Food Production, Processing and Nutrition

REVIEW

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Salmon processing discards: a potential source of bioactive peptides – a review

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Abstract

Salmon aquaculture generates 80% of the total revenue of finfish aquaculture across Canada. Salmon farming is carried out in a multilevel process, and at least 60% of the total production is considered as by-products, including skin, head, viscera, trimmings, frames, bones, and roes. These by-products are an excellent source of protein, which can be converted to protein hydrolysates through enzymatic hydrolysis and non-enzymatic processes such as chemical hydrolysis (acid and alkaline) in order to utilize them into value-added products. Several studies have reported that peptides from salmon protein hydrolysates possess bioactivities, including antihypertensive, antioxidant, anticancer, antimicrobial, antidiabetic, anti-allergic, and cholesterol-lowering effects. Incorporating in silico computational methods is gaining more attention to identify potential peptides from source proteins. The in silico methods can be used to predict the properties of the peptides and thereby predetermine the processing, isolation, and purification steps that can be used for the peptides of interest. Therefore, it is essential to implement robust, standardized, and cost-effective processing techniques that can easily be transferrable and scale up for industrial applications in view of circular economy and upcycling concept. This contribution summarizes the latest research information on Atlantic salmon, production statistics, growth lifecycle, processing, protein production techniques, nutritional and functional properties, peptide production and purification processes, as well as potential health benefits as a nutraceutical product.

Keywords Atlantic salmon, Aquaculture, Protein hydrolysate, Bioactive peptide, *In-silico* production, Health effect

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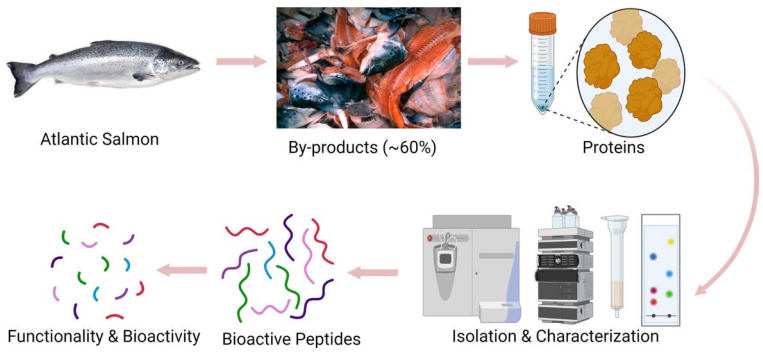
Graphical Abstract



Introduction

Fish is an important source of nutrition for millions of people around the world. Wild fisheries alone cannot sustain the global population growth; hence, aquaculture has provided a means to grow fish in a controlled environment. In both cases, the landed fish is processed, and the fillets so produced are sold for human consumption. However, fish processing produces various by-products, including head, skin, trimmings, fins, frames, viscera, bones, and roe, accounting for 60% of the total processing output (Chalamaiah et al. 2012; Dekkers et al. 2011). These processing by-products are often dumped in the ocean or landfills as waste in some parts of the world. In Canada, the USA, Norway, China, and many other countries, some of these by-products are sold at low cost to various industries for animal feed and fertilizer production. For example, Sandbakken et al. (2023) suggested that salmon protein hydrolysates were a promising novel feed ingredient for Atlantic salmon due to their excellent amino acid profile, high digestibility, and exclusion of identifiable prions. In addition, Canadian processors export fish heads to various markets as food delicacies (Coppola et al. 2021; Stevens et al. 2018).

These by-products are an excellent source of proteins, lipids, minerals, polysaccharides, and carotenoids. However, it is possible to increase the value of fish by-products by using a gentle, environmentally-friendly processes to produce high-quality products such as protein, oil, and minerals as primary products, which could be further processed and purified into omega-3 fatty acids, collagen, gelatin, bioactive peptides, enzymes, hydroxyapatite, and minerals (de la Fuente et al. 2023; Ghaly et al. 2013; Routray et al. 2018). Several proteolytic enzymes such as Alcalase, Flavourzyme, Pronase, Neutrase, Protamex, Sea-B-Zyme L200, Validase, bromelain, papain, pepsin, trypsin, chymotrypsin, pancreatin, and thermolysin can be used to hydrolyze fish by-products/ processing discards (Chalajmaiah et al. 2012, 2018, 2019; Harnedy & FitzGerald 2012; Kristinsson & Rasco 2000a, 2000b; Neves et al. 2017a, 2017b; Opheim et al. 2015; Thorkelsson & Kristinsson 2009; Yarnpakdee et al. 2014, 2015). Protein hydrolysates are also produced along with oil, bones, and sludge upon enzymatic hydrolysis of fish by-products. During hydrolysis, fish by-product proteins are solubilized, and the peptide bonds are cleaved, generating smaller peptides. These peptides have a smaller molecular size with more ionizable amino and carboxyl groups (Ishak & Sarbon 2018; Kristinsson & Rasco 2000c). The amino acid composition of fish proteins is well-balanced and provides high nutritional value for humans. In addition, the isolated protein hydrolysates have demonstrated various bioactivities, including antihypertensive, antioxidant, anticancer, antimicrobial, antidiabetic, anti-allergic, cryoprotectant, and cholesterol-lowering effects. Therefore, fish by-products play a vital role in developing various nutraceuticals and functional food products that can prevent or enhance the management and treatment of human diseases and maintain optimal human health (Ishak & Sarbon 2018; Opheim et al. 2015). To the best of our knowledge, this is the first literature review that attempts to thoroughly summarize the research work carried out on Atlantic salmon in order to produce bioactive peptides. It discusses the production status of Atlantic salmon in Canada, including Newfoundland, its production lifecycle, processing, and current utilization. It also reviews different methods of protein hydrolysate production and nutritional composition and functional properties of the resultant products. Finally, different bioactive production techniques, purification, separation, and characterization of peptides isolated from salmon

processing discards are reviewed. In addition, the important health benefits and the commercial approval process of salmon peptides are provided.

Salmon production

In Canada, salmon is produced by commercial fisheries (fresh and seawater), including wild capture and aquaculture. Canada is the fourth-largest producer of farmed salmon in the world. Three types of salmon, including Atlantic salmon (*Salmo salar*), Chi-nook salmon (*Oncorhynchus tshawytscha*), and Coho salmon (*Oncorhynchus kisutch*), are farmed in Canada. The United States of America is the largest export market for salmon farmed in Canada. Canada's aquaculture industry is a significant employer and economic driver in many coastal, rural, and Aboriginal communities. According to the Department of Fisheries and Oceans (DFO) Canada and Statistics Canada, the average annual finfish produced between 2008 and 2021 was 131,316 tonnes, with an average annual value of $947 million (Fig. 1) (DFO 2021). The corresponding average shellfish production was 38,719 tonnes, with an average annual value of $85 million. Salmon, along with mussels, trout, scallops, oysters, clams, and other finfish species, is farmed in various Canadian regions (Fig. 2). The production statistics between 2008 and 2021 indicate the dominanc of salmon among the majority of finfish aquaculture, with an average annual production of 111,752 tonnes valued at $781 million. Salmon aquaculture generates 80% of the total revenue of finfish aquaculture in Canada (DFO 2021). These statistics further reflect the importance of salmon aquaculture to the overall fisheries in Canada.

Newfoundland and Labrador's (NL) aquaculture has been steadily growing in the last decade, with a total finfish production of 19,635 tonnes and a value of $144 million in 2021 (Fig. 3) (DFO 2021). Newfoundland's aquaculture industry is dominated by salmon and along with small quantities of steel-head trout and mussels. The NL salmon aquaculture produced 15,904 tonnes, valued at $138 million in 2021 and accounted for 82% of the total production and 96% of the total aquaculture revenue (GovNL 2021).

Atlantic salmon production lifecycle

Salmon farming is carried out in a multilevel process, as illustrated in Fig. 4. In the first step of production, salmon eggs are collected from the broodstock (parent fish) selected from the best-performing fish on a sea farm and are moved into freshwater tanks or cages, usually during the autumn. The eggs are fertilized by mixing them with milt from mature male fish and nurtured at a hatchery in freshwater tanks for fifteen months. During the time inside the freshwater hatchery, the eggs hatch and young salmons are called "alevin". The size of the alevin is about 2 cm in length. Then the young salmon enters the second stage called "fry". In this stage, the salmon fry comes up

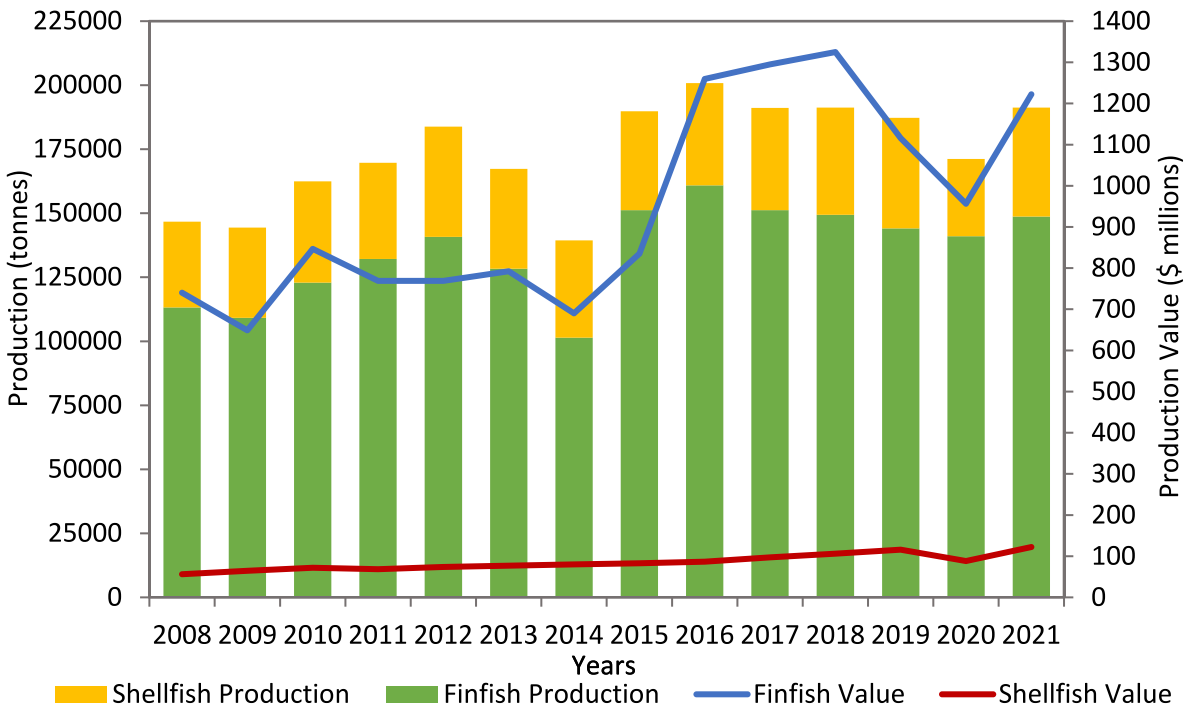


Fig. 1 Canadian finfish and shellfish production and values (2008–2021)

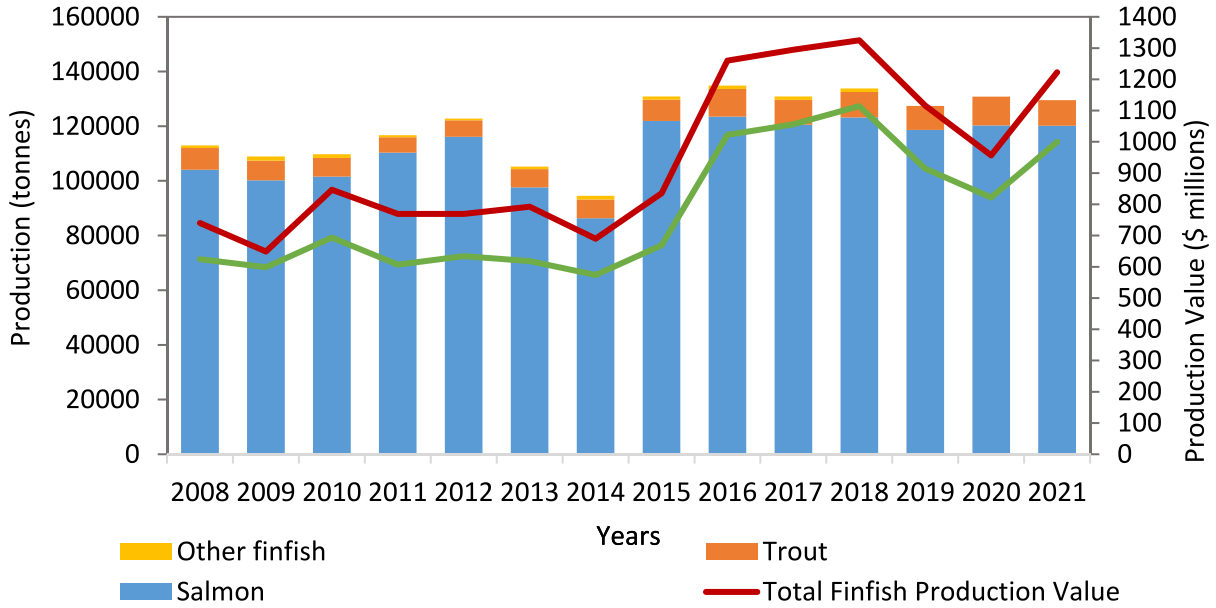


Fig. 2 Various types of Canadian finfish production and values (2008–2021)

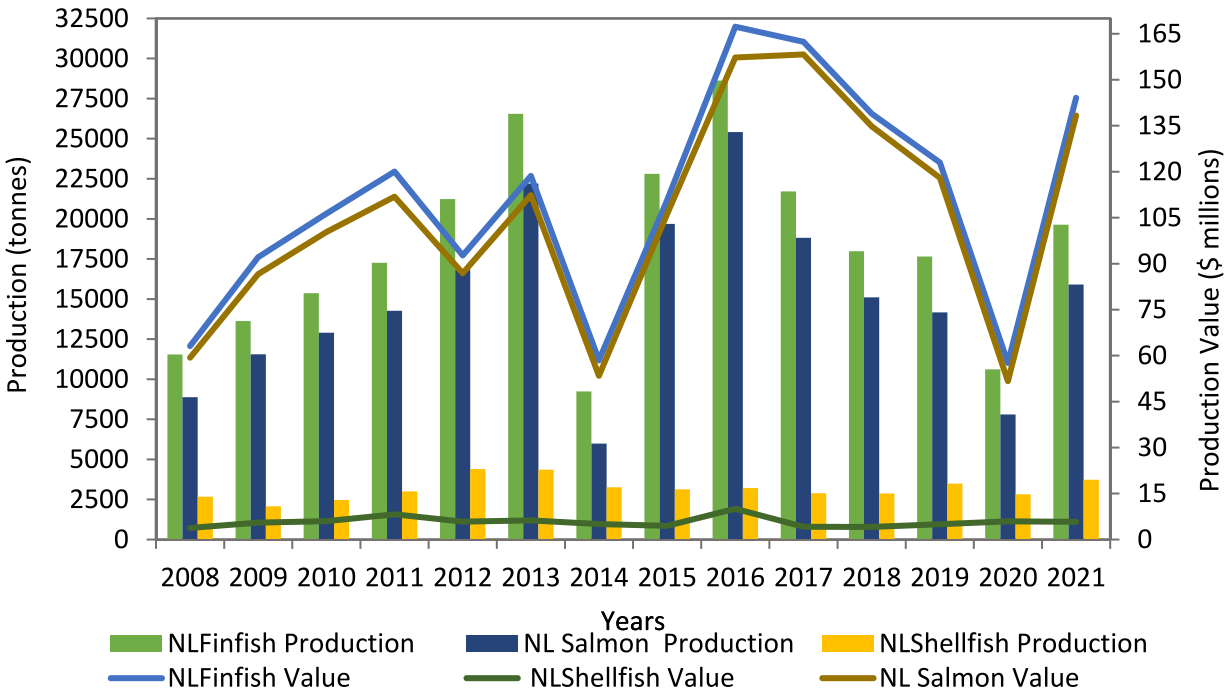


Fig. 3 Newfoundland and Labrador finfish, salmon and shellfish production and values (2008–2021)

from the gravel and starts feeding on microscopic life in the stream, and it grows to a length of 5–8 cm (Seafish 2011).

The fry matures and starts to feed (early spring), and then transferred to small tanks in the hatchery. At 12 months (second spring), salmons undergo physiological transformation to reach a weight of 80–120 g, and the process is called smoltification. The smolts are placed in specialized grow-out farms where they are raised to marketable size in sea pens. The biomass development of Atlantic salmon depends on the season and hence smolts are usually released into the

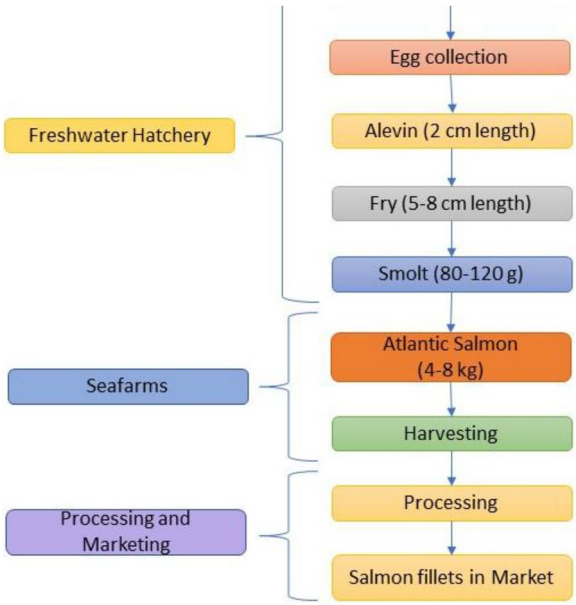


Fig. 4 The lifecycle of Atlantic salmon in aquaculture

seawater during the warmer half of the year. Usually, under normal circumstances, high mortality rates of smolts are observed in the first two months after release to the seawater. The mortality is due to the smolt body's inability to absorb salt at the release time. The salmon are fed for 12–18 months prior to harvesting. Salmon are harvested once they reach a marketable weight of 4–8 kg (ACCFA 2021; DFO 2019; MOWI 2019). During harvesting, fish are starved inside the sea farms for 2–3 days, and then transported to the slaughter plant alive. The fish is electrically stunned in the slaughter plant with a blow to the head, followed by bleeding and immersing in iced water. The fish is then gutted, washed, and chilled to maintain the flesh temperature at 3 °C. Then depending upon the market, the salmon is sold as a whole or filleted, and head and frames are sold as raw materials for animal feed or pet food manufacturing. In another type of harvesting, salmons are pre-processed on board before being brought to the processing plant. In this case, harvested salmons are directly immersed in carbon dioxide-rich water for a short time and processed onboard by quickly rupturing their arteries and transferring them into ice-filled containers. The bled salmons are transported in ice to the processing plant and gutted, washed, chilled, graded by weight and packaged for the market. The salmon is harvested before it reaches maturity at 28 months after the first hatch. Salmon do not die after reaching sexual maturity. However, there is a degradation in quality due to spawning. Upon spawning, harvesting has to be delayed for one year (Borderías & Sánchez-alonso 2011; Nesse & Frida 2014; Seafish 2011).

Atlantic salmon processing and utilization of by-products

After harvesting, the salmon is processed in the processing plant, and the fish is sold as head-on-gutted, butterfly fillets, skin-on fillets, or skinless fillets, depending on the market requirement. In the latter case, the salmon skins are discarded as waste. Salmon heads and frames are mostly shipped to food and feed processing industries for low-end utilization, such as pet food, mink feed, and fertilizer. Salmon guts are usually dumped as waste. However, these by-products can be utilized in a far better manner to produce various high-value nutraceuticals and other industrial products, including protein, fish oil, bio-diesel, collagen, gelatin, bioactive peptides, enzymes, hydroxyapatite and minerals (Dave & Routray 2018; Ghaly et al. 2013). Thus, valorization of fisheries resources could be achieved by upgrading them into high-value products (Fig. 5). The chain of events shown in Fig. 5 starts from salmon by-products to fish meal to fish protein isolates to fish protein hydrolysates and finally to bioactive peptides with potential health effects. Fish meal and silage already exist as high-volume products with relatively low prices (Thorkelsson & Kristinsson 2009).

Protein isolates can be produced by employing a pH shift technique. In this technique, fish mince is solubilized with acid (below pH 3.5) to extract myofibrillar proteins. The lipids are skimmed, denser impurities are sedimented, and soluble protein is extracted by centrifugation. The supernatant is subjected to isoelectric precipitation at a pH of 5.2–5.5 (isoelectric point) to precipitate myofibrillar proteins. Finally, protein isolates are extracted by washing. Protein isolates provide more value addition when incorporated into seafood products (Martín-Sánchez et al. 2009; Nolsøe & Undeland 2009). The goal of moving towards the value chain in the form of salmon protein hydrolysates and bioactive peptides is often challenging in most parts of the world. The production of protein hydrolysates and bioactive peptides is relatively expensive compared to low-end products. These products are low-volume and high-value with documented health effects in the various scientific literature. The transformation of salmon processing by-products into high-value products can revitalize many rural fish communities, fish producers, and processors. However, the production of these high-value products requires highly qualified personnel with technical expertise and skills in the field, which can be challenging at remote locations (Chalamaiah et al. 2012; Thorkelsson & Kristinsson 2009). Besides, by-products variation in terms of amount and composition is common during salmon processing due to a diverse set of operations at various steps. The variable quality of the by-products (raw materials), in some

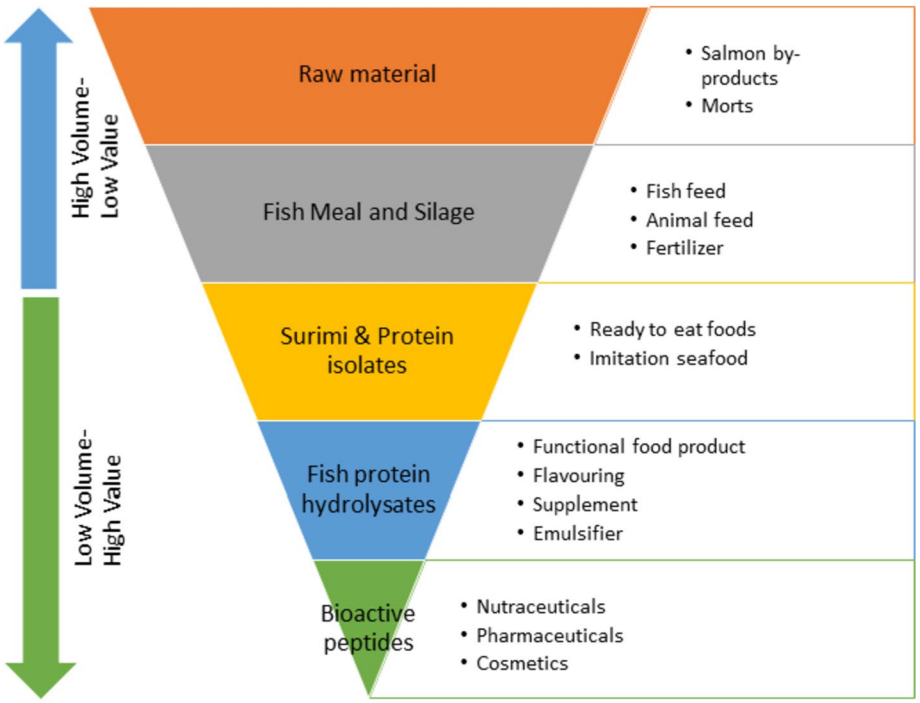


Fig. 5 Value-added utilization of salmon processing by-products

cases, makes them unfit to produce high-value products. Therefore, several steps need to be implemented to prevent raw material spoilage when the immediate production of protein hydrolysates is not possible. The spoilage of salmon by-products can be prevented by low-temperature storage (chilling), gutting, controlling water activity, or by adding salt and sugar (to bind free water molecules to create an osmotic balance) and the addition of food-grade antioxidants. Furthermore, to produce high-quality salmon protein and peptides, fresh raw materials without any oxidative changes, heating, pH change, and freezing are recommended. Any chemical and physical changes in the raw material during storage can lead to variations in amino acids, digestibility, and functional properties of the final product (Ghaly et al. 2010; Thorkelsson & Kristinsson 2009).

Chemical composition of Atlantic salmon

The proximate composition of salmon body parts is summarized in Table 1. The highest protein content (20.9%) is in the fillet portion of salmon. In contrast, the highest lipid (27%) and ash (1.94%) contents are present in salmon's frame/trimmings portion. Salmon fillets also contain a considerable amount of lipid (21.30%), with essential polyunsaturated fatty acids (PUFAs) and astaxanthin pigments responsible for the orange color of the fillets.

Lipid and fatty acid composition

The Atlantic salmon contains 17.30–22.12% lipid, which is mainly composed of both non-polar (neutral) and polar lipids. Neutral lipids, mainly triacylglycerols (TAGs), are dominant in salmon lipids. Meanwhile, salmon oil contains phospholipids (PL) as its intramuscular lipid (Kralovec et al. 2012). The salmon body parts, including the head, frame, and internal organs/ gut, contribute to the

Table 1 Proximate composition (%) of salmon body parts

Sample	Moisture	Protein	Lipid	Ash	References
Gut	50–60.45	10.38–13.8	22.12–30	1.8–1.94	(Aas, et al. 2022; Dave et al. 2014; Malcorps et al. 2021)
Head	62–63.35	11.31–14	17–21.86	3.51–4.4	
Frame/ trimmings	55–57.18	16.36–19	22.65–27	3.64–6	
Fillet	68.80–75	18.7–20.90	11.30–21.3	1.80–2.2	
Whole fish	64.50–75	16.7–17.90	17.30–22.12	1.88–2.2	

composition of salmon oil. According to a recent study, salmon oil extracted from the head, frames, and gut contained an average TAG content of 90.1 and 78.1%, respectively (Liu et al. 2020). In another study, the crude oil extracted from farmed Atlantic salmon viscera contained 92.83% TAG (Dave et al. 2014). Polvi and Ackman (1992) reported 84–90% TAGs and 0.2–5.2% free fatty acids (FFA) in total lipids in cultured Atlantic salmon muscle, depending on the diet. Depending on the geographic location, harvesting season, storage conditions, processing, oxidation parameters, and extraction technique, salmon oil has different levels of phospholipids and FFA. Liu et al. (2020) reported an average phospholipid content of 2.1 and 3.8% in the oil extracted from salmon heads and frames, as well as guts, respectively. The study also indicated that a higher amount of FFA was present in the oil extracted from the gut (11.7%) compared to that from head and frames (1.5%). Dave et al. (2014) reported 1.43% phospholipids and 1.23% FFA in the crude oil extracted from farmed Atlantic salmon viscera. On the other hand, Polvi and Ackman (1992) reported 10–12% phospholipids and 0.2–5.2% FFA in total lipids in cultured Atlantic salmon muscle, depending on the diet.

The Atlantic salmon oil is mainly composed of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Dave et al. (2014) studied the fatty acid composition of oil extracted from by-products, including gut, head, and frame of farmed Atlantic salmon. The results indicated that the salmon gut, head, and frame contained saturated fatty acids (SFAs; 19.21–21.93 g/100 g), MUFAs (36.82–39.58 g/100 g), and PUFAs (38.89–39.83 g/100 g). The highest SFAs, MUFAs, and PUFAs present in the gut, head, and frame were palmitic acid, oleic acid, and linoleic acid. The omega-3 fatty acids, including eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), were present in all three parts of salmon. Similar results were reported elsewhere (Głowacz-Różyńska et al. 2016; Haq et al. 2017; Liaset et al. 2003).

Protein and amino acid composition

The Atlantic salmon contains 19.1–20.4% protein, which is mainly composed of glutamic acid (5.96–6.46%), aspartic acid (4.63–4.92%), lysine (4.59–4.77%), leucine (3.78–4%), and valine (2.81–2.92%). Usually, farmed salmon has a lower total amino acid content than the wild salmon on a dry wet basis, though these are almost similar on a wet weight basis (Colombo & Mazal 2020). However, the content of amino acids is mainly dependent on the harvesting location, season, and processing techniques. For example, the protein contents of Atlantic salmon during different culinary treatments such as boiling, steaming, and oven-cooking were 16.69, 18.9, and 20.59%, respectively, while the raw fish had 14.73% protein (Fomena-Temgoua et al. 2022). They also reported that the major amino acids in cooked and raw salmon were aspartic acid and alanine. In particular, in cooked salmon, most essential amino acids, namely isoleucine, histidine, threonine, lysine, and valine, were significantly higher in raw salmon.

Production of protein hydrolysates

Traditionally, protein hydrolysates are prepared using several chemical extraction methods. However, techniques such as enzyme hydrolysis, microwave-assisted extraction, ultrasound-assisted extraction, high-pressure processing-assisted extraction, supercritical fluid extraction, pressurized solvent extraction, pulsed electric field-assisted extraction, and fermentation are preferred (Cheung et al. 2015; Senadheera et al. 2023). Protein hydrolysates obtained from these processes are used in the food industry as milk replacers, protein supplements, stabilizers in beverages, and flavor enhancers, among others.

Chemical hydrolysis

The chemical methods are carried out using acid and alkaline hydrolysis and salt extraction. One of the chemical methods still used in fisheries on a large scale is ensilaging. In the ensilaging process, spoiled fish, disease-affected morts, underutilized species, by-products from marine fishing, commercial fish waste, and industrial residues are mixed in large containers in the presence of various acids such as formic acid, which activates endogenous enzymes and the pH is maintained at 3–4 for several days. During this process, the oil produced is collected and used for industrial purposes and/or as a feed ingredient if the silage is protected with antioxidants. Fish silage is an excellent protein product mixed with other ingredients and used for feed and fertilizer purposes (Ghaly et al. 2013).

Enzymatic hydrolysis

Enzymatic hydrolysis is the process of adding enzymes to fish material to break down tissues and produce protein hydrolysates, which mainly improves physiochemical, functional, and sensory properties of the native protein. Enzymatic hydrolysis process is carried out under mild conditions and does not produce hydrolytic degradation products via racemization reactions common in acid and alkaline hydrolysis (Hossain et al. 2022a; Kristinsson & Rasco 2000d). Enzymatic hydrolysis process is usually carried out using different types of proteases. The proteases are categorized according to the specificity of their attack and mechanism against peptide bonds in the substrate. Based on the availability of structural and mechanistic information the proteases are classified into

six distinct classes: aspartic, glutamic and metalloproteases, cysteine, serine, and threonine proteases. Among these, aspartic, glutamic, and metalloproteases utilize activated water molecules as a nucleophile to attack the peptide bond of the substrate, whereas, in cysteine, serine, and threonine proteases, the nucleophile is an amino acid residue (C, S, or T, respectively) located in the active site (López-Otín & Bond 2008). According to the catalytic mechanism, they are categorized as endopeptidases and exopeptidases. The endopeptidases hydrolyze the peptide bonds within protein molecules to produce relatively large peptides. The exopeptidases break the peptide bonds from either the N terminus (also called aminopeptidases) or the C terminus (also called carboxypeptidases). In some cases, both endopeptidases and exopeptidases are used for complete and effective hydrolysis of peptide bonds (Kristinsson & Rasco 2000d; Senadheera et al. 2021; Shen & Chou 2009). However, enzymatic treatment is not always the choice of hydrolysis due to its long sample treatment time, high cost, and low analyte recoveries, among others (Kristinsson & Rasco 2000d).

Enzymatic processing of salmon by-products helps producing a broad spectrum of food ingredients and industrial products for many applications. Several proteolytic enzymes are often used to hydrolyze fish protein hydrolysates. Some commercially produced proteases include Alcalase, Flavourzyme, Pronase, Neutrase, Protamex, protease N, protease A, Orientase, Sea-B-zyme, thermolysin, and Validase. In addition, there are other various natural proteases such as chymotrypsin, pancreatin, papain, pepsin, trypsin, bromelain (Chalamaiah et al. 2012; Hossain et al. 2022a). The muscle proteins present in salmon by-products are sensitive to various process parameters, including temperature, heating rate, pH, salt, ionic strength, oxidizing agent, and non-meat ingredients. Improper storage of salmon by-products and lack of controllable environment leads to poor protein quality (Abdollahi & Undeland 2019; Xiong 2004).

The general hydrolysis procedure to obtain protein hydrolysates from salmon by-products is shown in Fig. 6. The first step in the enzymatic hydrolysis is the collection and processing of salmon by-products. During processing, salmon by-products are minced in a pilot-scale mincer producing particle size of less than 0.5 mm. During processing, salmon guts are always recommended to be processed separately due to relatively high amounts of endogenous enzymes, which could cause storage instability. To preserve the quality of raw materials and increase storage stability, food-grade antioxidants can be added before mincing. Minced salmon by-products are subjected to the hydrolysis process. During the hydrolysis process, various parameters are monitored and controlled ac-cording to optimum conditions. The hydrolysis parameters include reaction time, reaction temperature, type of enzyme, pH of the reaction system and enzyme concentration.

These parameters are optimized on a lab scale, and the process can be scaled up to the pilot level under optimum conditions. After hydrolysis, the reaction mixture is heated at 90 °C for 10 min, or the pH of the process is adjusted to deactivate the enzyme. The reaction mixture is subsequently subjected to separation techniques such as centrifugation on a lab scale or using a decanter on a large scale. After separation, two main products, salmon oil, and salmon protein hydrolysate, are obtained along with sludge and emulsion. The protein hydrolysate is then subjected to filtration to remove any suspended impurities and the oil. The filtered hydrolysate is then diafiltered to remove salt from the liquid. Some processes also include the pasteurization of protein hydrolysate to eliminate microorganisms. The diafiltered protein hydrolysate is then spray-dried to obtain purified salmon protein powder. The crude salmon oil obtained after hydrolysis is flushed with nitrogen, and food-grade antioxidants are added before storage at -30 °C. However, it is recommended to refine the oil immediately to preserve the quality of the oil. The traditional refining of salmon oil includes refining, bleaching, and deodorization, possibly followed by winterization and the addition of antioxidants (Abdollahi & Undeland 2019; Dave et al. 2014; Hou et al. 2017; Petrova et al. 2018; Ramakrishnan et al. 2013). Some of the outcomes reported in the literature on the preparation of protein hydrolysates from salmon by-products are shown in Table 2.

Ultrasound-assisted enzymatic treatment

The ultrasound-assisted enzymatic treatment is the process of using ultrasound waves exceeding 20 kHz. The application can be divided into low power-high frequency (100 kHz-1 MHz) and high power-low frequency (0–100 kHz) ultrasound. Low-power ultrasound is mainly used in medical diagnosis, whereas high-power ultrasound is used to disrupt cell walls and membranes (Grosso et al. 2015; Kadam et al. 2015). Ultrasound waves are mechanical waves that can be propagated by rarefactions and compression through solid, gas, and liquid media. It acts by generating bubble cavitation in the biological matrix.

For example, Kangsanant et al. (2014) studied the bioactivities of tilapia protein hydrolysates (TPI) extracted using ultrasound pretreatment and ultrasound-assisted enzymatic hydrolysis. The results from this study indicated that ultrasound-assisted enzymatic hydrolysis caused a reduction in the degree of hydrolysis, ranging from 23 to 35% relative to that of the

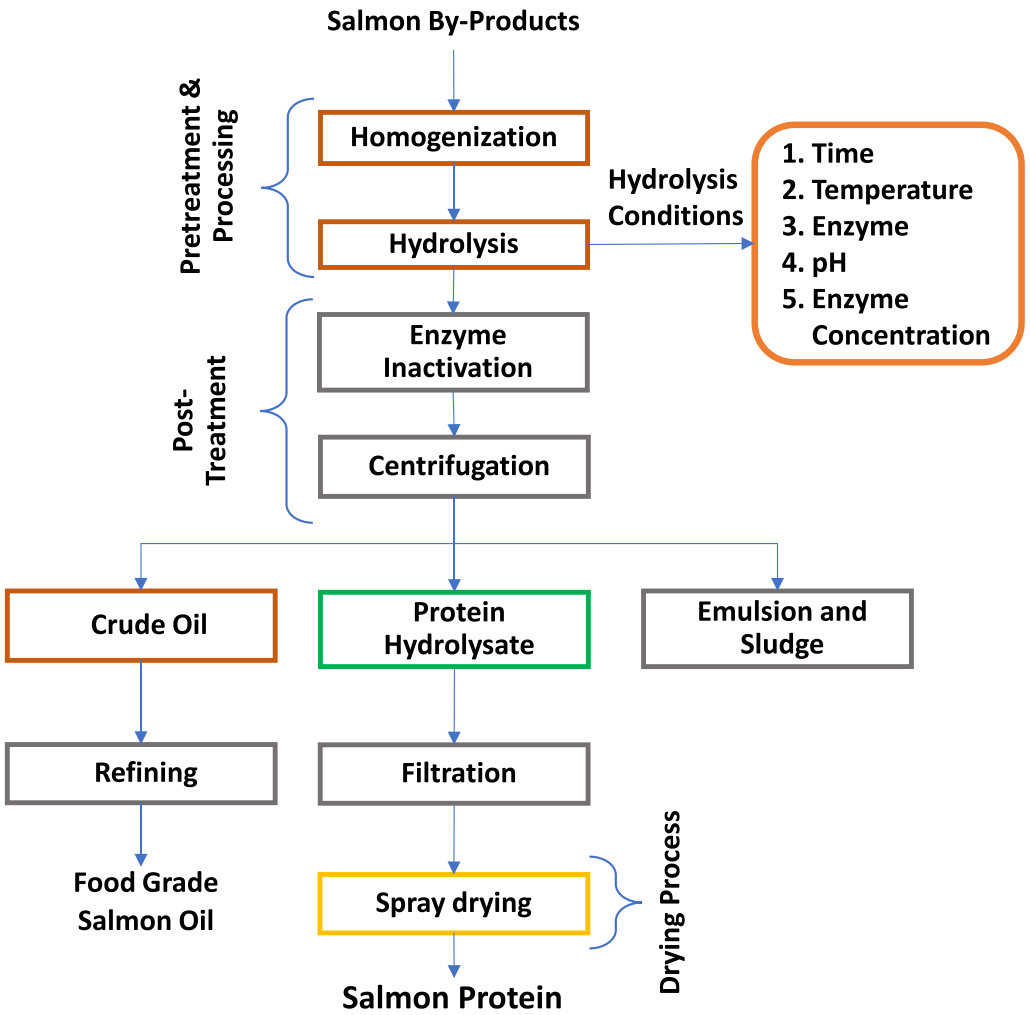


Fig. 6 Production of salmon protein via enzymatic hydrolysis process

conventional process. The ultrasound-assisted enzymatic hydrolysis at 70 W produced the highest in vitro antioxidant activity. The ultrasound-pretreated samples for 30 and 45 min produced the strongest nitric oxide inhibitory and antioxidative (macrophage cell lines) activities. In another study, Álvarez et al. (2018) studied the acid/alkali isoelectric solubilization/ precipitation (ISP) of proteins from mackerel using ultrasound. The study indicated that the traditional isoelectric solubilization/ precipitation process generated a lower yield than conventional enzymatic hydrolysis. However, the application of ultrasound to alkaline extraction helped recover more than 95% of total protein from mackerel by-products. Moreover, Misir and Koral (2019) studied the effect of ultra-sound treatment on the structural, chemical, and functional properties of protein hydrolysate prepared from rainbow trout by-products and reported that the application of ultrasound for hydrolysis might cause the unfolding of protein molecules, resulting in higher hydrophobic groups at the surface of molecules. This may bring about interactions among these groups that might lead to larger aggregates compared to smaller aggregates produced during conventional hydrolysis. The ultrasound hydrolysates also had significantly better foaming capacity, foaming stability, and oil-binding capacity, as well as a higher antioxidant activity. On the other hand, Tian et al. (2015) combined high-intensity ultrasound with an alkaline pH-shift process to develop TPI under various pH conditions. The ultrasound application resulted in a significant improvement in the consistency, protein solubility, and sediment size during the pH shift process. The ultrasound improved the yield at a lower pH (10.5) than the traditional alkaline pH process (pH 11.5).

High-pressure processing-enzymatic hydrolysis

During high-pressure processing, uniform pressure (100–1000 MPa) is applied instantaneously, independent of the size and geometry of food, on flexible packaging

Table 2 Enzymatic hydrolysis of salmon protein

Enzymes	Salmon Part	pH	Time (min)	Temperature (°C)	Enzyme Concentration	Protein Yield (%)	Reference
Alcalase	Muscle	7.5	180	40	7.5%	86.92–88.39	Kristinsson and Rasco (2000b)
Flavourzyme						78.95–84.26	
Corolase PN-L						72.88–79.32	
Corolase 7089						82.41–86.48	
Endogenous						71.67–79.12	
Alcalase	Head	8	120	55	5.5%	71.00	Gbogouri et al. (2004)
Alcalase	Skin	8.39	120	55	2.5%	77.03	See et al. (2011)
Neutrase Alcalase Flavourzyme and Protamex	Skin	6.7–8.0	240	53	10,000 U/g	45.11	Zhang et al. (2022)
Endogenous	Viscera	N/A	60 and 120	52	0.1%	77.40	Opheim et al. (2015)
Protamex + Endogenous	Viscera, head and frame					82.80	
Papain + Bromelain + Endogenous	Viscera, head and frame				0.05 + 0.05%	86.0	
Protamex + Endogenous	Viscera				0.1%	75.50	
Papain plus Bromelain + Endogenous	Viscera					76.60	
Papain plus Bromelain	Viscera					69.90	
Corolase PP	Backbone	N/A	120	50	0.1%	11.50	Slizyte et al. (2016)
Corolase 7089					0.1%	9.30	
Protamex					0.1%	9.40	
Bromelain 400 GDU/g + Papain 100TU/mg					0.05 + 0.05%	11.60	
Protex 6L					0.1%	8.50	
Seabzyme L200					0.1%	9.90	
Trypsin					0.1%	12.10	
Alcalase	Head and backbone	6.5	120	50	5.5—88 U/g	64.70	Aspevik et al. (2016a)
Promod 671L						66.00	
Protex 7L						62.20	
Protamex	Frame	6.5	60	50–56	10–90 AU/kg	43.00–61.00	Liaset et al. (2002)
Alcalase	Head, trimming, and frame	8.98	180	64.2	0.2%	84	Vázquez et al. (2019)
Alcalase and papain	Frame	8.0	240	85	1–3%	79.2–82.01	Idowu et al. (2018)
Bromelain BR1200 and FoodPro PNL	Backbone and head	N/A	60	50	10 U/g	89.3–92.1	Aspevik et al. (2021)

materials filled with liquid or solid food products. The pressure generation fluid is water, and the process can be carried out with or without heat (Elamin et al. 2015; Hossain et al. 2022b; Marciniak et al. 2018). During high-pressure processing, proteins are denatured where weak non-covalent chemical bonds such as hydrogen, hydrophobic, and ionic bonds are broken. This process is different from temperature or chemical denaturation, in which non-covalent bonds are broken, and the proteins are irreversibly unfolded and aggregated. Therefore, high-pressure processing modulates protein–protein and protein-solvent interactions. Upon low-pressure treatments (< 400 MPa), the number of hydrogen bonds is increased, whereas, under high-pressure treatments (> 400 MPa), it is broken. The structural modifications caused by high-pressure treatment may be reversible or irreversible depending on the pressurization parameters and the protein properties (Marciniak et al. 2018;

Rivalain et al. 2010). Proteins treated under high pressure have profound effects on both intramolecular and intermolecular interactions. The intramolecular interactions include ionic interactions, hydration, hydrophobic interactions, hydrogen bonds, van der Waals forces, and covalent bonds. The intermolecular interactions include protein–protein interactions, enzyme–substrate interactions, and protein-non-protein interactions (Boonyaratanakornkit et al. 2002).

Hydration of proteins plays a significant role in the high-pressure denaturation process. During high-pressure processing, the water fills crevices between amino acids, screens out repulsive forces, and facilitates side chains and polypeptide backbones. Water penetration under pressure leads to conformational transitions, resulting in protein unfolding. At this stage, pressure induces protein to adopt molten globule conformation, a compact, partially folded conformation without a specific tertiary structure. The modification of protein due to hydration under high pressure can be due to two factors. First, the opening of cavities allows a solvent to occupy an internal volume. Second, the surface area in contact with solvent is larger for unfolded proteins than native proteins (Boonyaratanakornkit et al. 2002; Marciniak et al. 2018; Rivalain et al. 2010; Royer 2005; Silva & Foguel 2009). The hydrophobic interactions in proteins induce non-polar chains to cluster inside proteins and significantly affect proper protein folding. Water molecules surrounding the non-polar groups have higher compressibility than both hydrophilic hydration and bulk water. Therefore, under high pressure, the hydrophobic core of globular protein is exposed to the solvent, resulting in decreased system volume. This effect results in the unfolding/denaturing process. The volume change reveals polar and non-polar groups, electrostriction, and elimination of cavities (Boonyaratanakornkit et al. 2002; Grigera & McCarthy 2010).

Under high pressure, the hydrogen bonds present within protein shorten in length, leading to the collapse of internal cavities. The collapse of cavities contributes to the compression of protein under pressure (Boonyaratanakornkit et al. 2002). During high pressure processing, hydrogen bond formation in the protein is promoted, and van der Waals forces are favored to maximize the packing density and reduce the protein volume. The phenomenon of protein stabilization under high pressure is due to the opposing effects of pressure and temperature on hydrophobic interactions and hydrogen bond formation (Knorr et al. 2006). Furthermore, low-contact-order hydrogen bonds (helical turns or beta hairpins) hidden between amino acids and close together in the polypeptide sequence are moderately affected by temperature. However, high-contact-order hydrogen bonds have larger sequence separations and are less stable against pressure and temperature changes (Nielsen & Schwalbe 2012).

Alemán et al. (2011) studied the enzymatic hydrolysis of catfish skin gelatin under atmospheric pressure and high pressure (100, 200, and 300 MPa for 15 and 30 min) using Alcalase at 50 °C, as well as collagenase, trypsin, and pepsin at 37 °C. This study's results indicated an enhanced degree of hydrolysis for the samples treated with enzyme under high pressure. The antioxidant activities evaluated by ferric reducing power (FRAP) and ABTS radical scavenging ability were improved for all hydrolysates treated under high-pressure. Moreover, Hemker et al. (2020) studied highpressure assisted enzymatic hydrolysis on tilapia by-products to produce protein hydrolysates. The results from this study indicated that pressure and holding time influenced the soluble protein content, which was increased with increasing pressure and holding time. The underlying mechanism is attributed to the activation of the enzyme and unfolding of protein substrate for improved results under high pressure. The study also showed that increasing pressure increased the release of soluble proteins and free amino acids, contributing to higher absorbance intensity. Under high pressure, the protein unfolding occurs that exposes hydrophobic amino acid residues like tyrosine and tryptophan. The pressure-treated protein hydrolysate had improved solubility and emulsifying properties. The high-pressure treated protein hydrolysate had improved antioxidant capabilities compared to hydrolysates treated under atmospheric pressure.

Composition of salmon protein hydrolysates

Several studies have reported that the salmon protein hydrolysates produced from various parts of salmon by-products consists of 69 to 89% protein, 0.06 to 16% lipids, 3.5 to 22% ash, and 0.9 to 5% moisture. The amount of protein and lipid is dependent on the type of salmon by-products used in the hydrolysis. The high amounts of ash in the final protein hydrolysates are primarily due to added acid or base to adjust the pH. During enzymatic hydrolysis, the degree of hydrolysis increases with the number of peptide bonds broken. During the breakage of peptide bonds, the pH of the reaction changes, and to maintain equilibrium, NaOH or HCl is added to the reaction system. Therefore, the ash content of the hydrolysates increases with increasing DH, with all hydrolysates having higher ash content than the unhydrolyzed protein (Chalamaiah et al. 2012). The final moisture content in the salmon protein hydrolysate is dependent on the type of sample and the temperatures employed during the drying process (Chalamaiah et al. 2012; Gbogouri

et al. 2004; Idowu et al. 2018; Kristinsson & Rasco 2000a; Opheim et al. 2015; See et al. 2011).

Salmon protein hydrolysates obtained after enzymatic hydrolysis are dominated by amino acids and short-chain peptides. The amino acid composition of salmon protein hydrolysates reported in various studies is shown in Table 3. There were significant differences in the amino acid composition of the protein hydrolysates obtained from the same species. The type of raw material used in the study plays an important role in the amino acid composition of the protein hydrolysate. Other factors which influence the amino acid composition include the type of enzyme and hydrolysis conditions. However, most of the salmon protein hydrolysates contain all the essential and non-essential amino acids and may serve as good sources of functional food ingredients (Chalamaiah et al. 2012; Opheim et al. 2015; Zhang et al. 2022).

Functional properties of salmon protein hydrolysates

The functional properties of salmon protein, such as foaming, emulsification, water-holding capacities, solubility, and gelation, are affected by the source of raw material, environmental, production, and processing factors. The production and processing factors include isolation, precipitation, drying or dehydration, concentration, enzymatic or chemical modification, and the environmental factors that include temperature, pH, and ionic strength (Thorkelsson & Kristinsson 2009).

Enzymatic hydrolysis of salmon protein generates a mixture of amino acids, di-, tri-, and oligopeptides, increasing the number of polar groups and the hydrolysate's solubility. The choice of substrate and enzymes and the degree of hydrolysis affect the resulting hydrolysate's physicochemical properties. The enzyme specificity strongly influences the molecular size and hydrophobicity of the hydrolysate. The control of the enzyme hydrolysis is essential; uncontrolled or prolonged hydrolysis may result in highly soluble peptides. The peptides produced in the uncontrolled reaction system totally lack the functional properties of the parent protein and may lead to undesirable bitter peptides. The physical and chemical properties that govern protein functionality include size, shape, amino acid composition and sequence, net charge and distribution of charges, hydrophobicity/hydrophilicity ratio, peptide structures, molecular flexibility, and ability to react with other components in a food system (Abdollahi & Undeland 2018; Kristinsson & Rasco 2000d).

Solubility of protein hydrolysates

The solubility of proteins is the most crucial functional property during developing and testing of new protein ingredients. Based on their solubility, fish muscle proteins, namely sarcoplasmic (most soluble), myofibrillar (soluble in high ionic strength solutions), and stroma proteins (least soluble) may be affected. The solubility of proteins influences many other functional properties,

Table 3 Amino acid composition of Atlantic salmon protein hydrolysates

Amino acids	Hydrolysates (mg/100 g)			Skin (g/100 g)	Trimmings (g/100 g)	Viscera + head + frame (mg/g)	Viscera (mg/g)
	Pepsin	Pepsin + pancreatin	Thermolysin				
Aspartic acid	0.4 ± 0.2	11.6 ± 1.1	12.4 ± 0.5	5.72	7.31	77.8 ± 0.8	77.0 ± 0.1
Threonine	2.9 ± 0.2	6.0 ± 0.3	17.8 ± 0.8	2.03	3.23	39.3 ± 0.1	42.4 ± 0.3
Serine	4.8 ± 0.2	6.4 ± 0.3	21.8 ± 0.6	3.92	3.19	39.2 ± 0.2	41.2 ± 0.9
Glutamic acid	3.1 ± 0.1	29.0 ± 1.2	54.2 ± 5.4	9.05	11.07	113.1 ± 1.3	109.6 ± 1.9
Glycine	13.7 ± 0.8	15.2 ± 1.0	33.0 ± 2.7	22.6	7.07	85.8 ± 1.4	66.5 ± 0.0
Alanine	24.7 ± 1.5	35.5 ± 0.9	95.0 ± 5.3	7.81	5.22	57.0 ± 0.0	53.4 ± 2.0
Valine	2.5 ± 0.1	15.2 ± 0.3	18.4 ± 1.7	1.71	3.83	42.6 ± 0.2	45.6 ± 0.1
Methionine	1.3 ± 0.1	11.8 ± 0.4	31.9 ± 1.6	2.46	2.66	24.3 ± 0.1	24.4 ± 0.8
Isoleucine	1.2 ± 0.1	21.9 ± 2.2	–	1.05	2.98	36.0 ± 0.2	37.4 ± 0.0
Leucine	2.4 ± 0.1	84.3 ± 1.4	69.8 ± 2.3	1.75	4.97	57.9 ± 0.4	62.6 ± 0.3
Tyrosine	1.9 ± 0.1	76.9 ± 4.3	11.4 ± 0.5	0.35	1.76	18.9 ± 0.5	22.2 ± 0.2
Phenylalanine	3.1 ± 0.3	76.3 ± 5.3	137.0 ± 4.0	1.71	2.53	31.2 ± 0.2	33.9 ± 0.3
Histidine	16.4 ± 0.9	21.0 ± 0.8	62.4 ± 2.7	1.33	3.88	26.3 ± 0.2	23.2 ± 0.7
Arginine	1.3 ± 0.6	196 ± 14	–	7.8	5.04	56.2 ± 0.3	50.8 ± 0.7
Tryptophan	–	18.3 ± 0.5	–			8.2 ± 0.0	10.4 ± 0.0
Lysine	–	–	–	2.93	6.4	71.2 ± 0.6	69.4 ± 0.4
Proline	–	–	–	10.63	3.98	49.2 ± 0.5	47.0 ± 0.3
Cysteine				0.49	0.94	9.4 ± 0.0	12.4 ± 0.0
Reference	Nakajima et al. (2009)			Harnedy et al. (2018)		Opheim et al. (2015)	

such as emulsification, water-holding capacity, and foaming properties. The salmon protein is mainly composed of myofibrillar proteins with myosin and actin as its main components. The myofibrillar proteins are soluble in high ionic strength solutions (Kristinsson & Rasco 2000b). Therefore, to increase the solubility of myofibrillar proteins in Atlantic salmon, enzymatic hydrolysis is recommended. Kristinsson and Rasco (2000b) studied the functional properties of Atlantic salmon muscle proteins hydrolyzed with various alkaline proteases. The results showed that alkaline proteases (Alcalase, Flavourzyme, Corolase PN-L, Coro-lase 7089, and endogenous extract) produced 92 to 99% soluble protein hydrolysates depending on the degree of hydrolysis. The results also indicated that the solubility of the hydrolysates was very high (above 90%) at pH 7.0 and 0.1 M NaCl. Electrophoresis results showed that the hydrolysates reached maximum solubility at 5% DH and remained unchanged at higher DH. The solubility is also believed to be the delicate balance of hydrophilic and hydrophobic forces of the peptides. Gbogouri et al. (2004) studied the influence of the degree of hydrolysis on the solubility of salmon byproducts hydrolysates prepared using Alcalase and reported higher solubility (above 90%) of hydrolysates at pH 6 to 7 while lower solubility at pH 3 to 4. The pH influences the charge on the weak acidic and basic side-chain groups. Therefore, protein hydrolysates display low solubility at their isoelectric point.

Water-holding capacity (WHC) of protein hydrolysates

The water-holding capacity of proteins is defined as the ability to maintain or imbibe water during the application of forces, pressing, centrifugation, or heating. Protein's water-holding capacity is essential in the food industry to improve the texture. The hydration properties of proteins determine their applications in food systems. This functional property is dependent on the water-protein interaction, which determines the functional properties of proteins, such as water binding and retention, swelling, solubility, emulsifying properties, viscosity, gelation, and syneresis. The interaction of protein and water depends on the composition and conformation of the protein molecules. The interactions between water molecules and hydrophilic groups of protein side chains occur via hydrogen bonding. Proteins containing a high percentage of charged amino acids can bind large amounts of water. Therefore, the water-holding capacity can be predicted from the amino acid composition of proteins (Chan et al. 2021; Ucak et al. 2021; Zayas 1997).

Proteins with low water-holding capacity are sensitive to storage humidity. Therefore, the choice of proteins with an appropriate water-holding capacity is essential in food formulation. The recommended maximum water content of fish protein hydrolysate for storage is 0.075 g/g at less than 15% RH. Salmon protein hydrolysates are highly hygroscopic, and therefore, proper packaging and low relative humidity of air during processing are important considerations. Polar groups such as COOH and NH_2 that increase during enzymatic hydrolysis substantially affect water and moisture absorption isotherm for fish protein (Kristinsson & Rasco 2000d). Kristinsson and Rasco (2000b) studied the water-holding capacity of salmon protein hydrolysates and reported that the degree of hydrolysis did not affect the property. However, the type of enzyme used for hydrolysis differentiated the water-holding capacity of salmon protein. Alcalase hydrolyzed samples have the highest water-holding effect compared to other alkaline proteases compared in the study. Samples hydrolyzed using Alcalase also produced the highest concentration of low-molecular-weight peptides than other enzymes. Corolase 7089 also produced similar concentrations of low-molecular-weight peptides as Alcalase; however, the water-holding capacity was the lowest. This phenomenon is due to the type of peptides produced by different enzymes as per their specificities. Therefore, the selection of enzymes plays an important role in the production of functional protein hydrolysates.

Emulsifying properties of protein hydrolysates

The emulsifying properties of proteins are evaluated by their ability to take part in emulsion formation and stabilize the newly created emulsion. Proteins are major components in most food emulsions (Ucak et al. 2021). During homogenization at high speeds, protein adsorbs to the surface of newly formed oil droplets and forms a protective membrane that prevents droplets from coalescing (Kristinsson & Rasco 2000d). Dried protein hydrolysates are surface-active molecules and possess good emulsifying properties due to the presence of both hydrophobic and hydrophilic amino acids. The surface-active nature of protein is an important property of its interfacial film-forming capacity (Lam & Nickerson 2013). Food emulsions are oil-in-water (O/W) and water-in-oil (W/O). When protein molecules are added to the oil in water emulsions, they migrate to the oil–water interface and realign to penetrate the oil droplets with their hydro-phobic groups. In contrast, the hydrophilic groups are aligned with the aqueous phase. Following alignment, a robust viscoelastic film is formed around the oil droplets. The protein-rich interfacial layers protect the emulsion against strong destabilization processes by acting as an electrostatic, structural, and mechanical energy barrier (Gbogouri et al. 2004; Haque et al. 2016; Kristinsson & Rasco 2000b). The emulsifying properties of proteins are described by emulsifying capacity (EC), emulsifying stability (ES), and emulsifying activity (EA).

The emulsifying capacity (EC) is defined as the amount of oil (mL) emulsified by the protein hydrolysate (g) before the phase inversion occurs. The emulsification stability (ES) is determined by the percentage decrease in the emulsion's interfacial area or the percentage volume of cream separated from the emulsion. The emulsifying activity (EA) is calculated as the interfacial area created per unit mass of protein. The protein-stabilized emulsions are affected by several factors such as pH, ionic strength, temperature, presence of low molecular-weight surfactants, sugars, oil phase volume, type of protein, and the melting point of the oil used during the process. Extrinsic factors such as type of equipment, energy input rate, and shear rate also the protein stabilized emulsions (Abdollahi & Undeland 2018; Kristinsson & Rasco 2000b; Zayas 1997).

Gbogouri et al. (2004) studied the emulsification capacity and stability of emulsions created using salmon protein hydrolysates. The study reported that the emulsification capacity varied between 0.02 and 0.2%. The results also showed that the emulsification capacity increased with increased protein concentration until reaching the maximum emulsification capacity and then decreased. The decrease of emulsification capacity was due to high protein concentration, increasing the adsorption rate higher than the spreading rate. Moreover, Kristinsson and Rasco (2000b) studied the emulsification properties of salmon protein extracted using alkaline proteases such as Alcalase, Flavourzyme, Corolase PN-L, Corolase 7089, and endogenous extract. The study indicated that salmon protein hydrolysates produced using endogenous extract had the highest emulsification capacity at all degree of hydrolysis than other hydrolysates. The changes in the degree of hydrolysis affected the emulsification capacity of protein. They also reported a positive correlation between the peptide length and surface activity, and a minimum of 20 amino acid residues had good emulsifying and interfacial properties. The endogenous enzymes in producing salmon protein extracts retained large peptides (~ 2000 Da) at all degrees of hydrolysis, and it was responsible for the high emulsifying activity. The peptides released during Flavourzyme hydrolysis were similar in size to endogenous extract hydrolysis. However, Flavourzyme hydrolysates had poor emulsification capacities. Therefore, more than the peptide size, the physicochemical makeup of the peptides may play an important role in the difference in the emulsification capacity of protein. Large differences in emulsification capacities were also observed between different hydrolysates at the same degree of hydrolysis. This property is due to the differences in enzyme specificity and hydrophobicity. Similarly, He et al. (2012) studied the emulsifying capacity of salmon protein hydrolysates using Alcalase, Flavourzyme, and Neutrase and found that the emulsifying capacity decreased with longer processing time and a higher enzyme-to-substrate ratio. This trend is attributable to low molecular weight peptides produced at a higher enzyme-to-substrate ratio and increased reaction time. The low molecular weight peptides are less efficient in decreasing the water–oil interface tension because they cannot unfold and reorient on the water–oil surface to stabilize the emulsion system. Flavourzyme hydrolyzed salmon produced the highest emulsifying capacity (51 ± 1.8 m^2/ g) at a 0.5% enzyme-to-substrate ratio and 30 min reaction time. Likewise, emulsion and surface-active properties of Atlantic salmon backbone hydrolysates were tested and found to have a negative impact on EA and critical micelle concentration (CMC), likely due to the generation of small peptides disrupting the amphiphilic balance (Steinsholm et al. 2021).

Fat absorption capacity of protein hydrolysates

The capacity of protein hydrolysates to absorb fat is an important attribute that influences the taste and texture of food. Fat absorption of proteins is affected by protein source, processing conditions, presence and composition of additives, particle size, and temperature. Fat absorption is a form of physical entrapment of oil by proteins. Low-density protein powder with small particle size entraps more oil than high-density protein powders. Insoluble and hydrophobic proteins have high oil absorption capacity. Oxidized lipids accelerate lipid-protein interactions. The proteins act as a trap for lipid peroxides and secondary lipid oxidation products. The breakdown products of oxidations can interact with terminal functional groups of amino acids in proteins and enzymes. These interactions influence the functional and nutritional properties of proteins and flavor. Four types of bonds involved in the protein-lipid interactions are hydrophobic, electrostatic, hydrogen, and non-covalent bonds (Kristinsson & Rasco 2000c; Ucak et al. 2021; Zayas 1997).

Gbogouri et al. (2004) studied the fat absorption capacity of protein hydrolysates pre-pared using Alcalase and reported that the best corn oil absorption was achieved at a degree of hydrolysis (DH) of 11.5%. The fat absorption capacity decreased with an increase in the DH, possibly due to the physical entrapment of oil and, thus, the higher bulk density of protein. On the other hand, Kristinsson and Rasco (2000b) studied the fat absorption properties of salmon protein extracted using alkaline proteases such as Alcalase, Flavourzyme, Corolase PN-L, Corolase 7089, and endogenous extract. This study also reported higher fat absorption at a 5% degree of hydrolysis for all extracts, but only limited research has been conducted on the fat absorption of fish protein hydrolysates. The

enzyme–substrate specificity also plays a major role in the fat-binding capacity of hydrolysates.

Sensory properties of salmon protein hydrolysate

The sensory properties, both taste and odor, of salmon protein hydrolysates should be acceptable to the consumers alongside their quality and functional properties. Significant problems associated with protein hydrolysates are the bitterness and fishy odor of the final product. The protein hydrolysate's bitterness is related to various factors such as hydrophobic amino acids, degree of hydrolysis, molecular weight, enzyme type, and peptide sequences. The amino acids responsible for the bitter taste are valine, isoleucine, phenylalanine, tryptophan, leucine, and tyrosine. The hydrophobicity (Q) values of more than 1400 Cal/mole and molecular masses of less than 6 kDa renders bitterness to the product (Benjakul et al. 2014; Cho et al. 2004; Idowu et al. 2018; Saha & Hayashi 2001; Sun 2011; Thorkelsson & Kristinsson 2009). Several methods have been used to mask the bitterness of protein hydrolysates, including the addition of glutamic acid, glutamyl-rich peptides, polyphosphates, gelatin, or glycine in the products (Benjakul et al. 2014). Recently, Xu et al. (2019) proposed a mechanism in which adding sodium chloride decreased the surface hydrophobicity. The screening of Na^+ reduced hydrophobic interactions between the protein hydrolysates and bitter taste receptors. The addition of sodium chloride creates a salting-in effect, causing the self-folding of the peptide and Cl^- to bind to the hydrophobic cavities and decrease the surface hydrophobicity. The study also indicated that the addition of sodium chloride decreased the particle size and turbidity of the protein hydrolysates. Specifically, the plastein reaction is carried out as a strategy to reduce the bitterness of peptides, and this is often carried out by the addition of glutamic acid, as was explained earlier. Other measures might also be considered in this regard (Gong et al. 2015).

Aspevik et al. (2016b) studied the sensory properties of protein hydrolysates prepared from Atlantic salmon using Alcalase, Promod, and Protex. The formation of bitter taste was attributed to both the degree of hydrolysis and enzyme specificity. The results indicated that protein hydrolysates obtained using Alcalase were more bitter and astringent than Promod and Protex hydrolysates. The subtilisin-activity of Alcalase releases more bitter-peptides from the salmon substrate than Protex and Promod enzymes. The study concluded that the degree of hydrolysis plays a significant role in several sensory attributes, such as bitter, umami, sea, fish, and pungent tastes and odors. The maximum intensity and threshold level of bitter taste were between medium to high based on the degree of hydrolysis. Similarly, Idowu et al. (2018) suggested that the hydrolysate obtained from salmon frames using Alcalase yielded higher bitterness than papain, which could be linked to hydrophobic groups toward their C-terminal. In contrast, Aspevik et al. (2021) suggested that salmon hydrolysates were linked with larger peptides (> 2 kDa), low flavor intensity, and pleasant flavors, including umami taste and sea flavor.

Bioactive speptides

Besides the nutritional aspect of proteins, bioactive peptides are also responsible for various physiochemical and sensory properties of foods and may act as functional and health-promoting ingredients (Chalamaiah et al. 2012; Rizzello et al. 2016; Shahidi & Zhong 2008). Most of the proteins' physiological and functional properties are attributed to the biologically active peptides encrypted in the protein molecules. Bioactive peptides are released from the parent protein source by the digestive enzymes (gastrointestinal digestion), food processing (ripening, fermentation, and cooking), storage, or by in-vitro enzyme hydrolysis (Daliri et al. 2017; Rizzello et al. 2016).

Empirical production of bioactive peptides and their functions

The bioactive peptides can be produced from protein sources using different methods, including enzymatic hydrolysis with digestive enzymes, enzymatic hydrolysis using proteases, fermentation, microwave-assisted hydrolysis, and other techniques, as mentioned earlier. Crude protein hydrolysates are subjected to various assays to screen for bioactivities. After detecting bioactivities, crude protein hydrolysates are fractionated based on peptide size via ultrafiltration. The fraction showing the highest bioactivity is further purified using various chromatographic techniques. The isolated peptides are then sequenced using tandem mass spectrometry and protein sequencing. Finally, based on the peptide sequence, peptides are synthesized, and the assays are repeated to confirm the bioactivities (Ryan et al. 2011; Wang et al. 2017b). The production of bioactive peptides is shown in Fig. 7. Some of the peptides produced from Atlantic salmon and their activities are given in Table 4.

Antihypertensive peptides derived from salmon

Angiotensin-converting enzyme (ACE) inhibitors are used to treat and manage hypertension. Several studies have been conducted on the bioactive peptides of Atlantic salmon and their beneficial actions in health promotion, including ACE inhibitory activity. ACE inhibitors could help to relax the arteries and veins, resulting in lower blood pressure. For example, Neves et al. (2017b) prepared bioactive peptides from salmon trimmings with ACE inhibitory and dipeptidyl peptidase IV (DPP-IV)

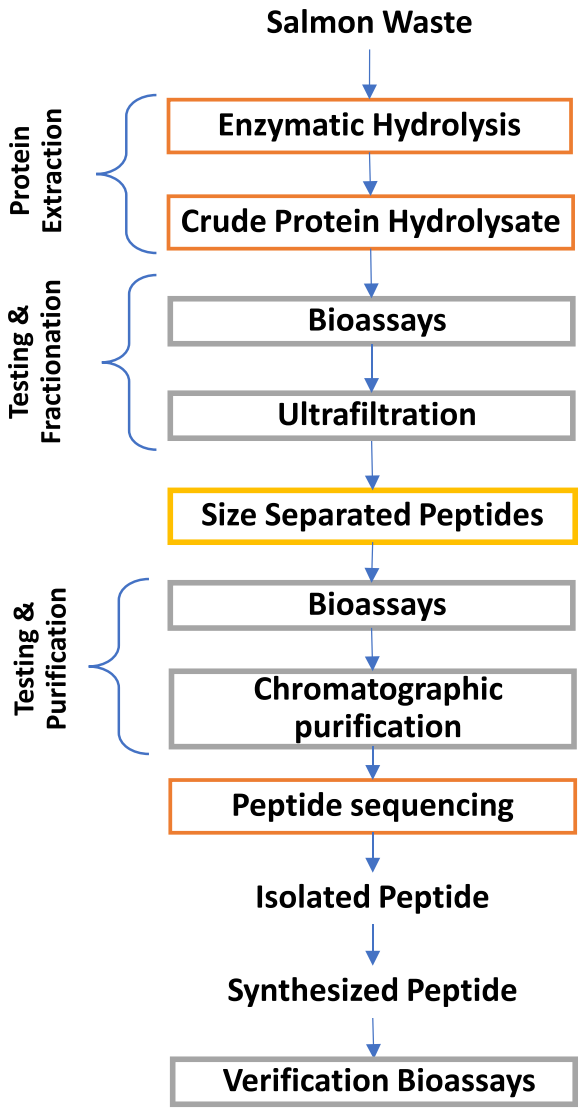


Fig. 7 Empirical production of bioactive peptides

inhibitory activities, as well as oxygen radical absorbance capacity (ORAC). Salmon protein procured from trimmings was isolated using the pH shift method and hydrolyzed with Alcalase, Flavourzyme, Promod, and Corolase PP. The IC_{50} values for ACE inhibitory activities of hydrolyzed salmon trimming protein ranged between 0.74 and 1.69 mg/mL. Alcalase-treated hydrolysates generated a more potent ACE inhibitory peptide with an IC_{50} value of 0.74 mg/mL. Promod hydrolysates produced the least potent ACE inhibitory peptide with an IC_{50} value of 1.69 mg/mL. The IC_{50} values for DPP-IV inhibitory activities prepared from salmon trimming protein ranged between 0.30 and 2.37 mg/mL. Corolase PP-treated hydrolysates generated a more potent DPP-IV inhibitory peptide with an IC_{50} value of 0.30 mg/mL. Promod hydrolysates produced the least potent DPP-IV inhibitory peptide with an IC_{50} value of 2.37 mg/mL. Similarly, Neves et al. (2017a) extracted gelatin from salmon trimming protein and hydrolyzed it with Alcalase, Alcalase + Flavourzyme, Corolase PP, Promod, and Brewer's Clarex. There were distinct differences in the peptide profiles of hydrolysates generated with different enzymes used. The increase in reaction time generated more low molecular weight peptides using all enzymes. More potent ACE inhibitory hydrolysates were generated using Corolase PP and Alcalase + Flavourzyme with IC_{50} values of 0.13 and 0.28 mg/mL, respectively. More potent DPP-IV inhibitory hydrolysates were generated using Corolase PP and Alcalase + Flavourzyme with IC_{50} values of 0.08 and 0.10 mg/mL, respectively. The ORAC values of antioxidant peptides extracted from salmon gelatin hydrolysates ranged between 103 and 540.94 µmol Trolox/g. The hydrolysates extracted using Corolase PP and Promod generated the most antioxidant peptides based on ORAC values. The salmon gelatin hydrolysate produced using Corolase PP was used for further analysis. The purified Corolase salmon gelatin hydrolysate was administered to spontaneously hypertensive rats (SHR). The in vivo study indicated improved mean arterial blood pressure (MAP), systolic (SBP) and diastolic blood pressure (DBP), and decreased the heart rate (HR) of SHR compared to the synthetic drug Captopril™. In addition, the ORAC values of antioxidant peptides prepared using Promod ranged from 587.41 to 882.58 µmol Trolox/g. Peptides generated by Alcalase, Al-calase + Flavourzyme, and Corolase PP had ORAC values higher than 601.47 µmol Trolox equivalents/g. In another study, peptides derived from salmon demonstrate strong ACE inhibition due to their high affinity to ACE active sites. In particular, peptides with molecular weight (> 1500 Da), C terminal peptide sequence, short chain length, hydrophobic amino acids, and presence of lysine or arginine at the C end exhibited higher ACE inhibitory activity (Phadke et al. 2021).

Ahn et al. (2012b) identified ACE inhibitory peptides from salmon protein by-products via enzymatic hydrolysis using Alcalase, Flavourzyme, Neutrase, pepsin, Protamex, and trypsin. The results indicated that Alcalase hydrolysates possessed the highest ACE inhibitory activity. The study also indicated that the VWDPPKFD peptide was a non-competitive inhibitor, and FEDYVPLSCF and FNVPLYE had mixed inhibition modes. The presence of phenylalanine, leucine, and tyrosine at the C-terminal appears to play an important role in their inhibition activity. In another study, low-molecular-weight ACE inhibitory peptides were isolated from Atlantic salmon skin by Gu et al. (2011). The peptides had a high quantity (90.79%) of oligopeptides below 1 kDa, with most

Table 4 Atlantic salmon derived bioactive peptides

Peptide/AA	Activities					Reference
	ACE IC_{50}	DPP-IV IC_{50}	ORAC (μmol TE/μmol peptide)	AIA (mM)	HRSA (μg/mL)	
GPAV	415.91 ± 2.65 (μM)	245.58 ± 7.15 (μM)	9.51 ± 1.40			Neves et al. (2017a)
VP	1215.15 ± 83.14 (μM)	758.15 ± 12.45 (μM)	19.45 ± 2.15			
VC	134.45 ± 5.15 (μM)	5413.45 ± 62.15 (μM)	3.45 ± 0.78			
YP	5.21 ± 0.94 (μM)	7564.02 ± 42.45 (μM)	17.18 ± 1.54			
FF	59.15 ± 0.54 (μM)	546.84 ± 8.15 (μM)	8.47 ± 1.05			
PP	1912.46 ± 63.15 (μM)	4343.48 ± 29.78 (μM)	12.48 ± 1.47			
W	135.48 ± 5.48 (μM)	438.15 ± 10.97 (μM)	4.31 ± 0.19			
F	125.15 ± 2.64 (μM)	295.15 ± 6.45 (μM)	4.59 ± 0.89			
Y	182.84 ± 0.52 (μM)	75.15 ± 0.84 (μM)	2.74 ± 0.65			
GF	178.14 ± 24.51 (μM)	1547.15 ± 34.15 (μM)	19.74 ± 1.01			Neves et al. (2017b)
GPVA	445.61 ± 6.94 (μM)	264.74 ± 1.59 (μM)	9.48 ± 0.94			
GGPAGPAV	673.16 ± 15.03 (μM)	8139.11 ± 134.68 (μM)	5.47 ± 0.94			
R	98.04 ± 0.15 (μM)	110.44 ± 0.47 (μM)	4.71 ± 0.09			
PAY				0.75		Ahn et al. (2015)
VWDPPKFD	9.12 ± 1.07 (μM)					
FEDYVPLSCF	13.72 ± 2.54 (μM)					
FNVPLYE	6.79 ± 0.78 (μM)					
AP	0.060 ± 0.001 mg/mL					Gu et al. (2011)
VR	0.332 ± 0.005 mg/mL					
GAAGR						
AGPS						
VDGK						
RER						
LN						
VTGK						
GHAGE						
VGGK						
GHGR						
GAPE		49.6 (μM)				Li-Chan et al. (2012)
GPGA		41.9 (μM)				
YYGYTGAFR	1.21 mg/mL					Jin et al. (2020)
LDKVFR	0.10 mg/mL					
VLATSGPG	0.18 mg/mL					
PR					91.3	Wang et al. (2008)
IVY	0.48 (μM)					Darewicz et al. (2014)
VW	1.4 (μM)					
IY	2.1 (μM)					
IW	4.7 (μM)					
VY	7.1 (μM)					
TVY	15 (μM)					
VFPS	0.46 (μM)					
VTVNPYKWLP	5.5 (μM)					
IWHHT	5.8 (μM)					
YALPHA	9.8 (μM)					
ALPHA	10 (μM)					

ACE Angiotensin-I converting enzyme inhibition, *DPP-IV* Dipeptidyl-peptidase IV (DPP-IV)-inhibitory activit, *ORAC* Oxygen radical absorbance capacity, *TE* Trolox equivalent, *AIA* Anti-inflammatory Activity, *HRSA* Hydrogen Radical Scavenging Activity

of them in the range of 132–576 Da (69%). The filtered salmon peptides had an IC_{50} of 1.165 mg/mL. To better understand and identify the peptides, the samples were subjected to RP-HPLC, and 11 different fractions were obtained. Two fractions out of 11 had high ACE inhibitory activities. A total of eleven peptides were identified from the two high ACE fractions, including three dipeptides, one tripeptide, five tetrapeptides, and two pentapeptides. Two dipeptides, AP and VR, exhibited an IC_{50} of 0.06 and 0.332 mg/mL ACE inhibition, respectively, which is 20 and 40-fold more potent than filtered salmon peptides (1.165 mg/mL).

Anti-inflammatory and anti-proliferative peptides derived from salmon

Anti-inflammatory properties of peptide fractions prepared from salmon by-products were studied by Ahn et al. (2012a). Salmon by-product protein from the pectoral fin was used for enzymatic hydrolysis. All salmon protein hydrolysates exhibited potent 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity in a dose-dependent manner. The purified pepsin-hydrolysate showed high DPPH scavenging activity (73% at 4 mg/mL) and hydrogen peroxide scavenging activity (87% at 4 mg/mL). The salmon hydrolysate was tested for inhibiting intracellular reactive oxygen species (ROS) and lipid peroxidation and its effect on glutathione (GSH) levels in Chang liver cells. The pepsin hydrolysate decreased intracellular ROS generation by 2.14-fold compared to the control group. It enhanced the GSH level in Chang's liver cells in a time and dose-dependent manner at a concentration of 1 mg/mL. The pep-sin hydrolysate also inhibited LPS-induced nitric oxide (NO) production (3.61 times) in a concentration-dependent manner, with the highest reduction at 400 µg/mL. The pepsin hydrolysate also inhibited proinflammatory cytokine production, including TNF-α (200 µg/mL), IL-6 (200 µg/mL), and IL-1β (200 µg/mL) in RAW264.7 macrophage cells in a concentration-dependent manner. In a follow-up study, Ahn et al. (2015) purified and studied the anti-inflammatory action of a tripeptide from salmon pectoral fin by-product protein hydrolysate. The purified anti-inflammatory peptide was identified as PAY, and it significantly inhibited the production of nitric oxide (NO) by 63% and prostaglandin E2 (PGE_2) by 45.33% in RAW264.7 cells at 0.75 mM concentration. The PAY tripeptide also inhibited LPS-stimulated TNF-α, IL-6, and IL-1β production in RAW264.7 macrophage cells at 0.75 mM concentration. On the other hand, Picot et al. (2006) studied the antiproliferative activity of fish protein hydrolysates against human breast cancer cell lines. Seven different types of fishes, including blue whiting, Atlantic cod, Atlantic salmon, Atlantic emporer, pollack, plaice, and Portuguese dogfish, were used to produce protein hydrolysates. The protein hydrolysates from blue whiting (35 and 28%), cod (40 and 20%), plaice (35 and 30%), and salmon (25 and 18%) exhibited significant growth inhibition relative to control against two human breast carcinoma cell lines, MCF-7/6 and MDA-MB-231 cells grown in vitro.

Dipeptidyl-peptidase IV (DPP-IV)-inhibitory peptides derived from salmon

DPP-IV degrades the glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), contributing to insulin secretion. Li-Chan (2012) studied the dipeptidyl-peptidase IV (DPP-IV)-inhibitory activity of peptides derived from Atlantic salmon skin gelatin hydrolyzed by Alcalase, Flavourzyme, and bromelain. The Flavourzyme hydrolysate prepared at 6% enzyme/substrate concentration exhibited the DPP-IV highest inhibition rate of 45.2%, followed by Alcalase (30%) and bromelain (23.1%). The peptides obtained within the < 1 kDa ultrafiltrate fraction exhibited the highest DDP-IV inhibition rate of 61.2%, whereas, the > 2.5 and 1–2.5 kDa fractions had 29.6 and 43.2% inhibition rates, respectively. The IC_{50} value of < 1 kDa fraction was 1.35 mg/mL. The < 1 kDa fraction was purified, and DPP-IV inhibitory activities were studied at a concentration of 100 µg solid/mL. The purified fraction had an IC_{50} value of 57.3 µg/mL. The peptides identified in the purified fraction were GPAE (372.4 Da) and GPGA (300.4 Da). The IC_{50} values of the two synthetic peptides, GPAE and GPGA, were 49.6 and 41.9 µM, respectively. Moreover, Jin et al. (2020) identified dipeptidyl-peptidase IV (DPP-IV)-inhibitory peptides from salmon skin collagen hydrolysate. The ultrafiltered sample analysis indicated that < 3 kDa fraction had the highest DPP-IV inhibitory activity with an IC_{50} value of 1.54 mg/mL. The study reported a novel hexapeptide (LDKVFR), and it also had the highest DPP-IV inhibitory activity. The molecular docking studies also revealed that six hydrogen bonds and eight hydrophobic interactions between LDKVFR and DPP-IV contributed to DPP-IV inhibition. Besides, Atlantic salmon processing by-products (skin) hydrolysates showed potent anti-diabetic effects similar to metformin in a genetically induced (ob/ob) mouse model, suggesting their potential to be used for the early management and prevention of Type 2 diabetes mellitus (Parthsarathy et al. 2021).

Antioxidative peptides derived from salmon

Atlantic salmon peptides have been reported to exhibit strong antioxidant potential. For instance, Wang et al. (2008) studied the purification and characterization of antioxidant peptides from salmon protamine hydrolysate.

Protamine is derived from fish milt, and it is usually discarded as an industrial by-product in fish plants. The salmon protamine hydrolysate was fractionated using size exclusion chromatography, and various fractions were analyzed for scavenging activity on hydroxyl radical, DPPH radical, and superoxide radical. The highest hydroxyl radical scavenging activity peptide (PR) had an IC_{50} value of 91.3 µg/mL. Likewise, Girgih et al. (2013) studied the antioxidant properties of peptides produced from salmon frame protein hydrolysates that were hydrolyzed sequentially with pepsin and trypsin + chymotrypsin, and the resulting hydrolysate was ultrafiltered through < 1 kDa membrane. Later, ultrafiltered protein hydrolysate was separated using reverse-phase HPLC into four peptide fractions (1–4). The results from this study indicated that fractions 2–4 exhibited higher ORAC values between 1315 and 1541 µM Trolox equivalent (TE)/g compared to non-fractionated protein hydrolysate (819.3 µM TE/g). A similar trend was seen for DPPH and superoxide radical scavenging activities. However, the non-fractionated salmon protein hydrolysates had higher metal chelating activity than the peptide fractions. The peptide fractions also strongly inhibited linoleic acid oxidation. In addition, the pressurized liquid extraction (PLE) technique was used to prepare antioxidant peptides from salmon muscle remains, skin, heads, viscera, and tailfins and found that both ORAC and Trolox equivalent antioxidant capacity (TEAC) assays exhibited strong antioxidant activity, mainly those prepared from viscera (de la Fuente et al. 2021). Hydrophobic amino acids, including alanine, proline, leucine, and valine, in salmon by-products could play an important role in showing antioxidant activity. Moreover, Hanachi et al. (2022) isolated salmon head peptides using membrane filtration, which exhibited strong ABTS and DPPH radical scavenging activities, ORAC, and metal chelation activity (MCA), as well as ACE inhibitory activity, with an IC_{50} value of 413.43 ± 13.12 µg/mL. The MCA could be linked to the presence of histidine and glutamic acid in salmon head peptides, providing more carboxylic groups and imidazole rings to bioactive peptides and increasing the electrostatic interaction with Fe^{2+} ions. Likewise, Rajendran et al. (2018) developed protein hydrolysates from Atlantic salmon processing waste (viscera) using lactic acid fermentation with the formic acid treatment and Flavourzyme and found that they showed higher metal chelation and ferric-reducing capacities. On the other hand, anti-allergic peptides were isolated from Atlantic salmon by-products using sephadex G-15 gel permeation chromatography, HPLC, and mass spectrometry (Wang et al. 2020). Results suggested that the isolated peptide (TPEVHIAVDKF) exerted excellent anti-allergic activity by inhibiting the release of β-hexosaminidase in immunoglobulin E (IgE)-mediated RBL-2H3 cell degranulation at IC_{50} value of 1.39 mg/mL. Slizyte et al. (2016) screened defatted salmon backbone protein hydrolysates using Corolase PP, Corolase 7089, papain, bromelain, Protex, Seabzyme L200, and trypsin for bioactivities. The highest DPPH radical scavenging activity was obtained from Protamex hydrolysates without oil separation before hydrolysis, followed by Corolase hydrolysates. The iron-chelating activities ranged between 54 and 87% for all hydrolysates. Bromelain + papain hydrolysates after 20 min had the best iron-chelating ability, indicating that larger peptides have a better ability to chelate iron, and this property weakens when the peptide size is reduced. Trypsin hydrolysates had the highest ACE inhibitory activity (IC_{50} = 0.9 mg/mL) after 120 min hydrolysis. Bromelain + papain hydrolysates (8 mg/mL) had the most significant glucose transport inhibiting activity (39% reduction) than other enzymes. The 250–300 Da dipeptides are reported to have a role in the regulation of glucose uptake.

In-silico production of bioactive peptides

The *in-silico* analysis involves computational methods applied to manage, curate, and interpret various biological systems. Protein databases such as UniProtKB, NCBI, and BIOPEP contain different protein sequences that can be utilized to analyze amino acid profiles of precursor proteins. Online tools such as BIOPEP and ExPASY-PeptideCutter can be used to select enzymes and proteins and predict theoretical bioactive peptide profiles. The *in-silico* hydrolysis results can be compared with the bioactive peptides reported in the literature and databases (Agyei et al. 2016; Senadheera et al. 2022; Tu et al. 2018; Vilas Boas et al. 2019). The *in-silico* approach for identifying and processing bioactive peptides is shown in Fig. 8. The different databases and tools are given in Table 5.

Darewicz et al. (2014) studied the ACE inhibitory peptides from salmon protein hydrolysates using three different methods: in silico, ex vivo, and in vitro. In the in silico analysis, the 52 amino acid sequences of salmon proteins were selected from the UniProt database. The proteolysis simulation using pepsin, trypsin, and chymotrypsin was conducted using the procedure built into the BIOPEP database. The *ex-vivo* digestion was carried out using human gastric juice (HGJ) and human duodenal juice (HDJ). The *in-vitro* digestion was carried out using pepsin and Corolase PP. The in silico digestion identified 11 ACE peptides from two types of salmon proteins (myofibrillar and sarcoplasmic). The two-step *ex-vivo* and *in-vitro* digestion were conducted to mimic the human digestion process. The results indicated that porcine enzymes more easily degraded salmon proteins than gastrointestinal enzymes. The sarcoplasmic proteins were

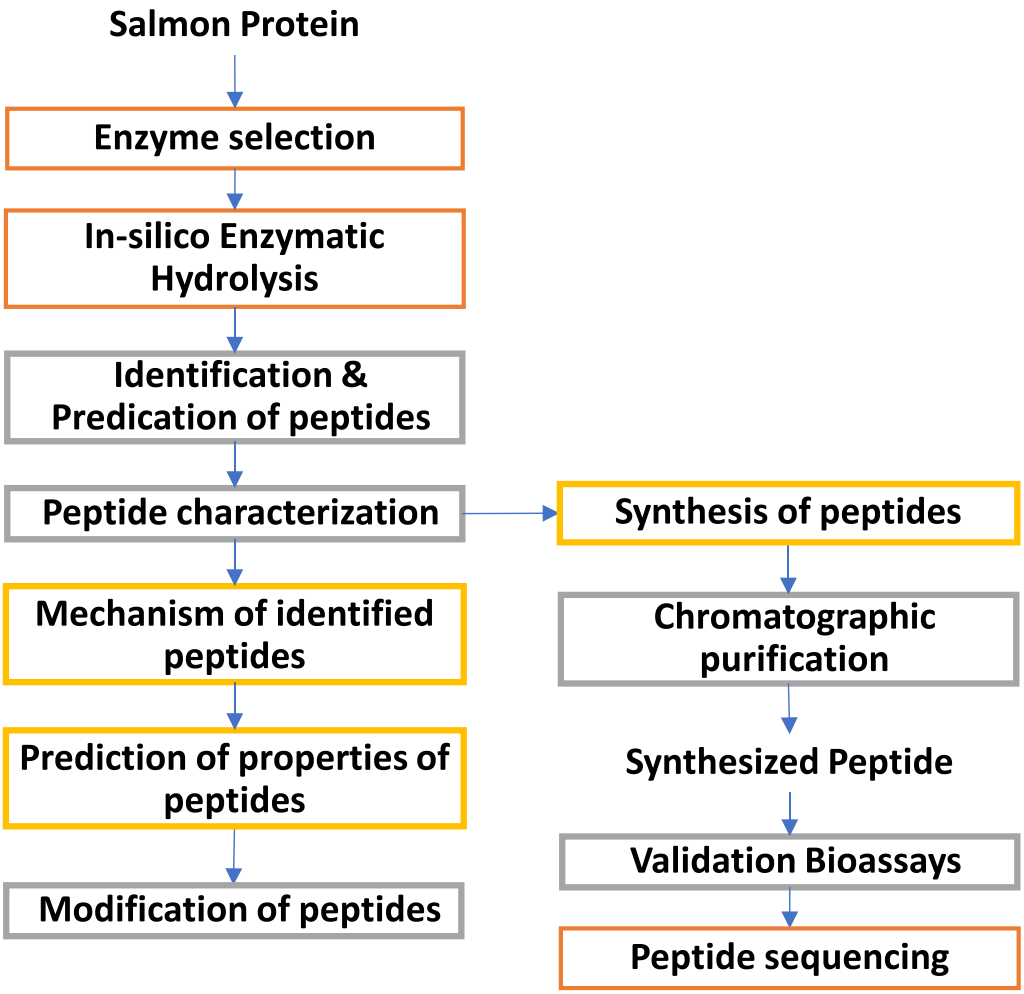


Fig. 8 In-silico production of bioactive peptides

broken down more easily than myofibrillar proteins. This study identified 9 and 7 peptides from *ex-vivo* and *in-vitro* hydrolysates, respectively, compared to 11 peptides identified in the in silico analysis (Table 4). The study indicated that results generated by in silico simulation of hydrolysis were not confirmed in the *in-vitro* studies due to the oversimplification assumed for the availability of all bonds susceptible to the enzyme in the polypeptide chain of the protein. Sometimes, incomplete data on the specificity of the enzyme can also lead to discrepancies (Senadheera et al. 2022).

Wang et al. (2017a) studied the potential of in silico approach for predicting DPP-IV inhibitory activity, *in-vitro*, of protein hydrolysates. In this study, 294 edible protein sequences (80 animal and 214 plant sources) and five commercial proteases were analyzed in silico. The authors hypothesized that protein hydrolysates with high contents of peptides having XP and XA might potentially be DPP-IV inhibitors. This study identified the frequency of specific amino acids by calculating the ratio of the number of peptides with proline, alanine, or proline + alanine as the second N-terminal residues to the total peptide fragments released by proteases. The in silico analysis was carried out on all combinations of protein sources and proteases. Based on these results, fifteen protein and protease combinations were selected for *in-vitro* analysis. The parvalbumin-beta 2, Atlantic salmon (*Salmo salar*; BIOPEP ID 1739), have the most alanine content (19.4 alanine/100 amino acid residues) and was suggested to have great potential as the precursor of DPP-IV inhibitory peptides. Unfortunately, the salmon proteins were not chosen for further *in-vitro* studies. However, the *in-vitro* studies from other proteins indicated that the DPP-IV inhibition rate increased with the degree of hydrolysis and hydrolysis time. The correlation studies between DPP-IV inhibition rates vs frequency of proline, alanine or proline + alanine (A%) showed that the selection of proline + alanine during in silico analysis

Table 5 Common *in-silico* databases reported in the literature

Databases	Name	Webpage	Purpose	Reference
Protein/peptide sequence databases	BIOPEP	http://www.uwm.edu.pl/biochemia/index.php/en/biopep	Protein and peptide database	Agyei et al. (2016); Cárdenas et al. (2020); De La Fuente et al. (2021); Senadheera et al. (2022); Tu et al. (2018); Vilas Boas et al. (2019)
	PepBank	http://pepbank.mgh.harvard.edu/	Peptide database	
	BioPD	http://biopd.bjmu.edu.cn/	Peptide database	
	SwePep	http://www.swepep.org/	Sweden peptide database	
	EROP-Moscow	http://erop.inbi.ras.ru/	Peptide database	
	MilkAMP	http://milkampdb.org/home.php	Dairy antimicrobial peptide database	
	UniProtKB	http://www.uniprot.org/	Primary sequence and structural information for proteins	
	NCBI Protein database	http://www.ncbi.nlm.nih.gov	Basic sequence information for proteins	
	PeptideDB	http://www.peptides.be/	Peptide database	
	AVPdb	http://crdd.osdd.net/servers/avpdb/index.php	Database of anti-viral peptides	
	CAMPR3	http://www.camp.bicnirrh.res.in/	Collection of anti-microbial peptides	
	HIPdb	http://crdd.osdd.net/servers/hipdb/index.php	Database of HIV inhibiting peptides	
	BACTIBASE	http://bactibase.hammamilab.org/main.php	Database of bacteriocins	
	APD	https://wangapd3.com/main.php	Antimicrobial peptide database	
		http://aps.unmc.edu/AP/		
	BitterDB	http://bitterdb.agri.huji.ac.il/dbbitter.php	Database for bitter peptides	
In silico digestion Tools	BIOPEP	http://www.uwm.edu.pl/biochemia/index.php/en/biopep	Tools for peptide predication	
	PeptideCutter	http://web.expasy.org/peptide_cutter/	Predict peptide cleavage sites	
	POPS	http://pops.csse.monash.edu.au/pops-cgi/index.php	Tool for modelling and predicting protease specificity	
		http://www.mybiosoftware.com/pops-2-1-1-prediction-protease-specificity.html		
	NeuroPred	http://stagbeetle.animal.uiuc.edu/cgi-bin/neuropred.py	Prediction of cleavage sites and mass from neuropeptide precursors	
	CAMPSign	http://www.campsign.bicnirrh.res.in/	Identification of AMPs using AMP family signatures	
	Enzyme Predictor	http://bioware.ucd.ie/~enzpred/Enzpred.php	Evaluation tool to determine the cleavage ability of enzymes	

Table 5 (continued)

Databases	Name	Webpage	Purpose	Reference
Potential bioactivity prediction	PeptideRanker	http://bioware.ucd.ie/~compass/biowareweb/	Pediction of bioactive peptides	
	BIOPEP	http://www.uwm.edu.pl/biochemia/index.php/en/biopep	Pediction of bioactive peptides	
	AntiBP2	http://www.imtech.res.in/raghava/antibp2/	Antibacterial peptide prediction tool	
	AVPpred	http://crdd.osdd.net/servers/avppred/submit.php	Collection and prediction of antiviral peptides	
	AVP-IC50Pred	http://crdd.osdd.net/servers/ic50avp/index.html	Prediction of peptide antiviral activity in terms of IC_{50}	
Toxicity/allergenicity prediction				
	ToxinPred	http://crdd.osdd.net/raghava/toxinpred/	Predicting the toxicity of peptides	
	BIOPEP	http://www.uwm.edu.pl/biochemia/index.php/en/biopep	Allergenic protein database	
	AlgPred	http://crdd.osdd.net/raghava/algpred/	Predicting allergenic proteins and peptides	
	Allerdictor	https://openebench.bsc.es/tool/allerdictor	Allergen prediction tool	
	EPIMHC	http://bio.med.ucm.es/epimhc/	Database of Naturally Processed MHC-restricted Peptide Ligands and Epitopes for Customized Computational Vaccinology	
Physicochemical characteristics prediction	Expasy-Compute pI/Mw	http://web.expasy.org/compute_pi/	A tool to compute the theoretical pI (isoelectric point) and MW (mol weight)	
	ProtParam	http://web.expasy.org/protparam/	A tool to compute the grand average of hydropathicity (GRAVY) and Instability index	
Peptide Structure Prediction Server	PepDraw	http://www.tulane.edu/~biochem/WW/PepDraw/	A tool to compute net charge and hydrophobicity	
	Pep-Fold	https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/	Predicting peptide structures from amino acid sequences	
	PEPstrMOD	http://osddlinux.osdd.net/raghava/pepstrmod/	Predicts the tertiary structure of small peptides with sequence length varying between 7 to 25 residues	
	I-TASSER	https://zhanglab.ccmb.med.umich.edu/I-TASSER/	Protein structure prediction and structure-based function annotation	

showed a strong correlation with *in-vitro* DPP-IV inhibition rates. However, the in silico selection of proline and alanine had a moderate and weak correlation with the *in-vitro* analysis. Similarly, Lacroix and Li-Chan (2012) evaluated the potential of various dietary proteins as precursors of DPP-IV inhibitors by an in silico approach. The study used three proteins from Atlantic salmon (actin-cytoplasmic 1, myosin regulatory light chain 2, and slow myosin heavy chain) and one protein from Chum salmon (Type 1 collagen alpha 2 chain) reported in the UniProt KB database. The in silico study identified 499 fragments matching sequences known to present an inhibitory activity against the DPP-IV enzyme. The GP and PG were the most frequently occurring sequences and were mainly found in the collagen proteins due to the high contents of proline and glycine. Collagen from Atlantic salmon and Chum salmon had a DPP-IV peptides occurrence frequency value of 0.110 and 0.305, respectively. Unlike other protein sources investigated in this study, salmon collagens contained relatively high numbers of DPP-IV inhibitory tripeptides APG and GPA. The study concluded that salmon proteins were one of the best potential precursors of DPP-IV inhibitors. In contrast, the plant commodity oat was the least promising potential source of DPP-IV inhibitors. In addition, de la Fuente et al. (2021) identified 67 peptides from salmon viscera and predicted their antioxidant activity using the BIOPEP-UMP database. Bioinformatics analysis exhibited a few small antioxidant peptides encrypted in amino acid sequences, mainly glycine-alanine-alanine and glycine-alanine-alanine. Most of these predicted antioxidant peptides were di- and tri-peptides in nature.

Computational characterization of peptides

The peptides obtained from in silico methods are subjected to computational models such as quantitative structure–activity relationships (QSAR) and quantitative structure–property relationships (QSPR). The QSAR methodology is used to describe the biological activity (ϕ) as a function of chemical structures (C) differentiated by molecular or physicochemical variables. In 1868, Crum-Brown and Fraser published the first equation for QSAR, as shown below (Gad 2014; Tichý et al. 2008).

$$\varphi = f(\mathrm{C})$$

The QSAR modeling starts with data collection and pre-processing. During this step, the bioactive peptide library is built by collating target peptide sequences for QSAR models. The data collected is transformed using smoothing, normalization, and aggregation. The data are further divided into two data sets: training and test. The training data set is used to formulate the QSAR model, while the test data is used to validate its predictability and accuracy. The molecular descriptors and features are selected. In this process, the peptides are described using selected physicochemical or amino acid descriptors. When a large set of descriptors is obtained, the features are filtered using filter and wrapper methods to reduce the dataset horizontally. This technique removes collinearity between the descriptor pairs. The heuristic methods used to select features are based on multiple linear regression, and it discards constant values and removes descriptors for all structures containing no value. The QSAR model is constructed using various statistical methods. The statistical techniques are classified into regression-based, classification-based, and machine-learning techniques. Once the model is built, it is validated using the test data. The parameters used in the model should be interpretable. The mechanistic interpretation is used to understand the influence of descriptors in the predicted activity. The applicability domain analysis is used to know whether the built models can be used for any set of compounds. In some instances, confirmatory studies allow for external validation of the QSAR model by synthetically manufacturing peptides for robustness and bioactivity (Nongonierma & Fitzgerald 2016, 2017; Peter et al. 2018). The schematic representation of QSAR modeling is shown in Fig. 9.

Over the years, amino acid descriptors have played a vital role in the peptide's structural variation. Amino acid descriptors help the modeling and prediction of biological activity as a function of molecular structure. The properties of the entire peptide can be forecasted with amino acid descriptors. The two-dimensional (2D) amino acid descriptors include hydropathic scale, amino acid z-scales for hydrophilicity/hydrophobicity (z1), molecular size/bulkiness (z2) and electronic properties/charge (z3), molecular electronegativity edge vector (MEEV) scales, divided physiochemical property score (DPPS) descriptor, the vectors of hydrophobic, steric and electronic properties (VHSE) scales. The three-dimensional (3D) amino acid descriptors use isotropic surface area and electronic charge index (ISA-ECI) and molecular surface-weighted holistic invariant molecular (MS-WHIM) scales (Agyei et al. 2016). In QPSR studies, the physiochemical properties of chemical compounds are determined based on the molecular structure information. Physiochemical properties such as melting point, boiling point, stability, dielectric constant, diffusion coefficient, thermodynamic properties, and hydrophobicity are tested for determining QSPR (Peter et al. 2018).

Purification and separation of peptides

The bioactive peptides are receiving increased interest to be produced commercially. The isolation and purification of bioactive peptides are essential for studying their

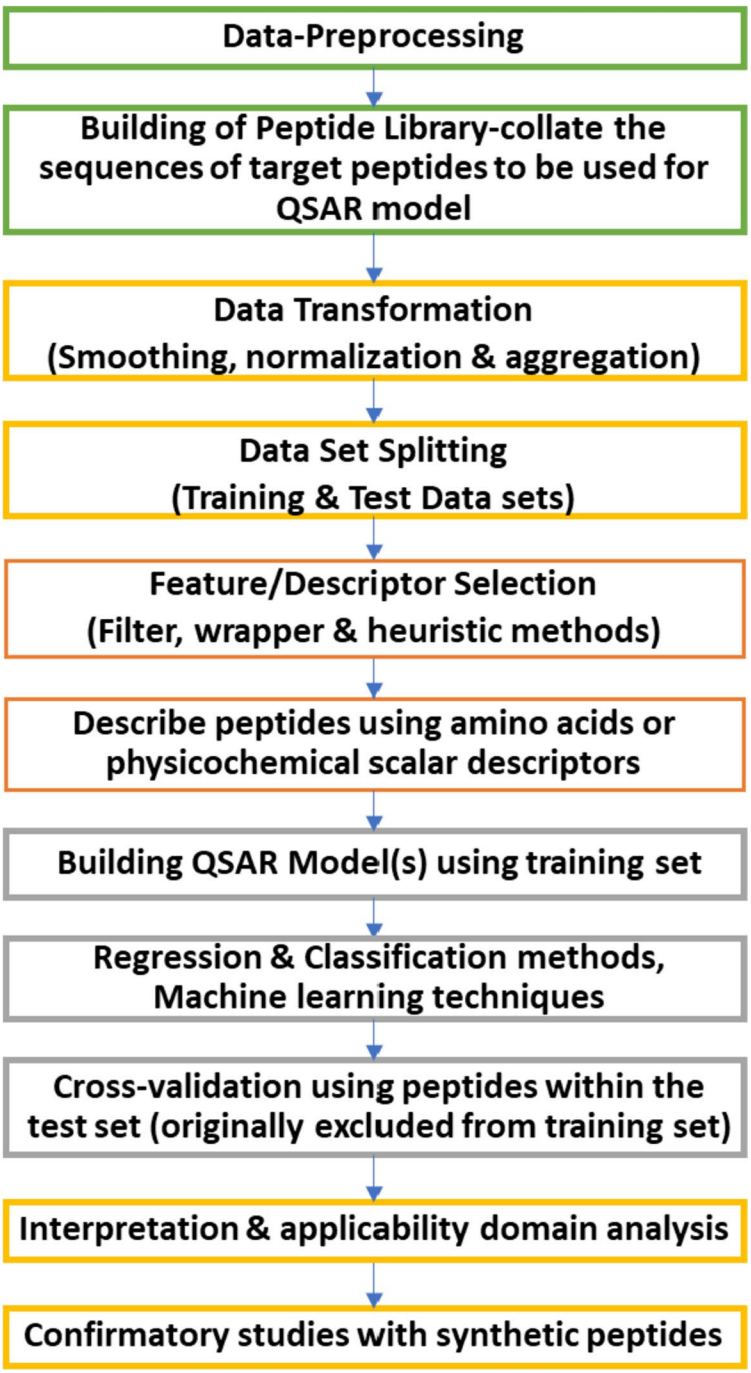


Fig. 9 Schematic representation of QSAR modelling

physicochemical properties and evaluating the bioactive properties by *in-vitro* and *in-vivo* assays. The three main factors that affect the purification process include purity, cost of production, and process time. Conventional purification of biological material involves various steps, including removing of the insoluble particles, isolation and concentration of products, purification, and polishing g (Lemes et al. 2016). It is estimated that about 70% of production cost is for separation and purification processes alone. Therefore, the purification steps should be straightforward, simple, and involve minimum processing steps. The most important is to know about the target peptide's structure, function, and properties to set up a correct purification strategy. Traditional isolation

and purification methods for peptides include selective precipitation, membrane filtration, and chromatographic techniques. The most common technologies used in the separation and purification of peptides are shown in Fig. 10 (Agyei & Danquah 2011; Agyei et al. 2016; Lemes et al. 2016; Thorkelsson & Kristinsson 2009; Udenigwe & Aluko 2012;).

Selective precipitation

The selective precipitation processes such as ammonium sulfate precipitation, isoionic precipitation, salting out, and solvent extraction can be used before the separation process to remove protein, enzymes, and other crude extract components (Lemes et al. 2016; Shahidi & Zhong 2008). These processes can be used at all stages of purification, from bulk recovery to selective isolation and are easy to scale up. The precipitation process is a cheap, simple, high throughput process that can be utilized in continuous operations. However, some precipitation processes are not effective for some proteins and peptides. Hydrophobic peptides with low solubility in aqueous and organic solvents are difficult to separate by this process. The precipitation process also requires a subsequent cleaning step to remove the precipitation agent from the peptide of interest (Agyei et al. 2016).

Membrane filtration

Membrane filtration uses selective barriers to transmit specific components while retaining other parts under pressure. The most common membrane filtration technologies include microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). The microfiltration membranes filter particles and globules with pore sizes between 100 and 10,000 nm. The ultrafiltration membranes filter protein and peptides with pore sizes between 2 and 100 nm. The nanofiltration membrane filters salt, solutes, and amino acids with pore sizes between 0.5 and 2 nm. The reverse osmosis process is mainly used to filtrate monovalent ions (Na^+, K^+, and Cl^-) with pore sizes between 0.1 and 1 nm (Bazinet & Firdaous 2013; Hakami et al. 2020; Lemes et al. 2016). Membrane filtration processes like ultrafiltration and nanofiltration are the most common for bioactive peptide filtration and concentration. These processes have several advantages, including (a) high throughput and scalable for continuous operation, (b) maintaining protein and peptide integrity, (c) improved enzyme yield and process productivity levels, (d) eliminating the use of any additives, (e) allowing selective transport and sound separation, and (f) performing at isothermic conditions and a fixed pH (Agyei et al. 2016; Bazinet & Firdaous 2013). Some of the disadvantages of using the membrane filtration process include (a) poor selectively while separating similar size peptides, (b) membrane fouling, (c) increased viscosity of the retentate and (d) low mass transfer rates for highly concentrated raw material (Agyei et al. 2016; Wang et al. 2017b). In recent years, applying the external electrical field as an additional driving force to the pressure gradient has been investigated to improve the separation efficiency of charged bioactive molecules. Some of these combination membrane processes include electromembrane filtration (EMF) and electrodialysis with UF membrane (EMUF) (Muro et al. 2013).

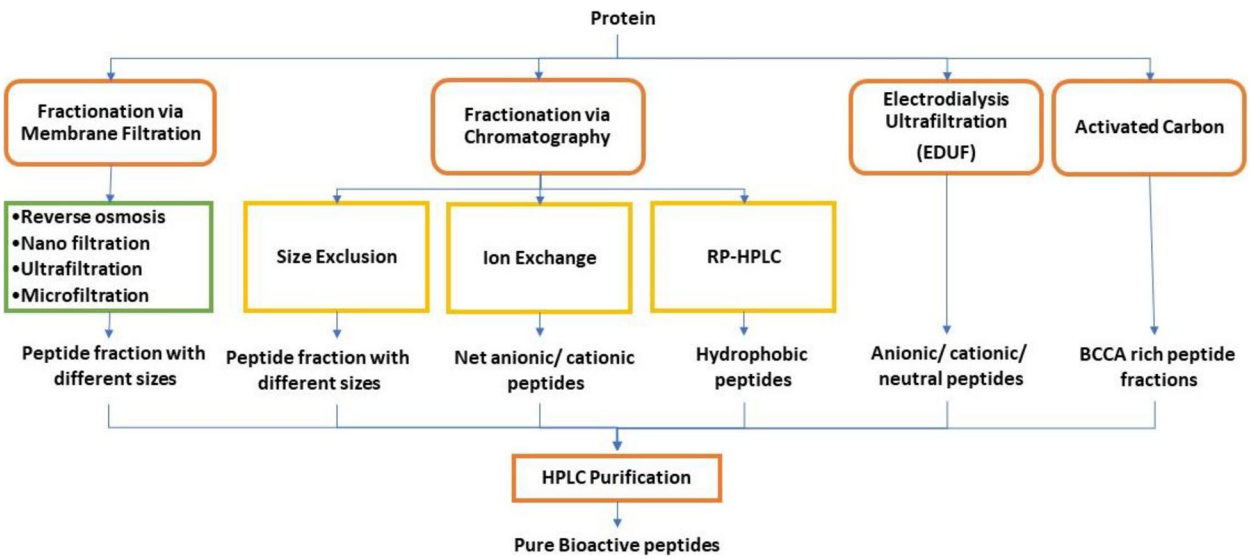


Fig. 10 Technologies for peptide purification

Electromembrane filtration combines membrane filtration and electrophoresis mechanisms, making it more selective than membrane filtration and less costly than chromatograph. In addition, operating parameters such as type of membrane, electrical field strength, salination, and hydrolysate concentration can be manipulated to improve the product transfer and rate of separation (Agyei & Danquah 2011). Suwal et al. (2018) extracted antioxidant peptides in a two-step process from rainbow trout protein hydrolysate using EMF. Electrodialysis with ultrafiltration membranes (EUDF) combines the charge selectivity feature of electrodialysis and size separation capacity (molecular weight cut-off) of ultrafiltration. This process also allows selective and simultaneous separation of anionic and cationic peptides. A study reported that the EUDF process allowed a selective and simultaneous separation of anionic and cationic peptides from the snow crab by-product protein hydrolysates. Two anti-cancer peptides were obtained in this study using the EUDF process (Doyen et al. 2011). Roblet et al. (2016) studied the separation of salmon frame bioactive peptides with antidiabetic effects using the EUDF process. In this study, two UF membranes and two ion-exchange membranes were used, and the researchers were able to obtain both anionic and cationic peptides.

Chromatographic techniques

Chromatographic techniques are considered the most powerful technique to isolate and purify bioactive peptides. The most used chromatographic techniques are re-versed-phase, ion exchange, size exclusion, and affinity chromatography. Some of the common advantages of using chromatographic techniques include (a) being highly selective and offering high resolution in a short time, (b) being an insoluble separating agent, and (c) concentrating and stabilizing target peptides. The disadvantages of using chromatography include (a) costly, especially during scale-up operations, (b) difficulty in treating viscous materials, (c) slow binding, (d) bead deformation during high pressure drops in scale-up operations, (e) solvent waste resulting environmental concern, (f) mass transfer limitation and steric hindrance can cause reduced capacity (Agyei et al. 2016).

Ion-exchange chromatography (IEC)

The ion-exchange chromatography is based on the principle of the attraction of oppositely charged molecules. Each protein molecule carries a surface charge depending on pI and pH. Each ion exchange membrane is made up of porous beads, which assist in the adsorption of the target molecule. Generally, protein above their pI is negatively charged and can bind to oppositely charged anion exchange beds. Whereas protein below their pI is positively charged and can bind to oppositely charged cation exchange beds (Jungbauer & Hahn. 2009; Lemes et al. 2016; Wang et al. 2017b). ACE inhibitory peptides were purified from salmon by-products using ion-exchange chromatography (DEAE FF ion-exchange column). The IEC fractions were monitored at 280 nm and investigated for the active ACE fraction (Ahn et al. 2012b). After SEC purification of salmon protamine hydrolysate, the highest antioxidant activity fraction was purified for the second time using a Macro-Prep High Q Support ion-exchange column. The IEC fractions were studied for antioxidant activity and were subjected to further purification steps (Wang et al. 2008).

Size Exclusion chromatography (SEC)

Size exclusion chromatography (SEC), also known as gel filtration chromatography (GFC), has been employed for the separation, desalting, and molecular weight estimation of peptides and proteins. The molecules fractionated using SEC do not bind to the chromatography medium, so the buffer composition does not directly affect the resolution (Lemes et al. 2016; Perez Espitia et al. 2012; Wang et al. 2017b). The SEC is used primarily in the early stages of the purification of peptides. The SEC's resolution is influenced by particle size, particle uniformity, bed height, column packing quality, flow rate, sample concentration, and volume (Wang et al. 2017b). ACE inhibitory peptides were purified from salmon by-product peptic hydrolysate using size exclusion chromatography (BioBasic SEC 60 column). The SEC fractions were monitored at 280 nm and investigated for active ACE fractions (Ahn et al. 2012a). The size exclusion chromatography was used as a second purification step for purifying salmon ACE fraction, which was previously purified using IEC. The SEC purification (Sephadex G-25 gel filtration column) was monitored at 280 nm and pooled, and the ACE inhibitory activity was determined (Ahn et al. 2012b). Wang et al. (2008) studied the antioxidant capabilities of salmon protamine hydrolysate. In this study, protamine hydrolysate was fractionated using size exclusion chromatography on the Sephadex G-35 column. These fractions were studied for antioxidant activity and were subjected to a further purification process. Picot et al. (2006) determined the molecular weight distributions of salmon protein hydrolysates using SEC in FPLC mode on a Superdex Peptides HR 10/30 column. Similarly, Slizyte et al. (2016) used SEC to separate salmon peptides based on molecular weight using Superdex Peptides HR 10/300 column.

Reversed-phase high-performance liquid chromatography (RP-HPLC)

The reversed-phase high-performance liquid chromatography (RP-HPLC) is a widely used technique for

fractionating peptides based on their hydrophobic properties. The RP-HPLC contains a stationary phase of lower polarity and a mobile phase of higher polarity. The main advantages of using RP-HPLC include ease of use, high resolution and sensitivity, and shorter run time than SEC and IEC (Lemes et al. 2016; Perez Espitia et al. 2012; Wang et al. 2017b). RP-HPLC was used as the third and final purification and fractionation of active salmon ACE inhibitory peptides after two prior purifications with IEC and SEC. The column used was ODS C_{18} (Hypersil Gold, 4.6 mm × 250 mm). The RP-HPLC purified salmon ACE inhibitory peptides were used for peptide sequencing and inhibition mode studies (Ahn et al. 2012b). RP-HPLC was used as the third and final purification and fractionation of protamine peptides using the YMC-Pack Protein-RP column. The highest antioxidant activity RP-HPLC fraction was collected and used in further characterization studies (Wang et al. 2008).

Identification and characterization of peptides

HPLC, as a separation technique, together with identification tools such as tandem mass spectrometric detection (HPLC–MS/MS), is the standard method for characterizing peptide sequences. The tandem MS/MS contains two or three quadrupoles and a TOF analyzer. The analysis of peptides in MS/MS happens in two stages: (a) a predetermined set of mass-to-charge (m/z) ions are separated from the rest of the ions from the ion source and fragmented via chemical reaction, and (b) mass spectra are produced for the fragments s (Lemes et al. 2016; Perez Espitia et al. 2012; Wang et al. 2017b). All peptides reported from Atlantic salmon shown in Table 4 were identified using tandem mass spectrometric detection (Ahn et al. 2012b, 2014, 2015; Gu et al. 2011; Neves et al. 2017b; Wang et al. 2008).

Challenges and commercial approval

In Canada, bioactive peptides, especially collagen peptides, are available in the market as natural health products (NHPs) for promoting skin health and other effects. The collagen peptides are sold under the Health Canada monograph as hydrolyzed collagen from bovine, porcine, chicken, fish, and shark (NPN: 80,108,792). ACE inhibitory peptides extracted from shrimp protein hydrolysate are also approved by Health Canada to be sold as a natural health product (NPN: 80,080,580). As per Health Canada's approved claim, ACE inhibitory peptides help to maintain healthy blood pressure and support cardiovascular health. The beneficial effects of bioactive peptides as functional foods can be represented with disease risk reduction claims or physiological function claims (Chalamaiah et al. 2019; Health Canada 2021).

The food health claims must be supported by substantial evidence from human studies. Health Canada assesses the substantiation of food health claims through ten guiding principles. The guiding principles include systematic approach, transparency, comprehensiveness, human evidence, high level of certainty, demonstration of causality, biological relevance of the claimed effect, feasibility of consumption of effective dose, health claim wording, and substantiation of one food-health relationship in a submission. *In-vitro* studies from animal studies can be used to support the results of human studies (Bureau of Nutritional Sciences 2009; Health Canada 2015). Other essential regulatory requirements for food health claims include a summary of the physical, chemical, and microbiological properties of peptides, manufacturing steps of the bioactive peptides, stability data of the peptides under suggested storage conditions, and the results of batch variability (Chalamaiah et al. 2019).

The necessary testing and research work for regulatory approval is very complex, time-consuming, and expensive. Most fish producers and processors operate in remote areas and are small-scale operations. It is challenging for small-scale producers and processors to take up such a complex process on their own. Some large-scale producers and processors are solely focused on producing and selling high-quality fish. Most of their revenue is from the edible portion of fish. At present, most of them are comfortable selling their by-products for cheaper returns as fish feed and fertilizer. However, with the proper foundational research work and testing, high-quality bioactive peptides can be produced with considerable economic returns than feed or fertilizers.

Conclusion

Atlantic salmon and its by-products have proven to be an excellent repository of potent bioactive molecules such as protein, omega-3 oil, collagen, gelatin, peptides, enzymes, hydroxyapatite, and minerals. Biomolecules such as bioactive peptides display potent antioxidant, antihypertension, antimicrobial, anticoagulant, and antidiabetic activities. However, these by-products are currently used mainly to produce low-value products such as fertilizer, silage, pet food, and fish meals. There are many reasons, including the cost of production, human resources with high technical knowledge and skills to work in remote places of the country, the quality control process for by-products handling during processing and infrastructure such as additional cold storage facilities, especially for by-products can impede many processors and producers to be involved in the production of high-value products. However, these biomolecules present in Atlantic salmon are low-volume and high-value

products. The transformation of the salmon processing by-products into high-value products can revitalize many rural fish communities, fish producers, and processors. Therefore, it is essential to look at different processing techniques, such as ultrasound and high-pressure, to assist the production processes to save time and money with no compromise on quality. Processes like ultrasound and high-pressure processing are already available on an industrial scale. They have been proven to effectively reduce costs and increase the performance and quality of the final product. Furthermore, a critical impeding factor in marketing these biomolecules, such as bioactive peptides, to human health and nutrition requires in vivo human studies. Most of the studies reported in the literature about bioactive peptides remain at the in vitro cell culture stage, and thus, extensive in vivo animal studies are required in order to use them as functional ingredients.

Acknowledgements
Not applicable

Authors' contributions
Conceptualization, V.V.R., D.D., and F.S.; investigation, V.V.R., A.H., D.D., and F.S.; resources, D.D., and F.S.; data curation, V.V.R. and A.H.; writing—original draft preparation, V.V.R.; writing—review and editing, V.V.R., A.H., D.D., and F.S.; visualization, V.V.R., A.H.; supervision, D.D. and F.S.; funding acquisition, D.D. All authors have read and agreed to the submitted version of the manuscript.

Funding
This research was funded by the Ocean Frontier Institute (OFI), through an award from the Canada First Research Excellence Fund.

Availability of data and materials
The dataset used and/or analyzed during this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
Dr. Fereidoon Shahidi is a member of Editorial Board of *Food Production, Processing and Nutrition* and he was not involved in the journal's review of, or decisions related to this manuscript.

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Received: 31 July 2023 Accepted: 31 October 2023
Published online: 07 January 2024

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