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Review **Derleme**

Novel Utilization of Fish By-Products and Wastes: Protein Hydrolysates

Balık Yan Ürünlerinin ve Atıklarının Yeni Kullanımı: Protein Hidrolizatları

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Abstract: The rapid growth of the world's population, urbanization, and increasing prosperity require better utilization of available protein resources. In addition, the development of new and sustainable resources is also very important. By 2050, the increase in protein demand in the world will cause it to double not only due to population pressure but also to the increasing awareness of the importance of proteins in a healthy diet for the elderly population. Fish and other aquatic products are important sources of protein. However, depending on the raw materials and the processes applied in the seafood processing sector, waste and by-products are generated at rates ranging from 20% to 75%. Many studies have shown that these products are important protein sources. However, the inadequate management of waste and by-products in the seafood processing sector is one of the biggest challenges facing the fish industry today. Various processes have been developed for the use of these products. An effective way to add value to these products is the production of protein hydrolyzates. Protein hydrolysates allow the release of peptides of different sizes with functional properties, various bioactivities such as antioxidant, antimicrobial, antihypertensive, anti-inflammatory, or antihyperglycemic. In this article, proteins belonging to fish by-products and wastes, their functional and technological properties, hydrolysis process, technological use of hydrolysates in the food industry have been reviewed.

Keywords
- By-products
- Fish
- Protein Hydrolysates

Özet: Dünya nüfusunun hızla artması, kentleşme ve artan refah, mevcut protein kaynaklarının daha iyi kullanılmasını gerektirmektedir. Ayrıca yeni ve sürdürülebilir kaynakların geliştirilmesi de oldukça önemlidir. 2050 yılına gelindiğinde, dünyadaki protein talebindeki artış, sadece nüfus baskısı nedeniyle değil, aynı zamanda yaşlı nüfus için sağlıklı beslenmede proteinlerin öneminin artan farkındalığı nedeniyle bunun iki katına çıkmasına neden olacaktır. Balık ve diğer su ürünleri önemli protein kaynaklarıdır. Ancak su ürünleri işleme sektöründe hammadde ve uygulanan işlemlere bağlı olarak %20 ila %75 arasında değişen oranlarda atık ve yan ürün açığa çıkmaktadır. Bu ürünlerin önemli protein kaynakları olduğu birçok araştırma ile ortaya konmuştur. Buna rağmen balık işleme sektöründe atık ve yan ürünlerin yetersiz yönetimi, günümüzde balık endüstrisinin yüzleşmek zorunda olduğu en büyük sorunlardan biridir. Bu ürünlerin kullanımına yönelik çeşitli süreçler geliştirilmiştir. Bu ürünlere değer katmanın etkili bir yolu protein hidrolizatı üretimidir. Protein hidrolizatları, fonksiyonel özellikleri, antioksidan, antimikrobiyal, antihipertansif, antiinflamatuar veya antihiperglisemik gibi çeşitli biyoaktivitelere sahip farklı boyutlardaki peptitlerin açığa çıkmasına izin verir. Bu makalede, balık yan ürünleri ve atıklarına ait proteinler, fonksiyonel ve teknolojik özellikleri ile hidroliz işlemi, hidrolizatların gıda sektöründe teknolojik kullanım olanakları derlenmiştir.

Anahtar kelimeler
- Yan ürün
- Balık
- Protein hidrolizatı

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1. INTRODUCTION

The term by-product is defined as the product that occurs outside the intended product during production. Small-deformed fish caught in the net during fishing, non-economic, non-target species other than the target species, and discarded species during processing; the head, tail, bone, shell, scales, internal organs, blood, and other fluids are by-products.

Numerous by-products emerging in the fishing and aquaculture industries around the world are either discarded or used for non-food purposes; in the production of low-value feed products such as fish silage, fish meal oil. This results in the loss of dietary essential constituents and an increase in economic costs (Olsen et al., 2014). Seafood is consumed fresh-chilled (44%) and processed with different technologies (54%, approximately 75 million tons) (FAO, 2020). Many by-products emerge depending on the structural characteristics of the raw materials used in the processing industry and the technologies applied. These products, namely the head, internal organs, spine, skin, fins, and clipping meat, are 50-75% in the production of salting and smoking processes. It is 30-65% canned fish. These rates are around 20-50% in bivalves and 50-60% in arthropods. Fish fillet processing by-products also consist of head, viscera, skin, caudal fins, and spine, and fillet trimmings that result during the filleting process (Dekkers et al., 2011; Shirai and Ramirez-Ramirez, 2011). By-products obtained from fish processing facilities are reported to be the source of numerous valuable compounds such as collagen, gelatin, high-quality proteins and peptides and lipids, including long-chain omega-3 fatty acids, chitin, vitamins A, D, B, and especially B12, minerals such as calcium, phosphorus, iron, zinc, selenium, and iodine, enzymes, and pigments (Disney et al., 1977; Pal and Suresh, 2016). The amount of protein in by-products ranges from 20 to 80 g/100 g on average (Khiari et al., 2015; Abbey et al., 2017). Esteban et al. (2007) determined the protein value 58% and lipid value 19% in the nutrient composition of the by-products that emerged in the fish market. They also revealed that there are significant levels of minerals in these by-products. Among these minerals, calcium, phosphorus, and zinc were calculated as 5.8%, 2.4%, and 6%, respectively. Besides, it is stated that the amounts of palmitic acid and oleic acid are reasonably high. Roslan et al. (2014) reported that the by-products of tilapia fish (*Oreochromis niloticus*) contain 14.60% crude protein, 66.57% moisture, 5.50% oil, and 8.93% ash.

Sustainable use of fish by-products for human consumption is one of the most priorities of the aquaculture industry, FAO (2020). Innovative technologies are used to produce bioactive compounds from seafood byproducts. These components have functional properties such as emulsification, foaming, oil binding, and water retention. They have also exhibited antioxidant and antimicrobial activity. With all these advanced properties, there have been reported that these bioactive components can be used in processed foods in different ways today. They increase the value of foods and give them a "functional food" feature (Haque and Mozaffar, 1992; Diniz and Martin, 1997; Sathivel et al., 2003; Kim and Mendis, 2006; FAO, 2017).

This study is aimed to discuss the processing and potential applications of fish waste and by-products as protein hydrolysate.

1.1. Proteins in Fish By-products

Proteins are polypeptides formed by the covalent bonding of twenty standard amino acids to each other in a characteristic straight chain in a certain type, number, and sequence. Principally, animal meats are similar in terms of protein content and amino acid composition. But the fish muscle is softer than terrestrial animal meats with a lower percentage of connective tissue (Helfman et al., 2009). Protein content in fish constitutes approximately 90% of the total dry matter. Fish proteins are complex systems consisting of three groups: myofibrillar, sarcoplasmic, and stromal proteins.

Many researchers have reported that there is a significant amount of protein in fish waste along with other components. Raghavan (2008) reported the protein rate in fish wastes between 10-20% of the total protein. Fish scales are an excellent source of collagen and gelatin from the skin and spine (Nurilmala et al., 2020).

1.2. Technological and Functional Properties of Fish Proteins

Fish proteins show high functional properties as well as nutritional value. The factors that reveal the functionality of proteins are due to some of their physical, chemical, and physicochemical properties. These are, respectively, due to the sizes, shapes, amino acid compositions and sequences of proteins, charge distributions, hydrophobicity/hydrophilicity ~~ratios~~, secondary, tertiary, and quaternary structures, the flexibility/hardness of their molecules, and their ability to interact with other molecules (Hanmoungjai et al., 2002). The technological properties of proteins include processing functions such as solubility, emulsification, gelling and foaming capacity. Proteins must be biologically denatured to show the desired functionality in foods. Also, this denaturation occurs using processes such as aeration, agitation, pressure, or heat treatments.

1.3. Fish Protein Hydrolysates and Hydrolysis Process

One of the promising industrial uses of fish by-products is the formation of water-soluble fish protein hydrolysate (FPH) (Giannetto et al., 2020; López-Pedrouso et al., 2020). FPH is produced by breaking down proteins into small peptide chains, and free amino acids by chemical or biological means. The main purpose of the hydrolysis process is protein recovery and increasing the yield of valuable components (Adler-Nissen, 1993). Hydrolysis of proteins includes chemically acid or alkaline hydrolysis, autolysis, bacterial fermentation, and enzymatic hydrolysis. Acid or alkaline methods are used in the chemical process. Acid hydrolysis is preferred to alkaline hydrolysis because of the decrease in nutritional value at the end of alkaline treatment. During thermal treatment in an alkaline pH environment, partial losses occur in amino acids such as terrine, cysteine, lysine, tyrosine, and arginine. This situation causes the conversion of L-amino acids to D-amino acids with the formation of toxic substances such as lysinoalanine (Friedman, 1978; Kristinsson and Rasco, 2000a). Acid hydrolysis is performed in vegetable protein hydrolysates production as a flavor enhancer, in bouillon manufacturing (Aaslyng et al., 1998). Chemical hydrolysis has several disadvantages; during neutralization, a large amount of salt formation is observed in both acid and alkaline environments. There is minimal solubility and precipitation of proteins in the isoelectric point adjustment process. Endogen digestive enzymes of fish can be used in the autolysis process. The concentration of these enzymes varies greatly within a species and between species. Bacterial fermentation occurs by the growth of lactic acid bacteria, but in this method, the lipids cannot be removed (Kristinsson and Rasco 2000b). Enzymatic hydrolysis enables proteins to be broken down into smaller peptides and amino acids by proteolytic degradation. There is no nutrient loss in the product obtained at the end of this process. Although enzymatic hydrolysis is preferred to chemical hydrolysis, enzyme inactivation is needed at the end of the process. Endogenous and exogenous enzymes can be used in the production of FPH. A typical hydrolysis process is characterized by a rapid initial phase due to the presence of many peptide bonds ready to break. An enzymatic hydrolysis is a suitable option for the production of amino acids and short-chain peptides, which do not require excessive chemical and physical applications.

Depending on the enzyme activity, the initial phase may differ. The reaction rate decreases as the process progresses. The decrease in enzyme activity with a decrease in pH and the presence of fewer peptide bonds in the medium can be explained by the inactivation of the enzyme by hydrolysis products, (Moreno and Cuadrado, 1993). And the possible content of protease inhibitors presents in the substrate (Hjelmeland, 1983). The enzymatic hydrolysis process is affected by;

Substrate (type and composition)

Enzyme (hydrolytic specification and efficiency)

Enzyme/substrate ratio (E/S)

Factors such as hydrolysis conditions (pH, temperature, hydrolysis time) (Adler-Nissen 1986)

Proteins can be separated to form rich fractions by enzymatic hydrolysis based on extraction with commercial proteases. Thus, undesirable reactions that damage valuable components of proteins are minimized using moderate temperatures and mitigating the effects of oxidation. Thus, functional properties (solubility, heat stability, water-binding ability, etc.) are preserved. The selection of enzymes is critical. Enzymes should be selected from the ones used in the production of foods, they should be reliable and if they are of microbial origin, the microorganism producing the enzyme should not be a pathogenic species (Beaulieu et al., 2009).

The enzymatic hydrolysis process generally comprises of three main stages;

Pretreatment,

Hydrolysis and

Recovery (He at al., 2013).

Pretreatment: the objective of this stage is to obtain a homogeneous mixture of minced raw material and water with low lipid content for hydrolysis. For this purpose, a homogeneous mixture is obtained by mixing the raw material and water in an equal ratio (1/1 w/w). Increasing the volume of water used does not increase protein recovery (Benjakul and Morrissey, 1997). The Food and Drug Organization (FDA) standardized the percentage of lipids in fish protein hydrolysates should be below 0.5% for human consumption (URL-1). Brown pigments resulting from lipid oxidation make a darker final product. For this reason, the amount of lipid in FPH should be well controlled. As a result, the raw material for FPH to be obtained from oily fish should be defatted before mixing with water. Organic solvents are used for this purpose. The defatting process not only reduces fat but also minimizes bacterial deterioration in FPH (Kristinsson and Rasco, 2000a).

Hydrolysis: after the pretreatment, the enzyme is added to the mixture homogeneously. Depending on the enzyme type, the process temperature and pH are adjusted to optimum conditions. The enzyme/substrate (E/S) ratio and the processing time are regulated depending on the desired functional properties and protein recovery in the final product. The hydrolysis is terminated by inactivating the enzyme at 90 °C for approximately 30 min. Thus, a liquid form of FPH is obtained. Enzyme selection is of great importance for the functional properties of protein hydrolysate. Enzymes such as pepsin, which was previously used in an acidic environment, were preferred because they also inhibit microbial growth in the environment. However, low acidity means a decrease in protein recovery, a decrease in nutritional value due to the breakdown of essential amino acids such as tryptophan, and a decrease in functional properties due to excessive hydrolysis (Kristinsson and Rasco, 2000a). As a result, enzymes such as alkalase, neutralase, and flavourzyme, which are active in neutral acidity under optimum conditions, are more preferred and used recently. When evaluated technically and economically, microbial enzymes are the most effective enzymes in the production of hydrolysate from fish proteins (Herpandi et al., 2011). The type of fish used is also of great importance in the relationship between FPH and enzymes. While protein recovery was 70.5% in FPH obtained from capelin using alkalase, it was 57.6% in FPH obtained using papain (Shahidi et al., 1995). Protein recovery rates of can be change by the effect of raw materials and enzyme types but the highest ratio have been obtained by using Alcalase compared with other enzymes, (flavourenzyme, neutralas etc.) (Alavi et al., 2018; Korkmaz and Tokur 2021). Recovery: in this process, FPH is pulverized. FPH in liquid form has a risk of rapid deterioration. Storing and transporting the powdered product is easier and its shelf life is longer. At this stage, the drying process is performed after the centrifugation process. Centrifugation is mainly conducted at 4000 g/min for 20 min. In this process, the hydrolysate separates into three layers: lipid, protein hydrolysate solution, and a semi-solid layer from top to bottom. First, the upper lipid layer is removed, then the protein hydrolysate is removed. While the freeze dryer is used in laboratory studies, and spray drying is applied in industrial use. The final product can be obtained in a creamy white with good water solubility and desired functions (Balcik Misir and Koral, 2019).

1.4. Enzymes used in the production of FPH

Enzymes are special biological catalysts that accelerate the reactions by bringing the necessary activation energy to the required minimum level for the realization of biochemical reactions that take place in the living structure and are left unchanged as a result of all these processes (Yıldız, 2007). The Enzyme Commission of the International Society for Biochemistry (IUB) has divided enzymes into 6 major classes: Oxidoreductases (EC1), Transferases (EC2), Hydrolases (EC3), Lyases (EC4), Isomerases (EC5), and Ligases (EC6) (Bergmeyer, 1979).

Enzymes are usually obtained from different plants, animal, and microbial sources. Plant enzymes that provide optimum hydrolysis in the pH range of 7 to 7.5 are papain (Gu et al., 2011;) and bromelain (Barbana and Boye, 2011; Forghani et al., 2012). Animal enzymes that provide optimal hydrolysis in the pH range of 2 to 8 are pepsin (Jumeri, 2011), trypsin, and α-chymotrypsin (Lee et al., 2011).

Microbial enzymes used for obtaining fish protein hydrolysates between pH 7 and 9 are alkalase (Gu et al., 2011; Ishak and Sarbon, 2016), protamex (Ngo et al., 2012), flavourenzyme, and neuterase (Je et al., 2009; Chi et al., 2015). Compared to enzymes of animal or plant origin, microbial enzymes have a wide range of catalytic activities and higher pH and temperature stability (Diniz and Martin, 1997). It has been reported that the most effective use of alkalase and flavorenzyme enzymes in fish protein hydrolysates is in the near-neutral pH range (7-9) (Herpandi et al., 2011).

1.5. Technological Applications of Fish Protein Hydrolysates

FPH has different properties depending on the raw material and production method. In addition, when combined with the properties of the main raw material to which it is added, a unique structure, nutritional value, and functional properties of the final product emerge.

FAO defines fish protein derivatives that can be available for human consumption because they contain higher amounts of protein than fish meat. Generally, FPH's contain 81–93% protein, less than 5% fat and 3–8% ash, and 1–8% moisture (Chalamaiah et al., 2012). The FDA determined that fish protein products can be utilized as a nutritional supplement for humans, provided that the hygienic conditions are met, with a protein content of more than 75% and a lipid content of below 0.5% (URL-1). Besides, FAO determined the maximum acceptable lipid value as 0.75%.

FAO and FDA guidelines emphasize that fish protein products can play an effective role in countries with protein deficiency. Nutritional studies have shown that adding these products to foods has definite positive effects.

It has been demonstrated that FPHs can be included in different food systems such as beverage fortification (Egerton et al., 2018), biscuit enrichment (Idowu et al., 2019), bread fortification (Vijaykrishnaraj et al., 2016), and ice-cream fortification (Shaviklo et al., 2011) to enrich the protein content.

They can be used as additives in various foods with their crucial and unique properties (protein enhancer, milk powder substitutes, emulsifying properties, water holding, and oil holding capacity, protein solubility, gelling activity, foaming capacity, and emulsification capacity (Chalamaiah et al., 2010).

Koç (2016) found that the hydrolysates produced from anchovy processing wastes contain 75% protein and 46% of these proteins are made up of essential amino acids. In addition to its nutritive properties, it has been determined that waste hydrolysates can be used as food additives with their emulsifying, foaming, water, and oil retaining properties. In terms of bioactive properties, hydrolysates were obtained from wastes at a concentration of 1 mg/ml; it was determined that it showed 60% ACE inhibition, 21% DPPH clearance, and 59% Fe^{+2} chelating activity. With these properties, it has been seen that there is a high level of use potential in the pharmacological fields. Fish protein hydrolysates improve the cooking yield of meat-based foods (Jenkelunas et al., 2018; Nur Ibrahim et al., 2020).

The patent number WO/2004/071202 reveals a method for injecting protein hydrolysates with 8%-20% concentrations obtained from salmon fish enzymatically into smoked salmon fillets with 18% brine and then the preservation of these fillets by cold and freezing. It has been reported that the added protein hydrolysate reduces the substantial loss of the product and delays the rancidity caused by lipid oxidation. In the sensory evaluation, it was stated that there was a slight change in smell and taste (Harald & Kjartan, 2004). With the patent numbered US 6,926,918 B2, a method was developed describing the use of fish hydrolysates instead of salt in foods. In this study, it was revealed that bonito hydrolysate improves the salty taste in foods and beverages and can be used instead of salt (Grossbier et al., 2014). Niko et al. (2014) emphasized that tetrapeptides isolated from sturgeon skin hydrolysate can prevent the degradation of myosin and actin during repeated freezing-thawing processes.

FPH can be used as a cryoprotectant agent having different peptides with different amino acid sequences in fish muscle protein-based systems by suppressing the denaturation of myofibrillar proteins in frozen products. High contents of free amino acids, especially Asp, Glu, Arg, and Lys, in hydrolysate or peptides proved to offer protection against freeze-induced denaturation of proteins and enhanced its cryoprotective effect (Cheung et al., 2009). Zhang et al., (2022) reported that the gelatin hydrolysates of flavourzyme from silver carp skin are promising candidates as natural and high-performance antioxidants and cryoprotectants in fish mince processing. In the study, researchers examined the Pacific hawthorn protein hydrolysate as a potential alternative to the 1:1 sucrose-sorbitol mixture commonly used as a cryoprotectant in frozen minced fish. They proved the high potential of using fish protein hydrolysates as a new generation cryopro-carrier to preserve frozen fish quality. Karnjanapratum and Benjakul (2015) determined that gelatin hydrolysate obtained from shark skin showed antioxidant and cryoprotectant effects in washed fish minced meat.

FPHs have the potential to be used as a coating material to delay lipid oxidation in seafood during cold preservation with natural antioxidants (Mirzapour-Kouhdasht et al., 2020; Balçık Mısır and Koral, 2021). In a master study titled "Determination of some quality changes during cold storage of trout fillets coated with powder protein hydrolysate obtained from trout waste". It was observed that hydrolysates were coated on the fillets of the trout fillet by dipping method and their changes were investigated during the cold storage. When the biochemical microbiological quality and sensory analysis were evaluated, the shelf life of the control group was 3 days, and the coated group was 7 days. According to the results of the study, it was determined that the edible coating made with protein hydrolysate obtained from the by-products of trout increased the shelf life of the trout fillets approximately 2 times Arslan (2016).

Edible protein films and coatings obtained from the hydrolysate of fish wastes have less carrying capacity (water vapor permeability) and are more flexible than other protein films (Dursun and Erkan, 2009). It has been determined that these products can be produced directly from fish meat and fish by-products, as well as from water-soluble proteins in surimi-washing water (Shiku et al., 2004).

FPH’s can be evaluated as an alternative to synthetic products with their antioxidant, antimicrobial, and antihypertension activity properties (Sohail et al., 2020). However, only limited clinical studies have been managed on FPHs to test their efficacy as a functional food in humans. Marchbank et al. (2008) investigated the protective effect of a commercial FPH derived from Pacific haddock and marketed in the US as an "over-the-counter" health food supplement in small bowel injuries. In this study, it was revealed that dyspeptic symptoms occurred in four of the eight subjects selected as the control group, but these symptoms did not occur in eight subjects when FPH was administered. It has been reported that protein hydrolysates produced from fish can be used as nutritional supplements to support various metabolic activities because they contain bioactive components and provide easy absorption (Idowu et al., 2020).

2. CONCLUSION

Fish by-products are valuable resources with great potential for human consumption. FPH production of foods is a promising alternative for improved and economical use of these resources. Such practices will also support and enhance the sustainability of the global aquaculture and fishing industry. However, the quality and functional properties of the product can vary by changing the raw materials, enzymes, and production conditions. They can be utilized in high-value products, and future research should focus more on these segments to ensure sustainable and economical use of marine resources.

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There was no conflict of interest in the preparation of this article.

AUTHOR CONTRIBUTION

Single Author

ETHICAL STATEMENT

All the applicable ethical rules were followed.

DATA AVAILABALITY STATEMENT

Research data is not shared.

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