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Valorisation of viscera from fish processing for food industry utilizations

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Abstract. Fish viscera is a valuable source of functional materials for the food industry, such as protein, oil, enzymes, protein hydrolysate, peptones, sterols, producing biodiesel, and other oleochemical industries. The major components of fish viscera are oil and protein, and their quantity depends on the fish habitats. Viscera oil from fish contains appreciable amounts of omega-3 fatty acids. The protein of fish viscera is a raw material for protein concentrates, hydrolysate, and bioactive peptides. All are valuable ingredients in food processing and product formulation. Most viscera are fish digestive tracts and organs responsible for producing enzymes, and several enzymes are found in high activity, such as lipases and proteases. The extraction of lipases and proteases from fish viscera and their utilizations have been intensively studied. Currently, the isolation of each major component from fish viscera is conducted separately. Therefore, it is challenging to obtain all valuable components from fish viscera to have a zero-waste process. This article reviews the separation of major components of fish viscera by conventional and emerging technology and the proposed simultaneous and integrative separation of all valuable major components.

1. Introduction

Increasing demand for manufactured foods leads to an increase in the waste of food industries. The global production of fish has been increasing over recent years. The fish industry has been growing continuously over the last decades and generates enormous by-products [1]. Fish industries such as filleting, canning, freezing, and manufacturing various fish-based products. These activities generate substantial fish by-products, often discarded or used as low-value ingredients in animal feed [2].

Fish by-products are the raw materials that are not used in the production of the primary product. The by-products are obtained from fish processing at an industrial level, either from fishing or aquaculture [1]. These fish processing operations generate significant volumes of by-products. Only 40 to 60% of the fish is used in the primary product, with the remainder being wasted or under-used [3]. The quantity of fish by-product is affected by species, fish size, season, and fishing zone [4]. The fish by-product contains fin, gills, backbones, belly flaps, heads, liver, skin, roe, viscera, and others [5]. Viscera is the highest quantity of fish processing by-products that occupies 12-18% of the whole fish

body weight. Other fish by-products are heads 9–12%, skin 1–3%, bones 9–15%, and scales 5% of whole fish body weight [6].

Fish by-products are a prominent potential source of valuable materials with important functional properties. These valuable compounds could be separated to give and add value in higher-end markets, such as ingredients for food industries. Currently, the valorization of fish by-products is a must to obtain clean and zero waste of fish processing industries. Several studies have explored the added values of fish by-products, including for agriculture (fertilizer/silage, compost, pesticide); energy (biofuel, oxidizer); animal feed (meal, oil, protein, silage, minerals); nutrition or supplement (oil, protein, mineral, amino acid); human food (gelatin, fish stock, fish sauce, liver oil); and pharmaceuticals (omega-3 fatty acids, calcium, chondroitin, collagen, bioactive peptides) [3].

High-quality compounds could be recovered and used for human consumption such as proteins, enzymes, oil, amino acids, hydroxyapatite, collagen, and gelatin have a great interest to valorize fish by-products. Currently, industries used fish by-products mainly for fish oil production and animal feed. Collagen is another important product from fish by-products. Other uses of fish by-products are producing biofuels or fertilizers, although is more limited than not feed or oil production, but these industrial activities are important for the fish by-products' valorization. Another important use of fish by-products is for obtaining enzymes since some marine enzymes have an important commercial application [1].

As the highest portion of fish by-products, fish viscera have a great interest to explore for having added-value products. Furthermore, fish viscera is a valuable source of several compounds which are essential for food industries. Among them, fish viscera oil, protein, digestive enzymes, and protein derivatives such as protein hydrolysate and bioactive peptide have been intensively studied. Several fish species have been explored, including deep seawater, demersal, pelagic, and freshwater fish. Some techniques also have been used on a laboratory scale to obtain valuable compounds from fish viscera. However, it is still challenging to obtain all the valuable materials from fish viscera simultaneously to have a clean and zero waste production. This article is aimed to review the method to obtain valuable compounds from fish viscera as the candidate for industrial food ingredients. In this review, some emerging technology will be discussed as a green technology for future development.

2. Integrative process for obtaining valuable materials from fish viscera

Fish viscera are obtained from fish's stomach contents, which consist of guts, liver, spleen, and pancreas [7]. The major components of fish viscera are water, protein, and lipid. Fish viscera is a protein and lipid source (Table 1), and its composition depends on the fish species. The composition of fish viscera compared to muscle and head has higher lipid and cholesterol, more saturated fatty acid but has higher ω-3 fatty acids [8]. Generally, fat is the highest component of fish viscera after moisture content. Some fish species have higher protein than fat (Table 1). Two major components of fish viscera, fat, and protein, are the great concern to separate from obtaining fish viscera oil and protein.

Establishing an integrative process to obtain all valuable material from fish viscera is a great challenge, mainly oil and protein. A simultaneous process is proposed to gradually separate valuable components from fish viscera, including fish oil, protein concentrate, and digestive enzymes. Protein could be further hydrolyzed to obtain fish protein hydrolysate. This hydrolysate might contain an appreciable quantity of bioactive peptides that could be further separated by several techniques such as gel filtration chromatography. Depending on the fish species, fish viscera oils contain omega-3 fatty acids that are potentially used as food supplements [9, 10, 11]. Fish viscera oils are also the raw material for biodiesel [12, 13]. Also, the lipase from fish viscera, such as from tilapia, has been studied for biodiesel [14].

Table 1. Fish viscera composition (%).

Fish Species	Moisture	Fat /Lipid	Protein	Ash	Ref
Gilthead sea bream (*Sparus aurata*)					[11]
Guts	47.70	34.11	12.89	1.09	
Liver	55.50	25.76	10.11	1.08	
Common carp (*Cyprinus carpio var. communis*)	63.96±0.25	22.98 ± 0.09	12.03 ± 0.10	1.03 ± 0.05	[15]
Pangasius (*Pangasianodon hypophthalmus*)	73.30±0.60	8.48±1.21	15.61±1.88	1.77±0.54	[16]
Tuna (*Euthynnus affinis*)	75.73±0.33	11.77±1.41	65.04±1.40	3.12±0.11	[17]
Yellowfin tuna (*Thunnus albacares*)	69.66±2.32	5.08±1.53	21.5±0.50	4.46±1.21	[18]
Rainbow trout (*Onchorhynchus mykiss*)	71.65±0.89	13.00±0.76	15.00±0.06	2.73±0.89	[19]
Parastromateus niger	74.00±0.50	3.90±0.30	14.40±0.50	3.40±0.40	[20]

Currently, the studies about the valorization of fish viscera by extracting oils and separating proteins are conducted separately. The first step in the recovery of all valuable major components from fish viscera is oil extraction (Figure 1). Fish oil extraction usually uses solvent extraction as probably the most common extraction method [15]. Some emerging technology in fish oil extractions have been studied, such as ultra-high-pressure pre-treatment before enzymatic hydrolysis [21], infra-red assisted extraction [15], and conventional ensilaging method by fermentation or acid hydrolysis [9]. According to Rai et al. [9]. Silage technology is recognized as being most useful to manage waste problems in the fish farming industry. The product of ensilaging, fish silage concentrate, contains a highly digested protein for animal feed.
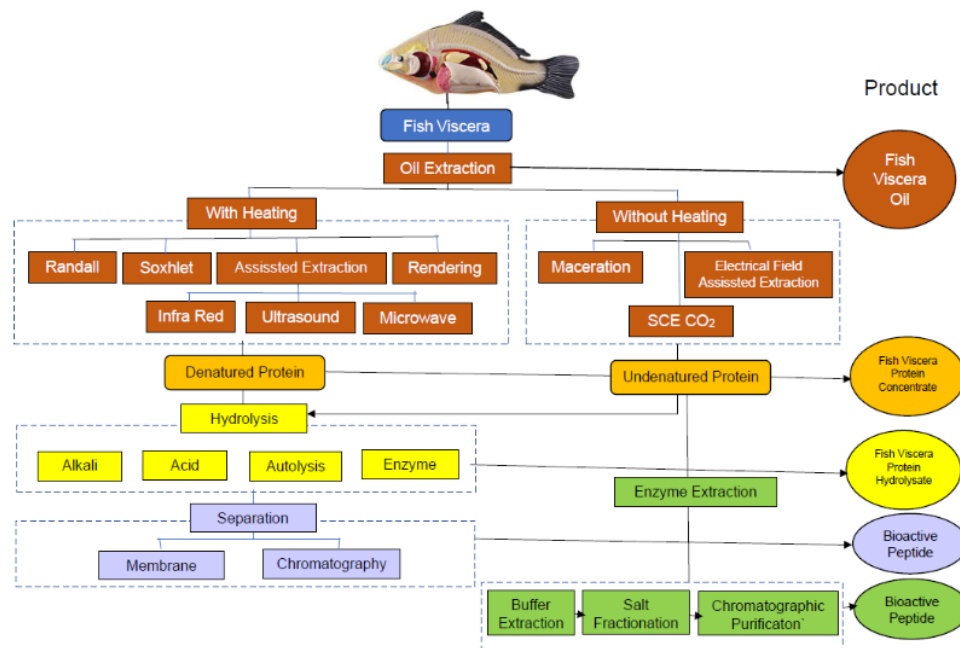

Figure 1. Integrative and simultaneous separation of valuable components from fish viscera.

In establishing an integrative and simultaneous process to recover all major components from fish viscera, the main consideration is that the protein residue after fish viscera oil extraction should be maintained undeniably. Denaturation of protein inactivates digestive enzymes. Therefore, heating should not be involved in fish viscera oil extraction if the residual protein is further used for enzyme separation. Therefore, maintaining the protein still in the native state is essential. However, involving heating in fish viscera oil extraction is allowed for the protein utilization for protein hydrolysates of bioactive peptides.

The residue from fish viscera oil extraction mainly consists of protein. In many cases, the concentration of protein is sufficiently high. Hisano et al. [22] reported that after fish viscera oil extraction, the residue is protein concentrate with a protein content of 88.4%. Unlike soy protein concentrate preparation, after oil extraction, protein is concentrated by precipitation in isoelectric pH. Digestive enzymes comprise lipase, protease, trypsin, and amylase, separated from protein by several techniques such as buffer extraction and slat precipitation. Purification sometimes is employed to have high enzyme activity.

Apart from digestive enzyme extraction, fish viscera protein hydrolysate is obtained by acid or enzyme hydrolysis [16]. Ensilaging by cultured fermentation or acid hydrolysis will produce fish viscera protein hydrolysate and oil simultaneously. However, ensilaging is not considered for digestive enzyme separation because microbial enzymes of acid hydrolyze protein during the process. Bioactive peptides from fish viscera protein hydrolysate are purified by membrane separation or chromatographic method. Fish peptides have some biological activities, including lipid homeostasis modulation, antioxidative, anticancer, anti-inflammatory, antihypertensive, and neuroprotective activities. In addition, these bioactive peptides are promising nutraceutical ingredients for food applications [23].

3. Fish viscera oil extraction

Fish oil is the most important ω-3 fatty acids source, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Both fatty acids play many beneficial roles in human health, with the benefits are the great interest of the food and pharmaceutical industries. One source of ω-3 fatty acids enriched fish oil is a by-product of fish processing. Therefore, the production of fish oil rich in ω-3 fatty acids is one way to valorize fish by-products, including fish viscera.

Table 2. Lipid composition of fish viscera from different species.

Fish Species	Unit	Lipid/FA	SFA	UFA	MUFA	PUFA	ω-3 PUFA	ω-6 PUFA	Ref
Tilapia (*Oreochromis niloticus*)	% body weight (ww)	13.86±0.83	3.35±0.03	3.55±0.04	1.44±0.02	2.11±0.02	0.81±0.01	1.29±0.02	[8]
Hybrid grouper (*Epinephelus lanceolatus* x *Epinephelus fuscoguttatus*)	% body weight (dw)	18.64±0.34	10.13±0.04	8.52±0.34	5.99±0.05	2.53±0.29			[27]
Trout (*Oncorhynchus mykiss*)	% fatty acid								[10]
• SCE CO₂ extraction			27.4±0.1	72.6±0.4	32.49±0.36	40.11±0.04	18.0±0.1	18.4±0.	
• Randall extraction			27.6±0.1	72.4±0.6	31.07±0.68	41.33±0.08	17.4±0.1	20.4±0.2	
Indian major carps (Rohu and Catla)	% fatty acid	89.37	40.50	48.87	23.63	15.86	11.66	4.20	[9]
Gilthead Sea Bream (*Sparus aurata*)	% fatty acid								[11]
• Gut			19.83	76.15	46.05	33.10	12.06	21.04	
• Liver			20.81	77.98	45.77	32.21	13.55	18.66	

FA=Fatty Acid, SFA=Saturated Fatty Acid, UFA=Unsaturated Fatty Acid, MUFA=Monounsaturated Fatty Acid, PUFA=Polyunsaturated Fatty Acid

Table 2 shows that all of the fat in the viscera contains an appreciable amount of ω-3 fatty acids. The concentration of ω-3 fatty acids depends on fish species. Also, viscera organ affects this concentration. A specific organ commonly used for fish oil extraction is the liver. Usually, big-sized fish have a large liver that is easy to separate and extract the oil. Several methods obtain crude fish oil from the viscera. The most common and conventional method is rendering, both wet or dry rendering [24, 25], and solvent extraction [10]. However, other methods are being developed, including infra-red

assisted extraction [15], Randall extraction [10], supercritical fluid extraction CO_2 (SCE CO_2) [10]. Purification after crude oil extraction from fish viscera is required to have an edible oil [26].

Basically, fish viscera are heated in rendering to coagulate protein, and the oil is liberated from the viscera organ tissue. Wet rendering used water as the media for heating, but the dry rendering does not use water. After rendering, fish oil and protein are separated by centrifugation and filtration. The different heating temperature during rendering affects the oil yield, and lower temperature produces less fish oil than higher temperature. The yield of fish oil is affected by fish species, and tilapia viscera have a higher yield than mackerel [25]. Dry rendering of catfish viscera with different temperatures showed that heating at 50°C for 2 hours produced good fish oil quality [24]. Low-temperature rendering is suggested to maintain omega-3 fatty acids, although the duration of heating is longer than high temperature. The disadvantage of rendering is the denaturation of protein due to high-temperature heating that is not suitable for digestive enzyme extraction. However, denatured protein is more susceptible to hydrolyze in preparation of fish viscera protein hydrolysate.

Another conventional fish oil extraction is solvent extraction by several methods such as maceration, Soxhlet extraction, and Randall extraction. This technique is based on the hydrophobicity of oil that is soluble in nonpolar organic solvents such as petroleum ether, hexane, methanol, and chloroform. However, the safety and eco-friendly issues restrict the use of solvent extraction. The choice of the solvent is affected by many factors: cost, low boiling point, availability, flammability, toxicity, and disposal procedures. Soxhlet extractor is usually used to reduce the time for extraction [28]. Randall extraction is the improvement of Soxhlet extraction. A conventional Soxhlet extractor is modified in some aspects to reduce the extraction time. In the Randall extractor, the sample container is porous and first immersed directly into the boiling solvent. In this way, fast wetting of the sample is possible, and soluble components are fast extracted [10].

Emerging technologies to extract fish oil from viscera is infrared (IR)-assisted extraction. Infrared is a part of the electromagnetic spectrum with wavelength ranges from 0.75 to 1000 μm. However, the oil yield is lower than Soxhlet extraction [15]. Principally, IR heating utilizes conductive and radiative heat transfer to generate and transfer heat. The heating by radiation occurs at the surface, and the samples' inside is conductively heated [29]. Compared to conventional heating, the benefits of IR are reduced heating time, high heat transfer coefficient, the small size of infrared equipment, low energy costs, and ease of process control [15]. However, the protein denaturation is possibly to occur due to the high-temperature heating.

Other emerging technologies to extract edible oil, in general, are ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), the use of supercritical fluids (SCE), and enzymatic methods. Microwaves, in MAE, warm the solvent, thus reducing the fish viscera moisture and increasing pressure. This pressure disrupts the cell membranes of the fish and leaches out the oil. This extraction uses a lower temperature and reduces extraction time but may induce oxidative degradation. UAE generates acoustic cavitation to break the cell walls, thus increasing solvent penetration and easing oil release. UAE decreases extraction time but has higher energy consumption and is difficult to scale up [28]. Guo et al. [30] developed an electrical treatment process in which cell membranes are disrupted due to an external electric field. Thus, the oil is easy to release. This method is promising because of low energy consumption and moderate operating conditions,

Among them, SCE CO_2 and enzymatic assisted extraction have been used to extract fish viscera oil (Table 3). SCE CO_2 is an effective fish oil extraction that is an uninvolved solvent, has no environmental hazards, and is safe to consume. Inconsistency data is found when comparing the oil yield to conventional solvent extraction, whether lower [31] or higher [10]. Extraction of oil from the fish liver before rendering increases the oil extractability twice [32]. Protease enzymes assist in protein hydrolysis that eases the release of oil from the tissues.

Table 3. Fish viscera oil extraction by several methods.

Fish Species	Extraction Method	Condition	Result	Ref
Common carp (*Cyprinus carpio var. communis*)	IR assisted extraction	An optimization of independent process parameters of input power (130–250 W), temperature (50–70 °C), and the radiation distance (1–30 cm), on oil yield using the response surface methodology	The optimal extraction conditions were the input power of 168.3 W, the temperature of 70 °C, and a distance of 6.08 cm. Oil yield was 23.73%.	[15]
Cobia (*Rachycentron canadum*)	Enzyme assisted extraction	Extraction by using alcalase, papain, pepsin, and trypsin pre-treatment	The maximum oil extractability was 38% by papain pretreatment. Enzymes improved the extraction, with the oil extractability is twice aqueous extraction (18.8%).	[32]
Not specified	Wet rendering	Comparing two different temperatures with and without centrifugation	High temperature wet rendering can omit centrifugation. Heating time has significant effect on the fish oil yield and the optimum was found at 60 min cooking.	[25]
Squid	Low-voltage direct-current electric field	Electrical treatments were carried out with various electric field strengths (5 V/cm, 10 V/cm, and 15 V/cm) for different time durations	By using this electrical method, the lipid extraction efficiencies reached 71.2 ± 1.0%, 72.1 ± 1.2%, and 71.7 ± 1.5% at electric field strengths of 5 V/cm for 12 h, 10 V/cm for 2.5 h, and 15 V/cm for 50 min, respectively	[30]
African catfish (*Clarias gariepinus*)	SCE CO_2	Optimum points were observed within the variables of temperature from 35 to 80°C, pressure from 10 to 40 MPa, flow rate from 1 to 3 mL/min, and soaking time from 1 to 4 h.	The optimized temperature was 57.5°C, pressure 40 MPa, flow rate 2.0 mL/min and soaking time 2.5 h. The highest oil yields were 67.0% (g oil/100 g viscera). The result was lower compared to solvent extraction by Soxhlet (78.0%)	[31]
Trout (*Oncorhynchus mykiss*)	SCE CO_2 and Randall extraction	SC CO_2 extraction at pressure and temperature of 500±3 bar and 60±1°C. Randall extraction by using Randall apparatus at atmospheric pressure and using n-hexane as a solvent.	The extraction yield of Randall extraction 57% and SCE CO_2 was 79%.	[10]
Indian major carps (Rohu and Catla)	Ensilaging by fermentation	Fermentation with added lactic cultures (*Enterococcus faecium* HAB01 and *Pediococcus acidilactici* K7), 10% inoculum was added to the homogenized viscera containing dextrose (10%) and salt (2%).	Cultured fermentation did not show any advantage over natural fermentation with respect to recovery of oil and no differences were observed in fatty acid composition	[9]

4. Fish viscera protein separation

The methods to produce fish viscera protein concentrate have not been established and soy protein concentrate preparation. Basically, the removal of oil by extraction before protein recovery will increase protein content. Therefore, the preparation of protein from fish viscera is simple by removing oil. The residue of fish viscera oil removal is high in protein.   Other components in the residue are in low quantity, such as ash, and carbohydrates such as glycogen and simple sugars.   Therefore, there is no further treatment to remove non-protein materials from the residue of oil extraction. However, the fish protein concentrate preparation from muscle involves pH adjustment to separate specific protein. The pH is adjusted to around 5.2-5.5 to obtain myofibrillar protein. A highspeed centrifuge is used in fish protein isolate preparation [33].

Hisano et al. [22] study showed that after oil extraction, the protein concentration was 88.78%, and it still contained ether soluble matters of 4,71%. However, the residual protein might consist of different proteins such as sarcoplasmic, myofibrillar, and stroma. Fish protein concentrate is usually prepared from fish muscle or flesh. Meanwhile, viscera and other fish processing by-products are the raw materials for fish protein hydrolysate [33].

5. Digestive enzyme from fish viscera

Enzyme utilizations in food industries are increasing during the last decades, with essential roles in some products such as in meat, dairy, confectionery and cereal products, beverage and bakery industries.

Enzymes are used to improve food quality and food production efficiency by reducing production time and cost. Traditionally, enzymes for food industries are derived from plant, animal, and microbial sources [34]. Microorganisms are being the most important source of commercial enzymes [35].

Other conventional sources of enzymes for the food industry are a great concern to explore. The by-product of food processing is an alternative source of enzymes, including the by-product of fish processing. Viscera has been intensively studied as the source of enzymes. The organs of the fish viscera are the guts, spleen, liver, and pancreas. Guts and pancreas have digestive enzymes, and the liver also contains enzymes, which can be extracted for application in food industries. These enzymes are including protease, trypsin, lipase, and amylase (Table 4). Most of the studies on the extraction of enzymes from fish viscera are the exploration of proteases, mainly trypsin and lipase. Exploration of amylase from fish viscera is restricted because fish limitedly consume carbohydrates. Fish viscera is a good source of protease; hence most of the feed for fish is animal.

Table 4. Extraction of digestive enzymes from fish viscera.

Fish Species	Enzyme	Condition	Result	Ref
Seabass (*Lates calcarifer*)	Lipase	Lipase with a molecular weight of 60 kDa, was purified using ammonium sulfate precipitation and a series of chromatography, including sepharose (DEAE) and Sephadex G-75 size exclusion columns.	The optimal pH and temperature were 8.0 and 50 °C.	[36]
Nile tilapia (*Oreochromis niloticus*)	Lipase	Lipase were partitioned and recovered using a thermo-separating aqueous two-phase system (T-ATPS)	T-ATPS was found to be an attractive technique for the recovery and partial purification of lipase	[37]
Nile tilapia (*Oreochromis niloticus*)	α-amylase	The viscera were homogenized in 10 mM sodium-phosphate buffer, pH 7.5 (200 mg/mL) and centrifuged at 10,000g for 25 min at 4 °C obtaining a crude extract. The enzyme was purified from the crude extract by a three-step procedure	α Amylase was highly stable for 24 h in the pH range 3.0–10.0, and to organic solvents. This enzyme was able to digest different carbohydrates, mainly showing endo-activity	[38]
Giant Amazonian fish pirarucu (*Arapaima gigas*)	Trypsin	The enzyme was purified by heat treatment followed by ammonium sulphate precipitation, molecular size exclusion chromatography (Sephadex G-75) and affinity chromatography (benzamidine-agarose).	The enzyme retained all of its initial activity after 180 min incubation at temperatures up to 45 °C. The presence of Ca^{2+} increased catalytic efficiency. Na^+, K^+ and Mg^{2+} inhibited pirarucu trypsin.	[39]
Vermiculated sailfin catfish, *Pterygoplichthys disjunctivus*	Trypsin	Viscera trypsin was purified by fractionation with ammonium sulphate, gel filtration, affinity and ion exchange chromatography (DEAE-Sepharose)	Trypsin MW was 27.5 kDa and exhibited maximal activity at pH 9.5 and 40°C,	[40]
Giant Amazonian fish pirarucu (*Arapaima gigas*)	Thermostable trypsin	Viscera was extracted by buffer at pH 8.0 and purified by ammonium sulphate precipitation, molecular size exclusion chromatography (Sephadex G-75) and affinity chromatography (benzamidine-agarose)	MW was 28.0 kDa, optimum pH and temperature were 9.0 and 65°C. The enzyme was stable for 30 min in a wide pH range (6.0–11.5) and at 55°C.	[41]
Hybrid catfish (*Clarias macrocephalus X Clarias gariepinus*)	24 kDa Trypsin	Trypsin was purified by ammonium sulphate fractionation and a series of chromatographies including Sephacryl S-200, Sephadex G-50 and DEAE-cellulose	Purified trypsin had MW 24 kDa.. The optimum pH and temperature were 8.0 and 60°C, stable up to 50 °C, and over a pH range o 6.0–11.0	[42]
Sardine (*Sardina pilchardus*)	Trypsin	Viscera was extracted with buffer pH 8.0, fractionated by ammonium sulphate, and purified by gel filtration Shepadex G-100	MW was 25 kDa, the optimum pH and temperature for the enzyme activity were pH 8.0 and 60 °C, at pH 9 the activity was 95.5%. The activity was inhibited by Cu^{2+} and Zn^{2+}	[43]
Farmed giant catfish	Alkaline protease	Enzyme was isolated by using three-phase partitioning (TPP). Viscera was extracted by buffer pH 8.0. The crude enzyme was partitioned by using salts; (NH4)2SO4, K2HPO4, and Na-citrate at the concentration of 50% (w/v) followed by adding t-butanol in the ratio of 1:0.5 (v/v)	The system consisted of crude enzyme extract:t-butanol 1:0.5 (w/v), 50% sodium citrate, pH 8.0 with incubation temperature of 25 C provided the highest enzyme recovery (220%)	[44]

Fish Species	Enzyme	Condition	Result	Ref
Farmed giant catfish	Acid and alkaline protease	The enzymes were extracted by using the aqueous two-phase system (ATPS). ATPS consisting of 20% polyethyleneglycol (PEG1500)–15% $MgSO_4$ or 15% PEG2000–15% $NH_3C_6H_5O_7$ was used for acid and alkaline proteases extraction.	The optimum pH and temperature for acid protease was 3.0 and 40℃, while alkaline protease was 9.0 and 60℃. High pH stability of the enzymes was found in the ranges of 1.0–5.0 and 8.0–12.0 for acid and alkaline proteases,	[45]
Farmed giant catfish (*Pangasianodon gigas*)	Proteases	Three-phase partitioning (TPP) was used to recover proteases. Different ratios of crude enzyme extract to t-butanol (1.0:0.5, 1.0:1.0, 1.0:1.5, and 1.0:2.0; v/v) and concentrations of ammonium sulfate (($NH_4)_2SO_4$) (20, 30, 40, and 50%; w/v) were investigated.	The best protease yield (163%) was obtained in the crude extract to t-butanol ratio of 1.0:0.5 in the presence of 50% $(NH_4)_2SO_4$. The MW was 25 kDa, corresponding to trypsin.	[46]
Sardinelle (*Sardinella aurita*)	Aspartic protease	The enzyme was extracted at pH 3.0 by adding 0.1 M HCl for 45 min. Crude enzyme was extracted by ammonium sulphate and purified with Sephadex G-100 gel filtration	The MW was 17 kDa, the optimum pH and temperature was pH stability between 2.0 and 5.0, the activity decreased 50% at 50℃ for 30 min.	[47]
Bolti fish (*Tilapia nilotica*)	Acidic protease	Protease was extracted from acetone powder in acidified distilled water and precipitated by ammonium sulfate followed by dialysis. The crude enzyme was purified using gel filtration	Purification activity were increased after purification. MW of 31.0 kDa. The optimal pH and temperature were 2.5 and 35℃. The pH stability between was 2 and 6.	[48]

Generally, to obtain enzymes from fish viscera is by extraction by using a buffer in a certain pH. The pH used determines the type of extracted enzymes. For example, trypsin is generally extracted at pH 8. Another protease is extracted at pH 3.0 to obtain aspartic protease. Most of the methods of enzyme extraction use ammonium sulphate to precipitate the enzymes. Purification is conducted by using gel filtration, ion exchange, or affinity chromatography (Table 4). Some modifications have been conducted to improve the enzyme extraction and purification from fish viscera. A series of chromatographic purification was employed to catfish viscera [40, 42] and giant Amazonian pirarucu [41]. Most of the protein separation by using salt precipitation after buffer extraction. This process produce crude enzyme and purification was used to increase their enzyme activity. Modification in general enzyme extraction from fish viscera have been performed such as by aqueous two-phase system (ATPS) [45], thermo separating ATPS [37], and butanol extraction [46]. All of this process required further study to establish the suitable industrial application in more large quantity extraction.

The characteristics of the enzyme from fish viscera are affected by fish species. The similar enzyme but different fish species reveals different characteristics. Therefore, the characterization of the enzyme is very important to establish the application of a specific enzyme from fish viscera. In general, the superiority of fish lipases over mammalian lipases is a cold-adapted property that is suitable to use in low-temperature processing [36]. In the last two decades, lipases have become increasingly important for industrial applications in the detergent, food, pharmaceutical, biodiesel, and bioenergy industries. Furthermore, lipases are costly enzymes; therefore, the use of by-products might reduce their price [37].

The uses of proteases in the food industry are in the dairy industry for cheese manufacturing and milk coagulation, baking industry to reduce protein content, brewing industry for malt and yeast growth improvement, and meat industry for many functions [35]. However, the most important proteolytic enzymes in the viscera of fish are serine proteases (chymotrypsin, trypsin, elastase, and collagenase) and aspartic protease (pepsin) [46].

The method of enzyme purification is expensive and difficult to scale up to the industrial level. Therefore, Ketnawa et al. [44] developed three-phase partitioning (TPP) for bio-separation that uses ammonium sulphate to precipitate protein. A three-phase layer was created by adding t-butanol to remove small molecular weight compounds. The enzyme would be in the interface. Meanwhile, contaminants primarily partition in either the top phase (the t-butanol) or bottom phase (the aqueous phase). This technique is scalable and directly can be used with crude suspensions. However, it is a great challenge to extract the enzymes from fish viscera at an affordable cost. The integrated process for

enzyme extraction and the utilization of the residue is interesting to explore.   Deep characterization is also required to establish a suitable application in industries.

6. Fish viscera protein hydrolysate

Fish viscera protein hydrolysate is the product of protein hydrolysis that cleavage the peptide bonds to obtain free amino acids or peptides with lower molecular weight.   Currently, protein hydrolysates are being produced at the industrial level in enormous quantities for several purposes, such as ingredients in foods with a particular functional property, nutritional supplements, and enrichment of food with protein.   Chemical and biological approaches are mainly applied to produce fish protein hydrolysate. Chemical methods by acid and alkaline hydrolysis are easy to use and comparatively cheap, but the disadvantages are unspecific peptides produced and a severe reaction [33].

Conventionally, acid hydrolysis is based on the treatment of fish viscera with excessively acidic solutions at high temperatures and might be accompanied by high pressures over a certain time.   The advantages of acid hydrolysis are quick, simple operation, and low cost; thus, it is applicable at the industrial scale. However, some essential amino acids, such as cysteine, methionine, tryptophan, and cysteine, might be destroyed.   The lack of this method is poor functional properties of protein hydrolysate because of salt formation after the neutralization.   Salt removal is proposed by several methods, such as ion exchange and nanofiltration.   Similar to acid hydrolysis, alkaline hydrolysis is also a simple process.   Fish viscera is mixed with alkaline solutions and heated at a certain temperature until hydrolysis is reached.   Due to alkaline conditions, some amino converts into lysinoalanine and lanthionine [6].

Biochemical methods are autolysis and enzymatic hydrolysis.   Autolysis involves endogenous digestive enzymes available in the fish.   This method is safe, economical, and simple, but the limitations are the occurrence of specific digestive enzymes and their concentration that is not easy to control. Digestive enzymes in the viscera are responsible for hydrolyzing the fish protein.   Exogenous enzymes are usually used to overcome the limitation of autolysis.   This technique is one of the most appropriate and highly applicable.   Numerous proteolytic enzymes commonly used are papain, pepsin, pancreatin, alcalase, trypsin, pronase, protamex, bromelain, validase, flavorzyme, neutrase, thermolysin, protease A, cryotin F, protease N, and orientate [33].

Different from chemical methods, enzymatic hydrolysis is easy to control and uses mild conditions. The specific activity of enzymes cleavages more precise peptide bonds.   The mild condition also controls undesirable side reactions.   This method also increases protein recovery and eases the purification of peptides.   The enzymatic method is currently of great interest for industrial applications because the products are more consistent and more defined with specific nutritional, biological, and functional properties.   Table 5 shows that most studies about preparing fish viscera protein hydrolysate involve exogenous enzymes or endogenous enzymes by autohydrolysis.

Table 5. Fish viscera protein hydrolysate preparation.

Fish Species	Enzyme	Condition	Result	Ref
Rainbow trout (*Oncorhynchus mykiss*)	Autolysis	Autolysis at fixed temperatures (40, 50, 60 C) or gradually increasing temperatures (40-60 °C) with different times)	Autolysis for 1 h at lower temperature (40 °C) was sufficient to produce autolysates rich in small peptides from rainbow trout by-products	[52]
Rainbow trout (*Oncorhynchus mykiss*)	Autolysis	Rainbow trout by-product was minced and subjected to different preparing methods including simple distilled water washing, calcium chloride + citric acid washing or their combinations, prior to hydrolysis.	Different pretreatments influenced oxidation of rainbow trout byproduct during hydrolysis. Oxidation was influenced by the washing methods.	[53]
Red tilapia (*Oreochromis* spp.)	Alcalase	A substrate concentration of 8 g protein/L, an enzyme / substrate of	Enzymatic hydrolysis with Alcalase is	[51]

Fish Species	Enzyme	Condition	Result	Ref
		0.306 (U/g of protein). The reaction was carried out at 60°C, at a pH of 10 and a constant agitation of 960 rpm	an efficient technique to produce hydrolysates with antioxidant capacity. The highest degree of hydrolysis of 42.6%. The most active peptides have a molecular weight of <1 kDa.	
Pangasianodon hypophthalmus	Papain, pepsin	pH of 2.0, 110 °C for 24 h. After completing hydrolysis process, pH was adjusted to 7.0.	Pepsin demonstrated the better results followed by papain	[16]
Common smooth-hound (*Mustelus mustelus*)	Esperase*	Viscera proteins were enzymatically hydrolyzed, using Esperase*, and the resulting hydrolysate was fractionated by ultrafiltration	Five fractions were obtained, FI (> 50 kDa), FII (5–50 kDa), FIII (3–5 kDa), FIV (1–3 kDa) and FV (< 1 kDa). Fractions with low MW peptides contained the highest amounts of hydrophobic amino acids (39.63 and 41.68%) and the strongest ACE-inhibitory activity	[52]
Persian sturgeon (*Acipenser persicus*)	Alcalase	Hydrolysis was performed at 35, 45 and 55°C, pH 8.5, using alcalase and an enzyme to substrate ratio of 0. AU/g viscera protein for a 205 min incubation.	Protein recovery and degree of hydrolysis ranged from 34.97% to 61.96% and 13.32% to 46.13%, respectively. The highest degree of hydrolysis was observed at 55°C after 205 min.	[53]

Table 5 shows the methods of protein hydrolysate preparation from the viscera of several fish species. The studies about fish viscera protein hydrolysate are objected to having bioactive peptides with a certain health function, such as the antioxidant activity of angiotensin-converting enzyme inhibitory activity. Therefore, an additional step is required to prepare fish viscera protein hydrolysate with specific activities. According to Gao et al. [23], these hydrolysates are purified by chromatographic and membrane separation methods to obtain bioactive peptides. Bioactive peptides from fish protein hydrolysate have biological activities, including lipid homeostasis modulation, antioxidative, anti-inflammatory, neuroprotective, antihypertensive, and anticancer activities. These bioactive peptides are promising nutraceutical ingredients for food application.

7. Conclusions
Valorisation of fish viscera by integrated and simultaneous separation of valuable components from fish viscera is a challenge to be further studied. Some emerging technologies are suitable to increase oil recovery in fish viscera oil extraction. Protein concentrate is the residual of oil extraction and as raw materials for fish viscera protein hydrolysate. The characterization of this hydrolysate is required for suitability in food application. Bioactive peptides from hydrolysate might be separated by advanced separation such as membrane or chromatography, and these compounds are very important in nutritional application. Maintaining of native protein during oil extraction is important for digestive enzyme extraction. It is still challenging to develop affordable, simple, and easy to scale up all valuable compounds extraction from fish viscera.

8. Acknowledgement

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