

Fish Protein and Its Derivatives: Functionality, Biotechnology and Health Effects

Esen Alp Erbay^{1,*,#} , Ahmet Faruk Yeşilsu^{1,#} 

¹Central Fisheries Research Institute, Vali Adil Yazar Cad. No:14, Kaşüstü, Trabzon, Turkey

[#]These authors share the first authorship.

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Corresponding Author

Tel.: +904623411053

E-mail: esen.alp@tarimorman.gov.tr
yesilsu@gmail.com

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Abstract

Animal derived proteins provide the essential amino acid requirements of a healthy diet. Among animal proteins, fish proteins have a well-balanced amino acid composition that are imperative for beneficial synthesizing and utilizing proteins in the body. Lack or low levels of any amino acid reduce the other amino acid utilization proportionately. This is why plant-derived proteins are potentially at a disadvantage for use in the body since they have relatively low levels of the essential amino acids methionine and lysine. Health studies have revealed that fish protein is suggested as a weight-loss diet to obese patients.

Introduction

Fish consumption reached to 20 kg per capita in 2014 in the world. Significant growth of fish consumption has significant effects on the diets of people in terms of health issues. Fish consumption was only about 17 percent of animal protein intake for global population and 6.7 percent of all protein sources in 2013. Fish has easily digestible high-quality proteins with essential amino acids and also includes fatty acids (*i.e.*, omega-3 fatty acids) and essential vitamins and minerals like A, B, D and calcium, selenium, zinc, iodine, iron respectively (FAO, 2020). Fish have well-balanced amino acid composition in their protein structure (Harnedy & FitzGerald, 2012) constitute an important

source of animal protein in the diet of many populations in the coastal areas. Human provide large part of the essential amino acid requirements from fresh, salted, dried and fermented fish products (Tamang, 2015; Majumdar *et al.*, 2016).

In general, fish include mainly lipids, water and proteins with the minor components such as nonprotein nitrogenous compounds and carbohydrates, minerals and vitamins. According to FAO, a fresh fish fillet has usually about 66-81 percent of (w/w) water, 16-21 percent of protein and widely ranged fat content depending on the feeding of the fish (URL-1). Fish is classified into two categories depending on their most important components, protein and fat. Two groups, medium fat high protein, low fat high protein and

comprise the most common types of fish and have protein contents from 15-20% in the edible parts of the fish (Finch & Liston, 1970). The fish protein can be classified into three groups according to FAO, 1) Structural proteins such as, actin, myosin, tropomyosin and actomyosin with a 70-80 % proportion in total protein content 2) sarcoplasmic proteins such as myoalbumin, globulin and enzymes with a 25-30 % proportion 3) connective tissue protein such as collagen; 3 % proportion in teleostei and 10 % in elasmobranchii) (URL-1).

Composition of the fish vary greatly between and within the species depending on the catch season, maturity and location where the protein content remains relatively constant and water and fat content alter. The crude protein content with contribution of non-protein nitrogenous (NPN) compounds like free amino acids, polyamines, peptides, nucleotides of fish, crustaceans and mollusks is slightly up to percentage 11-27 (Shahidi & Simpson, 2004). Fish protein is composed of 20-30 % sarcoplasmic composed of mainly albumins besides hemoproteins, 66-77 % myofibrillar such as myosin, actin, actomyosin and troponin and 3-5 % stromal proteins including collagens (Gates, 2016). Fish proteins have perfect water holding capacity and gelling capability supply peerless functional properties to fish processing industries (Taheri *et al.*, 2013).

The fish protein is highly sensitive to deteriorative changes however under proper conditions, these changes causing loss of fresh quality can be prevented or delayed (Shahidi & Simpson, 2004). During post-harvest storage, activity of proteinases in fish tissue including high proportions of free amino acids and other non-protein nitrogenous constituents, reduction of trimethylamine oxide to trimethylamine, oxidative deamination of amino acids and peptides to ammonia and degradation of proteins may cause quality losses of products and limit the storage life (Lougovois & Kyranas, 2005).

Fish and fish by-product proteins as well as enzymes which are key tools because of their catalytic nature are valuable in biotechnological applications of food industry (Fraatz *et al.*, 2014; Jemli *et al.*, 2016; Fernandes, 2016). In recent years, there has been an interest in recovering digestive enzymes from fish and shellfish processing discards for biotechnological applications in various sectors including acids for cheese making, herring fermentation, fish skinning, roe processing and production of specialty kits, as well as medical applications (Shahidi & Kamil, 2001).

In this review, protein structure of fish, effects of processing methods on protein, some functional properties and biotechnological applications with the health effects on human nutrition are mentioned.

Protein and Other Nitrogenous Compounds in Fish

Since proteins affect both sensory properties and nutritional value of fish products, they constitute an

important place in human health. Nine dietary essential amino acids including phenylalanine, histidine, leucine, lysine, threonine, iso-leucine, tryptophan, methionine and valine which are essential to the body and must be gotten by the diet are necessary for human. Fish and fish products' protein content is well suited for this purpose. Fish muscle has high proportion of proteins which are easily digestible and have lower collagen content leading to higher tenderness comparing to red meat (Savary-Auzeloux *et al.*, 2014; Islam & Joadder, 2005).

Food protein content generally is given as sum of both protein and NPN compounds (Sikorski, 2012). Nonprotein nitrogen (NPN) compounds including peptides, free amino-acids, amines, guanidine compounds, amine oxides, nucleotides, quaternary ammonium molecules, and urea are found mostly in the fiber sarcoplasm. Low molecular weighted non-protein nitrogenous compounds soluble in water like ammonia, dimethylamine, trimethylamine, free amino acids, creatine, nucleotides, and urea are existed in the cartilaginous fish. Some aquatic animals have relatively high percentage of NPN compounds comparing to the total nitrogen in the muscles whereas land animals generally have no more than 10% of total nitrogen. All these compounds have an effect on the sensory properties and play a significant role in the fish spoilage (Bandarra *et al.*, 2001). Most marine fishes include trimethylamine oxide (TMAO) which is degraded to trimethylamine ensuing a 'fishy' odor and causes consumer not to prefer. Land animals and freshwater species (except for Nile perch and tilapia from Lake Victoria) does not involve this compound. Creatine one of the non-nitrogenous component in fish muscle form the major NPN fraction. Phosphorylated form of this molecule plays an important role in fish muscle metabolism (Caballero, 2005).

Fish are divided into four groups depending on the percentage of crude protein: below 10%, 10-15%, 15-20%, and over 20% commercially (Sikorski, 2012). Studies revealed that variations occur in crude protein content depending on the variety, species, the state of nutrition, and the stage of the reproductive cycle of the animals. Boran & Karaçam, (2011) studied four species including horse mackerel, garfish, golden mullet, and shad in the Black Sea in Turkey and reported that protein content varied between 13.0-19.8% among the species studied on fresh weight basis related to the physiological processes in the fish body during the reproductive activities. The protein content of horse mackerel, garfish, golden mullet and shad varied as 13.01-18.60%, 13.99-18.45%, 13.31-17.20% and 14.30-19.80% respectively. The authors reported that when the average values were considered, the lowest protein content was in horse mackerel with 14.78% and the highest content of protein was in garfish with 16.89%.

Islam & Joadder, 2005 determined the protein content of *G. giuris* seasonally and reported that it varied from 14.09 % (February) to 16.03 % (August) in male individuals. Similarly, females had 13.88 % of

protein content in February and 15.56 % in November. They explained that, the protein content was low in the spawning season both in male and female where the average protein content of the female (14.61%) was lower than the male (15.23%).

Sarcoplasmic and Myofibrillar Proteins

Proteins composed of sarcoplasmic or myogen fraction of the muscle which have low molecular weight in the range of 40,000 to 70,000 kDa are soluble in water and dilute salt solution. Pelagic fish species like anchovy, sprat and horse mackerel have higher fractions of sarcoplasmic proteins comparing with demersal fish species like turbot, flounder etc... In general, white muscle of some fish species contain more sarcoplasmic proteins than dark muscles. Comparing with land animals, sarcoplasmic protein of fish incorporates myoglobin, albumins and enzymes where fish species from cold water oceans include antifreeze proteins and glycoproteins classified also in sarcoplasmic protein group (Shahidi & Botta, 2012). Enzymes especially glycolytic enzymes participating in the cell metabolism are the most found proteins in the sarcoplasmic proteins. Most fish species include heat-stable proteolytic enzymes, such as cathepsin in order to bind to the myofibrillars (Lanier, 2000). Many sarcoplasmic proteins are globular which are expected to bind more water upon denaturation. Sarcoplasmic proteins, consequently, has effects on water holding capacity (Wilson & Laack, 1999). The further group of sarcoplasmic proteins are the heme proteins that effect lipid oxidation and postharvest color change (Kim, 2002). In cold waters some fish species have proteins which are identified as antifreeze glycoproteins that lower freezing temperature (Sikorski, 2012).

Myosin, tropomyosin, actin, and the troponins T, C, and I regulating muscle contraction are myofibrillar proteins. Adenosine triphosphate (ATP) is broken down into adenosine diphosphate (ADP) to provide the chemical energy required for muscle contraction by catalyzing of myosin ATPase activity (Listrat *et al.*, 2016). The myofibrillar proteins of fish muscle are less stable in terms of thermal properties than those from mammalian muscles. Their stability depends on the fish species, habitat temperature, ionic strength and pH like in vitro properties of the proteins (Poulter *et al.*, 1985).

Changes in Protein in Frozen Stored and Heat - Induced Fish

Fish stored at about -20°C can be remain for several months without losing its flavors. However, solubility, water retention, lipid emulsifying properties and some other functional characteristics like gelling ability of muscle proteins can be changed.

Mitochondria and lysosomes lysis may occur after freezing and thawing process which can be lead an alteration in enzyme distribution (Karvinen *et al.*, 1982).

Freeze denaturation of proteins is the main reason for this change which follows the interactions between constituents of fish tissues and the denatured proteins. Denaturation of proteins, oxidation, re-crystallization of ice crystals and sublimation can cause quality losses of frozen fish (Haard, 1991). Protein denaturation contributes increase of drip loss leading mineral content diminish, loss of water holding capacity and textural changes (Nakazawa & Okazaki, 2020).

Heating the frozen fish needs time and temperature control in order to obtain products similar to unfrozen ones (Giang *et al.*, 1989). Heating process can lead reabsorbed of the water by the tissues and relation to ice crystals sizes and locations the structure of flesh can be changed. If the fish is not properly frozen and thawed, the protein will aggregate and water loss occurs during the thawing which cause hard product in texture (Nakazawa & Okazaki, 2020).

Rate of enzymatic processes are affected by the heating; it causes denaturation and resultant interactions of proteins increase chemical interactions of different tissue constituents. Thus, color and rheological properties of thawed fish fillets change and lose their water-retention capacity also develop some desirable flavor characteristics (Sikorski & Ruitter, 1994). Endogenous and bacterial enzyme inhibition by heating is the main purpose of the fish processing industry on the contrary of other food industries. Heating destroys activity of enzymes in fish easily. Sarcoplasmic proteins among other three muscle proteins do not affect the functional properties significantly. They are not main component of the histological structure of the tissues and can lose solubility easily when heated. Nevertheless, glyceraldehyde-3-phosphate dehydrogenase and aldolase like glycolytic enzymes have important effects on the rheological properties of fish gels. Toughness of the fish fillet can be increased by the thermal changes of the myofibrillar proteins and tenderness of the fish can be affected by the heated collagen transformation (Sikorski *et al.*, 1990). Only mild heating is required because of the muscle structure of fish and the low content of collagen so that the resultant loss of nutritional value is not high (Aitken & Connell, 1979). In heating under some conditions may lead the formation of different maillard products in seafood, and this can result with loss of nutritive value (Dworschak, 1980). Frying process of fish for about 4 min in fresh oil at 180°C may decrease the available lysine by about 18%. When highly oxidized oil is used, the loss is about 29% (Tooley & Lawrie, 1974). The effect of heat on nitrogenous components of fish may have also a food safety aspect because heating may serve to inactivate marine toxins (Gill *et al.*, 1985).

Effects of Processing Methods on Proteins

To extend the shelf life and improve the quality properties of fish canning, salting, drying, smoking and fermenting methods are used for decades (Figure 1).

Combined effects of low water activity, inhibition of bacterial growth, preservative effects of some chemicals and acetic acid, antioxidant and antibacterial effects of some smoking constituents develop extended storage life of fish products.

In salted fish, preservation is occurred due to the dry salt or brine which changes protein in result of effects of salt on proteolytic enzymes. The salt used for fish should not be finely grained due to rapid dissolving activity of the salt in the muscle and cause rapid withdrawal of moisture from the surface tissue. So that, protein denaturation occurs due to prevention of the penetration of the salt into the muscle known as "salt burn". This denaturation is generally proceeding slower than the penetration of salt in normal conditions (Tülsner, 1978). Ion-exchanging properties of proteins is another relevant issue which one of the effects of the salt. To obtain a soft and mushy texture of the fillet, Ca^{2+} and Mg^{2+} ions in the muscles should be substituted by the Na^+ from the brine. As this process is reversible, tough product may be obtained by accumulation of Mg^{2+} and Ca^{2+} from impure salt in the flesh (Sikorski, 2012).

In Southeast Asia, fermented fish sauce and paste are very common products consumed as a source of nutrients. Generally, whole, ungutted salted fish, is used for production of fish sauce or paste and protein hydrolysis plays a major role which amino acids' proteolysis decarboxylation occurs in achieving the desirable sensory properties (Ciou *et al.*, 2020).

Canning is one of the most important preservation techniques used in food industry including fish processing. In fish canning process, it is not recommended to canning process of lean fish due to

their flesh disintegrate under the high temperature leading texture and flavor lost. The preservation object is to inactivate the endogenous enzymes activity and reduce the moisture content. As a result of high temperature (121°C, 15 min.), proteins, vitamins, minerals and lipids go under alteration and sometimes nutritional and sensory values of these products could be reduced due to the formation of undesirable compounds (Aubourg, 2001).

Solar drying is one of the techniques to reduce water content whereby bacterial growth is inhibited mostly. Lower drying rate (1 m/s) has negative effect on protein content. In natural drying, fish dries faster and becomes harder although still some moisture remains inside which leads the drying process occur slowly and cause protein degradation. Also, extremely prolonged solar drying cause oxidize the lipids and nutritional value losts (Abraha *et al.*, 2018).

Smoking which provides antimicrobial smoking agents like phenols and formaldehydes to preserve the fish fillets is used for centuries for both preservation and flavoring. Smoking has reduced water activity effect by the heat which allows fish to be prevented from microbial spoilage. During the smoking process, temperature, type of wood and time must be controlled in order to obtain a high-quality resultant product. Basically, there are two types of smoking, hot and cold smoking. During smoking process, proteins and amino acids will be denatured depending on the intensity of heat applied. Overheating may lead loss of availability of essential amino acids like tryptophan, lysine and methionine (Adeyeye, 2019).

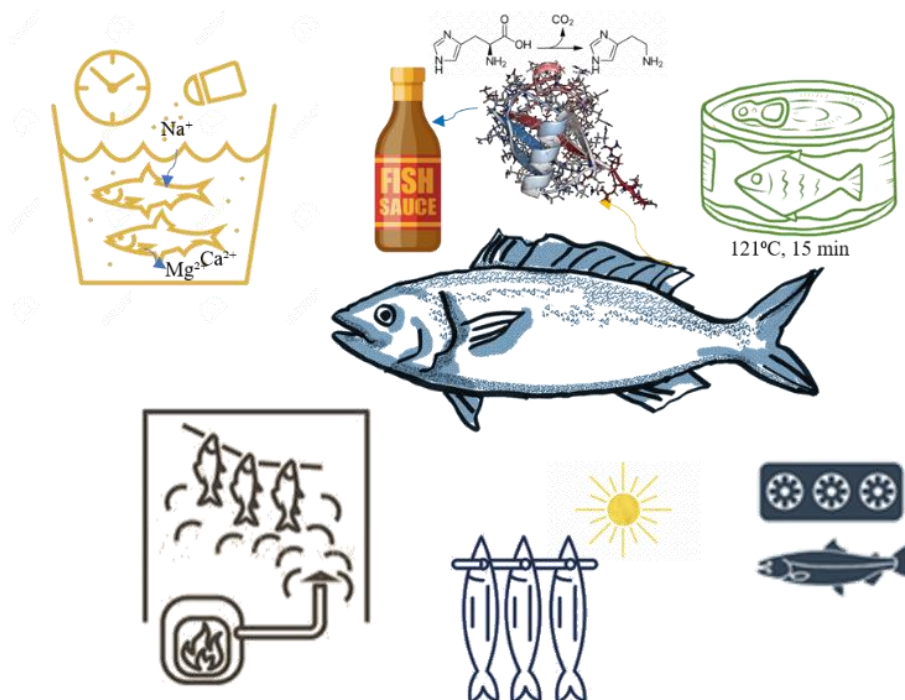


Figure 1. Fish processing methods

Fish Protein as a Functional Food Ingredient and Biotechnological Applications

The term "functional property" means the ingredients which lead to the appearance, texture and taste of a food. Isolating fish proteins for using in other types of foods as ingredient begins with the production of minced or mechanically deboned fish. This is also the common step of surimi production. Fish minces have functional advantages exhibiting a meat like texture than other type of meat minces like mammalian and poultry meats. Minced muscles from lean fish species are generally less functional in terms of gelling capability than surimi (MacDonald *et al.*, 1990), and also its constancy in frozen conditions is poor due to high capacity of enzyme activity (Suzuki, 1981). Fatty fish species are not stable in storage too due to potential of spoilage by oxidation of the lipids (Hultin, 1987; Shenouda, 1980).

Fish hydrolysates have been used as flavoring compounds, generally as fish sauce in the Eastern countries for centuries. This product is now also popular in Western countries as Eastern cooking techniques become widely known. Poor functional properties and solubility are the main disadvantages of the fish protein concentrates (FPC) (Meyer, 1965; Holden, 1971). The proteins are denatured highly to form gel even when solubilized by alkali treatment (Tannenbaum *et al.*, 1970), although reasonable foaming characteristic have been indicated (Baldwin & Sinthvalai, 1974).

Surimi is produced from minced fish such as underutilized species with little or no commercial value to provide opportunities to use different sources of protein. Wet concentrate of high-quality myofibrillar proteins from raw minced fish flesh are salt-solubilized and heated to generate surimi hydrogel. Alaska Pollock is used mostly in surimi production due to its good gelation properties, white color and desirable odor (Luo *et al.*, 2001).

Fish gelatins obtained from the cold-water fish species are less vulnerable to gel when comparing with the those from mammals but are excellent film-forming agents, crystal growth inhibitors and emulsion stabilizers (Norland, 1990). Fish gelatins are one of the succeed fish protein ingredient that take place in the marketing. Moreover, they can be used instead of mammalian gelatins where religious prohibitions exist (Karim & Bhat, 2009).

Marine biotechnology is a growing field that takes place in product chemistry, molecular biology, comparative biochemistry and gene technology. Recovery and/or transformation of "value-added" products from marine organisms is one of the main objectives of marine biotechnology. For instance, specific surfactants, emulsifiers, pharmaceuticals and flavorings can be obtained by treating the fish protein with particular proteinases. Natural products derived from marine organisms like vaccines, enzymes, antifreeze proteins, antibiotics, hormones and amino

acids can have incomparable properties (Figure 2). Industrial enzymes used in the depolymerization of natural substances are all almost hydrolases. Production of industrial enzymes are based on two methods (1) extraction from animal or plants and (2) microbial fermentation. Enzymes obtained from the fish processing waste or by-products are usually utilized by the industrial applications (Raa, 1990; Haard, 1991).

Egg albumin can be replaced by the those derived from crustaceans, mollusks and low-fat fish (Ockerman & Hansen, 1988). Also, cattle pancreas is the main source for the extraction of insulin used in the diabetes treatment. However, insulin isolated from fish can be also used as a replacement of bovine pancreas with its more stable properties (Sanlioglu *et al.*, 2013).

Fish Protein in Human Nutrition and Health Effects

The value of fish proteins is as high as meat protein and inferior to milk and egg protein. Fish protein has a stable composition of essential amino acids, with slight deficiencies of methionine and threonine, and an excess of lysine which is limited in cereals (Lozano & Hardisson, 2003). Fish protein includes essential amino acids such as tryptophan, cystine, lysine, methionine, and threonine which improves the digestibility. Aquatic protein contains essential amino acids and peptides much more than terrestrial meat protein such as lysine and methionine (Khan *et al.*, 2020). Fish has health benefits with its high-protein, low-fat content. In particular, white-fleshed fish, is lower in fat than any other source of animal protein, and oily fish are high in omega-3 fatty acids. Since the human body must supply these essential nutrients, fish are an important part of the diet. Also, fish are low in the "bad" fats commonly found in red meat, called omega-6 fatty acids (Schaafsma, 2008). Fish proteins are consisted of several peptides having functional properties which are beneficial for human health. These peptides show many biological activities such as antioxidant, anti-diabetic, anti-proliferative, antimicrobial, anti-coagulant, antihypertensive, and calcium-binding activities, thereby, improving the health (Khan *et al.*, 2020).

Protein is required for the construction of several essential compounds such as antibodies, enzymes, hormones and utilized for the structural formation of tissues and cells in the body. One of the main energy sources of our body is the protein content (1 g of protein provides 4 kcal). Infants, children, and pregnant women need dietary protein for depositing new protein in tissues and for the continuation of protein synthesis in adults' body. Therefore, inadequate protein intake can result diminished protein content in cells and organs and deterioration in the cellular capacity to perform normal functions. This leads to increased morbidity and mortality. On the other hand, excess protein intake is also disadvantageous (Young & Pellet, 1987). Therefore, an appropriate level of protein content is needed for an adequate diet for the assurance of long-term health.

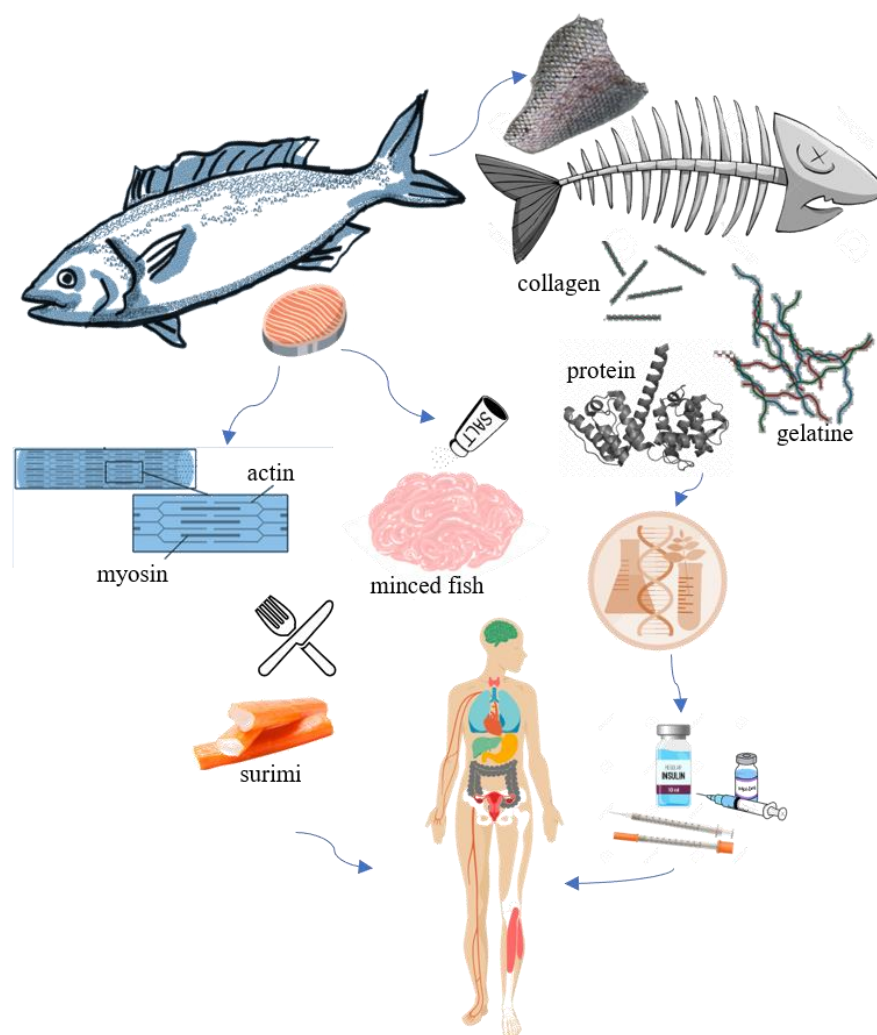


Figure.2. Utilization of fish by-products

One of the causes of death in children in developing countries is protein calorie malnutrition. Adults or children who have insufficient intake of protein or not be able to convert the vital nutrient to energy required for healthy tissue formation and proper organ function can have protein calorie malnutrition. As the insufficient intake of protein continues, loss of muscle mass, decreased immunity, and growth failure can be observed in the patient. Fish has greater satiety effect comparing to other animal protein sources and also more affordable when many varieties of fish species is concerned in the tropical countries (Mohanty *et al.*, 2019). Apart from their health effects, gelatine and collagen sort of fish protein is used in cosmetic industry with their fabulous moisturizing properties as anti-aging and anti-wrinkle (Guaadaoui *et al.*, 2014).

Author Contribution

Esen Alp Erbay: Conceptualization, writing original draft, editing.

Ahmet Faruk Yeşilsu: Conceptualization, writing original draft.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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