochromis niloticus*) viscera contain phospholipids comprising 6 types of phosphatidylcholines, 1 type of lysophosphatidylcholine, 1 type of phosphatidylethanolamine, and 1 type of lysophosphatidylethanolamine. The PUFA was lower in triacylglycerols (18.11–25.15%) than in phospholipids (30.35–52.05%). The saturated fatty acids (SFA) showed a preference to be incorporated at the sn-2 position; whereas MUFA (monounsaturated fatty acid) and PUFA were mainly bound in external positions in triglycerides (He *et al.,* 2021).

The cholesterol level was higher in the viscera than the muscle of tilapia (He *et al.,* 2021). Marine animals mostly have cholesterol as their major sterols. However, some phytosterols are also found as dietary origins from phytoplankton. Viscera from herring and mackerel contained phytosterols (campesterol and sitosterol), but the proportion was lower than cholesterol (93–98%). The abundance of phytosterols in herring viscera was affected by season, which was the highest in September (Souchet and Laplante, 2007). Souchet and Laplante (2007) reported the occurrence of cholesterol, demosterol, campesterol, and sitosterol in some fish pelagic viscera, with phytosterols found in much lower quantities.

All the lipid species contain fatty acids as the most determinant for their properties. The composition of fatty acids was affected and regulated by fish dietary sources. Thus, the fatty acid profile of aqua-cultured fish can be improved by optimizing the feed composition (He *et al.,* 2021), including the fatty acid composition of viscera.

4. FATTY ACID PROFILE OF FISH VISCERA

Fatty acid composition is an indicator of lipid quality and determines their properties. Fish lipids are typically rich in PUFA, although their composition differs among species. Furthermore, fatty acid composition in the same species of fish is affected by many factors such as fish diet, physiology, habitat, seasons, age, sex, and size. Sathivel *et al.* (2002) found that the predominance of linoleic acid in catfish was attributed to the fishmeal diet made from soy products.

Usually, the fish fatty acid compositional pattern is PUFAs > MUFAs > saturated fatty acids (SFAs) (Yin *et al.,* 2022). Conversely, He *et al.* (2021) indicated that SFAs were the dominant percentage over PUFAs and MUFAs in most lipid fractions of tilapia viscera. Sinanoglou *et al.* (2017) reported that MUFAs were predominant in the viscera of *D. labrax, S. aurata*, and *D. puntazzo,* followed by PUFAs and SFAs. Different patterns of fatty acid composition are found in fish viscera. Factors affecting the fatty acid composition of the whole fish body also affect the fatty acid composition of viscera.

Fish viscera have different fatty acid compositions compared to muscle and roe (Yin *et al.,* 2022). The highest quantity of SFAs was observed in viscera compared to their quantity in the head and muscle. Similarly, the PUFAs and MUFAs of viscera were higher than those of the muscle and head. The abundance of fatty acid from lipids in viscera resulted in all fatty acid classes to be higher than those in the muscle and head (He *et al.,* 2021).

The prominent fish fatty acids are EPA and DHA but their levels are highly dependent on species. He *et al.* (2021) reported that tilapia is not a superior source of EPA or DHA since their levels are quite low. Low levels of EPA and DHA were also observed in catfish viscera (Sathivel *et al.,* 2002). However, all seawater fish have significant levels of EPA and DHA. Low levels of EPA and DHA are typical of freshwater fish including their viscera. The belly fat in freshwater fish such as patin siam (*Pangasius hy-*

pothalamus) occurs in a semisolid state (Ayu *et al*., 2019), indicating a rich source of SFAs.

The composition of fatty acids in fish viscera is one of the important considerations for their utilization. Fish viscera oil rich in EPA and DHA is very suitable for health purposes. The rich SFA fish viscera oil is suitable for non-health uses such as for producing biodiesel. Therefore, a fatty acid compositional analysis is very important in the exploration of fish viscera oil.

5. EXTRACTION OF FISH VISCERA OILS

The extraction of oil from oil-bearing tissue including fish viscera is aimed to obtain crude oil for many purposes and further purification to obtain edible fish oils. Fish viscera management is very important to ensure that this material is feasible and safe for oil extraction. Fish viscera contain a considerable amount of water and are high in nutrients, therefore they are a good medium for bacterial growth. Cold-chain management of fish viscera is very important to prevent the formation of microbial toxins and the quality deterioration of fish viscera.

The common fish viscera oil extraction methods include organic solvent extraction, pressing method, ultrasound-assisted extraction (UAE), aqueous extraction (wet rendering), supercritical carbon dioxide extraction (SFE CO_2), and microwave-assisted extraction (MAE) (Wang *et al.,* 2020). Other reported methods for fish viscera oil extraction are electric field (EF) (Guo *et al.,* 2018), infrared-assisted extraction (Al-Hilphy *et al.,* 2020), extraction by high pressure (Zhang *et al.,* 2021), enzyme-assisted extraction (Garofalo *et al.,* 2023), and ensilaging (Rai *et al.,* 2010). The different extraction methods are shown in Figure 1.

Traditional extraction of fish oil involves wet and dry rendering, pressing by hydraulic and screw pressing, and solvent extraction. Another method to obtain fish oil is through the sedimentation of the liquor produced during fish canning and meal processing. The two latter are by-products of fish processing.

Fish oil solvent extraction is based on its hydrophobicity and involves the use of organic solvents. The solvent choice is affected by several factors: cost, flammability, low boiling point for oil recovery ease, disposal procedures, availability, and toxicity. The oil extraction efficiency is high but the food industry restricts the use of organic solvents in food due to environmentally unfriendly and non-sustainable processes (Alfio *et al.,* 2021).

5.1. Conventional methods

Normally, fish oil extraction by solvent uses the Soxhlet and Randall methods. Randall apparatus is a conventional Soxhlet extractor with some modifica-

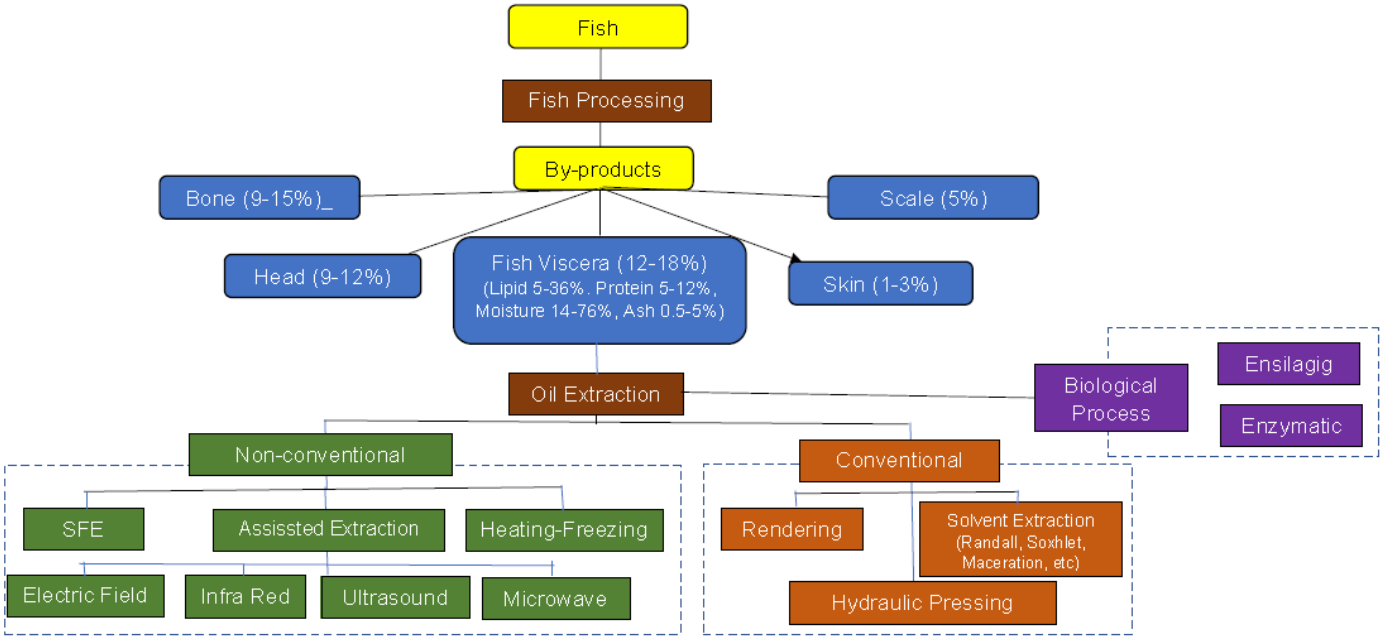

Figure 1. Fish viscera oil extraction by various methods

tions. This process is performed at atmospheric pressure by using n-hexane. The sample was put in a porous container and immersed directly into the boiling solvent. This immersion was aimed to immediately wet the sample and speed up the extraction of soluble components. The immersion step was followed by the washing process. The end of extraction is similar to the standard of Soxhlet extraction (Fiori *et al.,* 2012). The characteristics of trout viscera oil from the Randall process are shown in Table 2. This composition was not significantly different from the oil extracted by SFE CO_2 (Table 2). This might be related to the more nonpolar nature of hexane than liquid CO_2 in the solvent extraction by Soxhlet or Randall.

Wet rendering or aqueous extraction is a simple method for fish viscera oil separation. This method involves heating the fish viscera in the presence of water for a certain time and a definite temperature. During heating, protein denaturation and coagulation will release the bound fats, and fats will be melted and released into the aqueous media. Centrifugation is usually used to separate oil, water, and solid fish viscera. However, the oil yield was lower than that of the enzyme-assisted extraction method (Table 3). Ching-Velasquez *et al.* (2020) extracted the oil from a mixture of fish viscera by incubating the viscera with water at 95 °C for 1.5 hours. After heating, the slurry was filtered and the filtrate was centrifuged to obtain the upper layer containing the oily phase. The residual water in the oil was removed by vacuum drying and the oil yield was 75%.

Dry rendering uses high temperatures for oil extraction without water addition during heating. After grinding, Carp (*Cyprinus carpio*) fish viscera were cooked at 95-100 °C for 30 minutes. After sieving to remove spines and scales, the cooked viscera were

Table 2. Fatty acid class composition of fish viscera oil extracted by various methods

Fish Species	Extraction Method	SFA	MUFA	PUFA	ω-3 PUFA	ω-6 PUFA	References
Mix several fish*	Wet rendering	42.10	37.40	20.50	11.90	8.60	Ching-Velasquez *et al.* (2020)
Atlantic Salmon (*Salmo salar*)	Wet rendering	24.30	43.70	29.20	19.20	10.10	He *et al.* (2011)
Yellowtail Kingfish (*Seriola lalandi*)	Wet rendering	34.90	41.40	20.80	11.40	9.50	He *et al.* (2011)
Carp (*Cyprinus carpio*)	Dry rendering	26.86	41.89	25.54	13.61	11.93	Crexi *et al.* (2010)
Yellowtail fish (*Seriola quinqueradiata*)	Supercritical CO_2	41.16	34.46	24.38	20.14	-	Franklin *et al.* (2020)
	Soxhlet	38.63	35.72	25.65	21.38	-	
Trout (*Oncorhynchus mykiss*)	Supercritical CO_2	27.40	32.49	40.11	18.00	18.40	Fiori *et al.* (2012)
	Randall	27.60	31.07	41.33	17.40	20.40	
Indian mackerel (*Rastrelliger kanagurta*)	Supercritical CO_2	23.61	17.26	57.30	32.92	15.02	Sahena *et al.* (2010)
	Soxhlet	23.88	17.58	56.52	32.56	14.79	
Squid (*Sepioteuthis sepioidea*)	Electric field	8.87	13.09	17.30	16.00	0.95	Guo *et al.* (2018)
	Folch (solvent extraction)	10.45	14.26	17.48	16.13	1.04	
Red tilapia (*Oreochromis* sp.)	No treatment, centrifugation	32.78	36.76	30.40	6.15	0.82	Arias *et al.* (2022)
	Heating and freezing	37.08	30.26	32.03	6.72	0.92	
	Maceration	37.17	37.70	24.19	6.34	0.91	
Cobia (*Rachycentron canadum*) liver	Enzymatic (papain)	41.01	39.54	19.45	18.88	0.56	Wang *et al.* (2020)
	Enzymatic (pepsin)	41.82	39.21	18.97	17.86	0.54	
	Enzymatic (alcalase)	42.12	39.20	18.68	17.55	0.56	
	Enzymatic (trypsin)	41.58	39.32	19.10	21.26	0.55	
	Wet rendering	37.92	40.82	21.26	20.41	0.84	
Salmon (*Salmo salar*)	Microwave-assisted extraction	15.3	43.3	41.4	21.07	19.17	de la Fuente *et al.* (2022b)
	Solvent extraction	15.9	42.9	41.06	20.87	19.20	

*= Mexican snook (*Centropomus poeyi*) + Black seabream (*Spondyliosoma cantharus*) + King mackerel (*Scomberomorus cavalla*) + Striped mojarra (*Eugerres plumieri*).
SFA= Saturated fatty acids, MUFA= Monounsaturated fatty acids, PUFA= Polyunsaturated fatty acids.

Table 3. Extraction of fish viscera oil by different methods and their characteristics

Fish Species	Extraction Method	Oil Characteristics										References
		Yield (%)	Recovery (%)	FFA	PV	AV	TBA	FFA	AcV	TOTOX	Color	
Red tilapia (*Oreochromis* sp.)	*Control oil (CO)* Non-extracted oil from fish viscera				0.004±0.002							Arias *et al.* (2022)
	Oil extraction by heating and decanting (OEHD) The temperature of 67°C for 29 min. The oil was separated by decantation.	60.989±0.845			0.014±0.003							
	Oil extraction by heating and freezing (OEHF). The samples were allowed to stand until the temperature dropped to 45°C, then they were frozen at -18°C for 24 h to obtain a better separation of the oil since the phases separate as they solidify.	92.283± 0.327			0.014±0.001							
	Oil extraction with organic solvent (OES). 200 g of viscera and immersed for 2 h at 20°C in a dichloromethane-methanol mixture (2:1), in a 2:1 solvent-viscera ratio.	54.349± 0.378			0.039±0.006							
Carp (*Cyprinus carpio*)	*Dry rendering:* Heating the ground viscera at 95–100 ˚C, 30 min, and then centrifugation to separate oil.	-	-	3.3± 0.02	3.38±0.01	13.40±.4	6.70± 0.1	3.35 0.01	-	-	Lovibond 5.0 ± 0.1	Crexi *et al.* (2010)
	Ensilaging Spontaneous fermentation after acidification, fermentation for 15 days	-	-	6.6± 0.01	3.36 ± 0.02	10.3±0.3	1.10± 0.1	6.6± 0.01	-	-	Lovibond 16 ± 0.2	
Common carp (*Cyprinus carpio* var. *communis*)	*Infrared at pilot scale:* Optimum at 168.3 W, 70 °C, 6.08 cm, 3.5 kg.	23.73	-	0.68	2.83	-	1.55	-	-	-	-	Al-Hilphy *et al.* (2020)
Yellowtail fish (*Seriola quinqueradiata*)	*Supercritical CO_2:* 30 MPa, 40°C, flow rate 27 g/min, 3 h, 60 g.	40.87		4.56	2.50	13.65	-	-	-	18.65	L^*= 46.54, a^*= 15.23, b^*= 30.68, ΔE^*= 47.01.	Franklin *et al.* (2020)
	Soxhlet-ethanol: 75°C, 16 h, 5 g/100 ml.	56.13	-	7.41	8.33	20.91	-	-	-	37.57	L^*= 24.10, a^*= 0.40, b^*= 0.38, ΔE^*= 24.11.	
	Soxhlet-n hexane: 65°C, 16 h, 5 g/100 ml.	48.48	-	6.75	6.17	17.12	-	-	-	29.05	L^*= 25.03, a^*= 1.12, b^*= 1.70, ΔE^*= 25.11.	
Squid (*Sepioteuthis sepioidea*)	*Electric field:* 10 V/cm, 2.5 h, 20 g.	-	72.10	-	-	-	-	-	-	-	-	Guo *et al.* (2018)
Tilapia (*Oreochromis niloticus*)	*Wet rendering:* 100 ºC, 50 min, 1:1 (w/v)	20.00	-	3.00	-	-	-	-	5.97	-	-	El-Rahman *et al.* (2018)
Cobia (*Rachycentron canadum*) liver	*Enzymatic:* Papain 0.5 %, pH 6, 30 ºC, 120 rpm, 2 h. Then + 100 ml water, 95 ºC, 15 min.	38.00	-	-			-	-	-	-	-	Wang *et al.* (2020)
	Wet rendering: 1:2 (w/v), 15 min, 95 ºC.	18.80	-	-		-	-	-	-	-	-	

- = Not reported.
FFA = Free fatty acids (%), PV= Peroxide value (meq/kg oil), AV= Anisidine value, TBA= Thiobarbituric acid (mg malondialdehyde/ kg oil), AcV= Acid value (mg KOH/g).

centrifuged to separate the oil fraction (Crexi *et al.,* 2010). Dry rendered oil showed better peroxide value and free fatty acid levels compared to ensilaged oil. However, anisidine value as the indicator of advanced oxidation was higher in dry rendered oil. High-temperature heating might produce a secondary oxidation product. The ensilaging method was performed at low temperatures for a long time, involving microbes, so this method produced higher free fatty acid.

Arias *et al.* (2022) developed a new method for red tilapia viscera oil extraction by a simple and efficient heating-freezing method. Three oil extraction methods were studied: a) direct heating (69 °C, 29 min) followed by decantation; b) direct heating followed by subsequent freezing; and c) ultrasound-assisted extraction (UAE) using solvent. The results showed the yields of 92.12, 60.99, and 55.36%, respectively. The new method of heating-freezing extraction is a better alternative for producing high-quality fish oil than UAE. The fish oil quality of the three methods is comparable and all methods could preserve the oil from deterioration.

5.2. Biological extraction method

Low-temperature extraction of fish viscera oil has the advantage of preventing excessive peroxidation. To fasten the oil separation from the fish viscera, the liberation of oil from other viscera materials is very important. The methods to release the oil are by using biological processes such as fermentation and enzymatic reactions. Enzyme-assisted fish oil extraction was conducted by using protease, which assists the liberation of oil from protein compounds (Wang *et al.,* 2020). This enzymatic method was usually combined with other methods such as wet rendering to shorten the heating time.

The purpose of ensilaging is to ferment the viscera so the liberation of oil from the binding matrix will be easier. Spontaneous fermentation occurs as the result of high moisture content and the nutritious composition of the viscera which induce microbial growth. During ensilaging, the digestive enzyme in the fish viscera might also be active and involved in protein hydrolysis. Crexi *et al.* (2010), explained the process of carp viscera ensilaging by grinding the viscera and placing the ground viscera in plastic buckets. Acidification was conducted by adding glacial acetic acid and BHT antioxidants to reduce oxidation. During ensilaging, liquefaction occurred. After 15 days, the silage was heated at 50 °C and then centrifuged to separate the oil.

Besides spontaneous fermentation, ensilaging could be performed by using a starter culture. Rai *et al.* (2010) used lactic acid bacteria isolates comprised of *Pediococcus acidilactici* K7 and *Enterococcus faecium*. The silage mix was incubated for 72 hours at 37 °C. During ensilaging, there was an increase in acid value due to lipase activity produced by the bacteria. During ensilaging, the protein hydrolysis degree increased, so the oil yield increased. This process is useful as an integrative method to obtain fish viscera oil and protein hydrolysate concomitantly.

Wang *et al.* (2020) studied the various proteases (papain, pepsin, alcalase, and trypsin_ as pre-treatments to assist oil extraction from Cobia (*Rachycentron canadum*) viscera. The types of proteases affected the fish oil yield, and the highest was obtained from papain-assisted extraction. This pre-treatment was combined with aqueous extraction. Many factors should be established in the enzyme-assisted extraction of viscera oil such as pH, time, temperature, and other factors affecting enzyme activity. The data in Table 2 shows that the types of proteases slightly influenced the fish oil's fatty acid profile.

The study of Garofalo *et al.* (2023) showed the enzymatic hydrolysis of tuna viscera by using alcalase-produced high added-value products, such as good quality tuna oil and fish protein hydrolysate. This method is very suitable for the tuna processing industry such as canning, which produces a high quantity of tuna viscera by-products.

5.3. Extraction by non-conventional and advanced methods

The main disadvantage of fish oil solvent extraction from fish viscera is the degradation of susceptible compounds such as PUFA. Organic solvent has the potential to pollute the environment. In the last two decades, non-conventional and advanced extraction methods are known as promising alternative methods to solvent extraction. Despite the advantages of non-conventional and advanced methods, some drawbacks are also observed such as being costly, requiring high-power consumption and expensive equipment, and some methods are difficult to scale up. The non-conventional and advanced methods that have been explored to extract fish viscera oil are SFE, UAE, MAE, EF, IR, and sometimes en-

zyme-assisted extraction, which is also classified as a non-conventional method.

Extensive research has been conducted over the last decade to extract fish oil by SFE. The advantage of SFE in viscera oil extraction is the use of supercritical CO_2 to replace organic solvents. Low extraction temperature, commonly 40 °C, is another benefit. The viscera oil yield of 67% was obtained from the African catfish *Clarias gariepinus*, 79% from trout, and 44% from mackerel. The SFE fish oil quality was comparable to the oil extracted by the Soxhlet method (Melgosa *et al.*, 2020). Many factors affect the extraction efficiency of SFE, including temperature, pressure, solvent-to-feed ratio, and extraction time. The fatty acid profile of trout viscera oil extracted by SFE was almost similar to that extracted by Randall methods (Fiori *et al.*, 2012). However, the omega-3 fatty acid level was slightly lower in the SFE-extracted yellow tail fish (*Seriola quinqueradiata*) viscera oil compared to that extracted by Soxhlet (Franklin *et al.,* 2012). An almost similar result was shown for Indian mackerel (*Rastrelliger kanagurta*) viscera extracted by SFE and Soxhlet methods (Sahena *et al.,* 2010) (Table 2).

In general, UAE is recognized as an effective extraction method, by significant time minimizing, thereby increasing productivity and oil quality. UAE uses sound frequencies above human hearing level ranging from 20 kHz to 10 MHz. Acoustic cavitation and mechanical impact on the material matrix generated during UAE increase extraction efficiency. Both disrupt the matrix and facilitate the solvent to penetrate more easily, thus enhancing the yield (Al-Khawly *et al.,* 2019). To the best of our knowledge, no studies have reported the use of UAE for oil extraction from fish viscera. Our ongoing research is regarding the UAE extraction of fish oil from the goldband snapper viscera.

Electrical treatment is another method to improve fish viscera oil extraction. The direct-current electric field is a powerful way to disrupt the cell membrane of squid viscera thereby increasing the extraction efficiency. By this method, the oil extraction efficiencies reached 71 – 72%. This method uses a low-voltage direct-current electric field to provide moderate operational conditions, consumes low energy, and is highly effective for oil extraction (Guo *et al*., 2018).

Infrared-assisted extraction uses the infrared wavelength of 0.75 to 1000 μm. Infrared can heat either polar or non-polar solvents. However, activation reaction might occur in the infrared range, thus allowing structural modification of the samples (Xiang *et al.,* 2022). The advantages of infrared heating compared to conventional heating are high heat transfer efficiency, low energy consumption, small equipment, the process is easy to control, and shorter extraction time (Al Hilphy *et al.,* 2020).

The study of Al-Hilphy (2020) on the infrared-assisted extraction of carp viscera oil showed that this method could be used for viscera valorization on a large scale. Some independent variables affected oil yield and quality, including temperature, power, and radiation distance. The extracted oil has good quality as indicated by a TBA value of 1.55 mg malonaldehyde/kg oil, 0.675% free fatty acid, and a peroxide value of 2.83 meq/kg. However, the oil yield is quite low at 23.73%.

The MAE method has gained the interest of many researchers to explore the extraction of various biomaterials in recent years. Principally, the energy of a short electromagnetic wave disrupts the cell membrane and makes the oil easily leach out into the solvent. This technique results in higher oil recovery in a shorter extraction time. The short duration of extraction is able to protect the sensitive compounds from heat degradation (Afolabi *et al.,* 2018). This technique has been used to extract oil from sea bass and sea bream fish heads (de la Fuente *et al*., 2022a). Microwave energy rapidly heats the sample in contact with the solvent, thus improving the extraction. Increasing temperature causes an increase in the sample cell pressure, thus disrupting the samples massively and facilitating compound transfer into the solvent (de la Fuente *et al.,* 2022b). However, studies on the MAE method for fish viscera oil extraction are scarce. The study of de la Fuente *et al.* (2022b) on salmon viscera extraction by using MAE showed that the extraction parameter process comprising power, solid-to-liquid ratio, and time did not affect the lipid profile of extracted viscera oil. The data in Table 2 show that the composition of the fatty acid class is not different between salmon viscera oil extracted by MAE and Soxhlet. This means that MAE is more efficient than conventional extraction. Afolabi *et al. (2018)* found that MAE is a reliable and efficient extraction technique to obtain high-yield fatty acids from *M. albus* fish.

The data in Table 3 shows the fish viscera oil quality from different extraction methods. The extraction method affected oil quality, and in general, the non-conven-

tional method gave better oil quality than conventional solvent extraction. Extraction costs are one consideration in choosing a suitable extraction method. This cost is greatly influenced by the method because each method has different equipment and operational costs.

6. CONCLUSIONS

Fish viscera reveals seasonal variations in composition and easily deteriorates, which requires good handling management. Fast quality degradation could occur in fish viscera due to oxidation, enzymatic, and microbial degradation which start in a short time after fish catching. Viscera also consists of guts which are rich in digestive enzymes including lipase as the lipid-hydrolyzing enzyme. The different fractions of fish viscera have different compositions, so further studies are required to better understand the fractional composition of fish viscera. Inconsistent data were found about the fatty acid composition of fish viscera. PUFA is not always the predominant fatty acid in fish viscera oil. In some fish, MUFA is the most abundant fatty acid. The highest quantity of SFAs was observed in viscera compared to their quantity in the head and muscle. Many methods of fish viscera oil extraction have been explored. The emerging green methods are suitable owing to higher extraction efficiency than conventional techniques. The choice of extraction method highly depends on the purpose of fish viscera utilization. For digestive enzyme separation concomitantly with oil extraction, the non-destructive method and low-temperature process is suitable such as ensilaging. Some methods are still studied on a small scale and not all of the emerging green extraction techniques are suitable yet for a larger scale. Extensive future studies are needed to determine the suitable methods for fish viscera oil extraction on an industrial scale as well as refining to produce edible and safe fish viscera oils.

DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

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AUTHORSHIP CONTRIBUTION STATEMENT

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B. Susilo: supervising

F.C. Nisa: supervising.

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