

Enzymatic fish protein hydrolysates in finfish aquaculture: a review

Muhammad A.B. Siddik^{1,2} , Janet Howieson¹, Ravi Fotedar¹ and Gavin J. Partridge^{3,4}

1 School of Molecular and Life Sciences, Curtin University, Perth, WA, Australia

2 Department of Fisheries Biology and Genetics, Patuakhali Science and Technology University, Patuakhali, Bangladesh

3 Department of Primary Industries and Regional Development, Australian Centre for Applied Aquaculture Research, Fremantle, WA, Australia

4 Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute and School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA, Australia

Correspondence

Muhammad A. B. Siddik, School of Molecular and Life Sciences, Curtin University, Perth, WA 6102, Australia. Email: siddik@pstu.ac.bd and m.siddik@postgrad.curtin.edu.au

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Abstract

In intensive farming systems, fish are held at high densities, which may increase stress, leading to susceptibility to diseases that result in economic losses. Therefore, effective feeding practices incorporating health-promoting compounds such as proteins, hydrolysates and bioactive peptides that can stimulate the defence mechanisms of fish and achieve better growth are some of the priorities for sustainable aquaculture development. Globally, the fish processing industries generate and discard a large volume of waste every year, estimated at up to 60% of the harvested biomass. This waste can be converted to value-added products such as fish protein hydrolysate (FPH) with the addition of various proteolytic enzymes. FPH from fish processing waste including skin, heads, muscle, viscera, liver and bones is a good source of protein, amino acids, peptides and antioxidants and has been found to possess desirable functional and bioactive peptides. A moderate inclusion of FPH in aquafeeds has the potential to improve growth, feed utilization, immune functions and disease resistance of fish. Production of FPH, targeted to more precise molecular weight ranges, has superior functionalities that are in high demand. With interest in FPH as an aquafeed supplement, this review aimed to summarize the source, production processes and functional properties of FPH and the reported impact of FPH in aquafeed supplement on fish growth, survival, feed utilization, immune response and disease resistance. Possible limitations of using FPH and future research potential as an opportunity for the use of processing fish waste are also discussed.

Key words: antioxidant activity, aquaculture, bioactive peptide, enzymatic hydrolysis, fish protein hydrolysate, functional properties, immune response.

Introduction

Aquaculture is the fastest growing food production sector in the world, supporting the protein needs for an increasing human population (FAO 2018). However, this rapidly expanding sector is being marred by the occurrence of various diseases, leading to high mortalities (Hoseinifar *et al.* 2018; Kibenge 2019). In recent years, the use of antibiotics, disinfectants and chemotherapeutic drugs has been increasing to protect the farmed animals from invasive pathogens. However, such applications as an approach to combat pathogens have been questioned. As an example, in the case of bacterial infections, antibiotics are being commonly

used, and consequently, bacteria are developing strong resistance against antibiotics (Rico *et al.* 2017; Brunton *et al.* 2019). Furthermore, the practice of drug use against the target pathogens may cause risks to the aquaculture production systems and in the subsequent products destined for human consumption, which could impact on consumer health and cause unfavourable ecological and economic impacts (Brunton *et al.* 2019; Lulijwa *et al.* 2020). Therefore, to reduce the dependency on external medicines, the application of vaccines, microbial intervention (bioremediation, fermentation), probiotics and immune stimulants are key topics of aquaculture research (Panigrahi & Azad 2007; Dawood & Koshio 2019; Foysal

et al. 2020). Though vaccines against several harmful diseases such as bacterial disease, furunculosis and columnaris in finfish aquaculture have proven effective, vaccines have not been developed for many pathogenic viruses and bacteria (Pettersen *et al.* 2015; Dadar *et al.*, 2017). Thus, the use of dietary immune stimulants for boosting the innate immunity of farmed fish has received global attention as a disease and stress resistance strategy (Chaturvedi *et al.* 2018). However, finding a cost-effective, nutritionally balanced, growth-promoting and easily available immune supplements is a challenge for all aquaculturists.

Aquaculture industry relies on quality aquafeeds in which fishmeal (FM) produced from wild harvested fish is still considered the most effective protein source (FAO 2018). The indiscriminate use of marine pelagic fisheries, with possible ecological and environmental consequences, has resulted in concerns being raised over the sustainability of the aquaculture industry (Ahmed *et al.* 2019; Longo *et al.* 2019). Environmental concerns on its usage and the broadening gap between demand and supply of FM have resulted in extensive investigations focusing on identifying viable aquafeed protein alternatives to FM (FAO 2018; Siddik *et al.* 2018a). As an alternate protein source, utilization of conventional plant-based protein for finfish aquaculture faces a number of challenges due to undesirable characteristics including imbalanced amino acid profiles and antinutritional factors (ANFs) that can affect the growth performance, feed utilization, digestibility and overall health status of fish (Francis *et al.* 2001; Vo *et al.* 2020). Although animal by-products are considered a good source of protein, the utilization of these products in aquafeeds is still constrained by various factors including lack of some essential amino acids, high moisture, indigestible particles, microbial contaminants and the possibility of disease transmission (Mondal *et al.* 2008; Samaddar *et al.* 2015; Siddik *et al.* 2019a). Therefore, it is important to investigate economically viable, environmentally sustainable and readily available alternative to FM protein sources for sustaining the growth of aquaculture.

Waste streams from seafood processing industries can be in excess of 60% by weight of by-products including skin, fins, head, trimmings, viscera, frames and roe (Chalamaiah *et al.* 2012). These large quantities of processing by-products are commonly converted into low-value products such as animal feed, FM and fertilizer (Hsu 2010). There is significant potential to utilize these protein-rich waste materials by converting them into more valuable, bioavailable nutritional food products such as fish protein hydrolysate (FPH) (Benhabiles *et al.* 2012). FPH, a fish waste rendered product, can be produced in either liquid or powdered form and contains a larger proportion of smaller peptides of approximately 2-20 amino acids. In unhydrolysed product, these short-chain peptides are intact within the

sequence of the parent proteins but may be released by the action of enzymatic hydrolysis under accelerated conditions using proteolytic enzymes such as alcalase, protease, trypsin and pepsin (Sarmadi & Ismail 2010; Chalamaiah *et al.* 2012). It has been reported that FPH has excellent physico-chemical properties including increased solubility, emulsifying properties, foaming properties, water-holding capacity and fat binding capacity, which in turn increase feed palatability and simplify the biological nutrient uptake (Kasumyan & Døving 2003; Bhaskar *et al.* 2007; Gajanan *et al.* 2016). In addition, peptides derived from FPH have shown various physiological benefits including antioxidant, antihypertensive, antimicrobial, immunomodulatory and anticancer activities when consumed *in vivo* (Kang *et al.* 2019; Yaghoubzadeh *et al.* 2020).

In aquaculture, dietary inclusion of short-chain peptide-rich FPH, at appropriate levels, has been shown to induce growth performance, nutrient utilization, antioxidant activity and immune response of fish (Zheng *et al.* 2013b; Ospina-Salazar *et al.* 2016; Wei *et al.* 2016; Siddik *et al.* 2018b), especially for larvae and juveniles (Xu *et al.* 2016; Siddik *et al.* 2019b). In response to specific infections against viral, bacterial and parasitic infections, fish fed with FPH have also been found to increase innate immunity and disease resistance of fish (Kotzamanis *et al.* 2007; Siddik *et al.* 2019b; Chaklader *et al.* 2020).

Indeed, a great deal of research has been conducted using different types of FPH as a dietary ingredient evaluating growth performance, immune response and disease resistance of fish. However, no comprehensive review has been conducted so far on this subject. The aim of the present review was to summarize the sources, production processes and the results of the application of FPH as a feed supplement for different aquaculture species with respect to growth performance, feed utilization, digestibility, immunity and specific disease resistance of target fish.

Sources of FPH

Global seafood production has increased considerably over the years, reported at about 171 million tonnes in 2016, of which 47% came from aquaculture (FAO 2018). Fish fillets are often considered the most desired product in the market, even though this can result in up to 60% of the harvested fish volume being discarded. Hence, seafood processing operations may produce a large volume of filleting waste. For example, Australian seafood industries have been reported to produce over 100,000 tonnes of such processing by-product annually (Peter & Clive 2006; He *et al.* 2013). This by-product may include muscle, skin, fins, frames, head, viscera, trimmings and roe, which are classified as fish wastes and not used for human food. Profitable utilization of these fishery by-products is an important area

both for research and for fish industry. FPH is a possible outcome of such discards in both marine and freshwater fish, and hence have been the subject of many studies (García-Moreno *et al.* 2017; Giannetto *et al.* 2020; López-Pedrouso *et al.* 2020).

Dark muscle of fish rich in protein has limited value for human consumption due to the high possibility of oxidation and off-flavour, which may result in low consumer appeal and low market value of manufactured products (Chalamaiah *et al.* 2012). Therefore, some studies have utilized this dark fish muscle for production of FPH, converting it into a highly valuable product (Naqash & Nazeer 2013; Ghassem *et al.* 2014). Viscera from both fresh and saltwater fish have also been used to produce FPH. Many studies have been conducted on using this source as raw material for FPH production (Villamil *et al.* 2017; Bhaskar *et al.* 2008; Ovissipour *et al.* 2014). Fish skin, rich in collagen and gelatine, from the fish processing industry has also been used to produce FPH. Several studies reported the use of fish skin from different fish species to convert into hydrolysates (Blanco *et al.* 2017; Yin *et al.* 2010; Ngo *et al.* 2010). Fish heads generated from the fish processing industry are also a rich source of protein. Some previous studies have reported on the utilization of fish heads to convert into hydrolysates. These include black parrotfish *Chlorurus sordidus* and skipjack tuna *Katsuwonus pelamis* (Zhang *et al.* 2019; Prihanto *et al.* 2019).

Fish processing industries produce considerable amount of fish bone and fish frame every year globally, discarded or sold as cheap by-product (Arvanitoyannis & Kassaveti 2008). The increasing concern for environmental pollution and decreasing trend of natural resources emphasize the need to develop potential products with this waste material. Several studies described the production of FPH from fish bones and fish frames of different species, such as shortfin scad *Decapterus macrosoma*, cod *Gadus morhua* and ribbon fish *Lepturacanthus savala* (Šližytė *et al.* 2009; Nazeer *et al.* 2011; Kang *et al.* 2018). Finally, some studies use the whole body parts and by-products of various fish for hydrolysate production, including black scabbard fish *Aphanopus carbo*, round sardinella *Sardinella aurita*, herring *Clupea harengus*, Pacific hake *Merluccius productus* and pollock *Theragra chalcogramma* (Tang *et al.* 2008; Batista *et al.* 2010; Bougattef *et al.* 2010; Ho *et al.* 2014).

Production of FPH

There are several methods used to produce FPH, including chemical hydrolysis (acid and alkaline hydrolysis), autolysis, bacterial fermentation and enzymatic hydrolysis. Among them, enzymatic hydrolysis and chemical hydrolysis are the most commonly used methods due to a number of advantages. The chemical hydrolysis process is low cost,

is rapid and results in a high protein recovery; however, there is little control over the consistency of the hydrolysed products, with large variations in free amino acid profile due to the non-specific breakdown of peptide bonds (Celus *et al.* 2007). The autolysis process may be regulated by the action of endogenous digestive enzymes in the fish. But these endogenous enzyme concentrations vary greatly within a species and between species, as well as being highly seasonal and age-specific, resulting in end products of inconsistent molecular profiles (Kristinsson & Rasco 2000). Bacterial fermentation favours the growth of lactic acid bacteria that produce acid and antimicrobial factors, which inhibit competing bacteria, but under this method, removal of lipid is not possible (Kristinsson & Rasco 2000).

With the aim of producing better quality FPH, enzymatic hydrolysis is widely implemented to produce precise hydrolysates retaining the nutritive value of the source protein (Zamora-Sillero *et al.* 2017). This process works with a shorter reaction time and is beneficial for targeting specific peptide bonds and amino acids with optimal activity at specific conditions. Furthermore, enzymatic hydrolysis does not produce any residual organic solvents and toxic chemicals in the end products (Najafian & Babji 2012). Due to these advantages, the present review has focused on production of FPH based on enzymatic processes.

There are several proteolytic enzymes including alcalase, neutrase, papain, pepsin and trypsin, which are commonly used to produce FPH (Kristinsson & Rasco 2000). The alcalase, an alkaline enzyme obtained from *Bacillus licheniformis*, has been found to be a highly efficient enzyme for FPH production due to its high extraction ability under mild conditions and ability to produce FPH with small-sized peptides in a relatively short period (Kristinsson & Rasco 2000).

In the enzymatic process of pre-treatment stage, fish by-products and wastes are minced and homogenized with water (2:1 w/w) before being transferred to the reactor vessel where it is heated to the appropriate temperature. FPH should have well-controlled fat content (<0.5% w/w) as higher fat content may result in darkening of the final products due to lipid oxidation, producing brown pigments (Kristinsson & Rasco 2000). Defatting is therefore required for fatty fish before mixing with water, and commonly, organic solvents are used for this purpose. Treatment with organic solvents reduces extra fat and minimizes bacterial degradation (Kristinsson & Rasco 2000). As enzyme type, enzyme concentration, temperature, pH and time are influential parameters affecting product quality and function (Srichanun *et al.* 2014), it is necessary to optimize these parameters during the production process. The suitable ranges of these parameters include temperature (35.0–60.0°C), time (10.0–600.0 min), pH (1.5–11.0) and enzyme concentration (0.01–5.0%) for the various enzymes

used to produce FPH (Razali *et al.* 2015). The optimized enzymatic hydrolysis conditions (i.e. enzyme concentration, pH, time, temperature) to produce FPH from different fish and parts stated in various studies are summarized in Table 1.

In the hydrolysis step, the mince water slurry is subjected to homogeneous mixing with the selected enzyme. The enzyme selection plays a crucial role in production process as it allows better control of the hydrolysis process and the resulting product. A number of studies have reported that enzymes with microbial origin such as alcalase, neutrase and flavourzyme, with close to neutral pH reaction range (5.0–9.0), have advantages for producing FPH including temperature stabilities, greater pH range and a wide variety of catalytic activities resulting in products of high quality and nutritive value. Enzymes derived from animal sources such as pepsin, or plant sources such as papain, which have an acidic pH range, may lead to lower protein recoveries and less favourable nutritional values due to essential amino acid damage and low functionalities associated with excess hydrolysis (Kristinsson & Rasco 2000). Protein recovery also varies depending on the applied enzymes; for instance, the highest protein recovery from capelin *Mallotus villosus* of 70.5% was produced by alcalase compared to 57.6% with neutrase and 57.1% with papain.

Hydrolysis time and processing temperature are chosen according to the preferred protein recovery and functionalities of the final product. The size of the peptide fractions decreases with increased temperature and enzyme concentration until the temperature reaches the point of enzyme denaturation (Jamil *et al.* 2016). Following hydrolysis, the activity is terminated by inactivating the exogenous enzymes using heat at 85–95°C for 5–20 min (Ghassem *et al.* 2014). Liquid FPH may be dried in the recovery step to generate a powder form, because liquid hydrolysates can spoil quickly. FPH in powder form can be stored for a longer period of time and is easier to transport (He *et al.* 2013). The hydrolysed sample is centrifuged before drying. Centrifugation (10,000 ×g/30 min) separates the sample into three layers: a semi-solid layer at the bottom, the hydrolysed solution in the middle and a layer of fat on the top (Fig. 1). After removing the fat layer (defatting), the hydrolysed protein solution is transferred carefully without mixing with the bottom semi-solid layer. Then, the protein hydrolysis solution may be freeze-dried and the creamy white final product is stored at 4°C or lower, occasionally with vacuum packaging. In some cases, hydrolysates are dried using a spray-drying technique (Hassan *et al.* 2019). This process and optimized condition to produce FPH from fish by-products is presented in Figure 2.

Chemical composition of FPH

The chemical composition of FPH produced from various fish parts is displayed in Table 2. The protein contents of FPH vary between 60.0% and 90.0% depending on the types and sources of raw material and hydrolysis protocol followed (Kristinsson & Rasco 2000; Bhaskar *et al.*, 2008). The high protein content of FPH is due to the solubilization of protein during enzymatic hydrolysis and removal of lipid after hydrolysis and may be increased by the removal of insoluble fractions by centrifugation (Chalamaiah *et al.* 2012). However, the protein content of the FPH also varies with the temperature used for drying in the production process. According to Abdul-Hamid *et al.* (2002), the crude protein content of FPH was decreased to 37.7% from 49.6% when the drying temperature was increased from 150°C to 180°C. Thiansilakul *et al.* (2007) stated that the solubilized protein content in FPH depends on the amount of lipids in the raw materials used for FPH production. The raw materials having higher percentage of lipids produce lower amount of solubilized protein.

A number of studies have reported that lipid content of the FPH is <5% of total composition (Bhaskar *et al.* 2007; Pacheco-Aguilar *et al.* 2008; Ovissipour *et al.* 2009; Siddik *et al.* 2018b). The low lipid content of FPH may be due to removal of fat and insoluble protein fractions by centrifugation (Chalamaiah *et al.* 2012). A reduced lipid content in FPH may increase the stability of the final product towards lipid oxidation, which may increase the shelf life of FPH in storage condition. Researchers suggested various techniques to reduce the lipid contents in resultant FPH. Thiansilakul *et al.* (2007) defatted fish mince by isopropanol prior to hydrolysis and then cut off fat layer after hydrolysis for producing low lipid FPH. Kristinsson and Rasco (2000) used a separation process following fermentation to remove lipids and insoluble materials for getting less fat FPH. Hoyle and Merritt (1994) produced FPH with a low lipid content using ethanol from herring, *Clupea harengus*.

Several studies demonstrated that the ash content of FPH ranged between 0.45% and 27% of total composition (Choi *et al.* 2009; Yin *et al.* 2010; Chalamaiah *et al.* 2012). The high ash contents in FPH may be due to the addition of alkali for pH adjustment and/or largely contributed by breakdown of bones in the raw material (Choi *et al.* 2009; Batista *et al.* 2010). Also, the presence of shell, sand and small stones in the digestive tract of fish increases ash contents in FPH (Slizyte *et al.* 2005). A high moisture content has been regarded as drawback of FPH as it limits the applications of FPH. Many scientists reported the moisture content of FPH should be below 10% of total composition to retain its quality (Bhaskar *et al.* 2008; Chalamaiah *et al.* 2010).

Table 1 Enzymatic process to produce FPH from various fish and parts of fish and their optimized conditions using different enzymes

Fish species	Source	Applied enzyme	Optimized condition			Temperature (°C)	Outcome	Reference
			Enzyme conc. (%)	pH	Time (min)			
Sturgeon (<i>Acipenser persicus</i>)	Viscera	Alcalase	0.1 AU/g	8.5	30, 60, 120, 180, 205	35, 45 and 55	The highest degree of hydrolysis was observed at 55°C after 205 min	Ovissipour et al. (2009)
Beluga (<i>Huso huso</i>)	Viscera	Protamex	14, 22, 34, 46, 55	-	53, 80, 120, 60, 187	33, 38, 45.5, 53, 58	The optimum conditions to reach the highest degree of hydrolysis were 39.21°C, 114.2 min, and a protease (protamex) activity of 27.41 AU kg ⁻¹ protein.	Molla and Hovannisyan (2011)
Eel (<i>Monopterus</i> sp.)	Flesh	Alcalase	1.8	9.00	-	55.76	The optimum conditions were temperature of 55.76°C, enzyme concentration of 1.80% and pH of 9.0.	Jamil et al. (2016)
Tuna	By-product	Alcalase Neutrase	1.0 0.5, 1.0, 1.5	8.50 7,8,9	60 40, 100, 160	55 40, 50, 60	The optimum condition with alcalase at temperature 55°C, time 60 min, 1% enzyme concentration and pH 8.5	Saidi et al. (2013)
Small-spotted catshark (<i>Scyliorhinus canicular</i>)	Discards	Alcalase Esperase	0.5	6.0-12	37.3-80	60.8	The optimal conditions for the highest proteolysis were established with esperase in 60.8°C and pH 8.9	Vazquez et al. (2017)
Salmon (<i>Salmo salar</i>)	Frame	Alcalase Papain	1.0, 2.0, 3.0 1.0, 2.0, 3.0	8.0 7.0	240	40	Salmon frame hydrolysed with 3% alcalase and 3% papain provided the best result, while alcalase showed higher yield than that of papain.	Idowu et al. (2019)

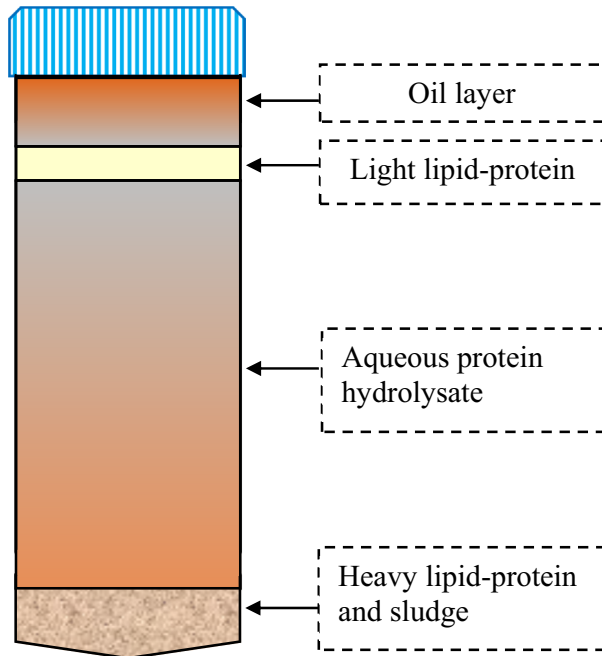


Figure 1 Fractions of soluble FPH produced from Australasian snapper *Pagrus auratus* by-products using alcalase enzyme (Kristinsson & Rasco 2000).

The amino acid contents of any food materials have a substantial role in various biological activities such as giving cells structure, carriers of oxygen, CO₂ and enzymes and serve as optimal storage of all nutrients including proteins, lipids, carbohydrates, minerals, vitamins and water (Wu 2013). The essential and non-essential amino acids needed for good health have been found abundant in FPH (Yin *et al.* 2010; Idowu *et al.* 2019). However, the FPH has been described in many studies to exhibit variation in their amino acid content (Wasswa *et al.* 2007). The disparity in amino acid contents of various FPH depends on several factors such as the raw material for producing the hydrolysate, enzyme used for hydrolysis, and the conditions and duration of hydrolysis (Klompong *et al.* 2007).

Functional properties of FPH

In food systems, the functional properties of any ingredients are important as these properties determine the quality and possible end use of the final product. During enzymatic hydrolysis, fish proteins are cleaved into a mixture of free amino acids and di-, tri- and oligopeptides. This process decreases the size of peptides and increases the number of carboxyl group of amino acids, thereby simplifying the protein structure to improve functional quality and bioavailability (Chalamaiah *et al.* 2012; Halim *et al.* 2016). Since the choice of enzymes and the degree to which the protein

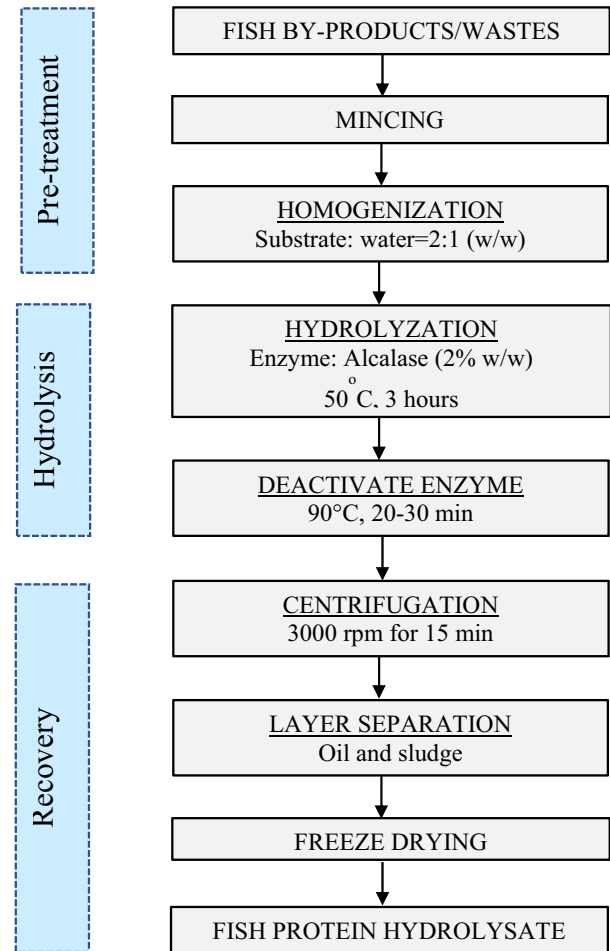


Figure 2 Schematic diagram of the processing method of FPH from Australasian snapper *Pagrus auratus* by-product using alcalase enzyme (He *et al.* 2013).

is hydrolysed strongly influence the functionality of FPH, manipulation of the reaction conditions during enzymatic hydrolysis is important to obtain hydrolysates with desired functional properties. The main functional properties attributed to FPH are solubility, and emulsifying and foaming capacities. The evaluation of these properties in regard to differing FPH preparations is therefore discussed in more detail below.

Solubility

Among various functional properties of the protein, enhanced solubility is considered a beneficial characteristics and an excellent index for the qualitative assessment of FPH functionality as many other functional properties such as emulsification and foaming of FPH are affected by solubility (Kristinsson & Rasco 2000; Naqash & Nazeer 2013). Protein with high solubility possesses increased

Table 2 The chemical composition of dried defatted FPH produced from whole fish and/or individual parts of fish

Fish species	Source	Applied enzyme	Nutritional composition (%)				Reference
			Protein	Lipid	Ash	Moisture	
Persian sturgeon (<i>Acipenser persicus</i>)	Viscera	Alcalase 2.4 L	65.82 ± 7.02	0.18 ± 0.4	7.67 ± 1.24	4.45 ± 0.67	Ovissipour <i>et al.</i> (2009)
Rainbow trout (<i>Onchorhynchus mykiss</i>)	Viscera	Alcalase	88.32 ± 0.07	0.80 ± 0.60	1.14 ± 0.88	3.45 ± 0.02	Taheri <i>et al.</i> (2012)
Yellowfin tuna (<i>Thunnus albacares</i>)		Alcalase	72.34 ± 3.20	1.43 ± 0.57	2.82 ± 2.74	22.34 ± 1.38	Ovissipour <i>et al.</i> (2012)
Pacific whiting, (<i>Merluccius productus</i>)	Muscle	Autolysis	85.6 ± 2.3	0.3 ± 0.1	16.6 ± 0.3	2.5 ± 0.6	Mazorra-Manzano <i>et al.</i> (2010)
Small-spotted catshark, (<i>Scyliorhinus canicular</i>)	Muscle	Alcalase Esperase and Protamex	89 ± 0.46	0.35 ± 0.06	1.11 ± 0.06	7.79 ± 0.70	Vazquez <i>et al.</i> (2017)
Tuna, anchovy and wild fish	Tuna frame, anchovy and wild fish in a proportion of 5:4:1	Papain and Bromelain	69.94	1.77	17.5	3.03	Wu <i>et al.</i> (2018)
Atlantic salmon (<i>Salmo salar</i>)	Head	Alcalase 2.4 L	82.3 ± 1.9	0.8 ± 0.02	10.4 ± 1.1	5.3 ± 0.2	Gbogouri <i>et al.</i> (2004)
Tuna	By-product	Flavourzyme	66.40 ± 0.27	2.37 ± 0.52	25.94 ± 0.04	7.25 ± 0.09	Nilsang <i>et al.</i> (2005)

dispersability of protein molecules and leads to the formation of improved colloidal systems (Zayas 1997). The potential applications of proteins in FPH can be expanded with higher solubility.

The level of degree hydrolysis (DH) and pH among other factors including temperature, ionic strength, type of solvent, and processing conditions strongly influence the solubility of protein (Damodaran, 2008; Egerton *et al.*, 2008). As the DH increases from 16.43% to 41.47%, the solubility of fish proteins increases from 35.68% to 90.18%. It is hypothesized that the high DH breakdown of large, complex protein molecules into smaller peptides with having more ionizable polar groups on their surface, and hence an increased ability to form hydrogen bonds with water molecules, results in a marked increase in solubility. The increased solubility may be further explained by conversion of hydrophobic to hydrophilic groups through the formation of two-end carbonyl and amino groups (Ghelichi *et al.* 2018). The biochemical mechanism of hydrolysis of macromolecules to peptides and amino acids is shown in

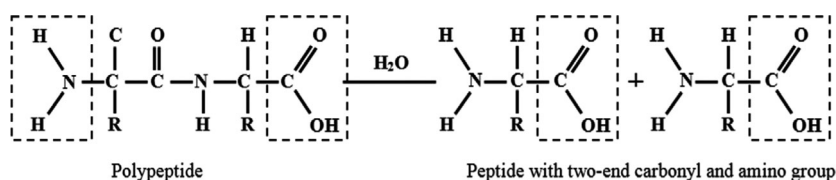
**Figure 3** Schematic diagram of the major conversion pathway of macromolecules into small peptides and amino acids during hydrolysis.

Figure 3. The low solubility of the hydrolysates with lower degree of hydrolysis may also be attributed to the structure of the rigid macromolecule with subunits bound by several intermolecular and intramolecular disulphide bonds and hydrophobic interactions (Paraman *et al.* 2007). Several studies have reported high solubility of FPH at various DH over a wide range of pH (Geirsdottir *et al.* 2011; Naqash & Nazeer, 2013; Gajanan *et al.* 2016). The higher solubility of proteins over a wide range of pH can have applications in many food formulations.

Emulsifying properties

The ability of FPH to form and stabilize emulsions can be measured by two emulsifying properties including emulsion activity index (EAI) and emulsion stability index (ESI). EAI is a measure of the ability of the protein solution to emulsify oil, whereas ESI measures the resistance of the protein solution to resist structural changes such as coalescence, creaming over a specific time (Thiansilakul *et al.*

2007). The emulsifying properties of FPH strongly influence by the degree of hydrolysis and the types of enzyme used during hydrolysis. Witono *et al.* (2016) found an inverse relationship between the extent of the enzymatic hydrolysis and the emulsifying properties. FPH with a lower DH can be attributed to a higher amount of large molecular weight peptides and higher surface hydrophobicity contributing to better flexibility and orientation at the oil–water interface. On the contrary, with a higher DH, a higher amount of smaller peptides are formed, which may result in a drastic loss of emulsifying properties. Enzyme selection also plays an important role in the emulsifying properties of FPH as it strongly influences the molecular size and hydrophobicity of the resulting peptides. Vieira *et al.* (2017) found that sardine protein hydrolysates with alcalase yield large molecular weight peptides with excellent emulsifying stability and activity, while protease produced smaller peptides and yielded hydrolysates with very poor emulsification properties. Therefore, a low DH and a careful choice of enzymes are a key issue if enhanced emulsifying properties are desired.

Foaming properties

Foaming properties are generally expressed as foam capacity (FC) and foam stability (FS). The FC of a protein is measuring the amount of interfacial area that can be created by whipping the protein, while the FS is measuring the time required to lose half of the volume from the foam (Kristinsson & Rasco 2000). There is a relationship between the DH, and the FC and FS of FPH. As DH increased, the FC and FS of FPH decreased. Gajanan *et al.* (2016) stated that higher DH increases levels of low molecular weight peptides in FPH, and as a result, more air can be incorporated into a solution of smaller peptides. These low molecular weight peptides with more air cells have less ability to maintain a stable foam. Latorres *et al.* (2018) produced FPH at 10 and 20% DH and found high molecular weight peptides at low DH have the strength to form a cohesive interfacial film capable of enveloping and retaining air. Foaming properties of FPH are found to be reliant on pH too. The lowest FC (72.33% from 93.13 to 103.16%) and FS (20.80% from 33.16 to 42.63%) of FPH are found close to the isoelectric point of the protein and increase at more acidic and alkaline pH (Ghelichi *et al.*, 2018). This may be due to the reduced net charge and peptide size via ionic repulsion.

Bioactive peptides from FPH

Molecular weight of peptides is considered to have a significant effect on the biological activities of FPH. Low molecular weight peptides are more easily absorbed and

assimilated by the gastrointestinal tract of animals than those with higher molecular weight (Martínez-Alvarez *et al.* 2015; Zamora-Sillero *et al.* 2018). Several studies recommend gel filtration (GF), nanofiltration (NF) and ultrafiltration (UF) to further refine FPH and to increase efficiency of by-products to make more efficient bioactive peptide dietary ingredients for human and animal consumption (Bourseau *et al.* 2009; Picot *et al.* 2010; Abejon *et al.* 2016). Pressure-driven membrane separation is sometimes used to separate peptides into different size groups (Bourseau *et al.* 2009). In line with the molecular weight cut-offs (MWCO), NF and UF of protein hydrolysates are recommended depending on the different outcome required. The NF can be used to concentrate hydrolysed products, while high MWCO (20.0–100.0 kDa) of UF membranes can be used to separate hydrolysed peptides from native proteins and proteolytic enzymes. The UF with intermediate MWCO (~4.0–8.0 kDa) allows fractionation of the peptide chain to enrich specific molecular sizes in the hydrolysate (Bourseau *et al.* 2009). Fast performance liquid chromatography (FPLC) may be used in GF to obtain small molecular weight (≤ 3.0 kDa) peptides (Centenaro *et al.* 2014). According to a report by Mahmoodani *et al.* (2014), the molecular weight of fish waste-derived bioactive peptides ranges between 0.2 and 2.0 kDa. Similarly, Sarmadi and Ismail (2010) reported that most purified peptide molecules from fish by-products range from 2.0 to 20.0 amino acids in sequence and are generally smaller in molecular size. However, Ngo *et al.* (2014) reported that the molecular weights for bioactive hydrolysate peptides were less than 3.0 kDa. A typical procedure for the isolation of bioactive peptide from FPH is shown in Figure 4.

A number of studies have revealed that fish-derived peptides have a myriad of bioactive potential including antihypertensive, antioxidative, antimicrobial and anti-inflammatory activity depending on the molecular weights of the peptides (Ishak & Sarbon 2017; Zamora-Sillero *et al.* 2017). For instance, following hydrolysis, the angiotensin-converting enzyme inhibitory activity (ACE-I-inhibitory) of *Channa striatus* protein hydrolysate increased to 0.058 mg mL⁻¹ with a molecular weight 10.0 kDa from 0.033 mg mL⁻¹ with a molecular weight 3.0 kDa compared with an unhydrolysed sample (Ghassem *et al.* 2014), while the antioxidant activity of tuna by-product hydrolysate protein increased from 11.0% with a molecular weight < 4.0 kDa to 75% with a molecular weight < 1.0 kDa following enzymatic hydrolysis (Saidi *et al.* 2014). According to Najafian and Babji (2012), peptides derived from animal muscles that have a molecular weight below 10.0 kDa and less than 50 amino acids in sequence exhibit antimicrobial activity. A study by Ahn *et al.* (2015) reported that the highest anti-inflammatory activity from salmon by-product protein hydrolysates was

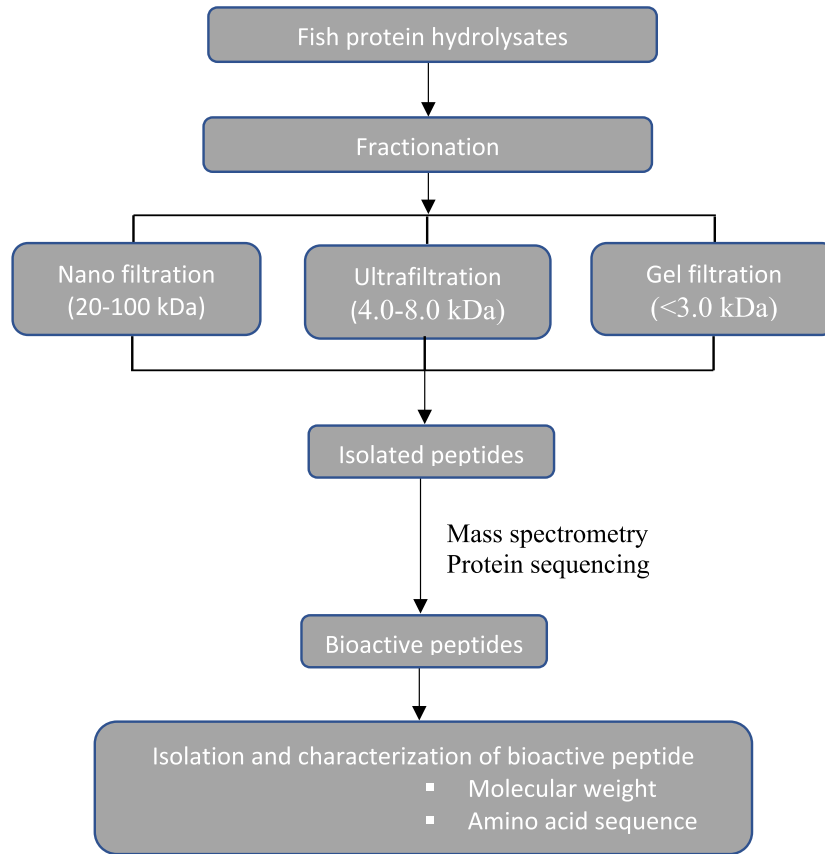


Figure 4 Schematic diagram of procedures for isolation and characterization of bioactive peptide from FPH (Ishak & Sarbon 2017).

derived with the molecular weight between 1.0 and 2.0 kDa.

Role of FPH in aquaculture production

Based on published information, the potential effects of supplementation of FPH in aquafeeds on feed intake and utilization, growth performance and biochemical responses in fish are summarized below.

Feed intake and utilization

The studies carried out using FPH and their effect on feed intake, feed utilization and nutrient digestibility in various fish species are summarized in Table 3. Biofunctional properties of FPH and their active compounds in facilitating better feed intake have been reported in many fish species (Chotikachinda *et al.* 2013; Ha *et al.* 2019). The enzymatic hydrolysis during production of FPH may cause formation of peptides with small molecular weight that may act as an attractant for fish (Chotikachinda *et al.* 2013). Feed palatability is often connected with the availability of small molecular weight peptides and free amino acids in

hydrolysed protein, which may stimulate feed intake of fish (Kasumyan & Døving 2003). Conversely, higher the feed intake was reported to decrease the feed utilization including feed efficiency, protein efficiency and protein retention in turbot *Scophthalmus maximus* (Xu *et al.* 2016). The reduced feed utilization may be due to the lower bioavailability of free amino acids, which may be due to the gastrointestinal absorption rate asynchronism between free amino acids and protein-bound amino acids (Espe *et al.* 1999; Langdon *et al.* 2007; Bodin *et al.* 2012). Refstie *et al.* (2004) reported that post-smolt Atlantic salmon *Salmo salar* had higher feed consumption when fed 10% and 15% FPH than the fish fed no FPH and 5% FPH, with no differences in feed efficiency ratio among dietary groups. In another investigation, inclusion of dietary UF FPH was significantly correlated with feed efficiency, protein productive value and protein efficiency ratio, but not significantly correlated with feed intake (Wei *et al.* 2016). In this study, it was demonstrated that the high-level (108 g kg⁻¹) UF FPH showed lowest feed efficiency, but the low-level (27–54 g kg⁻¹) UF FPH showed positive correlation with protein productive value and protein efficiency ratio. According to Hevrøy *et al.* (2005), the lower inclusion (60 g kg⁻¹)

Table 3 The effects of FPH on growth performance, feed intake and utilization and nutrient digestibility of farmed finfish

Tested fish	Source of hydrolysate	Enzyme used for preparing hydrolysate	Inclusion level	Duration of growth trial	Response	Reference
Barramundi (<i>Lates calcarifer</i>)	By-product of bluefin tuna <i>Thunnus maccoyii</i>	-	5, 10, 15 and 20%	56 days	(↑) Final body weight, specific growth rate at 5 and 10% (↔) Feed intake, feed conversion ratio and survival	Siddik et al. (2018b)
European sea bass (<i>Dicentrarchus labrax</i>)	White shrimp <i>Litopenaeus vannamei</i> and Nile tilapia <i>Oreochromis niloticus</i>	-	5%	70 days	(↑) Final body weight, specific growth rate compared to low FM diet	Leduc et al. (2018)
Barramundi (<i>Lates calcarifer</i>)	Bluefin tuna <i>Thunnus maccoyii</i>	Alcalase	10% with FM, PBM and BPBM	56 days	(↑) Feed intake and survival (↑) Final body weight at FM and BPBM (↑) Final body weight at FM	Siddik et al. (2019b)
Barramundi (<i>Lates calcarifer</i>)	Yellowtail kingfish <i>Seriola lalandi</i> Carp hydrolysate <i>Cyprinus carpio</i> Bluefin tuna <i>Thunnus maccoyii</i>	Alcalase	10% with PBM	70 days	(↔) Feed conversion ratio and survival (↑) Survival (↔) Final body weight, specific growth rate, feed conversion ratio and feed intake	Chaklader et al. (2020)
Barramundi (<i>Lates calcarifer</i>)	By-product of bluefin tuna <i>Thunnus maccoyii</i>	-	50 and 75%	70 days	(↓) Final body weight and specific growth rate at 50 and 75%	Siddik et al. (2018a)
Turbot (<i>Scophthalmus Maximus</i>)	By-products of pollock <i>Theragra chalcogramma</i>	-	UF: 5, 10, 15 and 20%	68 days	(↔) Condition factor and survival (↓) Final weight, specific growth rate and protein efficiency ratio at 20%	Wei et al. (2016)
Atlantic salmon (<i>Salmo salar</i>)	Whole herring	Alcalase	6, 12, 18, 24 and 30%	68 days	(↔) Feed intake, condition factor and survival (↑) ADC protein (↓) ADC dry matter at 20% (↑) Specific growth rate at 24 and 30% (↑) Thermal growth coefficient at 24% (↔) Final weight, weight gain, condition factor, survival and ADC lipid and dry matter	Høyevåg et al. (2005)
Japanese flounder (<i>Paralichthys olivaceas</i>)	Frames of pollock <i>Theragra chalcogramma</i>	Alcalase and flavourzyme	UF: 3.7 and 1.2% NUF: 3.7%	60 days	(↑) Feed conversion ratio and ADC protein (↓) Protein efficiency ratio	Zheng et al. (2012)
Japanese flounder (<i>Paralichthys olivaceas</i>)	Frames of pollack <i>Theragra chalcogramma</i>	Alcalase and flavourzyme	6, 11, 16, 21 and 26%	63 days	(↑) Feed efficiency both at UF and NUF at 3.7% (↑) ADC protein (↓) Protein efficiency ratio (↔) Survival and feeding rate per day (↑) Final body weight, specific growth rate at 11, 16 and 21% (↑) Feeding rate and feed efficiency ratio at 26% (↑) ADC protein (↔) Survival	Zheng et al. (2013b)

Table 3 (continued)

Tested fish	Source of hydrolysate	Enzyme used for preparing hydrolysate	Inclusion level	Duration of growth trial	Response	Reference
Turbot (<i>Scophthalmus maximus</i>)	Frames of pollock <i>Theragra chalcogramma</i>	Alcalase and flavourzyme	UF: 3.7 and 1.2% NUF: 3.7%	8 weeks	(↑) Final body weight, specific growth rate at UF 1.5% (↑) Feed efficiency, protein efficiency ratio, ADC protein and dry matter at UF 3.7% (↔) Hepatosomatic index (↓) Specific growth rate at 20% (↑) Feed intake at 20% (↓) Feed efficiency ratio and protein efficiency ratio at 10% and 20%	Zheng <i>et al.</i> (2013a)
Turbot (<i>Scophthalmus maximus</i>)	By-products of pollock <i>Theragra chalcogramma</i>	-	5, 10 and 20%	12-week	(↓) Specific growth rate at 20% (↑) Feed efficiency ratio and protein efficiency ratio at 10% and 20%	Xu <i>et al.</i> (2016)
Atlantic salmon (<i>Salmo salar</i>)	Raw body parts of pollock <i>Theragra chalcogramma</i>	Protamex	5, 10 and 15%	68 days	(↑) Specific growth rate, thermal-unit growth coefficients, body weights and feed intake at 10 and 15% (↑) ADC nitrogen, lipid and energy (↔) ADC protein and feed efficiency ratio (↑) Final body weight, specific growth rate, protein efficiency ratio and ADC protein at SH and KH (↓) Feed conversion ratio (↔) Feed intake, ADC dry matter and survival (↑) Final body weight at KH (↓) Feed conversion ratio at KH (↑) ADC protein (↔) Specific growth rate, feed intake, protein efficiency ratio, ADC dry matter and survival (↑) Final body weight at KH (↓) Feed conversion ratio at KH (↑) ADC protein and ADC dry matter (↔) Specific growth rate, feed intake, protein efficiency ratio and survival	Refstie <i>et al.</i> (2004)
Sea bream (<i>Pagrus major</i>)	Krill <i>Euphausia superba</i> Shrimp <i>Litopenaeus vannamei</i> Tilapia <i>Oreochromis niloticus</i>	-	SH: 4.80% TH: 4.23% KH: 4.03%	12 weeks	(↑) Final body weight, specific growth rate, protein efficiency ratio and ADC protein at SH and KH (↓) Feed conversion ratio (↔) Feed intake, ADC dry matter and survival (↑) Final body weight at KH (↓) Feed conversion ratio at KH (↑) ADC protein	Bui <i>et al.</i> (2014)
Red sea bream (<i>Pagrus major</i>)	Fresh tuna co-products Antarctic krill <i>Euphausia superba</i>	-	TH: 2% KH: 2%	12 weeks	(↑) Final body weight at KH (↓) Feed conversion ratio at KH (↑) ADC protein	Khosravi <i>et al.</i> (2015a)
Olive flounder (<i>Paralichthys olivaceus</i>)	Fresh tuna co-products Whole Antarctic krill <i>Euphausia superba</i>	-	TH: 2% KH: 2%	9 weeks	(↑) Final body weight at KH (↓) Feed conversion ratio at KH (↑) ADC protein and ADC dry matter (↔) Specific growth rate, feed intake, protein efficiency ratio and survival	Khosravi <i>et al.</i> (2015a)
Pike silverside (<i>Chirostoma estor</i>)	Fillets <i>Scomberomorus regalis</i> and shrimp tails <i>Pennaeus</i> sp.	-	15, 30 and 45%	8 weeks	(↓) Weight gain and specific growth rates at 30 and 45% (↑) ADC lipid and ADC dry matter at 30% (↔) Feed intake, feed conversion ratio, protein efficiency ratio and ADC protein (↑) Final body weight, feed conversion ratio at 15 and 25%	Ospina-Salazar <i>et al.</i> (2016)
Japanese sea bass (<i>Lateolabrax japonicus</i>)	Gut and head of pollock <i>Theragra chalcogramma</i>	Protease	5, 15 and 25%	60 days	(↑) Final body weight, feed conversion ratio at 15 and 25% (↑) Specific growth rate at 15%	Liang <i>et al.</i> (2006)
Yellow croaker (<i>Pseudosciaena crocea</i>)	Tissues of pollock <i>Theragra chalcogramma</i>	Flavourzyme and Alcalase	5, 10 and 15%	8 weeks	(↑) Weight gain (↑) Specific growth rate at 10 and 15%	Tang <i>et al.</i> (2008)

Table 3 (continued)

Tested fish	Source of hydrolysate	Enzyme used for preparing hydrolysate	Inclusion level	Duration of growth trial	Response	Reference
Persian sturgeon (<i>Acipenser persicus</i>)	Yellowfin tuna <i>Thunnus albacares</i> viscera	Alcalase	10, 25 and 50%	54 days	(↑) Final body weight, weight gain at 10 and 25% (↔) Survival and condition factor	Ovissipour <i>et al.</i> (2014)
Olive flounder (<i>Paralichthys olivaceus</i>)	Yellowfin tuna <i>Thunnus albacares</i> and skipjack tuna <i>Katsuwonus pelamis</i> by-products	-	5, 10, 20, 30, 40, 60, 80 and 100%	7 weeks	(↔) Weight gain and specific growth rate at 5–30% (↓) Weight gain and specific growth rate at 40–100% (↔) survival	Kim <i>et al.</i> (2014)

Increase, ↑; decrease, ↓; no change, ↔; compared to the control diet ($P < 0.05$); -, not mentioned. ADC, apparent digestibility coefficient; UF, ultrafiltered fish hydrolysate; NUF, non-ultrafiltered fish hydrolysate; FM, fishmeal; PBIM, poultry by-product meal; BPPIM, bioprocessed PBIM; SH, shrimp hydrolysate; TH, tilapia hydrolysate; KH, krill hydrolysate.

of FPH can increase the absorption of amino acids and protein, but higher inclusion levels (180–300 g kg⁻¹) of FPH resulted in oxidation and produced energy being stored in the body tissues, thus reducing anabolism availability and affecting feed utilization.

The diverse results of hydrolysate supplementation in diets may be related to the size of peptide fractions that originated from different filtration. Small fractions of low molecular weight peptides in fish diet are correlated with feed utilization. It has been demonstrated that the low molecular weight fractions of FPH can increase the utilization of amino acids by reducing gluconeogenesis (Li *et al.* 2009; Wei *et al.* 2016). Zheng *et al.* (2013a) tested two ultrafiltered (UF) (molecular weight < 1000 Da) and non-UF fish hydrolysate in turbot, *Scophthalmus maximus*, and found the highest feed utilization in fish fed with UF when compared to non-UF fish hydrolysate and the FM-based control. The higher feed utilization in the UF hydrolysate group suggests that small molecular weight fractions in fish hydrolysate are beneficial for feed utilization (Aksnes *et al.* 2006a; Aksnes *et al.* 2006b).

The nutrient digestibility of fish increases with dietary inclusion of FPH in diets containing small molecular weight fractions (Bui *et al.* 2014; Khosravi *et al.* 2015b; Ospina-Salazar *et al.* 2016). Although more investigations are necessary to explain the particular mechanism for improved apparent digestibility coefficient (ADC) of dry matter by dietary inclusion of hydrolysates, it could be assumed that the enhanced ADC of nutrients by dietary inclusion of FPH may be due to their higher absorption rate (Hevrøy *et al.* 2005; Liang *et al.* 2006; Kotzamanis *et al.* 2007; Zheng *et al.* 2012; Zheng *et al.* 2013a), as the functional properties of the supplementary proteins are improved by hydrolysis processes (Chalamaiah *et al.* 2012). The molecular form of protein in FPH could positively affect the assimilation of dietary protein by increasing the expression of intestinal amino acids and/or peptide transporter gene as presented in fish by Bakke *et al.* (2010) and in chicken by Gilbert *et al.* (2010). However, a number of studies found no significant effect between a FM-based diet and partially replaced FPH diets on ADC of dry matter, protein and energy in fish (Oliva-Teles *et al.* 1999; Swane-poel & Goosen 2018). Tonheim *et al.* (2007) explained the negative relation of FPH on protein digestibility by suggesting that the nitrogenous compounds in FPH are not as digestible as in FM protein. Furthermore, the higher inclusion of FPH may negatively influence the digestibility in fish. In juvenile turbot (*Scophthalmus maximus* L.), the ADC of dry matter was similar to the FM-based diet up to 15% FM protein substitute diets but at 20% inclusion of fish hydrolysate in the diet resulted in significant reduction in ADC of dry matter (Zheng *et al.* 2013b). Also, an inclusion level of 50% and higher fermented and non-fermented

tuna hydrolysate in juvenile barramundi, *Lates calcarifer*, resulted in significantly reduced ADC of dry matter, protein and lipid (Siddik *et al.* 2018a). In this study, it was suggested that the significant reduction of digestibility in juvenile barramundi may be due to the availability of excess amounts of free amino acids and free nucleotides, which may, in turn, have disturbed the normal process of digestion and metabolism of the ingested diets, resulting in poor digestibility.

Growth performance

A summary of studies carried out using FPH and their effect on growth performance indices such as final body weight (FBG), weight gain (WG) and specific growth rate (SGR) is illustrated in Table 3. The use of FPH at moderate levels (5–10% replacement of FM) for improving growth performance of fish has been well documented (Tang *et al.* 2008; Siddik *et al.* 2018b; Ha *et al.* 2019). The improved growth performance, when FM is replaced by FPH, may be due to increased palatability of the feed (Refstie *et al.* 2004; Hevrøy *et al.* 2005) and/or may be a result of the improved availability and subsequent uptake of free amino acids and suitable peptide fractions produced during the enzymatic process, which may be beneficial for the growth performance of fish (Xu *et al.* 2016). Amino acids can play a crucial role in a cell or organism having variety of protein syntheses with major physiological functions, such as carriers of oxygen, CO₂, vitamins, enzymes and structural proteins (Chalamaiah *et al.* 2012). FPH containing free amino acids and suitable peptides has a substantial role in maintaining good health of fish (Santos *et al.* 2009). Also, the molecular weight profile of FPH influences the growth performance of fish. The peptide fractions smaller than 10 kDa consist of biologically active peptides that act as growth and health promoters (Aksnes *et al.* 2006b; Bui *et al.* 2014; Ha *et al.* 2019). For example, Zheng *et al.* (2012) compared the effect of FPH and ultrafiltered FPH on growth performance of juvenile Japanese flounder, *Paralichthys olivaceus*, using four experimental diets designed as FM diet, FPH (non-ultrafiltered) diet and two ultrafiltered diets (UF1 and UF2, contained small molecular compounds). Results of this investigation indicated that the diet containing the higher proportion of small molecular weight peptides UF1 attained the best overall growth of experimental fish when compared to FM and other groups. The same authors also found a similar response of FPH and ultrafiltered FPH in turbot *Scophthalmus maximus* juveniles; that is that the small molecular weight peptides resulted in higher growth performance (Zheng *et al.* 2013a).

However, fish fed with higher FPH ($\geq 20\%$) diets have been reported to decrease growth performance (Xu *et al.*

2016; Siddik *et al.* 2018a). In Japanese flounder, *Paralichthys olivaceus*, 16% or higher inclusion of FPH in the diet resulted in significant reduction in growth performance (Zheng *et al.* 2013b). Also, an inclusion level of 20% FPH in turbot, *Scophthalmus maximus*, resulted in significantly reduced somatic growth of fish (Xu *et al.* 2016). The possible causes of reduced growth performance with higher hydrolysate levels may be due to an excessive number of short-chain peptides and free amino acids in these hydrolysed products, which could cause saturation of the peptide transport mechanism in the intestine (Carvalho *et al.* 2004; Tonheima *et al.* 2005; Ospina-Salazar *et al.* 2016). Also, higher amount of free amino acids could alter the absorption of amino acids leading to an increase of amino acid oxidation and reduced retention of dietary protein (Kolkovski & Tandler 2000; Aragao *et al.* 2004).

Biochemical responses

FPH supplementation stimulates various haematological and immunological parameters in fish, and a summary of these results is presented in Table 4. Haematological parameters are considered as vital physiological indicators for assessing general health and nutritional status of fish (Vazquez & Guerrero 2007; Siddik *et al.* 2019b). A number of studies reported that the improved functional properties of FPH inclusion result from the presence of biologically active peptides (Kotzamanis *et al.* 2007; Hermannsdottir *et al.* 2009; Harnedy & FitzGerald 2012; Ovisipour *et al.* 2014; Halim *et al.* 2016). A study on red sea bream, *Pagrus major*, showed that replacement of low FM diet by FPH led to an increase in haematocrit, haemoglobin, total protein and cholesterol levels, and the measured decrease in plasma glucose and triglyceride levels may indicate that the dietary inclusion of FPH leads to better absorption of the hydrolysed protein and enhancement of the general health condition of fish (Khosravi *et al.* 2015b). This contrasted with the results of another study on the same species, which found no significant differences in the haematological parameters of fish fed diets containing FPH including assessment of total protein, haematocrit, haemoglobin, glucose, total cholesterol and triglyceride (Bui *et al.* 2014). Goosen *et al.* (2015) found no significant effect of FPH on haematocrit and total protein level in mozambique tilapia *Oreochromis mossambicus*. Also, on juvenile coho salmon *Oncorhynchus kisutch* no significant variations were detected on haematocrit, leucocrit and total plasma protein level between FM and hydrolysate dietary groups (Murray *et al.* 2003). This discrepancy in results is possible due to a number of factors including fish size, experimental conditions and handling methods, as these factors may strongly affect the physiology of fish (Chatzifotis *et al.* 2010).

Table 4 The effects of FPH on haematological and immunological responses of fish

Tested fish	Hydrolysate and inclusion level	Response	Reference
Barramundi (<i>Lates calcarifer</i>)	Bluefin tuna <i>Thunnus maccoyii</i> (10% in FM, PBM and BPBM)	(↑) Lysozyme activity at FM and BPBM (↓) Blood glucose and glutamate dehydrogenase at BPBM (↔) Total protein, cholesterol, triglyceride and aspartate transaminase	Siddik et al. (2019b)
Barramundi (<i>Lates calcarifer</i>)	Yellowtail kingfish <i>Seriola lalandi</i> Carp hydrolysate <i>Cyprinus carpio</i> Bluefin tuna <i>Thunnus maccoyii</i> (10% in PBM)	(↑) Bactericidal activity at bluefin tuna hydrolysate (↓) Total bilirubin (↔) Serum protein, cholesterol, urea, creatine, aspartate transaminase, glutamate dehydrogenase and lysozyme activity	Chaklader et al. (2020)
Juvenile barramundi (<i>Lates calcarifer</i>)	Bluefin tuna <i>Thunnus maccoyii</i> (5, 10, 15 and 20%)	(↓) Blood glucose at 15 and 20% supplementation (↔) Serum lysozyme and complement activity, protein, aspartate transaminase, glutamate dehydrogenase and blood haematocrit	Siddik et al. (2018b)
Sea bream (<i>Pagrus major</i>)	Shrimp hydrolysate: 4.80% Tilapia hydrolysate: 4.23% Krill hydrolysate: 4.03%	(↔) Haematocrit, haemoglobin, glucose, total protein, cholesterol and triglyceride. (↑) Immunoglobulin (↑) Superoxide dismutase and antiprotease at krill hydrolysate (↔) Lysozyme activity, nitro blue tetrazolium activity and myeloperoxidase level	Bui et al. (2014)
Coho salmon (<i>Oncorhynchus kisutch</i>)	Boneless fish hydrolysate: 30.30% Hydrolysate with bones: 30.30% Cooked fish with bones: 29.13%	(↓) Haematocrit (↑) Leucocrit, at cooked fish with bones (↓) Total plasma protein at cooked fish with bones (↔) Lysozyme, complement, total serum immunoglobulin, myeloperoxidase, phagocytosis and nitro blue tetrazolium activity	Murray et al. (2003)
Red sea bream (<i>Pagrus major</i>)	Tilapia hydrolysate: 2% Krill hydrolysate: 2%	(↔) Haematocrit, haemoglobin, glucose, total protein, total cholesterol and triglyceride. (↑) Lysozyme activity (↑) Nitro blue tetrazolium activity at tilapia hydrolysate (↔) Immunoglobulin, myeloperoxidase activity, superoxide dismutase and antiprotease	Khosravi et al. (2015a)
Olive flounder (<i>Paralichthys olivaceus</i>)	Tilapia hydrolysate: 2% Krill hydrolysate: 2%	(↔) Haematocrit, haemoglobin, glucose, total protein, total cholesterol and triglyceride. (↑) Lysozyme activity at tilapia hydrolysate (↑) Nitro blue tetrazolium activity and superoxide dismutase at krill hydrolysate (↔) Immunoglobulin, antiprotease, myeloperoxidase activity	Khosravi et al. (2015a)
Olive flounder (<i>Paralichthys olivaceus</i>)	Shrimp hydrolysate: 3.34% Tilapia hydrolysate: 2.88% Krill hydrolysate: 3.12%	(↔) Haematocrit, haemoglobin, glucose, total protein, total cholesterol, triglyceride, aspartate aminotransferase activity and alanine aminotransferase activity (↑) Superoxide dismutase at shrimp hydrolysate (↔) Immunoglobulin, lysozyme activity, antiprotease activity, glutathione peroxidase activity, nitro blue tetrazolium activity and myeloperoxidase activity	Khosravi et al. (2017)
Japanese flounder (<i>Paralichthys olivaceus</i>)	Frames of pollock <i>Theragra chalcogramma</i> Ultrafiltered: 3.7 and 1.2% Non-ultrafiltered: 3.7%	(↑) Plasma IGF-I (insulin-like growth factor I) levels (↑) Liver IGF-I mRNA expression at ultrafiltered 3.7%	Zheng et al. (2012)
Turbot (<i>Scophthalmus maximus</i>)	Frames of pollock <i>Theragra chalcogramma</i> Ultrafiltered: 3.7 and 1.2% Non-ultrafiltered: 3.7%	(↔) Lysozyme activity, acid phosphatase activity, total antioxidative capacity, alkaline phosphatase activity and superoxide dismutase activity	Zheng et al. (2013a)

Table 4 (continued)

Tested fish	Hydrolysate and inclusion level	Response	Reference
Yellow Croaker (<i>Pseudosciaena crocea</i>)	5, 10, 15%	(↑) Lysozyme activity, complement activity and immunoglobulin at 10 and 15%	Tang <i>et al.</i> (2008)
Japanese sea bass (<i>Lateolabrax japonicus</i>)	5, 15, 25%	(↑) Phagocytic activity (↑) Lysozyme activity, complement haemolytic activity at 15 and 25% (↔) Number of nitro blue tetrazolium-positive cells	Liang <i>et al.</i> (2006)

Increase, ↑; decrease, ↓; no change, ↔; compared to the control diet ($P < 0.05$). FM, fishmeal; PBM, poultry by-product meal; BPBM, bioprocessed poultry by-product meal.

Lysozyme activity, active phagocytes, complement components and antibody molecules like immunoglobulins are considered as important parameters in the immune defence of fish (Saurabh & Sahoo 2008; Magnadottir 2006). Lysozyme, the leucocytic origin's mucolytic enzyme, is considered a vital indicator for immune response (Saurabh & Sahoo 2008). In fish, lysozyme acts against viral, bacterial and parasitic infections, and higher level of activity is found in fish blood as a response to infection (Puangkaew 2004). Lysozyme is also present as essential defence components for all vertebrates and invertebrates (Song *et al.* 2006). Phagocytes act as antibacterial response to remove dead and dying cells to maintain healthy tissues (Blander 2017). Fish complement activity (ACH50) has been found to have capacity to fight against foreign organisms and lyse foreign cells for destruction (Gasque 2004). Immunoglobulins are considered as one of the major body protection parameters for animals and humans, particularly for the teleost (Ross *et al.* 1998; Watts *et al.* 2001; Cuesta *et al.* 2004). Dietary inclusion of FPH in fish diets may trigger the immune system of fish (Murray *et al.* 2003; Kotzamanis *et al.* 2007; Khosravi *et al.* 2015b). Previous studies have demonstrated that the partial replacement of FM with FPH can improve the fish immunity (Liang *et al.* 2006; Kotzamanis *et al.* 2007; Tang *et al.* 2008; Bui *et al.* 2014; Chaklader *et al.* 2020). In Japanese sea bass *Lateolabrax japonicus*, it was reported that the dietary inclusion of 15% and 25% FPH significantly increased the lysozyme activity and complement haemolytic activity, whereas the phagocytic activity was significantly higher at all levels of FPH (5%, 15% and 25%) (Liang *et al.* 2006). Immunoglobulin M, lysozyme activity and complement C4 were significantly higher in fish fed with diets containing 10% and 15% FPH when compared to fish fed with the basal diet or diet containing 5% FPH (Tang *et al.* 2008). Enhanced lysozyme activity was found in juvenile barramundi, *Lates calcarifer*, fed poultry by-product meal diet supplemented with 10% tuna FPH when compared to the FM-based diet as control (Siddik *et al.* 2019b). Bui *et al.* (2014) found some

improvement in antiprotease activity, lysozyme activity, nitro blue tetrazolium activity and myeloperoxidase level in juvenile red sea bream *Pagrus major* fed with hydrolysates when compared to the FM-based dietary group, and immunoglobulin level was found to be significantly higher in hydrolysate groups. It has been suggested that the effect of hydrolysates on the fish immune system may be dependent on the size and concentration of peptides. FPH containing medium and small size peptides (molecular weight range 500–3000 Da) have been reported to stimulate the non-specific immunity of fish (Børgwald *et al.* 1996; Gildberg *et al.* 1996; Leduc *et al.* 2018). Superoxide anion production in Atlantic salmon *Salmo salar* was reported to be stimulated by peptides, in the size ranges from 500 to 3000 Da (Gildberg *et al.* 1996).

In some cases, no significant effect of FPH was found on fish immunological parameters. Zheng *et al.* (2013a) reported that the serum lysozyme activity, acid phosphatase activity and alkaline phosphatase activity of turbot *Scophthalmus maximus* were not affected by the levels of FPH inclusion in fish diet. Similarly, Goosen *et al.* (2015) did not find any significant effect of FPH on serum lysozyme concentration and immunoglobulin level in mozambique tilapia, *Oreochromis mossambicus*. Murray *et al.* (2003) suggested further studies on factors such as level and delivery route of dietary hydrolysate in fish before immunostimulatory effects are observed as they have found no variations in the immunological responses of coho salmon *Oncorhynchus kisutch* feeding diets with various hydrolysates against high FM-based control diet.

Studies demonstrating enhanced disease resistance in fish for the FPH addition in diets are summarized in Table 5. As described above, low molecular weight bioactive peptides in FPH may have immune-stimulating and antibacterial properties (Kotzamanis *et al.* 2007). The improvement in cellular and/or humoral immune function with heightened disease resistance of various fish due to bioactive peptides in FPH has already been established (Khosravi *et al.* 2015b; Siddik *et al.* 2018b). It is therefore assumed that

Table 5 The effects of FPH on disease resistances of fish

Tested fish	Used hydrolysate	Pathogen for challenge trial and dose	Methods and duration of challenge trial	Response	Reference
Barramundi (<i>Lates calcarifer</i>)	Tuna by-product hydrolysate (10% in FM, PBM and BPBM)	<i>Vibrio harveyi</i> 1.7×10^8 CFU mL ⁻¹	Injection 14 days	10% tuna hydrolysate supplemented in FM and BPBM diets resulted in higher survival of fish against <i>Vibrio harveyi</i> infection compared to fish fed FM-based control diet	Siddik <i>et al.</i> (2019b)
Barramundi (<i>Lates calcarifer</i>)	Tuna by-product hydrolysate (5, 10, 15 and 20% of FM replacement)	<i>Streptococcus iniae</i> 1.8×10^3 CFU mL ⁻¹	Immersion 14 days	5 to 10% FM replacement with tuna hydrolysate diets resulting in higher resistance to <i>Streptococcus iniae</i> infection	Siddik <i>et al.</i> (2018b)
Barramundi (<i>Lates calcarifer</i>)	Commercial tuna and carp hydrolysate, and yellowtail kingfish frame hydrolysate (10% in PBM)	<i>Vibrio harveyi</i> 1.1×10^8 CFU mL ⁻¹	Injection 14 days	All FPH supplemented PBM diets resulted in higher resistance against <i>Vibrio harveyi</i> infection when compared to FM-based control diet	Chaklader <i>et al.</i> (2020)
Sea bream (<i>Pagrus major</i>)	Shrimp hydrolysate: 4.80% Tilapia hydrolysate: 4.23% Krill hydrolysate: 4.03%	<i>Edwardsiella tarda</i> 1×10^5 CFU mL ⁻¹	Injection 21 days	Krill and tilapia hydrolysates in diets exhibited higher disease resistance compared to control in fish against <i>Edwardsiella tarda</i>	Bui <i>et al.</i> (2014)
Persian sturgeon (<i>Acipenser persicus</i> L.)	Tuna viscera protein hydrolysate (10, 25, 50% of FM replacement)	<i>Aeromonas hydrophila</i> 10^9 CFU mL ⁻¹	Immersion 5 days	None of the FPH included levels resulted in higher survival compared to control in fish against <i>Aeromonas hydrophila</i>	Ovissipour <i>et al.</i> (2014)
Coho salmon (<i>Oncorhynchus kisutch</i>)	Boneless fish hydrolysate: 30.30% Hydrolysate with bones: 30.30% Cooked fish with bones: 29.13%	<i>Vibrio anguillarum</i> , 7.71×10^5 CFU mL ⁻¹	Immersion 14 days	No differences were observed in fish survival between dietary groups and control	Murray <i>et al.</i> (2003)
Red Sea bream (<i>Pagrus major</i>)	Tilapia and krill hydrolysates (2% in FM)	<i>Edwardsiella tarda</i> 1×10^5 CFU mL ⁻¹	Injection 21 days	Tilapia hydrolysate diet resulted in higher disease resistance compared to control fish against <i>Edwardsiella tarda</i>	Khosravi <i>et al.</i> (2015a)
Olive flounder (<i>Paralichthys olivaceus</i>)	Tilapia and krill hydrolysates (2% in FM)	<i>Edwardsiella tarda</i> 1×10^3 CFU mL ⁻¹	Injection 10 days	FPH groups exhibited higher disease resistance to control in fish, but the differences were not significant among treatments.	Khosravi <i>et al.</i> (2015a)
Atlantic salmon (<i>Salmo Salar</i>)	Cod muscle protein: 10% Lactic acid bacteria: 10%	<i>Aeromonas salmonicida</i> 5.8×10^4 cells fish ⁻¹	Injection 4 weeks	No difference was registered between death rates of fish fed FPH to the control	Gildberg <i>et al.</i> (1995)

FPH, fish protein hydrolysate; FM, fishmeal; PBM, poultry by-product meal; BPBM, bioprocessed poultry by-product meal.

FPH has polypeptide fractions that may stimulate some mechanisms in fish that are essential for disease resistance. Juvenile barramundi *Lates calcarifer* fed 5-10% tuna hydrolysate in FM diets showed significantly higher resistance against *Streptococcus iniae* infection following a bath challenge containing 1.8×10^3 CFU mL⁻¹ of the bacteria due to the short peptides in hydrolysate, which may facilitated non-specific immune responses particularly lysozyme and complement activities in fish (Siddik *et al.*, 2018b). Red sea bream *Pagrus major* fed hydrolysate diets exhibited

significant improvement in disease resistance against *Edwardsiella tarda* injection of 1×10^4 CFU mL⁻¹ per fish due to the enhancement of innate immune responses including lysozyme activity and total Ig level (Khosravi *et al.* 2015b). Similarly, Bui *et al.* (2014) found significant improvement in survival rate of juvenile red sea bream *Pagrus major* fed krill and tilapia hydrolysates during a challenge trial with *Edwardsiella tarda* (1×10^5 CFU mL⁻¹ per fish). In that study, the disease resistance was assumed to be enhanced in fish fed FPH

diets because of the enhancement of antiprotease and immunoglobulin (Ig) levels. In contrast, there were no significant differences in survival rate in Japanese sea bass *Lateolabrax japonicus* fed with different levels of FPH when exposed to 10^8 cells of *Vibrio anguillarum* in sea water (Liang *et al.* 2006). The poor survival of juvenile coho salmon *Oncorhynchus kisutch* fed diets containing FM, FPH and cooked fish following challenge with *Vibrio anguillarum* (7.71×10^5 bacteria mL^{-1}) at 15°C by immersion suggested that the supplementary ingredients had no advantageous effect on the cellular defence mechanisms (Murray *et al.* 2003). Gildberg *et al.* (1995) reported that the survival rate of Atlantic salmon *Salmo salar* fed with diets containing hydrolysed fish protein was poor when challenged with *Aeromonas salmonicida*. The variation in the effect of FPH on disease resistance may result from the variation in peptide profile of hydrolysates, with such variation depending on the source of native protein, hydrolysis conditions, enzyme specifications, experimental period, level of dietary inclusion and species-specific differences (Klompong *et al.* 2007).

Short-chain peptides with lower molecular weight are considered more active compounds to play the important role of electron donors. These electron donors may prevent chain reactions by reacting with free radicals to make them more stable substances (Chi *et al.* 2014). Hydrolysis unfolds complex protein structure to produce low molecular weight peptides and amino acids, which improve antioxidant activity of hydrolysed protein in comparison with the intact protein (Sarmadi & Ismail 2010). FPH from yellowfin tuna *Thunnus albacores*, tilapia *Oreochromis niloticus*, shortfin scad *Decapterus macrosoma*, salmon *Salmo salar* and cod *Gadus morhua* have been reported to possess antioxidant properties (Farvin *et al.* 2014; Borawska *et al.*, 2016; Tejpal *et al.* 2017; Kang *et al.* 2018; Pezeshk *et al.* 2019). A study on red sea bream *Pagrus major* demonstrated an increase in superoxide dismutase in fish fed protein hydrolysate diets compared to the low FM-based diet; in addition, comparatively higher glutathione peroxidase activity (GPx) was observed in fish fed high FM-based diet and hydrolysate diets when compared to low FM-based diet (Khosravi *et al.* 2015b). With the same species, Bui *et al.* (2014) found improvement in the superoxide dismutase level in hydrolysate treatments when compared to the control group fed with FM-based diet. A study by Siddik *et al.* (2020) found that serum GPx activity in juvenile barramundi *Lates calcarifer* was significantly increased when fed fermented poultry by-product meal supplemented with 10% tuna hydrolysate compared to the FM-based control, while serum malondialdehyde and catalase activities were not altered feeding the same diet. However, Zheng *et al.* (2013a) reported that the superoxide dismutase activity of

turbot *Scophthalmus maximus* was not affected by the levels of fish hydrolysates inclusion, while total antioxidant capacity of fish was improved with increasing level of small molecular weight FPH.

Limitations

Despite the numerous and promising applications of enzymatic FPH derived from seafood processing residues in aquaculture feed, some limitations related to the raw material used and processing conditions must be taken into consideration. These limitations include the following:

- The composition of seafood processing by-products varies from batch to batch in the manufacturing process, and the resulting variable nutritional composition may create challenges in the production of a consistent end-product.
- The raw materials, particularly those with high fat content, are highly perishable, susceptible to oxidation and contain microorganisms that foster the release of putrid odour. Some of these microorganisms can be pathogenic to the host fish, if still present in the feed product.
- The production of high-yield and highly pure FPH and specific bioactive peptides from fish waste through enzymatic hydrolysis have generally been reported from small-scale or controlled laboratory systems. This process may require expensive processing, isolation, purification and characterization techniques in large-scale operation. Commercial technical and economic feasibility in large-scale systems hence needs to be tested.
- The heterogeneity of FPH, containing a diverse range of peptides with different molecular sizes, hydrophobic natures and surface properties, results in challenges in aquafeed formulation and in the design of stage-specific diets for individual species (Vázquez *et al.* 2019).
- The high moisture content (up to 90%) of FPH may make it unstable for long-term storage and difficulty in handling, and enhance microbial growth. Mechanical removal of water or drying can be possible solutions, but consideration must be given to waste disposal because of higher levels of organic matter in the water, and the drying may also lead to further microbial contamination.
- FPHs are generally processed with concluding high temperature to inactivate protease action before incorporation into aquafeeds. This high temperature may result in destruction and racemization of amino acids.
- The bioactivities of seafood by-product derived peptides mostly have been assessed only in vitro (Martínez-Alvarez *et al.* 2015). Thus, it is important to assess the peptide functionalities in fish after digestion to assess their usefulness, dose–response and safety prior to use as a functional feed ingredient.

Future research potential

Despite progress achieved in the production, utilization, nutritional significance and biological effects of FPH on finfish aquaculture, more studies are required to compare economic feasibility of using FPH. FPH produced through enzymatic hydrolysis from fish waste and fish by-products intrinsically has varying composition and functional properties. Most of the enzymes used in the process of hydrolysis have distinct types of specificities that are difficult to control, standardize and use in making products with specific characteristics and for specific purposes. Therefore, it is important to search for proteases with the narrow substrate specificity, which can produce FPH with standardized composition and functionality. Further, it is important to note that most of the investigations on the enzymatic processes for FPH production have been confined to small or laboratory scale due to the high cost of the proteases. More studies to reduce the burden of enzymatic process by advanced industry-scale process modification are needed to develop cost-effective commercial operations. There is also a need for species- and life cycle stage-specific determination of the exact threshold level in which dietary inclusion of FPH negatively impacts growth and health of fish. This knowledge is important for aquaculturists and feed producers to prevent adverse effects on farm production and species-specific feed formulation.

There is a concern that some FPH contains higher amounts of oligopeptides with a high abundance of basic amino acids that have a low palatability for fish especially for fry and fingerlings. Appropriate techniques or supplementation that can substantially alleviate this palatability issue should be investigated. In addition, the molecular structure of FPH peptides and identification of the exact sequence of amino acids responsible for the bioactivity in the FPH peptides should also be improved (López-Pedrouso *et al.* 2020). This knowledge can be used to optimize the production conditions, refine the generation of peptides of interest and to better understand the effective bioactive peptides in aquadiets on growth and health status of the target species. Delivery of bioactive rich microdiets, that is nanoencapsulation, is a further area of investigation. This technology can be successfully applied to entrap bioactive molecules with aquafeeds for the higher survival and enhanced growth performance of juvenile fish.

Furthermore, a number of studies have reported antioxidant, antimicrobial and antihypertensive properties in FPH (Abachi *et al.* 2019; Vázquez *et al.* 2019), and hence, the potential of FPH as alternative to dietary antibiotics should be explored. This will open new opportunities for the development of safe, efficient and cost-effective strategies for the prevention and alleviation of many diseases in aquaculture.

Conclusions

This work overviewed the production of FPH and bioactive peptides from fish waste, and their potential effects on growth, feed utilization, biochemical response and immune performance of finfish in aquaculture production. The data presented here suggest that fish waste-derived hydrolysates have promising implications in aquaculture feeds either as a source of proteins and amino acids or else as a source of peptides with bioactive potential. Every year, a considerable amount of discarded by-product is produced in the seafood industry worldwide. It is therefore important to carry out a broader and deeper investigation for new applications of this waste not only for the environmental safety caused by its disposal but also for the possible economic return converting this low-value waste into high-value products. Nevertheless, there are some challenges in assurance of quality raw material, development of cost-effective production processes, large-scale operation, and separation and isolation of exact peptides desirable for feed formulation and supplementation. Successfully addressing all these gaps may lead to the large-scale production of FPH with bioactive potentials for the development of new aquafeeds and formulation of species-specific diets for finfish aquaculture.

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