
Evaluation of fisheries wastes as protein hydrolyzate

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Abstract

In Turkey, it is known that, wastes of fisheries processing plants released to the environment and only a bit of that assessed as fish meal or fish feed by a few plant. It is expected that with the evaluation of these wastes which rich in nutrient content by converted to the various commercial products (chitin, protein hydrolyzate, carotenoprotein and pigment extraction etc.) to be beneficial in terms of both contribute to the country's economy and prevent to the environmental pollution. These wastes may be used in the food and feed industry, in pharmaceuticals and in the microbial studies as growth medium with the evaluated as protein hydrolyzate. In the World, although there are studies for the evaluation of shellfish and fish wastes as protein hydrolyzate; in Turkey, it is appear that such studies quite insufficient, so it is thought that researches on this issue should be increased.

Keywords: Fish, Crustacea, Waste water products, Protein Hydrolyzate, Enzyme

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Introduction

In the world, every year in parallel with the population increase, food production is becoming an important issue. For this purpose, there is a need for new protein sources. In countries especially which provide their protein need from foods of plant origin are experiencing problems because of the drought. Solid wastes of food industries are important source in terms of protein content. Particularly fish wastes have a high protein content. While the fish wastes maintaining with the old techniques this can lead to the loss of potential protein content in a significant manner Kılınç (2007). In recent years, in developed countries, water products and wastes have been used as bioactive materials because the rich content of protein, fat, chitin and mineral have increased the value of by-product processing technology. In developing countries, aquaculture sector has waste disposal problems at different levels on evaluation of fish wastes. In Turkey, in 2008, it has been reported that, a small amount of water processing wastes determined as 8,800 tons / year are

evaluated and the rest dumped into the environment. By-products emerging from fish processing are generally internal organs, skin, scales, muscle and muscle skeletons and these constitute 70% of the raw material (Atılğan 2008; Aylangan and Öztan 2008).

Materials and Methods

Preparation of Protein Hydrolyzate

Enzymes, acids and alkalis are used in order to hydrolyze proteins. Functional properties of proteins can be improved by enzymatic hydrolysis under controlled conditions. Substrate selection, protease and degree of protein hydrolysis affect physicochemical characteristics. Microbial enzymes such as alcalase works best at a alkali pH in terms of technical and economical. The material as hydrolyzate sample shown in Fig. 1, after passed a series of chemical treatment taken into flour by lyophilization process and are made ready for use. Enzymatic hydrolysis is used in development of functional component with maintaining nutritional value Aylangan and Öztan (2008).

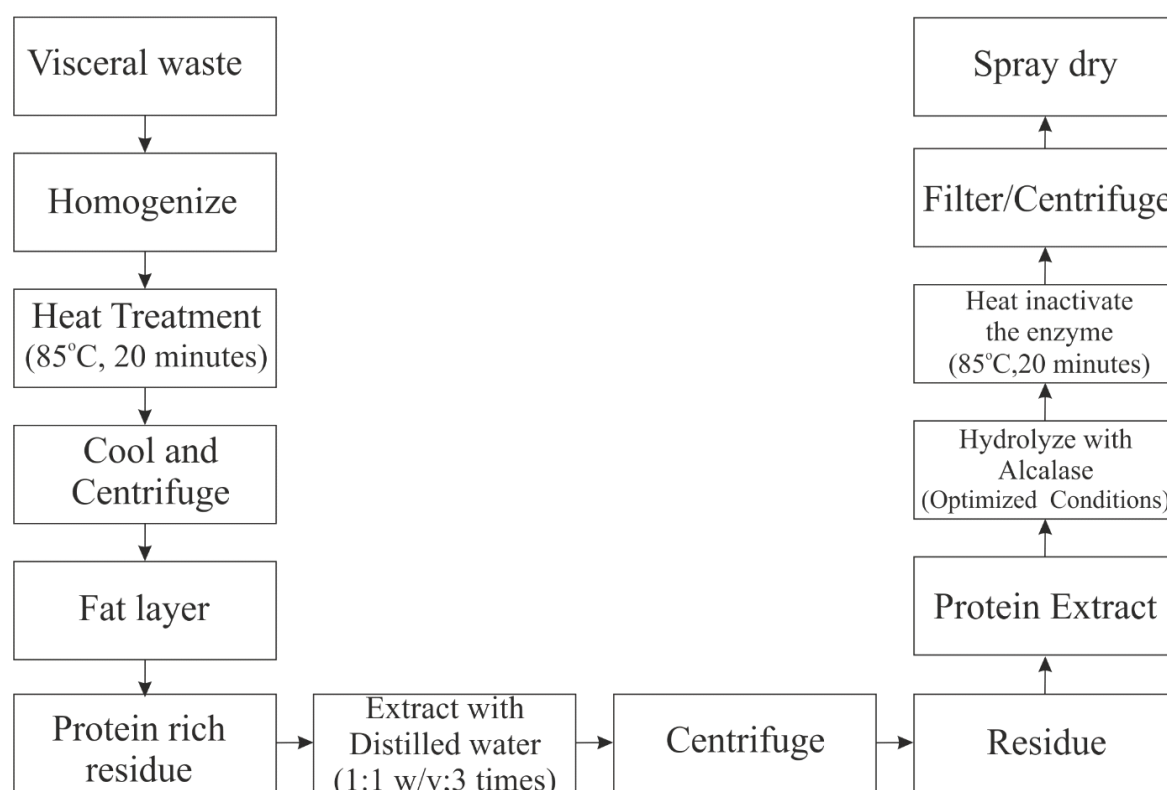


Figure 1: production protein hydrolyzate using alcalase enzyme from waste internal organs of Catla (*Catla catla*)

Applications of Protein Hydrolyzate

By-products are marketable and can be converted into appropriate form for use in the industry due to the hydrolysis process. Proteolytic enzymes are used to divide proteins into two fractions. The insoluble fraction is used as an animal feed and the part containing insoluble protein/peptide's as a functional food additive. In addition, due to an efficient nitrogen source, can be used as microbial growth area in microbiological studies. It also offers physiological advantages in activity and absorption rate of amino acids and is used widely in nutrition of young children who hypersensitive to food and in individuals who can't digest proteins. Due to its hygroscopic character, protein hydrolyzate can be used in

application where casein used as additive material (Martinez *et al.*, 2005; Aylangan and Öztan, 2008; Bhaskar *et al.*, 2008).

In recent times bioactive peptides are remarkable due to their antioxidant effect. It has been reported that protein hydrolyzates originated from plant and animal have antioxidant activity. It is referred that molecular size, the concentration of hydrolyzate and the degree of hydrolysis affect amino acid composition and together with antioxidant activity of food protein hydrolyzates Akıllıoğlu and Yalçın (2010).

Factors Affecting The Efficiency of Protein Hydrolyzate

In protein hydrolysis, the degree of hydrolysis (HD) is a key parameter in controlling the reaction. In literature too many methods described such as pH-stat, osmometri, soluble nitrogen content and trinitro-benzene-sulfonic acid (TNBS) for controlling the HD Bobuş (2010). During the enzymatic hydrolysis reaction degree of hydrolysis determined considering factors such as "Time, pH, temperature and enzyme concentration". When degree of hydrolysis increases the activity of emulsion, the emulsion capacity, foaming capacity and stability of foaming abilities decrease. In hydrolysis the molecular weight of protein is an important factor for using hydrolyzate as a functional material. Therefore, to achieve the appropriate molecular weight limited enzymatic hydrolysis is performed. However due to the inadequate incubation time, recovery of whole protein is not possible by limited enzymatic hydrolysis. It is reported that, for obtaining hydrolyzate that has desired molecular size and functional properties or peptide fraction it proposes use of ultrafiltration membrane system Aylangan ve Öztan (2008).

Production of Protein Hydrolysate From Shellfish

While processing waste of shellfish and species that have no commercial value are evaluated for this purpose, especially the shrimp and crab species have been studied for obtaining a protein hydrolyzate. In several countries

in the studies that have been made for shellfish, it is stressed that evaluated processing waste in this way is important in terms of obtaining food source and solution for emergence environmental pollution. In our country it is thought that, crabs and mussels that have no commercial value can be assessed with converting to the protein hydrolyzate. Processing waste of crustaceans consists of mostly shell, internal organ and a small amount of flesh. It is reported that industrial processing waste of crustaceans contains protein in proportion as 18-42% Shadidi and Synowiecki (1991).

In a study, when enzymatic hydrolysis processing wastes of *Portunus trituberculatus* was made with single factor and orthogonal array design method, maximum hydrolysis degree determined as 26,68% in condition of 55°C, pH 8,5, 1000U/g of the enzyme dosage in a 3 hour period. Also molecular weight of hydrolysis product was determined as less than 10kDa and total amino acid concentration as 1046,7 mg/L. Due to the high nutrition content of hydrolyzate product obtained from this study it is considered that it will perform possibility of widespread use Tao *et al.* (2010). In another study it is stated that the total dry weight by-product of *Chionoecetes opilio* was 87,4% and 78% of this raw material could be assessed and contain 42,9% protein, 14,8% lipid, 25,7% minerals and 16,2% chitin respectively. Molecular weight of hydrolyzate product obtained from this

study was determined as less than 35 kDa and has been recognized as relatively low value Beaulieu *et al.* (2009). Using the protease enzyme that isolated from the hepatopancreas of king prawn found in the Arctic Ocean, the protein hydrolyzate was obtained from the deep water prawn (*Pandalus borealis*) and king crab (*Paralithodes camtschaticus*). The most successful outcome provided in the condition of 8–8,5pH, temperature of 50–55°C and 5–6 h hydrolysis and hydrolysis degree are determined as 29,0% for crab and as 35,5% for shrimp. As a result it is suggested that, the protease complex isolated from hepatopancreas of king crab has a high proteolytic activity and wastes of North Arctic Ocean crustaceans to be used as an effective substrate for this purpose Mukhin and Novikov (2001). It is reported that Mexican Pacific Ocean has a rich source regarding to swimming red crab (*Langostilla*) that hasn't been considered as commercial yet. With a rich protein and carotenoprotein content, swimming red crab can be used as an alternative source of protein hydrolyzate as well as in the diet of salmonid, crustaceans and fowl. From the results of the study, it was determined that dried swimming crab contains of 43% protein, 8% lipid and 7,1% astaxanthin. During the hydrolysis processes several different protease enzymes were compared and the highest yield was obtained from alkalaz enzyme. It is reported that by the treatment of alkalaz enzyme at 0,5%

rate 20 minute, 73% of crude protein has been dissolved Fernando *et al.* (1999). Shadidi and Synowiecki (1991) reported that the shell wastes of crustaceans are rich in especially chitin at a rate of 14–32%. Protein hydrolyzates have been obtained from Crangoncrangon prawn during the enzymatic isolation of chitin. From this it is determined that the protein content of shell waste was 40,6% and the hydrolysis degree (HD) was at rate of 30%. Also these waste rich in regard to aspartic and glutamic acids Synowiecki and Al-Khateeb (2000). Dey and Dora (2011) have used four different commercial enzyme for head and shell waste of shrimp (*Penaeus monodon*). Alcalase, which showed the best result, was used to optimize hydrolysis conditions for shrimp waste hydrolysis by response surface methodology using a central composite desing. The HD were determined as 33.13% with the evaluation of temperature (59.37°C), pH (8.25), Enzyme/substrate (1.84) and time (84.42 min) factors. In a different study, Kjeldahl nitrogen taken up as criteria for the evaluation of protein hydrolyzate and 68,5% yield was obtained with the enzyme alcalase Gildberg and Stenberg (2001).

It is known that enzymes as well as certain chemicals are exploited for the hydrolysis process. In a study about this, for enhance the productivity of protein hydrolyzate obtained wastes of *Penaeus semisulcatus* shrimp with the alcalase enzyme, sodium sulphite and Triton X–100 were also used. As a

result the yield has been obtained with alcalase alone as 45,1%, 39% for alcalase + Triton X-100 and as 62% for alcalase+sodium sulfite (200mmol / L) and as 65,1% for three of them respectively Mizani *et al.* (2005).

Mukhin and Novikov (2001) have been prepared dry hydrolyzate by proteinaz complex which was obtained from hepatopancreas of king crab with protein of clam that found in Iceland. The resulting product contains at least 80% of the free amino acid and oligopeptide. Dominant amino acids that consist 50% of are aspartic acid, leucine, isoleucine, arginine and lysine. Protein hydrolyzate was estimated likely to be used as a nutrient culture of microorganisms and this could be used in the growth of the test cultures.

Fish wastes consist of internal organs, skin, bone and head. In the some studies different commercial enzymes have been used in the different fish substrates. For instance sardine, capelin, cuttlefish, round scad, Atlantic cod, catla, Persian sturgeon, beluga sturgeon and yellowfin tuna Nemati *et al.* (2012). Protein hydrolyzate was obtained using microbial proteases of Alcalase, Protamex and Flavourzyme from the by-products (head, skin and internal organs) of *Alosa caspia* which is an important species in the Caspian Sea. The results have shown that the protein hydrolyzate obtained from alcalase were higher protein content (78.91%), protein gain (80.42%) and degree of hydrolysis (21,06%). Martinez *et al.* (2005) have

characterized the effects of pH, temperature, substrate/buffer ratio and the concentration of enzyme to the protein recovery after treatment Flavourzyme with the wastes of *Carassius auratus*. The effect parameters of hydrolysis to the degree of hydrolysis described by Response Surface Analysis (RSA). When the critical values like as pH=5,9, T=53 °C, S: B=514,7 and E=80 Lapu (leucine aminopeptidase units) taken with mathematical model DH determined as >%26,6. Electrophoretic figures showed that there is a progressive decrease in the molecular weight of peptidic fraction from the 10th minute of hydrolysis. In their study Bhaskar *et al.* (2008) have prepared protein hydrolyzate from the internal organs of the freshwater carp *C. catla* found in India. Required hydrolysis condition (time, temperature, pH and enzyme to the substrate level) have been optimized with response surface methodolgy (RSM) using factorial design. For obtaining high degree of hydrolysis close to the 50% by using alcalase the optimum conditions determined as %1.5 (v/w) substrate level enzyme, 8.5 pH, temperature 50°C and time was 135 min. Amino acid composition of protein hydrolyzate obtained by using the optimum condition have revealed that protein hydrolyzate similar to FAO/WHO referance protein. Calculated chemical scores indicated that the methionine is a amount of minimum. It is reported that obtained hydrolyzate product may be a balancing

factor for feeding of fish and particularly that can meet amino acid needs of young carp. Salwanee *et al.* (2013) they have extracted Tuna fish's internal organs by diluting with water in a 1:1 ratio and than dehydrated after freeze. They found high rate of glycine (9,6%), arginine (9,2%), alanine (7%), lysine (7,2%) and leucine (7,0%) in the protein. After that, the study was continued by using single factor experiment looking effect of alkalaz concentration, temperature, pH and incubation time on the degree of hydrolysis. Degree hydrolysis of hydrolyzate has increased significantly ($p<0.05$) when enzyme concentration increased from 1,0 % to 1,5 %. But the concentration has started to set when

exceed the 1,5%. With the increase of temperature from 30 ° C to 40 the degree of hydrolysis increases also. The extension of incubation time from 60 min to 240 has increased degree of hydrolysis significantly ($p<0.05$) the important change wasn't observed for the pH. Protein hydrolyzates are good source of nutrition with higher raw protein concentration and rich amino acid profiles. It was reported that protein hydrolyzate obtained from *P.monodon* waste contained 529,93 mg/g amino acid and 54.67–55.93% of this amount contain essential amino acid and 39.27–38.32% of contain flavour amino acid Dey and Dora (2011).

Table 1: Dominant essential amino acid content of protein hydrolysates obtained from various Sources.

Essential Amino acids	<i>Penaeus monodon</i> (Shrimp)	<i>Penaeus borealis</i> (Shrimp)	<i>Chlamys islandica</i> (Scallop)	<i>Paralithodes camtschaticus</i> (Crab)	<i>Euthynnus affinis</i> (Tuna)
Valine	*	*		*	
Leucine	*	*	*	*	*
Isoleucine	*		*		
Lysine	*	*	*	*	*
Methionine					
Threonine					
Phenylalanine					
Tyrosine					
Histidine					
Tryptophan					
Arginine		*	*	*	*
References	Dey and Dora, 2011	Mukhin and Novikov,2001	Mukhin <i>et al.</i> ,2001	Mukhin and Novikov,2001	Salwanee <i>et al.</i> ,2012

Table 2: Dominant non-essential amino acid content of protein hydrolysates obtained from various sources.

Non-Essential Amino acids	<i>Penaeus monodon</i> (Shrimp)	<i>Penaeus borealis</i> (Shrimp)	<i>Chlamys islandica</i> (Scallop)	<i>Paralithodes camtschaticus</i> (Crab)	<i>Euthynnus affinis</i> (Tuna)
Asparagine/ aspartate	*		*		
Glutamine/ glutamate	*				
Serine					
Glycine	*				*
Alanine	*				*
Proline/ Hydroxyproline					
Cystine					
References	Dey and Dora, 2011	Mukhin and Novikov,2001	Mukhin <i>et al.</i> , 2001	Mukhin and Novikov,2001	Salwane <i>et al.</i> ,2012

From Tables 1 and 2, when looking at the amino acids content in hydrolysates that were obtained from different sources, essential amino acids Lysine, Leucine, Isoleucine, Arginine appears to be dominant both in shellfish and fish, Valine only in shellfish, Methionine, Threonine and Phenylalanine in fish and non-Essential Amino acids Asparagine / aspartate and glutamine / glutamate in shellfish, Glycine and Alanine both in fish and shellfish.

Discussion

In various country, researches that directed to the obtaining protein hydrolyzate from fisheries particularly from wastes of crustacean and fish processing come to the for seen. In different studies which carried for determine to the rate of protein hydrolysis of wastes of fisheries, HD value determined as 26-39% for crab wastes, as 30-65% for shrimp and as

21-50% for fish wastes respectively. It is expected to be evaluated of these products which gives high HD value in water processing industry. The most high yield is provided by the “Alcalase” enzyme which a proteolytic enzyme used in hydrolysis processes and this result is a highly significant finding can be give a direction to the other studies. Also the obtained protein hydrolyzate was found to be rich in essential amino acids. It is known that this have importance in nutrition as a balancing factor. As a result, in our country researches related to integrate the by-products of processing, fish discards and freshwater crustacea which have no commercial value to the economy are necessary to increase.

In recent years importance on studies about evaluation of fishes, crustaceans and by-products of processing that acceptable as waste product has increased. It is thought that these studies are important for the

commercial evaluation of aquaculture processing waste that both does not contribute to the economy in any way and causes ecologically damaging to the environment due to the increase of population and causing environmental pollution if not used. In addition it is considered that, protein hydrolysates derived from these products that have high protein value can be used as a growth media for microorganisms and can be converted into commercial products that used as an additive in pharmaceuticals, food and feed industry and thus is considered to contribute to the economy.

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