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Review

Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP)

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ABSTRACT

Considerable amounts of fish processing co-products (FPCP) are generated which currently impose a cost burden on the seafood industry in terms of waste disposal, with little benefit generated. The demand for the sustainable use of FPCP has led to the development of processes for the recovery and hydrolysis of proteins, the assessment of their functionalities, and application into different products. The aim of this review is to critically analyze the-state-of-the-art on the functions, applications and production processes of FPCP protein hydrolysates, and identify the key research trends and future research directions that will maximize the economic and environmental benefits for the fish processing industry. FPCP protein hydrolysates have been found to possess desirable physicochemical properties (e.g. emulsifying, foaming, oil and water binding capacities) and many interesting bio-activities (anti-oxidative, anti-hypertensive, anti-microbial and anti-anemia) with potential applications in food, nutritional and pharmaceutical products. Chemical hydrolysis has been the most common process for the production of crude FPCP protein hydrolysates, though with little ability to control product quality. The enzymatic hydrolysis process has emerged recently as the process of choice due to its mild reaction conditions, superior product quality and functionality. The enzymatic processes have been demonstrated at the laboratory scale, but not as in full industrial-scale operation, probably due to the high costs of the enzyme. Advanced cost effective processing technologies need to be developed for the production of high quality FPCP protein hydrolysates that possess specific functionalities for specific product applications. Protein hydrolysates with defined molecular weight ranges, tailor-made for superior functionalities are in high demand. With the discovery of new functions and applications for FPCP protein hydrolysates by refining the traditionally crude product mixture, the fish processing industry can be empowered with advanced value-added processing technology and next generation functional products to successfully turn the "cost center" for the removal of waste into a "profit center" for business growth.

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1. Introduction

Fish processing co-products (FPCP) are fish material left over from the primary processing of fish manufacturing process. The percentage of FPCP generated from fish processing is around 50% of the starting material by weight (Bechtel, 2003), and imposes a cost to dispose the material in the absence of value-added solutions. Australian seafood industries, for example, discard over 100,000 tonnes of these co-products annually (Peter & Clive, 2006). Due to their high organic matter content, fish processing co-products are classified as a certified waste which is more costly to dispose. Currently, it costs AUD $150 per tonne to discard the co-products as certified waste (Peter & Clive, 2006), which means that the Australian seafood industry spends over AUD $15 million per annum on disposal rather than generating benefits via productive utilization and value-adding. This inefficient business model has been identified to be not only cost-ineffective but also environmentally unfriendly.

In addition, global fishery production is expected to increase in the next few years based on the increase from about 134.3 million tonnes in 2004 to about 145.1 million tonnes in 2009 (Table 1). Utilization of FPCP to produce value-added products has been highlighted as one of the high priority areas for development within the global seafood industry.

FPCP have been used to produce fish silage, fertilizer and animal feeds (Arvanitoyannis, 2008), but these generate a low profit of about only USD 50 cents per tonne. This disappointing outcome is driving the seafood industry to develop higher value-add products. Characterization of the chemical composition on FPCP from many fish species (Bechtel, 2003; Sathivel et al., 2003) showed that the FPCP protein content is generally over 50% based on dry weight. Therefore, the key solution must be the utilization of FPCP protein.

The World Health Organization recommends fish protein as a significant source of essential amino acids (about 30% by weight) (Usydus et al., 2009). However, instead of utilizing FPCP protein directly, fish protein hydrolysates are becoming more popular. It is defined as fish proteins that are broken down into peptides of various sizes. The degradation can be carried out either chemically (using acid or alkali) or biologically (using enzymes) (Pasupuleti & Braun, 2010). These processes not only maintain a high essential amino acid content, but also generate many improved functions for food or pharmaceutical application. For example, improved capacities of oil-binding and emulsifying are required for meat products (Sathivel et al., 2004) and spread-texture food (Klompong et al., 2007), respectively; improved anti-oxidation and anti-hypertension activities can be used as natural anti-oxidants (Mendis et al., 2004) and to control high blood pressure (Fahmi et al., 2004), respectively, to replace synthetic products which may have negative side effects.

Previous studies produced FPCP protein hydrolysates using two different methods: a chemical process and an enzymatic process. The chemical process is conducted under high temperature (120 °C) and pressure (100 kPa) in an acid or alkaline condition; and is commonly used because of the low processing cost. However, the functionality obtained with milder processes is often lost; and these processes lead to corrosion of equipment. Enzymatic processes overcome these disadvantages by using processing conditions with lower temperature and pressure and a pH range between 5 and 8, which have received more attention recently using many fish species (Diniz & Martin, 1997; Sathivel et al., 2005; Slizyte et al., 2005). However, there is no report in scaling up enzymatic processes from laboratory to industry.

To better understand this field of research toward industrial development, the goal of this review comprehensively analyzes recent studies on functions, applications and processes of fish protein hydrolysates from FPCP, and points to future research trends in this field. This review indicates that FPCP protein hydrolysates are able to empower the fish processing industry to achieve higher profits by converting FPCP to high value FPCP protein hydrolysates, using advanced processes.

Table 1
World fisheries and aquaculture production in million tonnes (FAO, 2010).

	2004	2005	2006	2007	2008	2009
Total capture	92.4	92.1	89.7	89.9	89.7	90.0
Total aquaculture	41.9	44.3	47.4	49.9	52.5	55.1
Total world fisheries production	134.3	136.4	137.1	139.8	142.3	145.1

2. Functions and applications of FPCP protein hydrolysates in the food sector

Hydrolyzation can change the properties of FPCP proteins in three ways: decreasing the molecular weight, increasing the number of ionizable groups and causing exposure of hydrophobic groups. These interactions control the physicochemical properties of food formulations as they are directly responsible for their performance and behavior in food systems (Panyam & Kilara, 1996). Physicochemical properties in combination are important when the FPCP protein hydrolysates interact with other components of food such as oil and water.

There are extensive studies on the physicochemical properties of FPCP protein hydrolysates from different fish species, as summarized in Table 2. FPCP protein hydrolysates showed enhanced physicochemical properties, when compared with non-hydrolysed fish protein, or other commercial food-grade products having the same function. Among all of these physicochemical properties, solubility, emulsifying capacity and oil binding capacity are the three most important for food formulations.

2.1. Solubility

Good solubility is required for other properties such as emulsification and gelling. The increase in solubility is the most dramatic improvement of FPCP protein hydrolysates. Protein hydrolysates from Red salmon FPCP reached 95% solubility, after being hydrolyzed by alcalase in 2 h, with an E/S ratio of 5%, at 61 °C at pH 7.5, whereas, solubility of raw red salmon FPCP protein was only 20% (Gbogouri et al.,

Table 2
Enhanced physicochemical properties of FPCP protein hydrolysates.

Physicochemical properties	FPCP protein hydrolysates[a]	Reference sample	Fish species	Reference
Solubility (determined by Kjeldahl method, presented in nitrogen solubility index)	95%	55% (un-hydrolysed fish protein)	Shark	(Diniz & Martin, 1997)
	95%	22% (un-hydrolysed fish protein)	Salmon	(Gbogouri et al., 2004)
	70%	15% (un-hydrolysed fish protein)	Blue whiting	(Geirsdottir et al., 2011)
	75%	60% (soy protein)	Tilapia	(Foh et al., 2011)
Oil binding capacity	5.0 g oil/g protein	1.2 g oil/g protein (soy protein) 1.4 g oil/g protein (casein)	Cod	(Slizyte et al., 2005)
	3 g oil/g protein	1.9 g oil/g protein (soy protein) 2.8 g oil/g protein (milk protein)	Blue whiting	(Geirsdottir et al., 2011)
	3.38 ml oil/g powder	2.81 ml/g (soy protein)	Tilapia	(Foh et al., 2011)
Emulsifying capacity	128.3 g oil emulsified/g powder	17.0 g oil emulsified/g powder (casein)	Cod	(Slizyte et al., 2005)
	55 ml oil/0.5 g sample	39 ml/0.5 g of sample (un-hydrolysed fish protein)	Shark	(Diniz & Martin, 1997)
	40 g oil/50 ml 0.3% protein solution	24 g oil/50 ml 0.3% solution (Sodium caseinate)	Pacific whiting	(Pacheco-Aguilar et al., 2008)
	85.32%	55.27% (soy protein)	Tilapia	(Foh et al., 2011)
Water binding capacity	70.4 g water/g protein	68.4 g water/g protein (soybean protein)	Cod	(Slizyte et al., 2005)
	98%	87% (soy protein) 87% (whey protein) 87% (milk protein)	Blue whiting	(Geirsdottir et al., 2011)
Foaming capacity	50% of volume increased from initial volume sample	20% of volume increased from initial volume sample (un-hydrolysed fish protein)	Shark	(Diniz & Martin, 1997)
	150% of volume increased from initial volume sample	110% of volume increased from initial volume sample (un-hydrolysed fish protein)	Herring	(Liceaga-Gesualdo & Li-Chan, 1999)

[a] The highest value reported, which indicates the highest capacities in each study.

2004). The same trend was also observed in shark protein hydrolysates (Diniz & Martin, 1997). Longer processing times produced protein solutions with smaller molecular weights resulting in higher solubility. It is hypothesized that there is an increase in hydrophilic polar groups leading to an increase in their water-solubility (Kristinsson & Rasco, 2000). The improved solubility enables FPCP protein hydrolysates to be applied readily to formulated food systems (Thiansilakul et al., 2007).

2.2. Emulsifying capacity

Most processed foods contain oil which exists as an emulsions together with other constituents. The most frequent emulsion is an oil–water emulsion (Panyam & Kilara, 1996) in the form of spread-texture food such as vinaigrette, mayonnaise and hollandaise sauce. FPCP protein hydrolysates are good emulsifiers due to their improved amphiphilic nature, as they expose more hydrophilic and hydrophobic groups that enable orientation at the oil–water interface for more effective adsorption (Klompong et al., 2007). The emulsifying capacity of rockfish protein hydrolysates, obtained by hydrolysis for 1 h with Rhozyme, at an E/S ratio of 1/75 and pH of 6.5–6.7, increased to 231 g oil/g protein from 145 g oil/g intact Rockfish protein, (Spinelli et al., 1972). Similar results were also found with Herring protein hydrolysates (Liceaga-Gesualdo & Li-Chan, 1999). However, the extent of hydrolysis has to be carefully controlled, as excessive hydrolysis can decrease the emulsifying capacity of FPCP protein hydrolysates. The emulsifying capacity of Rockfish protein hydrolysates dropped from 231 g oil/g protein to 224 g oil/g protein when the hydrolysis time was extended from 60 min to 90 min (Spinelli et al., 1972). Protein hydrolysates of Pacific whiting (Pacheco-Aguilar et al., 2008), Yellow stripe trevally (Klompong et al., 2007) also showed a similar outcome. The reduced capacity is due to an excess of low molecular weight components which cannot fold so lose the ability of reorienting in the water–oil interface to stabilize the emulsion system (Klompong et al., 2007). Kristinsson and Rasco (2000) reported that protein hydrolysates should consist of at least 20 amino acids to possess good emulsifying capacity. The emulsifying capacity of FPCP protein hydrolysates was compared with other commercial food-grade emulsifiers such as soy protein powder, casein protein powder and sodium caseinate powder (Table 2), and was found to be more effective. This indicates the strong potential of developing FPCP protein hydrolysates as commercial emulsifying agents for food formulations.

2.3. Oil binding capacity

Oil binding capacity is an important function used in meat and confectionery products (Sathivel et al., 2004). The mechanism for this is attributed to the combination of physical entrapment of oil and the hydrophobicity of the material. Hydrophobicity of FPCP protein hydrolysates develops because hydrolysis cleaves the protein chain so more internal hydrophobic groups are exposed (Kristinsson & Rasco, 2000). Sathivel et al. (2005) found oil binding capacity of Red salmon FPCP protein hydrolysates increased during hydrolyzation within a certain time range whereas it dropped if hydrolyzation was further extended: the maximum value of oil binding capacity (7.8 ml oil/g protein) was demonstrated with a 50 min hydrolysis time using palatase, at an E/S ratio of 0.5%, 50 °C, but it dropped to 4.3 ml of oil/g protein when hydrolysis time was extended to 75 min. Similar results were also demonstrated with FPCP protein hydrolysates of grass carp (Wasswa et al., 2007). The excessive hydrolyzation compromises the integrity of the protein structure, and results in the degradation of the protein network formed to entrap oil. FPCP protein hydrolysates from many fish species were found to have a superior oil binding capacity compared to commercial food-grade oil binders such as soy protein powder, casein powder and milk protein powder (Table 2), and have the potential to be utilized as commercial oil binders in processed food.

In summary, the physicochemical properties, including solubility, oil binding capacity and emulsifying capacity of FPCP protein hydrolysates have been comprehensively studied, the mechanism of these properties have been determined, and their potential applications have been demonstrated. However, to the best of our knowledge, these studies were mainly limited to laboratory-scale tests and studies involving FPCP protein hydrolysates in commercial food formulations based on these properties have rarely been reported.

3. Functions and applications of FPCP in the nutritional and pharmaceutical sector

The high essential amino acid content of FPCP protein hydrolysates enables it to be used in the formulation of nutritional supplements. Shen et al. (2012) suggested that fish protein hydrolysates from *Collichtchys niveatus* can be incorporated as supplements in health-care

food as the total content of essential amino acids was 970 ng/ml whereas nonessential amino acids were 709.1 ng/ml.

Besides the high essential amino acid content, bio-active peptides can also be produced from FPCP protein hydrolysates. The primary source of bio-active peptides currently has been dairy products. However the marine environment represents a myriad of protein resources including algae by-products and FPCP, and the environment in which they are found makes these resources a new and relatively untapped source for new bio-active compound generation (Rustad & Hayes, 2012). The bio-active peptides contained in FPCP protein hydrolysates can be potentially used in pharmaceutical products, whereas they are inactive within the sequence of the parent protein (Sarmadi & Ismail, 2010). These bioactivities are summarized in Table 3. It can be seen that the value of these bioactivities derived from fish protein hydrolysates of FPCP is comparable or exceed the reference samples, which mostly are commercial products. The most commonly evaluated bioactivities are anti-oxidation and anti-hypertension.

3.1. Anti-oxidative activity

Free radicals are formed during the metabolism of oxygen. They are oxygen atoms with an unstable structure of unpaired electrons, which are highly reactive with adjacent molecules (Klompong et al., 2007). Under normal conditions, endogenous anti-oxidative defense systems can eliminate free radicals, but under certain circumstances are not able to suppress all the free radicals. This results in cellular damage, which, in turn, initiates diseases such as atherosclerosis, arthritis, diabetes and cancer (Sarmadi & Ismail, 2010). Synthetic antioxidants such as α-tocopherol and butylated hydroxyanisole are used as supplements, but their side-effects are a potential health hazard which cannot be ignored. Nowadays there is considerable interest in finding anti-oxidants from natural resources that have little or no side effects (Mendis et al., 2004).

FPCP protein hydrolysates derived from many fish species such as hoki (Mendis et al., 2004), cod (Guerard & Sumaya-Martinez, 2003) and mackerel (Wu et al., 2003) have demonstrated anti-oxidative activities (Table 3) that are higher than that of commonly used synthetic anti-oxidants such as α-tocopherol and butylated hydroxyanisole. This is due to their high content of Tyr, Trp, Met, Lys, Cys and His that act as donors of protons or hydrogen with a positive charge to react with the unpaired electrons of free radicals. Hydrolyzation unfolds protein structure to expose more of these amino acids, and leads to improved anti-oxidative activity of FPCP protein hydrolysates compared to the intact protein (Sarmadi & Ismail, 2010). The anti-oxidative activity of fish protein hydrolysates can be further improved using the mallard reaction to form fish protein hydrolysate–glucose complexes (You et al., 2011).

This shows the potential of replacing synthetic antioxidants with FPCP protein hydrolysates.

The anti-oxidative activity of FPCP protein hydrolysates can also be applied to food products to extend their shelf life. Lipid oxidation leads to the development of undesirable off-flavors and potentially toxic reaction products. Antioxidants not only improve the stability of lipids and lipid-containing foods but are also used to preserve food products by retarding discoloration and deterioration resulting from oxidation, which also results in enhancing shelf life (Herpandi et al., 2011). Dekkers et al. (2011) found that dip treatment of mahi mahi fillet in whole tilapia protein hydrolysates showed significant lower levels of the oxidation product of malonaldehyde, compared to the control, and improves the stability and enhances the shelf life of the processed mahi mahi fillet.

3.2. Anti-hypertensive activity

One form of hypertension is caused by the increase in activity of angiotensin I converting enzyme (ACE), a blood pressure regulator. It converts inactive decapeptide angiotensin I to octapeptide angiotensin II, a potent vasoconstrictor, by cleaving its C-terminal His-Leu bond. In addition, ACE also degrades bradykinin, a vasodilatory peptide (Fahmi et al., 2004). Currently commonly used synthetic ACE inhibitors, such as captopril, have strong side effects such as cough and skin rashes (Waeber, 2001), so interest in finding new ACE inhibitors with little or no side effects is growing.

FPCP protein hydrolysates from many fish species have been found with good anti-hypertensive activity. Jung et al. (2006) tested the change of systolic blood pressure of spontaneously hypertensive rats by administering protein hydrolysates of yellowfin sole and captopril. They found the blood pressure of these rats dropped to the same level of 165 mmHg after 9 h. Fujita and Yoshikawa (1999) found that both 100 molar captopril and 90.6 molar Bonito protein hydrolysates could decrease systolic blood pressure by 50 mm Hg (Table 3). These results indicate the possibility of developing FPCP protein hydrolysates as natural ACE inhibitors. Hosomi et al. (2012) compared the anti-hypertensive activity of fish protein hydrolysates and other protein sources such as casein, and suggested that fish protein hydrolysates can provide better health benefits than casein by decreasing the cholesterol content in the blood, which would contribute to the prevention of circulatory system diseases such as arteriosclerosis.

These bioactivities of FPCP protein hydrolysates have been comprehensively studied before by different methods, such as cell-based assays and animal-based experiments using cholesterol-fed rats (Ben Khaled et al., 2012). However, no clinical trials of these bio-active peptides have been carried out, therefore FPCP protein hydrolysates are not available as pharmaceutical drugs yet.

Table 3
Bio-activities of FPCP protein hydrolysates compared with other reference samples.

Bio-activities	FPCP protein hydrolysates[a]	Reference sample	Fish species	References
Anti-hypertensive activity	75%[b]	9.4%[b] (un-hydrolysed fish protein)	Blue whiting	(Geirsdottir et al., 2011)
	90.6 molar[c]	100 molar[c] (captopril)	Bonito	(Fujita & Yoshikawa, 1999)
	−35 mm Hg[d]	−35 mm Hg[d] (captopril)	Yellowfin sole	(Jung et al., 2006)
Anti-oxidativity (measured by DPPH method)	93%	87% (α-tocopherol)	Hoki	(Mendis et al., 2004)
	22%	10% (butylated hydroxyanisole)	Cod	(Guerard & Sumaya-Martinez, 2003)
	80%	60% (autolysed fish protein)	Mackerel	(Wu et al., 2003)
Antimicrobial activity(measured by MIC assay)	0.218 mg/l vs *B. cereus*	0.297 mg/l for *B. cereus* (Tetracycline)	Leatherjacket	(Salampessy et al., 2010)
	0.222 mg/l vs *S. aureus*	0.340 mg/l for *S. aureus* (Tetracycline)		
Anti-anemia activity (measured by hematology analysis)	3.82×10^9/l	2.74×10^9/l	Saurida elongate	(Dong et al., 2005)

[a] Data of the most significant value in each study was selected to present in this column.
[b] Measured by ACE inhibitory method.
[c] Measured by minimum effective molar basis (mol/l) for anti-hypertensive activity.
[d] Measured by change in systolic blood pressure of spontaneously hypertensive rats by administering ACE inhibitory fish protein hydrolysates and captopril with the dose of 10 mg/kg body weight, 9 h after the administration.

4. Production processes of FPCP protein hydrolysates

The first commercial fish protein hydrolysates were produced in the 1940s in Sweden using a chemical process (Kristinsson & Rasco, 2000). In that process, fish protein is cleaved into peptides of variable molecular weights by either acidic or alkaline treatment using high temperature (121 °C) and high pressure (100 kPa) (Sanmartin et al., 2009). It was popular due to the high protein recovery, fast processing and low cost. However, it operated with little ability to control the quality of fish protein hydrolysates, resulting in weak aforementioned functionalities, both physicochemical and bioactive. These disadvantages significantly limited the high value-applications of these protein hydrolysates for food and drug. They are currently used for low-value products such as fertilizer (Kristinsson & Rasco, 2000) with a profit of only US50 cent/ton, or as a nitrogen source for the growth of lactic acid bacteria (Gao et al., 2006).

With the aim of using FPCP protein hydrolysates in high value-added products, enzymatic processing was employed to produce well-defined FPCP protein hydrolysates. The proteins can be easily deactivated in mild conditions of temperature and pH. The availability of various enzymes from different sources enables the manufacturer to choose the best one based on the desired final product (Pasupuleti & Braun, 2010). For example, enzymes can be employed to systematically remove amino acids from either the N- or C- terminus (Sanmartin et al., 2009). The enzymatic processes have a number of advantages (Table 4) and hold the most promise for the future of FPCP protein hydrolysate production. Due to this, this review has focused on enzymatic processes. The enzymatic process can be divided into 3 processing steps: pre-treatment, hydrolyzation and recovery (Fig. 1).

4.1. Pre-treatment

The purpose of the pre-treatment step is to prepare homogenized water–mince mixtures with low fat content for the subsequent step of hydrolyzation. FPCP are minced then mixed with equal amounts (w/w) of water to form a homogeneous water–mince slurry. Increasing the amount of water does not increase the protein recovery of FPCP protein hydrolysates but reducing water decreases it (Benjakul & Morrissey, 1997). The fat content of fish protein hydrolysates need to be well-controlled. The Food and Agriculture Organization of the United Nations set up a standard that the fat content of fish protein hydrolysates has to be below 0.5% (w/w) for human consumption. A higher fat content darkens the final products, due to the release of brown pigments from lipid oxidation (Kristinsson & Rasco, 2000). Therefore de-fatting FPCP of fatty fish is required prior to mixing with water. Organic solvents are commonly used for this purpose. Hoyle and Merritt (1994) de-fatted fatty herring by ethanol (90%) treatment with a mince:ethanol ratio of 1:2 at 70 °C for 30 min, and produced FPCP protein hydrolysates with an acceptable fat content. A similar process was also successfully conducted on another fatty fish species, sardines (Quaglia & Orban, 1987). Organic solvent washing not only removes excess fat but also minimizes bacterial degradation of fish protein hydrolysates (Kristinsson & Rasco, 2000).

Table 4
Comparison of the chemical process and enzymatic process to produce FPCP protein hydrolysates (Kristinsson & Rasco, 2000; Sanmartin et al., 2009).

Processing method	Advantages	Disadvantages
Chemical process (acid and alkali)	High protein recovery Short processing time Low processing cost	Absence of homogeneous hydrolysates Bitterness Poor functionalities High salt content Metal corrosion of equipment Reaction is hard to control Form toxic substances like lysino-alanine Form D-amino acids, which are not absorbed by humans
Enzymatic process	Less bitterness of final hydrolysates Maintain functions and nutritive value of final hydrolysates Low salt content in final products Produce homogeneous hydrolysates	High processing cost Long processing time

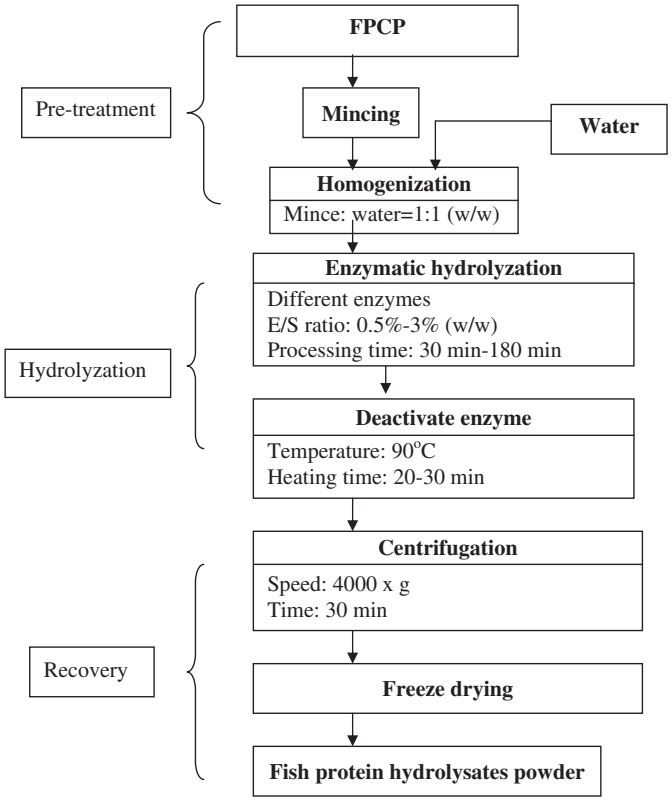

Fig. 1. Flow diagram of the enzymatic processing method to produce fish protein hydrolysates.

4.2. Hydrolyzation

The selected enzyme is mixed homogeneously with the mince–water slurry after the pre-treatment step. Processing temperature and pH are adjusted to the optimal values of the selected enzyme. The E/S ratio and processing time are set up according to desired functionalities and protein recovery of the final protein hydrolysates. Hydrolyzation is terminated by deactivating enzymes at 90 °C for about 30 min. This process has produced fish protein hydrolysates from many fish species (Table 5). The origin and specificity of the enzymes used in the studies listed in Table 5 are presented in Table 6.

Enzyme selection is essential for the preparation of functional attributes of protein hydrolysates. Enzymes with an optimal working pH in the acidic range such as pepsin were preferred in earlier times as the low pH could also inhibit microbial growth. However the acidic pH atmosphere also lead to low protein recoveries, low nutritional values due to the destruction of the essential amino acid tryptophan and low functionalities due to excess hydrolyzation (Kristinsson & Rasco, 2000). Therefore, enzymes with an optimal reaction pH close to neutral, such as alcalase, neutrase and flavourzyme, are now used more extensively. Most of enzymes in Table 6 are of microbial origin. In comparison with animal or plant-derived enzymes, microbial enzymes have several advantages, including greater pH and temperature stabilities. From a

Table 5
Enzymatic process to produce FPCP protein hydrolysates from different fish species using different enzymes.

Fish species	Applied enzymes	Outcome	Reference
Shark	Alcalase	Enzymatic process was responsible for the improvement of protein functionalities. However, excess hydrolyzation decreased functional properties.	(Diniz & Martin, 1997)
Red salmon	Alcalase	1. Optimal processing condition based on achieving the highest Degree of Hydrolysis (DH) was determined. It was: E/S = 5.2%, temperature of 57 °C, processing time of 2 h and pH of 8.0 for DH of 17.2%. 2. Hydrolysates with the highest DH had the best solubility, but oil binding capacity was better when DH was low.	(Gbogouri et al., 2004)
Pacific whiting	Alcalase Neutrase	Optimal processing condition based on achieving the highest DH was determined. It was: E/S = 20 AU/kg raw material, processing time of 1 h, temperature of 55 °C substrate/butter = 1:1 (w/v), by using alcalase.	(Benjakul & Morrissey, 1997)
Persian sturgeon	Alcalase	Optimal processing condition based on achieving the highest DH was determined. It was: E/S = 0.1 AU/g protein, processing time of 205 min, temperature of 55 °C with highest DH of 61.96%.	(Ovissipour et al., 2009)
Grass carp	Alcalase	Hydrolysates had better oil binding capacity and emulsifying capacity at low DH, better water holding capacity at higher DH.	(Wasswa et al., 2007)
Herring	Alcalase Papain	1. Alcalase hydrolysates had higher DH than papain hydrolysates, under the same processing conditions. 2. Papain hydrolysates were more bitter than alcalase hydrolysates.	(Hoyle & Merritt, 1994)
Capelin	Alcalase Neutrase Papain	1. Alcalase was best based on achieving the highest DH. 2. Incorporation of hydrolysates (up to 3%) in meat model systems resulted in an increase of 4% in cooking yield and inhibition of oxidation by 17.7%–60.4%.	(Shahidi et al., 1995)
Yellowfin sole	α-Chymotrypsin	A peptide of Met-Ile-Phe-Pro-Gly-Ala-Gly-Gly-Pro-Glu-Leu was identified with the highest ACE inhibitory activity, after enzymatic process, membrane fractionation, ion exchange, gel penetration and protein sequence test.	(Jung et al., 2006)
Mackerel	Protease N	Hydrolysates with molecular a weight of approximately 1400 Da possessed stronger anti-oxidative activity than that of the 900 and 200 Da.	(Wu et al., 2003)

technical and economical point of view, microbial enzymes such as alcalase operating at alkaline pH have been reported to be most efficient in the hydrolyzation of fish proteins (Herpandi et al., 2011).

Interaction between enzymes and functions of FPCP protein hydrolysates also varies based on fish species. Alcalase produced capelin protein hydrolysates with a high protein recovery of 70.5% compared to the lowest of 57.6% with papain (Shahidi et al., 1995), whereas the protein recovery (86%–88%) of yellowtail kingfish hydrolysates produced by alcalase, flavourzyme and neutrase was not significantly different (He et al., 2012). Cod protein hydrolysates produced by using flavourzyme possess higher oil binding capacity (4.1 g/g protein) than that produced by neutrase (3.1 g/g protein) whereas emulsifying capacity was in contrast (8.5% of the initial emulsion of flavourzyme and 10.5% of initial emulsion of neutrase). Results from many studies demonstrated that alcalase generally produced FPCP protein hydrolysates from the same fish species with the highest anti-oxidative activity (Sarmadi & Ismail, 2010). However, the relationships between enzymes and other functions of FPCP protein hydrolysates were not determined.

The effectiveness of hydrolyzation was indicated by degree of hydrolysis (DH), which was defined as the percentage of peptide bonds cleaved (Adler-Nissen, 1979). So far, DH has been recognized as the most effective, and perhaps the only indicator of hydrolyzation. Higher DH leads to higher protein recovery because more cleaved peptide bonds result in protein hydrolysates with smaller molecular weights, which are more soluble in water, thereby increasing protein recovery of hydrolysate powder. Protein recovery of Pacific whiting hydrolysates increased from 48.6% at DH of 10% to 67.8% at DH of 20% (Pacheco-Aguilar et al., 2008). Similar results were also found with capelin protein hydrolysates (Shahidi et al., 1995). Functionalities were also correlated to DH. When DH increased from 11.50% to 17.30%, emulsifying stability and oil binding capacity of red salmon protein hydrolysates dropped from 86.6% to 74.7%, and 3.55 ml oil/g protein to 2.8 ml oil/g protein, respectively. Anti-oxidative activity of loach protein hydrolysates increased from 80% at a DH of 18% to 90% at a DH of 23% but dropped back to 80% when increasing DH to 33%. The reason for this is that DH affects the molecular weight distribution of FPCP protein hydrolysates, while they are associated with various functionalities.

Table 6
Enzymes used to produce fish protein hydrolysate (Vercruysse et al., 2005; Xu et al., 2011).

Enzymes	Origin	Specificity
Alcalase	*Bacillus licheniformis*	Narrow, mainly for hydrophobic amino acids
Neutrase	*Bacillus amyloliquefaciens*	Narrow, mainly for Leu and Phe
Papain	Papaya	Broad, endoprotease
α-Chymotrypsin	Bovine pancreas	C-terminus of Thr, Trp, Phe, Leu
Flavourzyme	*Aspergillus oryzae*	Endoprotease and Exoprotease mixture

4.3. Recovery

FPCP protein hydrolysates were dried to a powder in the recovery phase. Liquid forms of fish protein hydrolysates can spoil quickly due to the high water content and the ease with which bacteria utilize proteins as substrates. The powder form of fish protein hydrolysates has a definite advantage in that it is lighter and easier to transport than the liquid form and can be stored for longer periods of time. The recovery steps were centrifuged followed by drying. Centrifugation at 4000 *g* for at least 20 min generally separates the hydrolysed mince–water slurry into three layers: the oil layer on the top, the protein hydrolysate solution in the middle and a semisolid layer at the bottom. After the fat layer was removed the protein hydrolysate solution was decanted, without disturbing the semi-solid layer. For the next step freeze drying was used in previous laboratory studies though it can be predicted that spray drying will be used if the production is scaled up from laboratory to industry scale. The final product of FPCP protein hydrolysates is creamy white in color with good water solubility and desired functions.

4.4. Membrane fractionation and further purification

In some previous studies (Bougatef et al., 2010; Rajapakse et al., 2005) membrane fractionation was applied between fat removal after centrifugation and freeze drying in recovery. The purpose of this was to fractionate fish protein hydrolysates with certain molecular weights that possess the strongest functions. It has been determined that bioactivities associated with molecular weights of FPCP protein hydrolysates. Tilapia and cod protein hydrolysates possessed the strongest anti-oxidative activity in the molecular weight range 3.5–10 kDa (Raghavan & Kristinsson, 2008), and <10 kDa (Jeon et al., 1999), respectively; anti-hypertensive activity of cod protein hydrolysates increased from 59.6% with a molecular weight range of 10–30 kDa to 87.62% with a molecular weight below 1 kDa (Je et al., 2004). Molecular weights of

100–500 Da and 1000–3500 Da were the two ranges with the most bioactive peptides (Vandanjon et al., 2009). The influence of key factors in membrane fractionation including the concentration factor, relative recovery in the retentate and final retention factors, on the outcome of this process has been studied by Bourseau et al. (2009).

Furthermore, some purification studies used after membrane fractionation followed by gel permeation chromatography to obtain pure peptide fragments with the highest bioactivities among all fragments. This level of purity is required for the development of pharmaceutical products. Several peptide fragments have been purified from FPCP protein hydrolysates, for example, Ser-Pro-Arg-Cys-Arg from lizard fish with strong anti-hypertensive activity (Wu et al., 2012) and Leu-Lys-Pro-Asn-Met from Alaskan pollack with strong anti-oxidative activity (Je et al., 2004). However, both membrane fractionation and purification are difficult to scale up to an industrial scale due to the high production cost.

4.5. Storage of FPCP fish protein hydrolysates

Dried fish protein hydrolysates were normally stored at a temperature of 4 °C or lower, sometimes with vacuum packaging. Pacheco-Aguilar et al. (2008) stored fish protein hydrolysates of Pacific whiting in vacuum packed polyethylene bags which were maintained at −20 °C until use. Liceaga-Gesualdo and Li-Chan (1999) stored fish protein hydrolysates of herring in desiccated plastic containers at 4 °C for further use. Lower temperatures and removal of oxygen (air) reduce the oxidative reaction rate of food, therefore extend its shelf life. Lin et al. (1998) stated that the absence of air during the drying process could inhibit oxidation. Jakobsen and Bertelsen (2000) found that a low temperature of below 4 °C almost prevents oxidation of beef regardless of the oxygen level; however the oxygen level becomes more critical when the temperature is raised.

Though there are many advantages of enzymatic processes when compared with chemical processes, it would be difficult for the industry to scale up this process based on current studies, due to the high production cost as a result of the following: (1) using large quantities of enzymes; (2) low protein recovery: protein recovery of the enzymatic process is lower than the chemical process for the same processing time; and (3) long production cycles: the enzymatic process needs more than 2 h, whereas the chemical process can be done in 20 min. However, previous studies mainly focused on functionalities of FPCP protein hydrolysates produced by enzymatic processes, rather than process modifications to reduce the production cost of enzymatic processes.

5. Future research and development directions

Functions of FPCP protein hydrolysates have been comprehensively studied before. Future research should focus on overcoming barriers to the transfer of positive laboratory outcomes to industrial production. Research can be undertaken in process modifications to reduce production costs. Several key future research directions are below.

5.1. Influence of molecular weights on physicochemical properties of FPCP protein hydrolysates

The relationship between molecular weight of FPCP protein hydrolysates and bioactivities has already been studied. However, the relationship between molecular weight and physicochemical properties has not been studied. Studying this relationship should be undertaken so that physicochemical properties of FPCP protein hydrolysates can be further improved to make them more suitable as functional ingredients of food.

5.2. Optimization of enzymatic processing conditions based on protein recovery and functionalities

Previous optimization studies of enzymatic processing conditions only focused on increased protein recovery. However, high protein recovery might be achieved, but with a reduction of functionalities, whereas optimization based on both protein recovery and functionalities of FPCP protein hydrolysates has not been done yet. This needs to be undertaken as both protein recovery and functionalities are important for FPCP protein hydrolysates. Though it has not been done with FPCP protein hydrolysates, similar optimization studies have been conducted on other food products, such as kamaboko, a Japanese seafood product made by fish mince in paste form: both the white color and firm texture are important quality parameters for kamaboko, but enhanced washing processing conditions increase whiteness but reduce firmness. Shan et al. (2010) successfully optimized washing processing conditions to produce kamaboko from common carp with both market acceptable whiteness and firmness, by using a response surface methodology experimental design.

5.3. Microwave intensified enzymatic hydrolyzation for reducing cost of enzymatic process

The high cost of enzymatic processing and a long processing time can be a barrier for industrial production; whereas, microwave-intensified processing is able to afford higher yields in shorter reaction times. The popularity of this method is increasing due to the advantages of it being clean, cheap and convenient (Corsaro et al., 2008), and has been used to produce a variety of food products, including the food productions using enzymes. For example, microwave intensification has been used to enhance enzymatic hydrolyzation of cellulosic materials in rice straw, and as a result enhanced markedly the accessibility of the cellulosic materials for enzymatic hydrolyzation. The enhancement was 1.6 times more using microwave intensification at 170 °C for 5 min, compared with enzymatic hydrolyzation without the microwave intensification treatment (Ooshima et al., 1984). Izquierdo et al. (2008) analyzed the effects of microwave intensification on hydrolyzation of a commercial whey protein concentrate by seven food grade enzymes, and found that microwave intensification increased the proteolysis of all enzymes. Microwave intensification has not been used with enzymatic processing for producing fish protein hydrolysates to-date, whereas based on the previous positive outcomes with other products, it would be interesting to apply microwave-intensification to shorten processing times for producing FPCP protein hydrolysates, thereby significantly reducing processing costs.

5.4. Applications of FPCP protein hydrolysates in food formulations

FPCP protein hydrolysates have been found with various beneficial functions for the food industry. However, to date there are very few information on applying FPCP protein hydrolysates in food formulations. Studies can be undertaken for the following applications: FPCP protein hydrolysates can be used as a high quality protein to enhance the protein content of food due to its high essential amino acid content; as a milk powder replacement due to its the high efficiency ratio (PER) value, as an oil binder in meat products such as sausages due to its high oil binding capacity; as an emulsifier in spread texture food due to its high emulsifying capacity, and as a water binder to increase cooking yield of boiled food due to the high water binding capacity.

5.5. Food safety tests of FPCP protein hydrolysates

While there are assumed food safety concerns on the application of FPH in various food applications, however there is no scientific report on this topic. For the enzymatic hydrolyzation process, the condition of deactivating enzyme after hydrolyzation process at 90 °C

for 30 min is equivalent to pasteurization. This process may effectively eliminate 90% or more of harmful microorganisms as shown in milk processing, however scientific tests are required.

Besides the concern of microorganisms, as seafood related product, the histamine content of FPH cannot be ignored. Australian kingfish were determined to have high levels of histamine by Italian authorities (Food recalls and alerts in the European Union, 2010). This has raised the food safety concern of histamine in fish-related products. FPH is a concentrated fish protein related product; the histamine content could also be concentrated from fish body to FPH. Due to this, the histamine content of FPH should be paid special attention.

In addition, allergies to seafood products are present in 22% of all patients with a diagnosis of food hypersensitivity (Pascual et al., 1992). As FPCP protein hydrolysates are derived from intact fish protein, they might also induce fish allergies. The allergens analysis is essential to complete the food safety profile of FPCP protein hydrolysates.

To support FPH applications in food or nutritional and pharmaceutical products for human uses, food safety tests on FPCP protein hydrolysates must be carried out if fish protein hydrolysates are launched as market products. The comprehensive food safety tests, mainly including histamine content test, microbiological test and allergy test are necessary to ensure that the FPCP protein hydrolysates produced meet food safety standards.

5.6. Economic feasibility analysis of FPH industrial production and business case for producing FPCP protein hydrolysates

A major outcome of the FPCP protein hydrolysates research is to enable their commercial utilization. The barriers are not only the aforementioned lack of applied research, but also the lack of business case for production. Therefore, a comprehensive business case is required for the commercialization of FPCP protein hydrolysates including clearly understanding the market situation, development of a market strategy, technical operation plan, management and personnel, legal concerns, finance plan, action plan, risk analysis and exit opportunities.

Economic feasibility analysis of FPH production on industrial scale is the fundamental information for the FPH business plan. The economic feasibility analysis should comprehensively analyze the influence of key production parameters, such as production scale, equipment cost, raw material cost, operation cost, product selling prices on total investment and return on the investment payback time and the profit return on investment. A range of process simulation software such as SuperPro Designer can be applied for this assessment. Superpro Designer is a powerful tool for economical evaluation, which can be utilized to mathematically evaluate the process economic performance. This process simulator offers the opportunity to shorten the time required for process development, and allows the comparison of process alternatives on a consistent basis so that a large number of process designs can be synthesized and analyzed interactively in a short time (Rouf et al., 2001). SuperPro Designer has been widely used to simulate industrial production of various bio-processing products for economic feasibility analysis, such as beta-galactosidase (Chodori, 2003), molasses (Michael, 2008), and antibody (Husin, 2009). The SuperPro Designer can also be applied to carry economic feasibility analysis of FPH production on industrial scale, and build up the fundamental knowledge for the FPH business plan. However, to the best of our knowledge, the economic feasibility analysis of FPH industrial production has not been done before. This gap should be filled in order to successfully market FPH as commercial products.

6. Conclusions

Considerable amounts of money are spent on discarding FPCP annually by the global seafood industry. This inefficient business model increases the cost burden of seafood industry. With the aim of changing this “cost center” into “profit center”, this can be turned around with value-added functional products such as FPCP protein hydrolysates which can be produced from various fish species. The functional properties that can be applied to the food industry are oil binding, water binding and emulsification. Other bioactivities, such as antioxidation and anti-hypertension have a role in human health, where the pathway to market is highly regulated. Driven by this, more effective processing methods for producing FPCP protein hydrolysates are being developed with enzymatic processes becoming more popular because they generate FPCP protein hydrolysates with better functions. However, so far the enzymatic process is only used on a laboratory scale rather for industrial production due to the high cost of the enzymes. Studies to reduce enzymatic process costs by advanced process modification are required toward the development of cost-effective industrial processes. The other key research gaps are lack of process optimization toward both high protein recovery and better functionalities, lack of food safety data, lack of production of controlled molecular weight products with specific functions, lack of process economic assessment at industrial scale, and lack of demonstration of applications in food and nutritional products. Successfully addressing these research gaps would lead to the commercial development of fish protein hydrolysates as functional ingredients for the formulation of daily-consumed food, functional food, and nutrition supplements.

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