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Aquatic Products as Functional Food Sources

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CHAPTER 1

Fishery Products as Functional Food Sources of Selenium: Extraction and Potential

Endang Trowulan, Asep Awaludin Prihanto

Abstract

Selenium, an essential trace mineral element, plays various physiological roles in the human body. Its functions include acting as a potent antioxidant, anti-inflammatory agent, and providing antiviral protection against oxidative stress, thereby reducing the risk of several diseases. The limited scope of prerequisites and harmful consequences associated with selenium led to the development of the Selenium-Health Benefit Value (Se-HBV) as a metric for seafood safety. A positive Se:Hg ratio can help reduce the risk of mercury (Hg) toxicity. Fishery products typically contain both inorganic forms (selenite and selenate) and organic forms (selenoprotein, selenocysteine, and selenomethionine) of selenium simultaneously. The relatively high total Selenium content in fishery products presents an opportunity to develop Selenium-enriched functional foods. The methods for extracting selenium in total and quantifying species-specific Selenium content in these products are predominantly conducted in the liquid phase, often in combination with other techniques, to enhance efficiency while preserving the integrity of the various Selenium species. When considering the potential development of fishery products as viable sources of selenium in the diet, it is crucial to consider factors such as bioaccessibility and bioavailability to optimize their health benefits.

Keywords

Extraction, Functional food, Fishery products, Selenium

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1.1 Introduction

Selenium (Se), as a nutrient, is an essential trace mineral required by the body for several biochemical reactions (Natasha et al., 2018). It was initially discovered as a by-product of sulfuric acid and nitric acid industries (Kieliszek & Błazejak, 2013). It derived its name from the Greek word "Selene," meaning moon (Zhang, 2019). The growing interest in studying this element arises from its vital functions within the body, notably its role as a potent antioxidant, anti-inflammatory agent, and antiviral contributor (Wrobel et al., 2016). Within the body's biological spectrum, selenium is a cofactor for various enzymes, including triiodothyronine deiodinase, in the prevention of goiter (Adadi et al., 2019). It supports enzymes like glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) in safeguarding against free radical damage and lipoperoxides (Rai et al., 2019). This mineral is also instrumental in protecting the body against oxidative stress (Kieliszek, 2019). Maintaining an adequate selenium nutritional status has been linked to improved prognostic outcomes and a reduced risk of various diseases, including cancer, Alzheimer's disease, diabetes, cardiovascular disorders, mental disorders, fertility disorders, infection, and inflammation (Barchielli et al., 2022). This also extends to preventing and treating SARS-CoV-2-infected patients with selenium deficiency (Hekmatnia et al., 2021).

Selenium is a common element in the Earth's atmosphere, lithosphere, biosphere, and hydrosphere (Matos et al., 2017). The extensive utilization of selenium across various industries and its relevance in medical biotechnology and human health have elevated the commercial value of this metalloid, leading to increased research in recent years (Funari et al., 2021). Selenium deficiency poses a significant global challenge, with numerous instances of poverty linked to low selenium levels in specific terrestrial environments (Ullah et al., 2019). Selenium nutrient requirements can be met through dietary sources, and one well-known food rich in selenium is the Brazil nut (Bertholletia excelsa), containing a concentration of 19.91 μg/g (Adadi et al., 2019). The daily selenium intake recommended by the World Health Organization (WHO) is 40 μg/day (Sakr et al., 2018), with a tolerable intake limit of 400 μg/day (Tóth & Csapó, 2018). According to Tan et al. (2016), approximately 15% of the population is estimated to be selenium deficient, with an average daily intake of only 7 to 11 μg/day (Combs, 2001). Therefore, it is essential to implement strategies to address selenium deficiency, as its absence can lead to severe health problems and increase susceptibility to various diseases. These strategies may include developing functional foods enriched with selenium or supplementation (Adadi et al., 2019).

Functional foods are defined as foods that contain ingredients with health functions and have been approved to exert physiological effects on the human body, with documented health benefits (Crowe & Francis, 2013). The demand for functional foods containing selenium (Se) is also rising, driven by increasing knowledge of Se's health functions. While plant products can serve as a source of Se, those cultivated in lowlands typically have low Se content (Rayman, 2012). Animal-based foods can offer an alternative source of Se, as they can accumulate Se from Se-enriched feed or Se-fertilized plants (Meyer et al., 2014).

Selenium naturally exists in inorganic and organic forms, including selenate and selenite (Budiyanto, 2014). In our diets, selenium can be found in organic or inorganic forms, with common dietary selenium compounds comprising selenomethionine (SeMet), selenocysteine (SeCys), Se-methyl selenocysteine (MeSeCys), and selenite. SeMet is the dominant form in vegetables, grains, legumes, nuts, and yeast, rich in organic selenium. Inorganic selenium is also present in select foods and water. SeMet can be found in various food products, including grains, meat, eggs, dairy items, legumes (especially Brazil nuts), and yeast. Animal protein sources are the primary suppliers of SeCys in our diets, while MeSeCys can be sourced from Brassica and Allium species like garlic and onions. Selenite is naturally present in foods in small quantities, and the primary origin of inorganic selenium compounds in human diets is supplementation (Dobrzyńska et al., 2023).

Selenium in food is often bound to proteins, so products with high protein content, such as meat, fish, and cereals, tend to indicate a high selenium content (Holben & Smith, 1999). Seafood, in particular, serves as a good source of selenium, with marine fish containing approximately 1.11 to 0.97 µg/g and freshwater fish containing about 0.18 to 0.97 µg/g (Kieliszek & Błazejak, 2016). Fish and various fishery products have been recognized as selenium sources, including fish, mollusks, crustaceans, and algae. Selenium found in proteins exists in the form of the 21st amino acid, selenocysteine (SeCys or Sec), which is prevalent in fish and other seafood. The content of selenoproteotomes (the form of SeCys found in proteins) is more significant in seafood than in terrestrial mammals. Seafood contains organic and inorganic selenium, making fish and shellfish the primary selenium source for the Japanese population (Yoshida et al., 2011). Efforts have been made to develop fishery products as functional food products to enhance their health benefits. Therefore, this review will discuss the potential of fishery products as food sources of selenium, the chemical forms of selenium in natural sources, methods for extracting selenium from fishery products, and their development as functional food products and nutraceuticals.

1.2 Fishery Products as Source of Selenium

Selenium (Se) enters living cells, including microorganisms, plants, animals, and humans, in various inorganic forms and is biologically converted into organic forms, primarily as two selenoamino acids: selenocysteine (SeCys) and selenomethionine (SeMet). SeCys is essential in catalytic metabolic processes as an enzyme, while SeMet can be incorporated into the selenoproteome (Hossain et al., 2021).

The selenium content in food depends on geological and geographical factors. In plants, the selenium content is influenced by soil type, natural selenium levels, use of selenium-enriched fertilizers, and bioavailability (Titov et al., 2022). In animal products, selenium content is lower and depends on the selenium content in the consumed feed. Selenium is most abundant in fish, ranging from 0.4 to 4.3 µg/g, as well as in liver and kidney organs (0.2-2.0 µg/g) and muscle tissue (approximately 0.3 µg/g) (Rayman et al., 2008).

The high selenium content in fish makes seafood a primary source of natural selenium. The difference in selenium content between fish and terrestrial animals is attributed to their living environments and diets. Selenium is absorbed directly from the water through the gills and the

intestine in an inorganic form (selenite and selenate), which is then biologically converted into its organic form. Laboratory studies have shown higher bioaccumulation of organic selenium forms such as SeMet (Mackay, 2006). Therefore, selenium bioavailability depends on its chemical structure, especially in selenium-rich animal sources like fish and seafood, where it is predominantly found as SeCys and SeMet (Moreda-Piñeiro et al., 2013b).

Numerous studies have examined selenium content in marine organisms and fishery products. Selenium in fish primarily exists in the form of selenoproteins, which include derivatives of not only selenomethionine (SeMet) and SeCys but also non-protein organic selenium. Consequently, marine animals typically have higher selenium content than terrestrial animals (Yoshida et al., 2011). Analysis of selenium content in various seafood, including Atlantic cod, Greenland halibut, Atlantic salmon, Atlantic herring, common crabs, blue mussels, calanus, clams, and Euphasia super, revealed variations. For example, farmed Atlantic salmon had the lowest selenium content at 0.17 mg Se/kg dry weight. In comparison, blue mussels (Mytilus edulis) had the highest at 8.23 mg Se/kg dry weight, primarily in the form of selenomethionine (Bryszewska & Måge, 2015). Carnivorous fish contained approximately 1.63 ± 0.45 μg/g of selenium, higher than non-carnivores at 1.39 ± 0.074 μg/g and crustaceans at 1.08 μg/g. However, selenium levels in fish vary significantly, depending on the fish species, habitat, and season (dos Santos et al., 2017). The total selenium content in tilapia was measured at 3.30 ± 0.394 μg/g, mullet at 3.9 ± 0.826 μg/g, and crab at 5.8 ± 0.850 μg/g (Moatkhef et al., 2020). Additionally, selenosugar (selenosugar 1, methyl-2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside) was identified in aqueous extracts of muscle tissue from sardine *(Sardina pilchardus)*, mackerel *(Scomber scombrus)*, and tuna *(Thunnus albacares)*, with total selenium contents of 0.42 mg/kg, 0.51 mg/kg, and 0.74 mg/kg wet weight, respectively (Kroepfl et al., 2015). In Japanese seafood, selenium content in various tissues ranged from 110 to 24.8 mg/kg in alfonsino *(Japanese bluefish)*, with the highest content in edible muscle tissue at 1.27 mg/kg. In contrast, other fish muscles typically contained selenium levels between 0.12 to 0.77 mg/kg (Yamashita et al., 2013). Shijimi clams *(Corbicula japonica)* had a relatively high concentration of selenium measured at 3.5 μg/g dry weight (Yoshida et al., 2017).

Some scientists propose using the Selenium-Health Benefit Value (Se-HBV) as a scientific measure of seafood safety. This concept is based on research indicating a "toxicological antagonism" relationship between mercury and selenium, where MeHg toxicity is considered an adverse effect of mercury. In contrast, selenium is associated with physiological benefits. Regardless of the mercury content in the fish, if the selenium level exceeds that of mercury, the fish is deemed safe for consumption (Perry et al., 2012). Positive HBV values for seafood indicate a higher molar amount of selenium than CH3Hg, whereas negative values suggest a higher content of CH3Hg compared to selenium (Ralston et al., 2014). With few exceptions, marine fish generally contain more selenium than mercury, making them safe and beneficial for health. Consequently, most saltwater fish, being selenium-rich sources, protect against the potential adverse effects of CH3Hg (Ralston et al., 2019). Research on HBV in seafood has been conducted in various regions, and the selenium-mercury molar ratio differs among fish species. For instance, in some fish species in Taiwan, the average selenium-mercury molar percentage ranks as follows: red tilapia (166.8) > abura (87.9) > river shrimp (82.4) > white shrimp (64.2) > butterfish (44.6) > milkfish (37.0) > tuna (15.6) > grouper (13.9) > ayu (13.4) > back coral (13.0)

> weever (11.8) > saury (9.0) > shark (7.8) > marlin (4.2) (Fang et al., 2011). Se/Hg molar ratios for pelagic fishes from the central North Pacific Ocean near Hawaii were evaluated as follows: marlin (17.6) > yellowfin tuna (14.1) > mahimahi (13.1) > skipjack (12.8) > spearfish (11.4) > wahoo (10.8) > scythe pomfret (6.7) > albacore tuna (5.3) > bigeye tuna (5.2) > blue marlin (4.1) > escolar (2.4) > opah (2.3) > thresher shark (1.5) > swordfish (1.2) > mako shark (0.5). Yellowfin tuna exhibited the highest total selenium content at 1.59 ± 0.17 μg Se/g (Kaneko & Ralston, 2007). For ratios less than 1, these species should be consumed with care to avoid health risks. Se content in fresh seafood, measured using cold vapor atomic absorption spectroscopy (CVAAS) and hydride-generation atomic absorption spectrometry (HGAAS) methods, ranged from 0.073 mg/kg in perch to 0.743 mg/kg in tuna and from 0.067 mg/kg in spiny lobster to 0.605 mg/kg in scallops (Plessi et al., 2001).

The selenium content in fish is also influenced by its habitat and the cooking method used. Generally, marine fish have higher selenium content. Fish's edible portion (EP) contains varying selenium levels depending on the cooking treatment. For example, fresh fish has a selenium content of 37.1-198.5 μg/100 g EP, fried fish contains 52.9-262.4 μg/100 g EP, and boiled fish contains 48.0-154.4 μg/100 g EP. In contrast, freshwater fish has lower selenium content, with 6.9-29.4 μg/100 g EP in fresh fish, 13.7-43.8 μg/100 g EP in fried fish, and 10.1-26.5 μg/100 g EP in boiled fish. Based on these cooking effects, most fish retain a high percentage of selenium (64.1-100% accurate retention) when boiled or fried. Long-tailed tuna, Indo-Pacific Spanish mackerel, short-bodied mackerel, tilapia, and red tilapia are recommended to be cooked by boiling and frying for optimal selenium intake (Singhato et al., 2022a).

Algae, as low trophic level organisms, also play a vital role in absorbing and accumulating selenium, which can be essential or toxic for algal growth, depending on the dose and species. The unicellular green alga Haematococcus pluvialis, known for natural astaxanthin production, can accumulate up to 646 μg/g total selenium and 380 μg/g organic selenium per dry weight (Zheng et al., 2017). Algae can accumulate selenium as organic compounds due to detoxification mechanisms and adaptations to selenium levels in their environment, using it as a storage method within their cells (Bodnar et al., 2015). Green algae can also metabolize inorganic selenium into various inorganic selenium intermediates through selenomethionine and selenocysteine, utilizing the excess as a detoxification response (Neumann et al., 2003). Studies have investigated the total selenium content in several edible seaweeds, including Dulse (Palmaria palmata) at 96±10 ng/g, Nori (Porphyra umbilicalis) at 57±6 ng/g, Kombu (Laminaria ochroleuca) at 60±1 ng/g, Wakame (Undaria pinnatifida) at 230±10 ng/g, Sea spaghetti (Himanthalia elongata) at 68±1 ng/g, Sea lettuce (Ulva rigida) at 49±1 ng/g, and Canned seaweed (cooked Himanthalia elongata and Saccorhiza polyschides) at 57±2 ng/g (Moreda-Piñeiro et al., 2013a).

3.3 Selenium Extraction in Food

Naturally, the primary source of selenium is obtained from soil derived from carbonaceous debris and volcanic ash, and the geological processes influence its quantity in the ground (Zhou et al., 2021). Several extraction methods are used for obtaining Se from soil samples, and these methods are adapted from techniques used to determine the total content of certain elements in soil or solid materials. According to Supriatin et al. (2018), these methods involve partial

deconstruction using concentrated acids. However, to minimize environmental impact and ensure laboratory safety, it is advisable to use environmentally friendly concentrated acids while obtaining information about the total Se content in the sample. These methods include deconstruction using HNO3 and HCl (EPA, 2001; Keskinen et al., 2009), deconstruction using HNO3, HClO4, and HF (Spackman et al., 1994), deconstruction using HNO3 and HClO4 (Soil Research Center, 2009; Gao et al., 2011), and deconstruction using HNO3 and HF (Imran et al., 2016).

Measuring selenium content in complex food matrices is traditionally challenging due to the element's low concentration. This challenge arises from the presence of numerous other factors in the material. Some solutions include separating or pre-concentrating selenium from the material matrix before quantification (Hagarov & Nemˇ, 2022). Techniques for determining total Se content in various samples, such as water, food, beverages, plants, and seafood, include inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), electrothermal atomic absorption spectrometry (ETAAS), hydride generation atomic fluorescence spectrometry (HG-AFS), flame atomic absorption spectrometry (FAAS), and hydride generation atomic absorption spectrometry (HG-AAS) (Altunay et al., 2021). However, FAAS, ETAAS, and ICP-OES may lack the sensitivity required for samples with low ionization energy (9.7eV); the ICP-MS method is sensitive but expensive and not yet available in many laboratories. To address these limitations, the Hydride Generation (HG) technique is employed as an efficient atomic absorption spectrometry (AAS) method. The HG technique efficiently analyzes samples without needing catalysts by converting them into volatile components (Zam et al., 2019).

Measurements of Se in various matrices have been carried out with multiple food samples such as plants, fish, meat, eggs, and so on. Equipment availability and researcher experience are factors considered when choosing a method for extracting and identifying Se species in samples (Pedrero & Madrid, 2009). The best extraction method is one with high efficiency while maintaining the integrity of the Se species, ensuring accurate measurement results. Various methods are currently employed for extracting Se species, including the use of aqueous solutions (such as water, water-MeOH, and neutral solutions), acid-alkaline hydrolysis (involving HCl, methanesulfonic acid, and tetra-methyl-ammonium hydroxide - TMAH), and enzymatic hydrolysis (using enzymes like proteinase K, pepsin, protease, and subtilisin, or a combination of protease and lipase enzymes). Acid extraction, however, is associated with challenges such as low SeMet recovery and, in some instances, the complete loss of SeCys (~100%) in extracts that contain basic Se0 (Jagtap & Maher, 2016).

Ultra-trace Se in a sample requires an effective preconcentration procedure before quantitatively determining Se. Among the most popular preconcentration techniques are extraction methods that separate different species through selective chemical reactions, falling into two categories: liquid-liquid and solid-phase extraction (Viana et al., 2021). A straightforward technique for Se extraction from dried fish, shellfish, meat, and seaweed samples was conducted by Ohki et al. (2016) using subcritical water (SW), with Se determination carried out via inductively coupled plasma mass spectrometry (ICP-MS). This technique is similar to the conventional method involving microwave-assisted acid digestion—the optimal treatment involved SW extraction at 120°C for 120 minutes.

Traditional liquid-liquid extraction (LLE) and solvent extraction techniques have seen significant advancements, including the adoption of miniaturization strategies. The transition from LLE using larger volumes to microextraction has led to the development of liquid-phase microextraction (LPME), solvent microextraction (SME), and liquid-liquid microextraction (LLME), as described by Kamal El-Deen et al. (2023). These techniques have found practical application, as illustrated by Bağda & Tüzen (2017), who employed an environmentally friendly vortex-assisted ionic liquid-based microextraction method on various food samples, including shellfish. This approach yielded Se concentrations ranging from 2.0 to 7.0 μg/L, with a detection limit of 9.8 x 10-2 μg/L in the form of SE(IV). Altunay et al. (2021) also applied the vortex-assisted ionic liquid dispersive liquid-liquid microextraction (VA-IL-DLLME) to extract Se(IV) from food samples, achieving exceptional extraction efficiency, speed, and lower detection limits without the necessity for heating steps. Furthermore, this method employs an environmentally friendly solvent, C8mim NTf2. In the case of milk samples, an extraction technique was developed for the determination of Se(IV) and Se(VI) using the magnetic dispersive micro-solid-phase extraction (MDMSPE) technique, followed by graphite furnace atomic absorption spectrometry (GAAS), resulting in detection limits of 1.0 and 1.3 pg mL−1 (Chen et al., 2021).

An alternative separation approach is Cloud Point Extraction (CPE), a surfactant-based method utilizing a non-ionic surfactant as the extraction medium. Changes in experimental conditions, such as temperature, pH, pressure, and ionic strength, induce micelle formation, separating the aqueous surfactant solution into two isotropic phases. The separation and preconcentration of inorganic ions via CPE typically involve several sequential steps, optimizing experimental conditions to establish a reliable procedure. Triton X-114 (polyoxyethylene-7.5-octylphenoxy ether) is the most commonly utilized non-ionic surfactant in CPE, while various complexing agents are employed (Hagarov & Nemˇ, 2022).

Various methods have been implemented for extracting Se from protein-containing or protein-enriched foods, encompassing liquid-phase extraction, enzymatic extraction, and auxiliary extraction. The liquid-phase extraction techniques, as delineated in Xiong et al.'s (2023) review, include the following:

Table 1.1 Liquid phase extraction methods of Se from Se-protein enriched food

Extraction method	Specialized process	Example of application on sample	Advantages and disadvantages
Water extraction	The process involves crushing the sample and combining it with distilled water in a precise ratio. This mixture is then extracted at a specific temperature with stirring for a defined duration, followed by centrifugation. The resulting clear liquid is gathered. Ammonium sulfate is introduced to reach the desired level of precipitation and separate the	Cordyceps militaris *Edode Lentinus* Beras, kacang brazil and garlic	Advantages: Enhanced preservation of SeP's biological activity, minimal environmental impact, and straightforward operation. Disadvantages: Reduced extraction efficiency and time-intensive process.

Extraction method	Specialized process	Example of application on sample	Advantages and disadvantages
	precipitated fraction. Subsequently, dialysis is performed before the final step of drying, resulting in the acquisition of a water-soluble protein sample.		
Alkaline extraction	The process involves crushing the sample and subjecting it to extraction with a specific alkali concentration at a designated temperature with stirring. Afterward, the supernatant is separated through centrifugation, and the pH is adjusted to reach the isoelectric point before centrifugation and drying to yield a protein sample.	Cordyceps militaris, Edode Lentinus, Mushrooms, rice, beans, buckwheat, and quinoa	Pros: Achieves a high extraction rate and is suitable for most plant SePs. Cons: Elevated alkali concentration may lead to lysine production in proteins, altering their physiological function, and the process can be time-consuming.
Salt extraction	The sample is crushed and extracted with a specific alkali concentration at a certain temperature with continuous stirring. The supernatant is subsequently centrifuged, and the pH is adjusted to the isoelectric point before centrifugation and drying to obtain a protein sample.	Cordyceps militaris, *Edode Lentinus, rice*, *Ganoderma lucidum*	Advantages: Appropriate for extracting salt-soluble proteins; frequently employed for residual processing following the extraction of water-soluble proteins; and well-suited for removing animal-origin SePs. Disadvantages: Limited extraction efficiency
Alcohol extraction	The samples were crushed and combined with 75% ethanol at a predetermined ratio. They were then subjected to extraction at room temperature with stirring, followed by centrifugation at 4°C. The supernatant was mixed with a specified amount of distilled water and left to settle overnight. Afterward, it was centrifuged at 4°C to collect a residue, which was subsequently dried to yield a protein sample.	Cordyceps militaris, rice, *Ganoderma lucidum*	Advantages: Appropriate for extracting alcohol-soluble proteins and highly effective when using an alkaline method. Disadvantages: Low extraction efficiency, protein loss, and potential environmental pollution due to the use of organic solvents.

Extraction method	Specialized process	Example of application on sample	Advantages and disadvantages
Buffer solution extraction	The sample is first crushed and then subjected to extraction using a defined liquid buffer concentration (such as PBS or Tris-HCl) at a low temperature with continuous stirring. The supernatant is centrifuged, the pH is adjusted to induce protein precipitation, and the resulting residue is dried to obtain a protein sample.	Mushroom, Buckwheat Quinoa, Flammulina velutipe	Advantages: Minimizes protein damage and is well-suited for extracting soluble proteins. Disadvantages: Requires organic solvents or other agents for protein precipitation treatment.

(Xiong et al., 2023)

Enzymatic extraction is a method for extracting Se known for its ease of use, reasonable specificity, and excellent activity, which makes it a commonly employed technique. Enzymes like proteases, including trypsin, pepsin, proteinase K, and XIV, are utilized. However, a drawback of this method is its time-consuming nature, often necessitating a combination of multiple enzymes. Sometimes, it is combined with other ways to accelerate the reaction rate. Enzymes play a vital role in Se-protein extraction by breaking down proteins into low molecular weight peptides. In specific cases, a hybrid approach is adopted, involving initial alkaline extraction for sample preparation followed by enzyme treatment to separate SeProtein (SeP) into SePeptide-protein (SePPs) (Xiong et al., 2023). For example, in rice extraction, a combination of alkaline protease, neutral protease, trypsin, and pepsin is employed to hydrolyze Sep and SePPs (Fang et al., 2019).

Auxiliary extraction techniques are used to enhance the efficiency of Se-protein extraction by addressing the limitations of liquid-phase extraction and enzymatic extraction methods. These additional techniques often encompass heating, stirring, microwave treatment, ultrasound, and high-pressure modes. The simultaneous use of enzyme extraction and ultrasound in SeP extraction from Cardamine violifolia has shown promising results, achieving a protein purity of 77% and a total Se content of 9097.33 ± 35.66 mg/kg (Wu et al., 2020).

1.4 Selenium Extraction from Fishery Products

The ability of aquatic organisms such as algae, fish, shellfish, crabs, and shrimps to bioaccumulate selenium from their environment results in a high Se content in fishery products. These products contain inorganic Se (selenite and selenate) and organic Se (selenoproteins, selenopeptides, selenoamino acids, and selenopolysaccharides). Since Se typically binds with proteins, Se in fishery products is primarily in the form of selenoproteins (SeP). Therefore, the extraction of Se from fishery products primarily involves the SeP extraction method. The liquid-

phase extraction method of Se from Se-enriched protein foods was reviewed by Xiong et al. (2023).

Several conventional methods using liquid-liquid extraction have been employed on various seafood samples to quantify total selenium and selenium species present in fishery samples, as detailed in Table 1.2. These methods indicate that the liquid-phase extraction method utilizing solvents is preferred for extracting fishery products due to the availability of instruments and techniques considered more accessible to researchers. Additionally, the identification of total selenium and selenium species in fresh seafood was performed using microwave-assisted acid digestion and the measurement of complete Se in the dialyzable fraction. This method detected selenocysteine at low concentrations, while inorganic Se and methyl selenocysteine were not detected (Moreda-Piñeiro et al., 2013b).

Solid-phase extraction, as described by Mendil et al. (2017), involved the following procedure: 50 ml of the sample solution (containing 3 µg Se(IV)) was adjusted to pH 5, then TZ-SG (300 mg) was added to a glass column (100 mm long and 10 mm in diameter) that had been conditioned with a buffer solution. Subsequently, the test buffer solution was passed through the column at a flow rate of 4 ml of 0.5 mol/L HNO3. GF-AAS then determined the Se concentration. This method was successfully applied to microwave-assisted water-extracted meal samples.

Altunay et al. (2020) introduced a simple, rapid, environmentally friendly, and cost-effective method that can also be applied to seafood. This method combines alcohol-DES-based vortex-assisted liquid-liquid microextraction (Alcohol-DES-VAHLLME) with hydride generation atomic absorption spectrophotometry (HG-AAS). The technique offers reasonable detection limits and a broad calibration range, making it an attractive choice for determining and extracting total selenium in food samples.

1.5 Prospects for the Development of Fishery Products as Functional Food Sources of Selenium

The increasing awareness of the importance of maintaining health through natural ingredients has led to a rising demand for products that meet these criteria in recent years. Fishery products, whether from land or sea, are recognized for their high selenium content, attributed to their ability to accumulate selenium through metabolism. Selenium is pivotal in various physiological functions essential for overall health, making it a vital nutrient. However, selenium as a nutrient source is primarily found in food. Nevertheless, the selenium content in food can vary by region. As a result, one alternative approach involves enriching animal food products with selenium through dietary supplementation. The effectiveness of this technique is influenced by factors such as duration, dosage, and oxidation state, which determine its dual targeting modalities with pro-oxidant and antioxidant potentials (Gu & Gao, 2022).

The Selenium Health Benefit Value (HBV) criterion, developed to assess the risk or benefit of consuming seafood, exhibits a positive trend for fishery products, particularly those consumed frequently. Consumption of seafood with an excess of molar CH3Hg relative to selenium helps maintain selenoenzyme activity, reducing the risk of mercury (Hg) exposure.

This criterion also provides a dependable basis for distinguishing seafood that should be limited during pregnancy from those consumed for their health benefits (Ralston et al., 2019).

Fishery products sourced from animals with naturally higher selenium content have the potential to serve as a natural selenium source without the need for selenium-enriched feed supplementation. Consequently, seafood samples from the market often exhibit relatively high total selenium levels. Nevertheless, to understand whether the selenium source is effective within the body, it is crucial to investigate the mechanisms of absorption and metabolism. Therefore, evaluating the bioaccessibility and bioavailability of fishery products becomes essential. Bioaccessibility pertains to the release of bioactive compounds from the food matrix through the digestive tract, eventually absorbed by the intestine for further processing. Bioavailability, conversely, refers to the compounds absorbed through the body's mucosa for use in physiological functions or their storage in the circulatory system after intestinal metabolism and digestion. Experiments simulating the human gastrointestinal tract, in vitro studies with cell cultures, and investigations involving mice have been conducted to assess bioaccessibility and bioavailability (Yang et al., 2022).

The assessment of bioaccessibility in various commonly consumed fish in Thailand depends on factors like their type and the preparation method. Among these fish, longtail tuna exhibited the highest total Se content at 262.4 µg/100 g of product. The average total Se measurements ranged from 43.8 to 115.6 µg/100 g of product. Interestingly, when compared to longtail tuna, cork fish and Indo-Pacific Spanish mackerel demonstrated significantly higher bioaccessibility rates of 70.0% and 64.6%, respectively. The preparation method, whether boiling or frying, did not considerably affect bioaccessibility, underlining the suitability of fish as a high-bioaccessibility Se source (Singhato et al., 2022b).

Table 1.2 Liquid phase extraction methods of fishery products

Extraction Method	Extraction Process	Fishery Products	Results	Reference
Water extraction with supercritical water solvent	A dry powdered sample weighing 0.1 g is blended with pure water at a liquid-to-solid ratio 100. Subsequently, it is transferred into a sealed container encased in a pressure-resistant stainless steel outer layer. The container's atmosphere is purged with argon, and the mixture is heated within the range of 180°C to 220°C for a specified duration. After cooling and centrifugation, the aqueous phase is filtrated to eliminate any solid residues. The resulting filtrate is then appropriately diluted and subjected to analysis using ICP-MS.	Bluefin tuna, big eye tuna, mackerel, scalp seaweed, green laver, hijiki, wakame, mozaku	Ranges from 1.06 to 20.4 µg/g dry weight	(Ohki et al., 2016)
Microwave-assisted Acid Digestion	The dried sample, in powdered form, is meticulously weighed to a specific amount and transferred into a pressure-resistant container. Subsequently, HNO3 and H2O2 are introduced into the container. The container is sealed and then positioned on a rotating microwave oven carousel to initiate the extraction process, which is conducted for a designated period. Following cooling and filtration, the resulting filtrate is appropriately diluted and subjected to Se content analysis using ICP-MS.	Bluefin tuna, big eye tuna, makarel, scaloprumput laut klep, green laver, hijiki, wakame, mozaku	Ranges from 1.7 - 17.2 µg/g dry weight	
Acid extraction	Samples weighing 0.1 to 0.2 grams were combined with 1 milliliter of nitric acid and perchloric acid mixture in a 1:2 ratio. This mixture was then heated within the 200 to 220 degrees Celsius range for a specific duration. Subsequently, the selenium concentration was quantified using hydride generation atomic absorption spectroscopy.	Fish and shellfish in Japanese waters	Lowest 0.12 mg/kg for Japanese eel Highest 1.27 mg/kg for alfosino fillet	(Yamashita et al., 2013)

Extraction Method	Extraction Process	Fishery Products	Results	Reference
Acid extraction	Fish samples weighing 1 gram each were placed into containers and mixed with 15 milliliters of HNO3 (65%). The mixture was then heated to 200°C. After cooling, an additional 15 milliliters of H2O2 was introduced, and the mixture was heated to 150°C. Following another cooling period, the mixture underwent filtration, and the resulting liquid was analyzed for total selenium content using ICP-MS.	Mackerel, mullet, tilapia, shrimp	Mackerel 2.4 ± 0.448 µg/g, mullet 3.9 ± 0.826 µg/g, tilapia 3.30 ± 0.394 µg/g, shrimp 6.8 ± 0.035 µg/g	(Moatkhef et al., 2020)
water extraction	The process began by isolating the fish samples from their bones and skin and then homogenizing them using a food processor. Subsequently, 1 gram of the homogenized sample was measured and transferred into 50 ml polypropylene tubes, which were then stored in a frozen state. For extraction, 1 gram of the sample was combined with 10 milliliters of water and subjected to three rounds of sonication, each lasting 5 minutes, in an ultrasonic bath. Throughout the extraction, manual shaking of the tube was performed. The resulting extract was then filtered using a 0.2 µm filter, and the filtrate was subsequently analyzed for selenium content via HPLC.	Sardines and tuna meat	Total Se in mackerel was 0.42 mg/kg, in sardines 0.51 mg/kg, and in tuna 0.74 mg/kg in wet weight. Se species were identified in the class of cello-sugar and Se-methylseloneine.	(Kroepfl et al., 2015)
water extraction	The meat samples underwent a series of steps for analysis. Initially, they were freeze-dried and subsequently pulverized using a food processor. Two grams of the powdered sample were then accurately weighed and enclosed within a non-woven fabric bag. This bag containing the sample was heated in the	Shijimi mussels	1.006±0.040 µg/g freeze-dried scallop meat.	(Yoshida et al., 2017)

Extraction Method	Extraction Process	Fishery Products	Results	Reference
	presence of 20 milliliters of water for 2 hours. Following this, the mixture underwent centrifugation at 750 ×g, maintained at a temperature of 4°C. The resulting supernatant was subjected to filtration through a 4.5 µm membrane. The filtrate obtained from this process was further analyzed for selenium content using ultrafiltration, chromatography, paired ion extraction, and mass spectrometry techniques.			
Enzyme extraction	Fresh or powdered samples were precisely weighed at 0.2 grams in the analysis process. These samples were then introduced into a phosphate buffer solution with a concentration of 1 mM. This buffer solution also contained a non-specific protease enzyme, specifically protease type XIV derived from Streptomyces griceus, at an 8g/L concentration. The mixture was subsequently placed in a rotator tube and left to incubate for 24 hours at room temperature. Following the incubation, the resulting extract was combined with 2.0 milliliters of 65% nitric acid and 0.5 milliliters of 30% hydrogen peroxide. This combination led to the digestion and dissolution of the sample, resulting in a final volume of 25 milliliters with the addition of Milli-Q water. The resulting extracts were then analyzed using an inductively coupled plasma-mass spectrometer (ICP-MS).	Atlantic cod (fillet) (Gadus morphed) Atlantic salmon (Salmo salar) Greenland halibut (Reinhardtius hippoglossoides) Atlantic herring (Clupea harengus) Blue mussel (Mytilus edulis) Common crab (Cancer Pagurus cephalothorax) Claws Scallop	The total Se content ranged from 0.17 mg/kg dry weight for Atlantic salmon to 8.23 mg/kg for blue mussels. The efficiency of the total extract by phosphate buffer was 2.5 times higher in marine plankton and shellfish than in fish. Selenite, selenate, and selemethionine were detected.	(Bryszewska & Måge, 2015)

In vitro, bioavailability assessments of total Se and Se species from raw seafood relied on simulated gastric and intestinal digestion/dialysis methods. These experiments revealed selenocysteine in low concentrations, while Se-(Methyl)selenocysteine and inorganic Se (selenite and selenate) were notably absent in the dialysate. The bioavailability of total Se was approximately 6.69% + 3.39% for fish samples and 5.45% + 2.44% for mollusks (Moreda-Piñeiro et al., 2013b). Meanwhile, seaweed displayed a substantially higher bioavailability of 66.7% + 31.1%. This suggests a positive association between bioavailability and carbohydrate and dietary fiber content (Moreda-Piñeiro et al., 2013a).

Given the advantages of fishery products as sources of Se, characterized by their favorable SE-HBV, high bioaccessibility, and bioavailability, alongside their resistance to alteration due to preparation and cooking methods, the potential for developing selenium-based functional foods from fishery products appears promising. The design of such functional foods must adhere to six crucial factors outlined by (Adadi et al., 2019) as stated below:

1. Fortification should not disrupt the original food's sensory properties, including flavor, color, texture, and odor.
2. The stability of the selenium active ingredient in the fortified food must meet acceptable standards.
3. A thorough examination is required for potential interactions among micronutrients in the fortification system and between the active ingredient and the food carrier, which might impact metabolic absorption.
4. Adding supplementary ingredients, such as encapsulated binders and stabilizers, to improve active ingredient retention should not necessitate alterations in existing technology.
5. Functional foods should be efficiently absorbed from the food carrier at consumption levels compatible with a healthy diet.
6. The significant costs associated with designing functional foods should maintain the food's capabilities and make it more competitive compared to less favorable alternatives.

In developing selenium-source functional foods from fishery products, careful attention to these factors is essential to create innovative solutions addressing selenium deficiency and enhancing the nutritional and health benefits offered by fishery products, which are known for their rich nutritional content.

1.6 Conclusion

The capability of fishery products, ranging from algae to fish, to bioaccumulate selenium is highly advantageous. This inherent ability results in these food items having a higher selenium content than other selenium sources. Fishery products contain both inorganic and organic selenium species, with organic selenium, such as selenium cysteine and selenium methionine, being the most readily absorbed. Fishery products can also metabolize inorganic selenium, like selenite and selenate, into organic selenium. The SE-HBV parameter, which exhibits a positive trend with typical species, mitigates concerns about mercury toxicity associated with fishery products. Current selenium extraction techniques from fishery products often employ conventional methods to quantify both total selenium and selenium species within these

materials. Future research endeavors should prioritize the development of commercial extraction methods characterized by high efficiency and yield. This approach would enable the exploration of fishery products as functional foods in economically significant species and the by-products of the fishing industry that typically possess relatively lower economic value. Furthermore, the extracted products could be enhanced raw materials for selenium-source food supplements.

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CHAPTER 2

Taurine from Aquatic Resources: Extraction and Functional Properties

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Abstract

Taurine is a neutral β-amino acid that originates from metabolizing sulfur amino acids. It distinguishes itself by having a sulfonic acid group in its chemical structure instead of a carboxylic acid group. Taurine can be obtained from a substance through an extraction process. As a functional compound, taurine serves various physiological functions in humans, such as protecting against tissue damage caused by free radicals, facilitating efficient fat absorption and dissolution, reducing the risk of hypertension, enhancing immune endurance, and serving as a critical antioxidant scavenging reactive oxygen species. This review provides an overview of extraction methods, physiological functions, and the prospective development of functional food products derived from taurine. The appropriate selection of extraction methods significantly influences the production of taurine compounds. Non-conventional methods, particularly Ultrasound-Assisted Extraction, stand out as environmentally friendly and highly effective strategies for extracting taurine compounds from materials. The physiological functions encompass antioxidant, anti-cancer, anti-inflammatory, and neuroprotection activities. Despite the limited utilization of taurine in functional foods, there is an opportunity to develop helpful food products by incorporating or supplementing taurine into foods.

Keywords

Extraction methods, Physiological functions, Taurine

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2.1 Introduction

Taurine, a neutral β-amino acid, is a product of the metabolic processes involving sulfur-containing amino acids, as elucidated by El-Sayed in 2014. Its chemical structure sets taurine apart, which features a sulfonic acid group instead of a carboxylic acid moiety. This unique characteristic means that taurine is not integrated into proteins but rather exists freely within the body, as highlighted by studies conducted by Huxtable in 1992 and Suresh et al. in 2011. Taurine is classified as a semi-essential amino acid in humans because it is synthesized endogenously from methionine and cysteine, facilitated by the presence of vitamin B6, as documented by Sirdah et al. in 2002.

The biosynthesis of taurine exhibits significant variability among individuals and is contingent upon factors such as nutritional status, protein consumption, and the accessibility of cysteine. The availability of cysteine, in turn, hinges on the metabolic equilibrium between homocysteine and methionine, a process influenced by folic acid, vitamin B12, and the enzymatic activity of methyltetrahydrofolate reductase, as elucidated in a study by De Luca et al. in 2015. Due to limited taurine biosynthesis capacity in humans, alternative sources include dietary intake from meat and seafood (Salze & Davis, 2015). Taurine deficiency does not directly pose health problems, but long-term insufficiency can impact numerous metabolic pathways (Huang et al., 2014).

As a functional compound, taurine provides various physiological functions in humans, such as offering protection against tissue damage from free radicals (Jagadeesan & Pillai, 2007), facilitating efficient fat absorption and dissolution (Devi et al., 2009; Schaffer et al., 2014), reducing hypertension risk (Yamori et al., 2010), enhancing immune endurance (Miyazaki et al., 2004; Zhang, Izumi, et al., 2004), and serving as a primary antioxidant scavenging reactive oxygen species (Oja & Saransaari, 2017).

Taurine is primarily sourced from fish, shellfish, and animal proteins (Stapleton et al., 1997). Shellfish contain higher taurine concentrations than finned fish, with taurine content reaching 800 mg/100 g in shellfish (Laidlaw et al., 1990). Additionally, red algae are considered a good taurine source for consumption (Kawasaki et al., 2017).

The extraction process is used to obtain taurine compounds from a substance. Non-conventional extraction methods have emerged to address the limitations associated with traditional extraction techniques, including prolonged extraction durations, excessive solvent usage, high energy requirements, and the potential degradation of sensitive compounds, as highlighted in a study by Ummat et al. in 2021. Various non-conventional extraction approaches, such as Microwave Assisted Extraction, Ultrasound Assisted Extraction, Enzyme Assisted Extraction, Supercritical Fluid Extraction, and Pressurized Liquid Extraction, have been applied to extract a range of bioactive substances, as discussed by Kadam et al. in 2014. These technologies offer advantages such as reducing or eliminating the need for harmful chemical solvents, improving extraction yields and quality, shortening extraction times, and minimizing energy consumption, as emphasized by Ummat et al. in 2021.

The utilization of taurine compounds in functional food products needs further exploration, considering its beneficial physiological functions pharmacologically for humans and animals. This review provides an overview of physiological processes, extraction methods, and the prospective development of functional food products derived from taurine compounds.

2.2 Taurine Extraction

The extraction of taurine from a substance can be carried out using conventional and non-conventional methods, namely environmentally friendly methods (Green Extraction Methods). Traditional taurine extraction methods often reported and performed include water boiling methods. However, this method requires a long time, has high energy consumption, and can lead to taurine compound degradation (Plaza & Turner, 2015). Table 2.1 provides an overview of taurine compound extraction using the water boiling method and illustrates that the taurine compound obtained from a substance is influenced by the type of material, extraction temperature and time, and the quantity of sample used.

Table 2.1 Taurine Extraction Using Conventional Method

No	Sample	Treatment	Result			References
			Taurine (mg/g)	% Amino Acid	Yield (%)	
1	Squid *(Loliolus beka)*	50 g sample in 1 L distilled water; temperature 100^0C; duration 4 hours	904.82	22.97		(Han et al., 2019)
2	Squid *(Loliolus beka)*	20 g sample in 1 L distilled water; temperature 100^0C; duration 4 hours		38.22		(Lee, Han, Shin, et al., 2019)
3	Octopus *(Octopus ocellatus)*	20 g sample in 1 L distilled water; duration 24 hours	4351.15	29.66		(Lee, Han, Oh, et al., 2019)
4	Octopus *(Octopus vulgaris)*	4 g sample in 200 mg distilled water; duration 24 hours	1430.05	39.84	48.22 ±0.17	(Lee, Han, Park, et al., 2019)
5	Freshwater snail	Sample:distilled water 10:1; temperature 100^0C; duration 90 minutes	0.06	0.95		(Kim et al., 2019a)
6	Atrina pectinate/atrina bivalvia	Sample:distilled water 10:1; temperature 100^0C; duration 2 hours	0.04	0.76		(Kim et al., 2019b)
7	Atrina pectinate/atrina bivalvia	50 g sample in 1 L distilled water; duration 24 hours	410.533	21.69		(Shin et al., 2019)

Standard environmentally friendly methods used for compound extraction from materials include Microwave Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE), Ohmic Heating Assisted Extraction (OHAM), and Ultrasound Assisted Extraction (UAE) (Dranca & Oroian, 2016). These methods can reduce extraction time and energy consumption and improve the extract's yield and quality (Ummat et al., 2021). However, extraction using microwave waves may damage the molecular structure of the section due to its heat transfer mode, and the extraction costs for Ohmic Heating and Supercritical Fluid Extraction are relatively high (Cruz et al., 2020; Saberian et al., 2017).

The utilization of the Ultrasound-Assisted Extraction method has been reported for obtaining taurine compounds from a substance, as shown in Table 2.2. This method offers advantages compared to other environmentally friendly extraction methods, including reduced extraction time, solvent use, and processing costs (Ummat et al., 2021). The parameters that need to be optimized when using this method include frequency, power, temperature, time, and the ratio of sample to solvent (Heleno et al., 2016).

Table 2.2 Taurine Extraction Using Ultrasound-Assisted Extraction (UAE)

No	Sample	Treatment	Results		References
			Yield (mg/g)	Conclusion	
1	Nori *(Porphyra yezoensis)*	Solid-to-liquid ratio 1:4; time 38.5 minutes; temperature 40.5^{0}C; power 300 W	13.2	The application of this method improves the efficiency of the extraction process, reduces extraction time, and operates at a lower temperature to obtain taurine yields comparable to those obtained from conventional extraction	(Wang et al., 2015)
2	Cow Liver	Solid-to-liquid ratio 1:4; time 12.8 minutes; temperature 40^{0}C; power 205 W	6,20	This method reduces extraction time and exhibits good reproducibility compared to conventional methods.	(Guo et al., 2020)

Ultrasound-assisted extraction is a non-thermal physical processing method well-suited for extracting bioactive compounds from diverse sources, particularly natural bioactive. This approach, as documented by Dumitrascu et al. in 2019 and Saberian et al. in 2017, involves using ultrasound waves featuring frequencies ranging from 20 kHz to 100 kHz. These waves generate bubbles as a consequence of pressure variations. Subsequently, the collapse of these bubbles induces cavitation, resulting in the disruption of interfaces around particles. This, in turn, leads to the release of bioactive compounds into the matrix, as elucidated by Kadam et al. in 2015.

The use of environmentally friendly extraction methods for taurine compound extraction from a substance is limited; thus, further research is needed to explore using these extraction methods to obtain taurine compounds. Considering its more effective and efficient extraction process than conventional methods, the UAE method stands out as one of the most effective and efficient environmentally friendly methods for obtaining taurine compounds from a substance.

2.3 Physiological Function of Taurine

Taurine as an Antioxidant

Reactive oxygen species (ROS) and free radicals, which are generated due to irregular metabolism or immune responses, refer to oxygen compounds that exhibit instability and possess unpaired electrons, as outlined by Porter et al. in 1995. In the presence of pathological conditions, there is a disruption in the equilibrium between ROS production and elimination, which consequently affects biomacromolecules. Typically, the creation of free radicals includes species such as superoxide anion (O2−), hydroxyl anion radicals (OH−), oxygen (O2), and hydrogen peroxide (H2O2), all of which are highly unstable and tend to engage in reactions with other substances within the human body. In most cases, these free radicals induce oxidative stress, damaging cell membranes, lipids, and proteins, ultimately leading to processes like cell swelling, denaturation, and apoptosis, as discussed by Kang in 2013.

Until now, several synthetic antioxidants like BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate), and TBHQ (tertiary butyl hydroquinone) have been commonly employed to mitigate damage caused by oxidative stress, as reported by Khan et al. in 2010. However, excessive usage of these synthetic antioxidants can result in adverse side effects, including toxicity, cancer, and liver damage, as indicated by Ko et al. in 2012. Consequently, extensive research efforts have been dedicated to finding natural antioxidants that do not induce harmful side effects.

Marine organisms present a promising source of biological compounds compared to terrestrial plants. The marine environment possesses distinctive metabolic processes and an array of advantageous components such as fatty acids, minerals, vitamins, polysaccharides, and bioactive compounds, as discussed by Holdt & Kraan in 2011. Biologically active proteins play a pivotal role in metabolic systems and hold potential as materials due to their structural composition and amino acid sequences. Additionally, these proteins participate in various physiological functions, as highlighted by Najafian & Babji in 2012. Among the bioactive compounds suitable for use as antioxidants, taurine stands out due to its advantageous properties, including antioxidant defense, cellular osmoregulation, and facilitation of fat digestion, as reported by Li et al. in 2009.

Table 2.3 Taurine as an Antioxidant

No	Taurine Sources	Methods	Results	Reference
1	Squid meat	*ABTS* and *DPPH*	Provides hepatoprotective effect against H_2O_2-induced oxidative stress and apoptosis by inhibiting ROS formation	(Han et al., 2019)
2	Octopus meat	*ORAC Assay*	Exhibits beneficial effects against H_2O_2-induced oxidative stress	(Lee, Han, Oh, et al., 2019)
3	Octopus meat	*ABTS*	Shows potential hepatoprotective function	(Lee, Han, Park, et al., 2019)
4	Clam meat	*FRAP* dan *ORAC*	Has antioxidant capacity twice as high as BHT	(Kim et al., 2019b)
5	Clam meat	*ABTS* dan *ORAC*	Protects hepatocytes exposed to H_2O_2 by triggering antioxidant mechanisms within the cell	(Shin et al., 2019)

Taurine in clam extract using the ABTS method exhibits intense free radical activity, where this antioxidant activity is expressed as Trolox equivalents antioxidant capacity (TEAC), or mM equivalent Trolox per mg of extract (mM Trolox eq./mg extract), with a TEAC value of 1.146 ± 0.002 in clam extract and a TEAC value of 0.654 ± 0.004 in Butylated Hydroxytoluene (BHT) as a control (Kim et al., 2019a). Furthermore, the free radical scavenging activity with ABTS from clam extract can scavenge more than 85% of free radicals (Shin et al., 2019).

Taurine as an Anti-Cancer Agent

Taurine, also known as 2-aminoethanesulfonic acid, is a naturally occurring amino acid widely distributed in mammalian tissues, as highlighted in studies by Schaffer & Kim in 2018. Several investigations have demonstrated that taurine exhibits anti-tumor properties by impeding cell proliferation and promoting apoptosis in specific cancer types. This effect is achieved by regulating anti-apoptotic and pro-apoptotic proteins (Choi et al., 2015; Ibrahim et al., 2018; Tang et al., 2015; Tu et al., 2018; Zhang et al., 2014).

Taurine has been shown to effectively hinder cell proliferation and induce apoptosis in nasopharyngeal carcinoma cell lines, as elucidated by He et al. in 2018. Notably, taurine demonstrates a significant capacity to inhibit the proliferation of nasopharyngeal carcinoma cells without exhibiting toxic effects on normal nasopharyngeal epithelial cells when employed at concentrations below 32 mM taurine over 48 hours. Furthermore, taurine induces apoptosis in nasopharyngeal carcinoma cells through the mitochondrial pathway, as revealed in He et al.'s study in 2019. An innovative taurine peptide vaccine has also been identified for its potential to delay, restrain, and/or treat spontaneous tumorigenesis (Han, 2019).

The proliferative ability of tumor cells was 100 ± 10.63 in untreated Ehrlich ascites carcinoma (EAC) carrier animals. Tumor cell proliferation was reduced to 74.71 ± 7.45, 71.76 ± 4.89, and 64.82 ± 5.12, respectively, in rats treated with Cyclophosphamide (CTX), levamisole plus CTX, and Taurine plus CTX (Ibrahim et al., 2018). The addition of Taurine to CTX (where CTX is a chemotherapy drug used to treat various cancers, including breast cancer, blood cancer,

ovarian cancer, lymphoma, neuroblastoma, and retinoblastoma) exhibits superior anti-tumor properties by controlling tumor cell proliferation and apoptosis. Taurine suppresses the growth of ectopic xenograft tumors derived from lung cancer cells in rats. Treatment involving the exogenous upregulation of the p53 modulator of apoptosis (PUMA), the administration of taurine, and the combination of exogenous PUMA and taurine leads to average tumor suppression ratios of 42.94 ± 1.99, 50.09 ± 2.35, and 78.52 ± 1.46%, respectively. Notably, the tumor suppression ratio observed in the combined treatment group is significantly higher than in the groups subjected to single treatments with either taurine or PUMA alone, as demonstrated in Tu et al.'s study in 2018. These findings suggest a synergistic effect when taurine and exogenous PUMA are administered together, suppressing xenograft tumor growth in rats. The ability of taurine to induce apoptosis in the human lung cancer cell line A549 is noteworthy. A549 cells exposed to varying concentrations of taurine exhibit a notable increase in the proportion of apoptotic cells, significantly surpassing the levels observed in the control group (Tu, 2018).

Taurine as an Anti-Inflammatory Agent

Inflammation is widely recognized as a primary defense mechanism against infections. One of the key features of chronic inflammation is the persistent activation of macrophages. Macrophages play a vital role in the body's immune response, including tasks such as antigen presentation, phagocytosis, and immunomodulation, as highlighted in the work of Fujiwara and Kobayashi in 2005. When macrophages release excessive pro-inflammatory mediators, they can contribute to chronic inflammation and severe tissue damage, potentially leading to diseases like cancer, diabetes, rheumatoid arthritis, and neurodegenerative disorders (Heller et al., 1997).

Under normal physiological conditions, high concentrations of nitric oxide (NO), produced by inducible nitric oxide synthase (iNOS), play a significant role in host defense, cytoprotection, inflammation, and nerve transmission. However, NO overproduction can lead to inflammatory and autoimmune diseases (Pautz et al. 2010).

It's worth noting that elevated levels of reactive oxygen species (ROS) have been demonstrated to increase the expression of nuclear factor-kappa B (NF-κB), as shown in the research by Chen et al. in 2008. When cells are exposed to Lipopolysaccharide (LPS), ROS production within the cells increases, and an excess of ROS is associated with the activation of various inflammatory pathways. This intricate relationship between anti-oxidative and anti-inflammatory activities suggests that anti-oxidative assays can serve as markers for evaluating anti-inflammatory properties.

NF-κB activation plays a crucial role in both the acute phase and the overall inflammatory response by regulating the expression of numerous inflammatory mediators and cytokines, as indicated by Otterbein et al. in 2000. Consequently, various therapeutic strategies targeting the inhibition of NF-κB activation have been explored to prevent and treat inflammatory diseases. Taurine, a sulfur-containing non-essential amino acid synthesized endogenously from methionine, is a potential candidate for modulating these processes.

2.4 Prospects for the Development of Functional Food Products with Taurine Compound

Being a natural amino acid, taurine is associated with minimal side effects, and current research findings have not uncovered any genotoxic, carcinogenic, or teratogenic effects, as discussed by Ripps and Shen in 2012. Its excellent safety profile has led to widespread use as a supplement in various food and beverage products, with a notable presence in meat and seafood items naturally rich in taurine, as noted by Mendivil in 2021. Taurine serves multiple beneficial roles in metabolic and physiological processes, including the regulation of glucose and lipids, energy metabolism, anti-inflammatory, and antioxidant functions, reduction of oxidative stress and lipid peroxidation, and the enhancement of lipid absorption and metabolism (Yang et al., 2015; Zhang et al., 2017).

Taurine boasts a range of well-documented physiological and pharmacological functions, encompassing activities such as bile acid conjugation, modulation of endoplasmic reticulum stress, osmoregulation, stabilization of cell membranes, neuromodulation, participation in energy metabolism, antioxidation, anti-inflammatory properties, and maintenance of calcium homeostasis, and all discussed in-depth by Salze and Davis in 2015. Additionally, as explored, taurine can enhance immune responses and contribute to the regulation of neuroendocrine functions (Ommati et al., 2018). Table 2.4 provides a comprehensive overview of taurine's applications as a supplement in both human and animal food and beverages

Table 2.4 The Utilization of Taurine as a Food Supplement

No	Sample	Taurine Supplementation	Results	Reference
1	Turbot Fish *(Scophthalmus maximus)*	Addition of taurine at 0,4%, 1,2%, and 2,0% in feed	1.2% taurine supplementation significantly protects turbot fish from oxidation, ER stress, and inflammation (anti-inflammatory)	(Zhang et al., 2021)
2	Grouper Fish	Addition of taurine at 1,05%, 1,2%, 1,31%, and 1,6% in feed	Supplementing hybrid grouper diets with 1.31% and 1.05% taurine levels results in optimal growth rates and feed efficiency. Taurine plays a crucial role in augmenting the overall antioxidant capacity of hybrid grouper, mitigating lipid peroxidation resulting from heightened nutrient metabolism.	(Qian et al., 2021)
3	Europe Seabass *(Dicentrarchus labrax)*	Addition of taurine at 0,2%, 0,5%, 0,7%, dan 1,2% in feed	Taurine supplementation at 0.2% shows maximum antioxidant value	(Martins et al., 2021)

No	Sample	Taurine Supplementation	Results	Reference
4	Turbot Fish *(Scophthalmus maximus)*	Addition of taurine at 0,4%, 1,2%, and 2,0% in feed	Taurine diet supplementation enhances muscle growth in turbot fish by promoting hyperplasia of muscle fibers, where 1.2% taurine addition significantly increases hyperplasia fiber growth and texture properties in turbot fish muscles.	(Sampath et al., 2020)
5	Quail Birds	Taurine (0, 2,5, atau 5 g per kilogram of feed) added to feed	Taurine supplementation, especially at higher doses (5 g/kg), for quail layer feed results in improved productive performance and nutrient digestion	(Orhan et al., 2020)
6	Giant Grouper Fish *(Epinephelus lanceolatus)*	Gradual taurine levels (0,1, 0,3, 0,5, dan 1 g/kg) in fish meal	Taurine supplementation at 1 g/kg in feed enhances growth and nutrient digestibility in giant grouper fish.	(Lin & Lu, 2020)
7	Broiler Chicken	5 g/kg taurine in feed	Taurine can improve intestinal morphology in broiler chickens exposed to chronic heat stress up to a specific limit	(He et al., 2019)
8	Swamp Eel	Taurine 0,1% dan 0,2% in feed	Adding taurine to the diet fosters stability in the gut microbiota, mitigating the adverse impacts of diets containing oxidized fish oil on microbial composition and interactions among different species within tissues. Furthermore, it helps restore the gut microbiota's normal functioning in swamp eels.	(Peng et al., 2019)
9	European seabass *(Dicentrarchus labrax)*	Taurine at 1% in feed	Taurine supplementation can modulate antioxidant responses in the liver and intestines of European seabass fish	(Coutinho et al., 2017)
10	Male Rats	TAU injected at a dose of 50 mg/kg for 60 days	The safeguarding impact of taurine on sexual dysfunction in diabetic male rats could be ascribed to its anti-oxidative characteristics and its capacity to maintain the stability of cell membranes.	(Mohamed & Gawad, 2017)

No	Sample	Taurine Supplementation	Results	Reference
11	Flatfish	Microcapsules supplemented with taurine (3% of the total composition)	Taurine diet supplementation may be crucial during this stage, contributing to enhanced larval growth potential and metamorphosis improvement	(Pinto et al., 2010)
12	Obese Woman	Capsules containing 3 g pure taurine powder consumed 2 hours before physical exercise and during fasting on other days	Taurine supplementation can increase irisin levels in obesity conditions and result in higher resting metabolic rates	(Batitucci et al., 2019)
13	-	Addition of taurine at 25, 35, and 50 mg in beverages	High selectivity, optimal analytical parameter values, and a wide range of applications for pharmaceutical and toxicological purposes	(Draganov, 2014)

Taurine possesses physiological functions that humans can effectively utilize. Nonetheless, the utilization of taurine in human dietary supplementation remains constrained compared to its application in animal feed. This is despite the human body's need for an external source of taurine due to the limited activity of the rate-limiting enzyme (cysteine sulfinate decarboxylase) in taurine biosynthesis (Chang et al., 2013; Vitvitsky et al., 2011). Therefore, further research is needed to explore taurine supplementation for human food, which could provide opportunities and development for existing functional food products.

2.5 Conclusion

The selection of appropriate extraction methods greatly determines the taurine compounds produced. The use of non-conventional methods, particularly ultrasound-assisted extraction (UAE), is one of the environmentally friendly extraction methods that is highly effective and efficient in extracting taurine compounds from a material. The physiological functions of taurine can be harnessed to create functional food products such as antioxidants, anti-cancer, anti-inflammatory, and neuroprotective agents. The utilization of taurine compounds in functional foods still needs to be improved, presenting opportunities for developing helpful food products by adding or supplementing taurine in foods.

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CHAPTER 3

The Potential of Protein Hydrolysate from Fisheries By-Products as Therapeutic Food for Energy-Protein Malnutrition in Children

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Abstract

This article explores the possibility of protein hydrolysates obtained from fisheries by-products as a medicinal remedy for addressing energy-protein deficiency in children. Energy-protein malnutrition is a major global health problem, especially in areas with limited availability of varied and nourishing food options. The study centers on the sustainable exploitation of fisheries by-products to transform these underutilized resources into valuable, easily absorbed therapeutic foods. The research uses thorough nutritional analysis to evaluate the hydrolysates' protein composition, amino acid profile, and bioactive components. It emphasizes the potential of these hydrolysates to be used as effective nutritional therapies. The paper highlights the significance of considering both the nutritional effectiveness and sustainable acquisition of resources when devising strategies to address malnutrition. The research examines the bioavailability and digestibility of protein hydrolysates to determine their appropriateness for meeting the unique dietary requirements of malnourished children. This study adds to the ongoing discussion on creative and sustainable methods to address childhood malnutrition. It highlights the significance of using fishery by-products as a helpful resource in creating therapeutic foods. The results promote additional investigation and advancement of these protein hydrolysates to generate significant interventions worldwide to enhance infant nutrition.

Keywords

Dietary, Energy, Fish by-product, Malnutrition, Protein

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3.1 Introduction

The per capita fish consumption has continuously increased over the past five years. Statistical data from the Ministry of Marine Affairs and Fisheries of the Republic of Indonesia (2014) indicates that fish consumption between 2009 and 2013 increased from 29.08 kg per capita to 35.14 kg per capita, representing a growth of 20.83%. In 2010, the cumulative volume of captured fisheries production reached 5,384,418 tons and witnessed significant growth until 2013, culminating in 5,863,170 tons. This growth potential corresponds directly to the potential yield of generated by-products (Rohmah et al., 2015).

Fishery by-products comprise bones, skin, fins, heads, scales, and internal organs. Fishery by-products represent one of the most significant challenges in the fish processing industry (Atma, 2016). Improper management of these by-products can lead to environmental issues, especially when the organic content in fish processing waste becomes a nutrient source for microbial growth (Syahrul and Dewita, 2016).

Fishery by-products have significant economic value when appropriately handled. These by-products still contain relatively high levels of protein and amino acids. Generally, fish waste contains various elements such as nitrogen, potassium, phosphorus, and others that contribute to the composition of fats and proteins. Based on this analysis, fishery by-products have the potential to be developed into fish protein hydrolysate products (Utomo et al., 2014). Transforming fishery by-products into products such as fish protein hydrolysates can reduce environmental pollution and enhance the value of fisheries products (Atma, 2016).

Fish Protein Hydrolysate (FPH) is derived from fish and fishery by-products through protein hydrolysis techniques. This process entails decomposing proteins found in fish tissues into smaller peptides, ultimately forming amino acids (Petrova et al., 2018). FPH is a fundamental component in food additives and dietary supplements, primarily enhancing protein consumption and mitigating protein-related malnutrition (Utomo et al., 2014).

According to Gunawan et al. (2011), malnutrition is when the body experiences energy, protein, and nutrient deficiencies. Protein-energy malnutrition (PEM) or Malnutrition with Energy and Protein Deficiency (MEP) are specific conditions related to defects in energy and protein. Nutritional deficiencies, especially energy-protein ones, remain a significant health issue in Indonesian society. The prevalence of undernourished or underweight children is still high. According to the Basic Health Research Ministry of Health of the Republic of Indonesia results of 2016, from 2007 to 2013, there has been no improvement and even a slight increase in these numbers. The province with the highest percentage of underweight children is East Nusa Tenggara (33%). Widjajanti et al. (2019) state that acute MEP is very dangerous, with a high risk of death if not properly managed.

Over the past two decades, hospitals have typically treated and managed acute MEP. However, recently, certain groups have approached this issue differently. This approach includes researching alternative products to prevent and treat uncomplicated MEP cases. One such product is Ready-to-Use Therapeutic Food (RUTF) (Schoonees et al., 2019). RUTF was first developed in Nigeria as a paste, primarily using peanuts (a local ingredient). Peanuts are high in fat but low in protein (Komari and Astuti, 2012). Therapeutic food products are typically made from main ingredients like rice or cereals enriched with plant-based rather than animal protein.

However, animal protein contains higher levels of protein and amino acids, more comprehensive vitamins and minerals, and saturated fatty acids and cholesterol, which are necessary for children's physical and cognitive development (Aprilia and Hati, 2016). According to Alvares et al. (2018), fishery by-products can be used in therapeutic food products due to their excellent nutritional content safe for human consumption.

Research on fishery by-products as fish protein hydrolysates has been extensively conducted, including studies on waste from kakatua fish heads (Prihanto et al., 2018), carp fish eggs (Chalamaiah et al., 2015), catfish skin (Yin et al., 2010), tuna fisheyes (Mutamimah et al., 2018), and trout fish offal (Wald et al., 2016). However, the food sector's use of protein from fishery by-products still needs to be improved. Yet, these by-products contain high levels of protein and amino acids. Alvares et al. (2018) state that fishery waste can be a safe and promising source of protein and amino acids for human consumption. However, its utilization has remained suboptimal until now. Thus, fish protein hydrolysates derived from fishery by-products have significant potential for use as supplements and additives for protein malnutrition patients. Therefore, this chapter aims to explain and overview the protein and amino acid content of fish protein hydrolysates derived from fishery by-products as an alternative nutritional source for energy-protein malnutrition in children.

3.2 Protein-Energy Malnutrition

Protein-Energy Malnutrition (PEM) is one of the significant nutritional disorders in Indonesia as well as in other developing countries. The highest prevalence is found in infants, lactating, and pregnant mothers. Individuals with PEM experience various pathological conditions caused by deficiencies in energy and protein in varying proportions. These deficiencies lead to degrees of PEM ranging from mild to severe (Adriani and Wijatmadi, 2012).

Protein-Energy Malnutrition (PEM) is a prevalent nutritional ailment frequently observed in developing countries like Indonesia, Africa, Central America, and South America. This healthy condition predominantly impacts children under 5 (infants) and expectant or nursing mothers (Victoria, 2015). As assessed based on weight and age criteria, 113.4 million children are grappling with Protein-Energy Malnutrition (PEM). Most PEM cases manifest in developing countries, with 70% occurring in Asia and 26% in Africa (Hussein and Adam, 2015).

3.2.1 Causes and Types of Protein-Energy Malnutrition

The direct causes of PEM are calorie and protein deficiency, indicating insufficient consumption of foods containing calories and protein and impediments to nutrient utilization. Infectious diseases and worm infestations can hinder nutrient absorption and utilization, which are fundamental to the development of PEM. Indirect causes of PEM encompass several dominant factors, including low income and reduced purchasing power for food, especially low-protein foods. Another indirect cause is the state of the country's economy; during a monetary crisis, the cost of goods, including energy and protein sources such as rice, chicken, meat, and eggs, tends to rise. Other influential indirect causes of deficient consumption of energy and protein-rich foods include low general and nutritional education, resulting in a lack of understanding of the

role of nutrients in human health. Additionally, inadequate food production to meet demands, many children, poor hygiene conditions, and inefficient or uneven trade and distribution systems contribute (Adriani and Wijatmadi, 2012). There are three types of PEM: marasmus, kwashiorkor, and marasmus-kwashiorkor. Each type presents distinct clinical and biochemical symptoms (Rabinowitz et al., 2013). According to the Indonesian Ministry of Health of the Republic of Indonesia (2010), the classification of Protein-Energy Malnutrition (PEM) is based on the following indicators: weight-for-age (W/A), weight-for-height (W/H), height-for-age (H/A), and body mass index-for-age (BMI/A). The categories and threshold values for a child's nutritional status are presented in Table 3.1.

Table 3.1 Categories and threshold values for child nutritional status based on indices

Index	Nutritional Status Category	Threshold (z-score)
Weight-for-Age (W/A) Children Aged 0 – 60 Months	Severely Underweight	< -3 SD
	Underweight	-3 SD s/d < -2 SD
	Normal	-2 SD s/d 2 SD
	Overweight	> 2 SD
Length/Height-for-Age (L/H or H/A) Children Aged 0 – 60 Months	Very Short	< -3 SD
	Short	- 3 SD s/d < -2 SD
	Normal	-2 SD s/d 2 SD
	Tall	> 2 SD
Weight-for-Length/Height (W/L or W/H) Children Ages 0 – 60 Months	Very Thin	< -3 SD
	Thin	- 3 SD s/d < -2 SD
	Normal	-2 SD s/d 2 SD
	Overweight	> 2 SD
Body Mass Index-for-Age (BMI/A) Children Ages 0 – 60 Months	Very Thin	< -3 SD
	Thin	- 3 SD s/d < -2 SD
	Normal	-2 SD s/d 2 SD
	Overweight	> 2 SD

Source: Decree of the Minister of Health of the Republic of Indonesia Number: 1995/MENKES/SK/XII/2010 concerning Anthropometric Standards for the Assessment of Child Nutritional Status

Marasmus is the most common severe form of malnutrition in nutritional deficiency emergencies. The word "marasmus" originates from Greek meaning "to waste away." It is characterized by excessive metabolism of fats and muscles to produce energy. Children affected by this condition exhibit an appearance resembling that of older men, with visible skin and bones. Children suffering from marasmus appear more alert and active. As the disease progresses, they may become irritable, less active, and refuse to eat (Shakur et al., 2018).

Kwashiorkor is an edematous form of malnutrition, almost similar to marasmus, and is known as a wasting syndrome. Children with this disease usually experience body fat depletion, low body weight, and height. Other characteristics of this disease include dry and thin skin, an

enlarged and bloated head appearance, emaciation, weakness, hypotension, and hypothermia (Benjamin and Lappin, 2019).

Individuals with kwashiorkor experience gastrointestinal disturbances, often rejecting all food and sometimes requiring a gastric tube. Kwashiorkor patients are prone to a distinctive skin disorder called "crazy pavement dermatosis," characterized by the gradual appearance of red spots mixed with patches that darken and peel over time. This phenomenon generally occurs on the back, buttocks, and around the vulva, which remains moist due to sweat or urine. Liver enlargement also occurs, sometimes extending to the navel due to fat accumulation in liver cells. Kwashiorkor patients also suffer from anemia. Serum albumin and globulin levels decrease, sometimes falling below 2 and occasionally reaching 0. Serum cholesterol levels are low, possibly due to poor nutritional intake or disrupted cholesterol synthesis (Par'i, 2016).

Marasmic-kwashiorkor arises due to a daily diet lacking in both energy and protein. Children's weight drops below -3 SD, resulting in a thin appearance. Still, there are also signs of edema, hair abnormalities, dry and dull skin, weakened muscles, and decreased blood protein (albumin) levels (Par'i, 2016). Patients with marasmus and kwashiorkor can be observed in Figure 3.1.

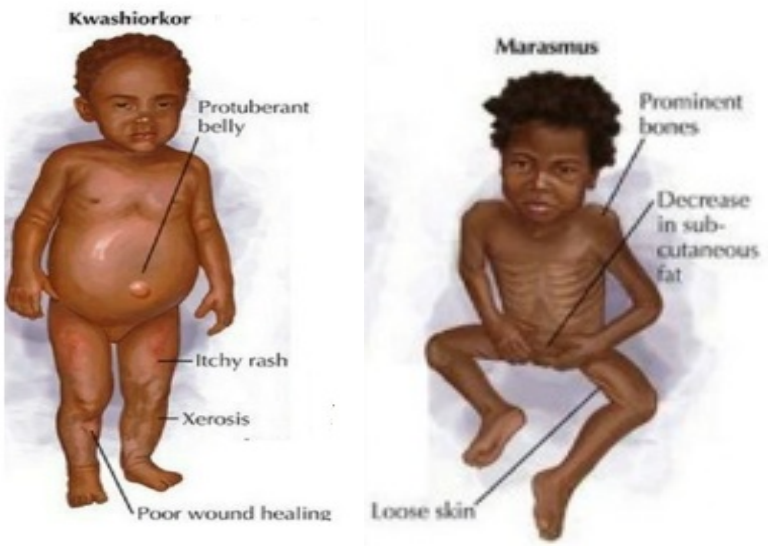

Figure 3.1 Patients with Marasmus and Kwashiorkor

3.3 Fish Protein Hydrolysate (FPH)

Fish protein hydrolysate was introduced in Japan and China around 1990 as a by-product of Monosodium Glutamate (MSG) production. Fish protein hydrolysate is hygroscopic and can be liquid, paste, or powder. Liquid fish protein hydrolysate contains 30% solids, while the paste contains 65% solids. Fish protein hydrolysate is produced by breaking fish proteins into simple peptides and amino acids through enzymatic and chemical hydrolysis processes (Annisa et al., 2017).

3.3.1 Definition of Fish Protein Hydrolysate (FPH)

Fish Protein Hydrolysate (FPH) is derived from breaking fish proteins into shorter-chain compounds, accomplished through enzymatic, acidic, or alkaline hydrolysis. The primary source of fish protein hydrolysate is underutilized fish by-products that retain substantial protein content (Bernadeta et al., 2012). FPH represents a collection of water-soluble proteins that remain uncoagulated even when exposed to high temperatures. It consists of a mixture of polypeptides, dipeptides, and individual amino acids. The production of fish protein hydrolysate involves subjecting high-protein content materials to either acid or enzymatic hydrolysis processes (Saputra and Nurhayati, 2016). A visual representation of the FPH product can be found in Figure 3.2.

Figure 3.2 Commercial fish protein hydrolysate

3.3.2 Methods for Production of Fish Protein Hydrolysate (FPH)

Fish Protein Hydrolysate (FPH) can be produced using several methods, including chemical methods (acid or base) and biochemical methods (enzymes from microorganisms or from the fish itself) added at appropriate levels (Ovissipour et al., 2012). Over the past few decades, the primary production procedures for protein hydrolysates have remained relatively similar. Attention is still given to breaking down fish peptides or specific combinations of amino acids that can produce the desired effects for each intended application (Pasupuleti and Demain 2010).

The process of producing fish protein hydrolysate is also cited in Wisuthiphaet and Kongruang (2015), where economically low-valued fish catches are minced and then mixed with equates at a 2:1 w/w ratio, homogenized to enhance the hydrolysis reaction. Next, the mixture is conditioned to the optimal pH and temperature for the enzyme. Once the temperature and pH of the mix are at the enzyme's optimum conditions, the next step is to add the enzyme and homogenize the mixture. The hydrolysis is then carried out in a shaker incubator for 5, 10, and 15 hours at 200 rpm. After that, inactivation is done at 90°C for 30 minutes. Subsequently, the mixture is centrifuged at 3000 rpm for 30 minutes, and the supernatant is collected and then dried using a freeze-dryer.

According to Kurniawan et al. (2012), the incubation process of producing fish protein hydrolysate should be stopped when signs of a decrease in hydrolysis rate appear. The decrease in hydrolysis reaction rate can be caused by several factors, such as the decrease in specific peptide bonds for the enzyme, product inhibition, enzyme inactivation, and enzyme molecule stability, which affect the enzyme's binding with the substrate, either directly or indirectly, resulting in a decrease in the concentration of the products produced. The fish protein hydrolysate production principle follows the flowchart shown in Figure 3.3.

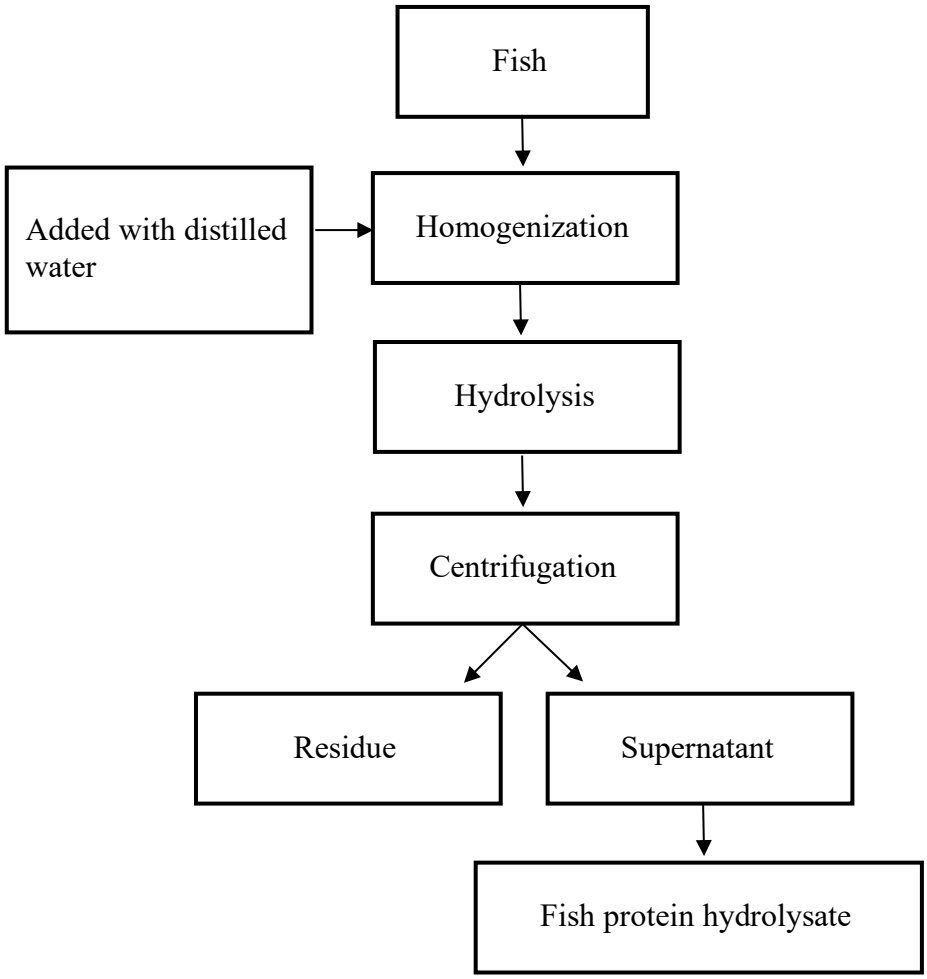

Figure 3.3 The principle of fish protein hydrolysate production

The process of producing FPH commences with the preparation of the raw materials. Water, chemicals, or enzymes are introduced into the raw materials to facilitate chemical or enzymatic hydrolysis. Once the desired degree of hydrolysis has been attained, the process must be halted through chemical or thermal treatments, depending on the specific hydrolysis method employed. Following the cessation of hydrolysis, the separation of solids from liquids within the protein mixture occurs, most commonly using centrifugation techniques. Subsequently, the liquid fraction, enriched with peptides, undergoes drying and is stored at a temperature of 4°C or lower (Petrova et al., 2018).

In producing Fish Protein Hydrolysate (FPH), various hydrolysis methods are employed to break fish proteins into simpler peptides. The production of FPH using different hydrolysis methods can be seen in Table 3.2.

Table 3.2 Production of FPH with different hydrolysis methods

Species	Used part	Treatment / Enzyme used	References
Cynoglossus macrosomic	Meat	Pepsin enzyme	Bhingarde *et al.,* 2018
Rastrelliger kanagurta	Fish bones	Pepsin enzyme	Sheriff *et al.,* 2014
Salmon	Fish bones	Papain enzyme	Slizyte *et al.,* 2016
Cod	Meat	Bromelain enzyme	Himonides *et al.,* 2011
Oreochromis niloticus	Meat, bones, fins, and heat	Alcalase enzyme from *Bacillus licheniformis*	Roslan *et al.,* 2014
Merluccius productus	Meat	Flavourzyme from *Aspergillus oryzae*	Jenkelunas *et al.,* 2018
Eubleekeria splendens	Meat, bones, fins, head, and gills	HCl	Wisuthiphae dan Kongruang, 2015
Thunnus albacores	Viscera	Protamex enzyme from *Bacillus sp.*	Pezeshk *et al.,* 2017
Upeneus teniopterus	Meat, head, gills, viscera, bones, and fins	Formic acid	Narikimelli *et al.,* 2019
Monopterus sp.	Meat	Alcalase enzyme *Bacillus licheniformis*	Halim dan Sarbon, 2016
Paralichthys olivaceus	Meat, head, gills, viscera, bones, and fins	Alcalase and flavourzyme	Zheng *et al*., 2012
Oreochromis niloticus	Meat	Alcalase enzyme	Yarnpakdee *et al*., 2012
Patinopecten yessoensis	Meat	Papain enzyme	Zhou *et al*., 2012
Snapper	Scales	Flavourzyme	Lin *et al.,* 2020
Channa striata	Meat	Alcalase enzyme *Bacillus licheniformis*	Zakaria dan Sarbon, 2018
Oreochromis niloticus	Meat, head, gills, viscera, bones, and fins	Alcalse enzyme	Alvares *et al*., 2018
Sardine pilchardus	Meat	Alcalase enzyme	Moreno *et al.,* 2016
Horse mackerel	Meat	Trypsin enzyme	Moreno *et al.,* 2014

Species	Used part	Treatment / Enzyme used	References
Sardinelle	Meat	*B. Subtilis* fermentation	Jemil *et al.,* 2014
Halibut	Meat	Protamex enzyme	Yang *et al.,* 2015

3.3.3 The Utilization and Benefits of Fish Protein Hydrolysate

Fish Protein Hydrolysate (FPH) can be added to dietary supplements in the food industry. In the pharmaceutical industry, it is used to produce dermatological products such as facial cleansers and skin moisturizers. Additionally, FPH can function as an emulsifying agent. Its current applications include adding the necessary nutrients to fermentation systems to support microorganisms in producing primary and secondary metabolites optimally, making it a valuable tool in biotechnology development for industrial purposes and its incorporation in biopharmaceuticals (Pasupuleti and Demain, 2010). The utilization of FPH over the decades is illustrated in Figure 3.4.

(Source: Pasupuleti & Demain, 2010)

Figure 3.4 The utilization of fish protein hydrolysate

Peptides resulting from protein hydrolysis are in high demand in the food and pharmaceutical industries. Fish Protein Hydrolysate (FPH) is an ideal protein source for human nutrition, given its well-balanced amino acid composition and favorable impact on gastrointestinal absorption. Recent research has illuminated that FPH exerts physiological effects beyond providing amino acids and energy. These studies indicate that FPH is an antihypertensive, antioxidant, and immunomodulator and can lower plasma cholesterol and triglyceride levels (Nobile et al., 2016). FPH comprises peptides rich in amino acids, and its bioactive properties include antioxidant, antimicrobial, immunomodulatory, antihypertensive, and mineral and hormone-regulating capabilities (Wangkheirakpam et al., 2019). The various functional properties of FPH are outlined in Table 3.3.

Table 3.3 The various functional properties of fish protein hydrolysate

Sources	Hydrolysis method	Bioactive compounds	Function	References
Engraulis ringens	Protease enzyme	Short peptides	Growth	Costa *et al.,* 2020
Sardinelle	*B. Subtilis* fermentation	Bioactive Peptide	Antibacterial and antioxidant	Jemil *et al.,* 2014
Zosterissessor ophiocephalus	Protease enzyme	Bioactive Peptide	ACE-inhibitor	Nasri *et al*., 2013
Horse mackerel	Trypsin enzyme	Bioactive Peptide	Antioxidant	Moreno *et al.,* 2014
Capros aper Linnaeus	Papain enzyme	peptides	Antihypertensive	Hayes *et al*., 2016
Sardine pilchardus	Alcalase enzyme	Bioactive Peptide	Emulsifier	Moreno *et al.,* 2016
Pacific hake	Flavourzyme enzyme	Amino acids and peptides	Cryoprotectant	Jenkelunas *et al.,* 2018
Zosterissessor ophiocephalus	Protease enzyme	Peptides and amino acids	Antioxidative	Nasri *et al*., 2013
Oyster	Bromelain, trypsin, and papain enzyme	Peptides	Immunomodulatory	Bingna *et al*., 2013
Cod	Protamax enzyme	Bioactive peptide	ACE-inhibitor	Ween *et al.,* 2017
Halibut	Protamax enzyme	Bioactive Peptide	Anti-allergy	Yang *et al.,* 2015

The relatively high protein content in fish can be used as a protein source to address the issue of protein inadequacy. Protein plays a crucial role in tissue regeneration during the growth phase of children and helps prevent malnutrition and protein-energy malnutrition (Asare et al., 2018). Protein hydrolysates have evolved as promising nutritional supplements. Fish protein hydrolysate is used in clinical conditions, weight loss, and malnutrition (Nesse et al., 2011).

3.4 Fishery By-Products

In several developing countries, the impact of fishery industry waste has become a severe problem. Fishery by-products, such as internal organs, skin, scales, muscles, heads, and bones, obtained from 70% of the raw material, can contaminate the environment if not correctly handled (Cilbiz and Hanol, 2015). Most fishery industries only utilize about 40% of the fish, which is the flesh, while the remaining 50%-60% of the total fish weight is considered fishery by-products (Utomo et al., 2014). Fishery by-products can be classified into several types, including 1) waste from non-target catch; 2) waste from processing leftovers, including offal, fins, heads, trimmings, skin, bones, and frames; 3) waste due to overfishing; and 4) waste during distribution (spoiled fish) (Irianto et al., 2014).

3.4.1 Availability of Fishery By-Products

Management and processing of by-products are a priority to prevent environmental issues. The food industry generates over 48 million tons of products annually. Fishery by-products account for 215,000 tons (0.4% of the total waste generated). Due to their significant functional value, fishery by-products contain numerous valuable compounds across various sectors. However, these fishery by-products still need to be utilized (Penven et al., 2011). Every year, a substantial amount of marine fish catch is used as raw material in the seafood industry, resulting in approximately 100,000 tons of fishery by-products. These by-products are often discarded or sold as low-value products despite containing several components of protein and essential amino acids (Chamcheun, 2015).

Over the past half-decade, per capita fish consumption has witnessed a consistent upward trajectory. According to statistical data from the Ministry of Marine Affairs and Fisheries of the Republic of Indonesia (2014), fish consumption surged from 29.08 kg per capita in 2009 to 35.14 kg per capita in 2013, marking a substantial growth of 20.83%. Concurrently, the total volume of capture fishery production in 2010 stood at 5,384,418 tons and continued its rapid ascent until 2013, eventually reaching 5,863,170 tons. This promising trend is closely linked to the potential for generating fishery by-products (Rohmah et al., 2015).

3.4.2 Nutritional Content of Fishery By-Products

Proper handling of fishery by-products can yield substantial economic benefits. These by-products still retain significant levels of protein. Generally, fish by-products encompass essential elements like Nitrogen, Phosphorus, Potassium, and others, which are fundamental constituents of protein and fat formation (Lepongbulan et al., 2017). Upon evaluating these components, the potential exists to transform fishery by-products into fish protein hydrolysate products (Utomo et al., 2014). Notably, waste generated by the fishery industry contains notable quantities of protein and unsaturated fats. The protein content within these by-products stands as a valuable natural protein source. These fishery by-products can be employed as raw materials to produce hydrolysate protein, concurrently addressing environmental concerns (Nurhayati et al., 2014). By repurposing fishery by-products for the creation of products such as fish protein hydrolysate, the dual benefits of curbing environmental pollution and augmenting the value of fishery outputs can be achieved (Atma, 2016). The nutritional composition of fishery by-products applicable for fish protein hydrolysate production is illustrated in Table 3.4.

Table 3.4 Nutritional content of fishery by-products

Raw materials	Protein	Fat	Ash	Moisture	Reference
Post rigor fish waste (*Cyprinus carpio)*	18,4%	3,52%	1,07%	76,7%	Saputra dan Nurhayati, 2016
Head (*Chlorus sordidus)*	20,37%	3,92%	4,19%	71,68%	Prihanto *et al.,* 2019

Raw materials	Protein	Fat	Ash	Moisture	Reference
Waste meat, head, fins, and bones (*Eubleekeria splendens)*	15,69%	2,56%	3,51%	79,67%	Wisuthiphae dan Kongruang, 2015
Viscera (*Thunnus albacares)*	19,11%	7,4%	0,71%	72,77%	Pezeshk *et al.,* 2017
Viscera (*Acipenser persicus)*	15,48%	15,68%	5,76%	39%	Ovissipour *et al.,* 2010
Head (*Loligo* sp.)	57,27%	1,67%	6,31%	28,42%	Sukkhown *et al*., 2017
Viscera (*Oncorhynchus mykiss)*	32%	55%	-	-	Wald *et al.,* 2016

3.5 Protein Hydrolysate Derived from Fishery By-Products

The increasing consumption of fish signifies a rise in the volume of fishery by-products that will be generated. Fishery by-products comprise various fish species or secondary products with low commercial value, accounting for over 50% of the fish body, including fins, gills, heads, skin, and viscera, which are discarded as waste (Caruso, 2016). According to Utomo et al. (2014), fishery by-products hold high economic value when appropriately managed. These by-products still contain significant levels of protein and amino acids. Generally, fish by-products contain nitrogen, potassium, phosphorus, and others that constitute protein and fat building blocks. Based on this content, these by-products have the potential to be developed into fish protein hydrolysate products. Using Fish Protein Hydrolysate (FPH) often serves as a base material for food additives and dietary supplements to enhance protein intake and prevent malnutrition.

3.5.1 Protein and Amino Acid Content of FPH Derived from Fishery By-Product

A substantial amount of fishery by-products is discarded or used for low-value products. However, there is significant potential in well-managed fishery by-products. There is a great potential to reduce these by-products into value-added and human-consumable products. Through enzymatic technology, it is highly feasible to produce quality food products. One such application is creating Fish Protein Hydrolysate (FPH) using fishery by-products as raw materials (Muzaifa et al., 2012).

Leveraging products derived from fishery by-products represents a significant production avenue for the fish processing and seafood industries, unlocking opportunities for increased revenue and enhanced nutritional offerings. A prevailing strategy for by-product utilization entails transforming underutilized fish components into fish protein hydrolysates. The exploration of protein sourced from by-products traces its roots back to the 1960s, primarily driven by the mission to furnish a cost-effective and nourishing source of fish protein. Fishery by-product-derived fish protein hydrolysates (FPH) manifest exceptional functional and nutritional worth, particularly in their protein and amino acid content (Nurdiani et al., 2017).

FPH products derived from fishery by-products contain high protein and nutritional value due to their essential amino acid composition. Protein from these by-products presents a compelling and promising alternative due to its suitable physiological functions for application in health and pharmaceutical products (Roslan et al., 2014). The characteristics of hydrolysates directly influence their functional properties and usage in food products. Fish protein hydrolysates from fishery by-products have demonstrated potential for application in food and pharmaceuticals. Protein hydrolysates produced from results using enzymes like alcalase, flavourzyme, protamex, pepsin, and trypsin can become a primary protein source of good quality (Muzaifa et al., 2012). A comparison of protein content, degree of hydrolysis, and amino acid composition of hydrolyzed protein from various fishery by-product wastes can be found in Table 3.5.

Table 3.5 Comparison of Protein Content and Degree of Hydrolysis of FPH Derived from Fishery By-Product

Fish	Fish part used	Hydrolysis method	Moisture	Protein	DH %	Reference
Freshwater						
Cyprinus carpio	Post rigor fish waste meat	Papain enzyme	- (*Dry basis)*	5,20%	-	Saputra dan Nurhayati, 2016
Oreochromis niloticus	Viscera and bones	Alcalase enzyme	- (*Dry basis)*	58,48%	41,66%	Silva *et al.,* 2014
Cyprinus carpio	Egg	Trypsin enzyme	4,59%	73,63%	-	Chalamaiah *et al.,* 2015
Oreochromis niloticus	Head, bones, and viscera	Protamex enzyme	3,5%	69,8%	22,1%	Robert *et al.,* 2015
Oreochromis niloticus	Red flesh, skin, bones, and fin	Alcalase enzyme	7,1%	80,6%	-	Alvares *et al*., 2018
Ictalurus punctatus	Skin	Alcalase enzyme	6,10%	39,73%	-	Yin *et al.,* 2010

Fish	Fish part used	Hydrolysis method	Moisture	Protein	DH %	Reference
Sea water						
Oncorhynchus mykiss	Viscera	Pepsin enzyme	- (*Dry basis)*	68%	30%	Wald *et al.,* 2016
Eubleekeria splendens	Meat, head, fins, bones	Papain enzyme	- (*Dry basis)*	72,10%	35,56%	Wisuthiphae dan Kongruang, 2015
Paralichthys olivaceus	Red flesh, skin, bones, and fins	Alcalase enzyme	- (*Dry basis)*	46,54%	-	Zheng *et al*., 2012

Fish	Fish part used	Hydrolysis method	Moisture	Protein	DH %	Reference
Sea water						
Chlorus sordidus	Head	Endogenous enzyme	4,19%	20,37%	30,65%	Prihanto *et al.,* 2019
Acipenser persicus	Viscera	Alcalase enzyme	4,45%	65,82%	46,13%	Ovissipour *et al.,* 2010
Sepiida sp.	Viscera	Endogenous enzyme from *M. Mustelus* viscera	4,45%	85,18%	12,51%	Cudenne *et al*., 2015
Loligo sp.	Head	Flavourzyme	8,13%	76,42%	21,27%	Sukkhown *et al.,* 2017
Aphanopus carbo	Head, bones, skin and viscera	Protamex enzyme	3,16%	77,52%	-	Batista *et al.,* 2010

The highest protein content of Fish Protein Hydrolysate (FPH) derived from freshwater fish by-products, based on dry basis analysis, was obtained from the hydrolysis of viscera and bones of *Oreochromis niloticus* using the enzyme alcalase, with a content of 58.48% (Silva et al., 2014). The lowest protein content was derived from the hydrolysis of post-rigor phase flesh of *Cyprinus carpio* using the enzyme pepsin, with a content of 5.20% (Saputra and Nurhayati, 2016). The highest protein content of FPH from all freshwater fish by-products was obtained from the hydrolysis of red flesh, skin, skeleton, and fins of *Oreochromis niloticus* using the enzyme alcalase, with a content of 80.6%. In seawater fish by-products, the highest protein content, based on dry basis analysis, was obtained from the hydrolysis of residual flesh, head, fins, and bones of *Eubleekeria splendens* using the enzyme papain, with a content of 72.10% (Wisuthipae and Kongruan, 2015). The lowest protein content was derived from the hydrolysis of red flesh, skin, head, bones, and fins of *Paralichthys olivaceus* using the enzyme alkalase, with a content of 45.54% (Zheng et al., 2012). The highest protein content from all marine fish by-products was obtained from the hydrolysis of *Sepiid*a sp. viscera using the enzyme alcalase, with a content of 85.18% (Cuddene et al., 2015), and the lowest from the hydrolysis of *Chlorus sordidus* head using the endogenous enzyme of the fish, with a content of 20.37% (Prihanto et al., 2019).

The variation in protein content of FPH is due to differences in fish species, fish parts, types of enzymes used, and applied hydrolysis conditions (Jamil et al., 2016). Saputra and Nurhayati (2016) state that the raw materials for FPH production from viscera waste contain only 0.95% protein, which is low and affects the final protein value of FPH. The selection of enzymes in FPH production is crucial, as different enzymes have varying specifications and yield other product qualities (Hou et al., 2011). Enzymatic hydrolysis is preferred over chemical hydrolysis due to benefits such as stable reaction conditions, fewer unwanted products, and higher product quality. The highest protein values are generally obtained from the hydrolysis of fish viscera. However, per Regulation (EU) No. 1069/2013 of the European Parliament and Council, fish viscera fall into category 3 and cannot be used as human food due to potential disease risks.

According to Wijayanti et al. (2015), protein hydrolysis involves adding water, causing the water volume to exceed the substrate volume. Water use increases the contact area between the enzyme and the substrate, potentially leading to more significant hydrolysate products within a specific time frame. The water content produced in various studies varies due to different sample types and evaporation temperatures, where the samples lose a significant amount of moisture. Witono et al. (2014) explain that this variability might be due to differences in enzyme types, incubation times, and analysis methods used for estimation. The water content in FPH can influence the final protein value of the hydrolysate.

The degree of hydrolysis (DH) indicates the protease's proficiency in degrading proteins, and it compares the quantity of amino nitrogen and the total nitrogen present. A heightened DH signifies a more efficient hydrolytic process in which peptide bonds are effectively cleaved (Nurilmala et al., 2018). Nurhayati et al. (2013) point out that a substantial DH results from an increase in peptides and amino acids that are soluble in trichloroacetic acid (TCA), resulting from the cleavage of peptide bonds during protein hydrolysis. In the context of Thunnus albacares head protein hydrolysis, Ovissipour et al. (2010) highlight that the choice of enzyme type can lead to variations in DH values. The Alcalase enzyme, renowned for its remarkable efficiency, can generate hydrolysates with a notably high DH within a relatively short timeframe and under stable conditions. This enzyme yields fish protein hydrolysates boasting excellent nutritional and functional attributes, well-suited to fulfilling human protein needs (Roslan et al., 2014). Kurniawan et al. (2012) further elaborate that the DH value is influenced by the quantity of peptide and amino acid compounds produced through enzyme-driven protein degradation. Consequently, a heightened degree of protein breakdown into short-chain compounds, encompassing amino compounds, translates to a more excellent DH value in measurements comparing amino nitrogen to total nitrogen.

The essential amino acid content obtained from enzymatic hydrolysis of various fish by-products is presented in Table 3.6. For freshwater fish by-products, the highest values for isoleucine and arginine were obtained from the hydrolysis of *Ictalurus punctatus* skin using the enzyme alcalase, with contents of 4.78% and 8.43%, respectively (Yin et al., 2010). The highest values for leucine, methionine, phenylalanine, threonine, tryptophan, valine, lysine, and histidine were obtained from the hydrolysis of red flesh, skin, skeleton, and fins of *Orechormis niloticus*, with contents of 7.56%, 6.61%, 9.17%, 12.6%, 5.72%, 8.28%, 7.79%, and 17.87%, respectively (Alvares et al., 2018). The lowest values for essential amino acids in freshwater fish by-products, including isoleucine, leucine, methionine, phenylalanine, threonine, arginine, and histidine, were obtained from the hydrolysis of *Pangasius* sp. head and bones using the enzyme papain, with contents of 1.84%, 2.72%, 0.74%, 2.03%, 1.05%, 1.55%, and 0.49%, respectively (Nirmala et al., 2018). Tryptophan, valine, and lysine were not found in the digestive tract hydrolysis of *Orechormis niloticus* using the enzyme alcalase (Silva et al., 2014).

Table 3.6. Comparison of amino acid content in FPH derived from various fishery by-products

Reference		Yin *et al.,* 2010	Silva *et al.,* 2014	Roslan *et al.,* 2014	Robert *et al.,* 2015	Nirmala *et al.,* 2018	Alvares *et al*., 2018
Raw Material (Freshwater Fish)		Skin from *Ictalurus punctatus*	Viscera from *Oreochromis niloticus*	Head from *Oreochromis niloticus*	Head, bones, and viscera from *Oreochromis niloticus*	Head and bones from *Pangasius* sp.	Red flesh, skin, bones, and fins from *Oreochromis niloticus*
Hydrolysis method		Alcalase enzyme	Alcalase enzyme	Enzymatic Hydrolysis	Protamex Enzyme	Papain Enzyme	Alcalase Enzyme
Amino acid (Total Amino %)	Arginine	8,43	2,2	3,22	4,91	1,55	7,40
	Histidine	2,49	9,1	1,16	1,53	0,49	17,87
	Isoleucine	4,78	3,8	2,14	2,66	1,84	4,63
	Leucin	7,38	6,7	3,92	4,68	2,72	7,50
	Methionine	1,35	3,2	1,48	1,71	0,75	6,61
	Phenylalanine	4,69	4,2	2,23	2,64	2,03	9,17
	Threonine	3,91	4,6	2,44	3,03	1,05	12,6
	Tryptophan	-	4,2	-	0,66	-	5,72
	Valin	5,39	-	2,46	3,14	1,95	8,28
	Lysine	6,05	-	-	5,09	2,30	7,79
	Alanine	6,56	9,3	4,56	5,02	2,30	4,05
	Asparagine	-	-	-	-	-	-
	Cysteine	-	1,7	0,08	0,53	-	-
	Glutamine	-	-	-	-	-	-
	Glutamic acid	11,80	16,7	7,96	9,57	6,27	42,49
	Glycine	11,96	15,0	6,78	7,00	4,04	8,46
	Proline	5,68	8,5	4,18	4,82	-	-
	Serine	3,40	4,8	2,33	3,01	1,21	12,28
	Tyrosine	3,74	2,9	1,58	1,94	0,84	-
	Aspartate	8,87	10,4	4,58	6,26	3,27	30,05
	Hydroxylysine	0,66	-	-	-	-	-
	Hydroxyproline	3,00	-	-	2,4	-	-

Reference		Pezeshk *et al.,* 2017	Swanepoel dan Goosen, 2018	Mutamimah *et al.,* 2018	Prihanto *et al.,* 2019	Lin *et al.,* 2020	Jafarpour *al.,* 2020	Ovissipour *et al.,* 2010
Raw Material (Freshwater Fish)		Viscera from *Thunnus albacares*	Head from *Lophius vomerinus*	Eye from *Thunnus* sp.	Head from *Chlorurus sordidus*	Skin from *Lutjanus sp.*	Bones from *Gadus morhua*	Viscera from *Acipenser persicus*
Hydrolysis method		Protamex enzyme	Alcalase enzyme	Papain enzyme	Endogenous enzyme	Flavourzyme	Alcalase enzyme	Alcalase enzyme
Amino acid (Total Amino %)	Arginine	8,54	6,54	2,90	6,12	7,04	0,55	7,28
	Histidine	1,45	1,95	3,50	2,85	1,05	0,26	2,08
	Isoleucine	8,35	3,46	3,01	4,34	1,07	0,81	3,8
	Leucin	2,35	7,77	8,17	8,48	2,19	0,98	7,13
	Methionine	8,91	4,46	4,50	-	2,09	0,42	10,3
	Phenylalanine	5,36	3,98	2,80	5,53	2,05	0,63	3,14
	Threonine	1,24	4,44	3,83	6,80	2,10	0,54	3,5
	Tryptophan	-	-	-	-	-	-	-
	Valin	3,04	4,66	1,80	5,38	2,36	1,17	5,79
	Lysine	0,49	10,17		8,30	3,06	0,67	6,8
	Alanine	9,14	6,52	2,67	7,41	8,88	0,66	6,3
	Asparagine	-	-	-	-	-	-	-
	Cysteine	-	-	1,60	-	0,18	-	-
	Glutamine	-	-	-	-	-	-	-
	Glutamic acid	12,69	15,42	19,70	14,43	9,66	-	13,7
	Glycine	13,75	6,60	6,27	7,63	21,19	1,29	5,4
	Proline	-	5,04	6,67	5,64	13,41	0,75	3,46
	Serine	5,22	4,85	3,06	1,81	2,96	0,49	4,2
	Tyrosine	2,78	3,76	3,01	4,22	1,09	0,22	2,34
	Aspartate	10,54	10,39	7,26	11,06	4,79	-	8,3
	Hydroxylysine	-	-	-	-	-	-	-
	Hydroxyproline	-	-	-	-	-	-	-

Among non-essential amino acids essential for children due to growth needs, arginine and histidine are included. The highest values for isoleucine in marine fish by-products were obtained from the hydrolysis of *Thunnus albacares* viscera using the enzyme protamex, with a content of 8.35% (Pezeshk et al., 2017). The highest values for leucine, phenylalanine, and threonine in marine fish by-products were obtained from the hydrolysis of *Chlorurus sordidus* head using the endogenous enzyme of the fish, with contents of 8.84%, 5.53%, and 6.80%, respectively (Prihanto et al., 2019). The highest values for methionine and valine in marine fish by-products were obtained from the hydrolysis of *Acipenser persicus* viscera using the enzyme alcalase, with contents of 10.3% and 5.79%, respectively (Ovissipour et al., 2010). The highest lysine content in marine fish by-products was obtained from the hydrolysis of *Lophius vomerinus* head using the enzyme alcalase, with a content of 10.17% (Swanepoel and Goosen, 2018). The lowest values for essential amino acids in marine fish by-products, including isoleucine, leucine, phenylalanine, threonine, and valine, were obtained from the hydrolysis of *Gadus morhua* bones using the enzyme alcalase, with contents of 0.81%, 0.98%, 0.63%, and 1.17%, respectively (Jafarpour et al., 2020). The lowest lysine value was obtained from the hydrolysis of *Thunnus albacares* viscera using the enzyme protamex, with a content of 0.49% (Pezeshk et al., 2017). Therefore, the highest amino acid values among all fishery waste come from the hydrolysis of red flesh, skin, bones, and fins of *Orechormis niloticus* (Alvares et al., 2018), while the lowest values are from the hydrolysis of *Gadus morhua* bones using the enzyme alcalase (Jafarpour et al., 2020).

Assessing amino acid content can be instrumental in gauging the quality of produced FPH, particularly when examining the ratios of amino acids present within the protein (Prihanto et al., 2019). Amino acid compositions exhibit variability due to intrinsic factors such as species, gender, age, and extrinsic factors linked to the environment. The specific arrangement of amino acids in hydrolysates is contingent on the types of enzymes employed and the conditions governing the hydrolysis process. The quantity of essential amino acids found in each hydrolysate can fluctuate based on several factors, including fish species, the sections of fish utilized, enzyme varieties used, and the conditions governing hydrolysis. The amino acid composition represents a critical nutritional facet, and the quantity of amino acids found in FPH determines its quality. In specific investigations, chemical values are computed based on protein and amino acid content (Hao et al., 2011). The presence of essential amino acids within the hydrolysates signifies the potential of these compounds to offer valuable nutritional benefits for health (Prihanto et al., 2019).

Protein hydrolysates are produced from various protein sources through processes like heating or enzyme addition. Hydrolyzed proteins are easier to digest and absorb than intact proteins, as they increase plasma amino acid levels, promoting muscle synthesis. Due to their high protein and amino acid content, fish protein hydrolysates have emerged as promising nutritional supplements. They meet nutritional needs and prevent malnutrition in various clinical conditions (Nesse et al., 2011).

3.5.2 Method of Producing FPH from Fishery By-Products for PEM in Children

Based on Table 3.13 and Table 3.14, the treatment used to produce the best protein and amino acids is enzymatic. Enzymes such as alcalase, papain, protamex, flavourzyme, pepsin, trypsin, endogenous enzymes from fish, and protease enzymes from the digestive tract of *M. mustelus* are used. According to Witono et al. (2014), FPH can be produced chemically and enzymatically. Enzymatic hydrolysis is the safest and most advantageous method compared to chemical processes, as it generates free amino acids and short-chain peptides with varying lengths. These products have a wider range of uses in the food industry. Protease enzymes are capable of hydrolyzing peptide bonds in proteins. Factors influencing the hydrolysis process include enzyme-substrate ratio, different types of enzymes, pH, time, and temperature.

Based on the comparison of FPH from freshwater and marine fishery by-products to produce FPH with protein and amino acid quality suitable for human consumption, the use of FPH from the hydrolysis of fresh fishery by-products of *Oreochromis niloticus* using alcalase enzyme as conducted by Alvares et al., 2018 is recommended. This study is the only application of FPH from fishery by-products tested on humans. Alvares et al. (2018) state that FPH from fishery by-products contains active peptides that can potentially treat cardiovascular diseases. This study was tested on 9 individuals who consumed 5 grams of FPH. From the results of this study, changes in FMD (Flow-Mediated Dilation) values did not significantly affect the subjects. However, producing FPH from fresh fishery by-products of *Oreochromis niloticus* using alcalase enzyme can be used as a natural source of protein and amino acids safe for human consumption. Considering the results of previous research, to produce FPH safe for human consumption and promising as a source of protein and amino acids, the method from the study by Alvares et al. (2018) can be utilized as the best method for FPH production to prevent protein-energy malnutrition in children. The process of producing FPH can be seen in Figure 3.5.

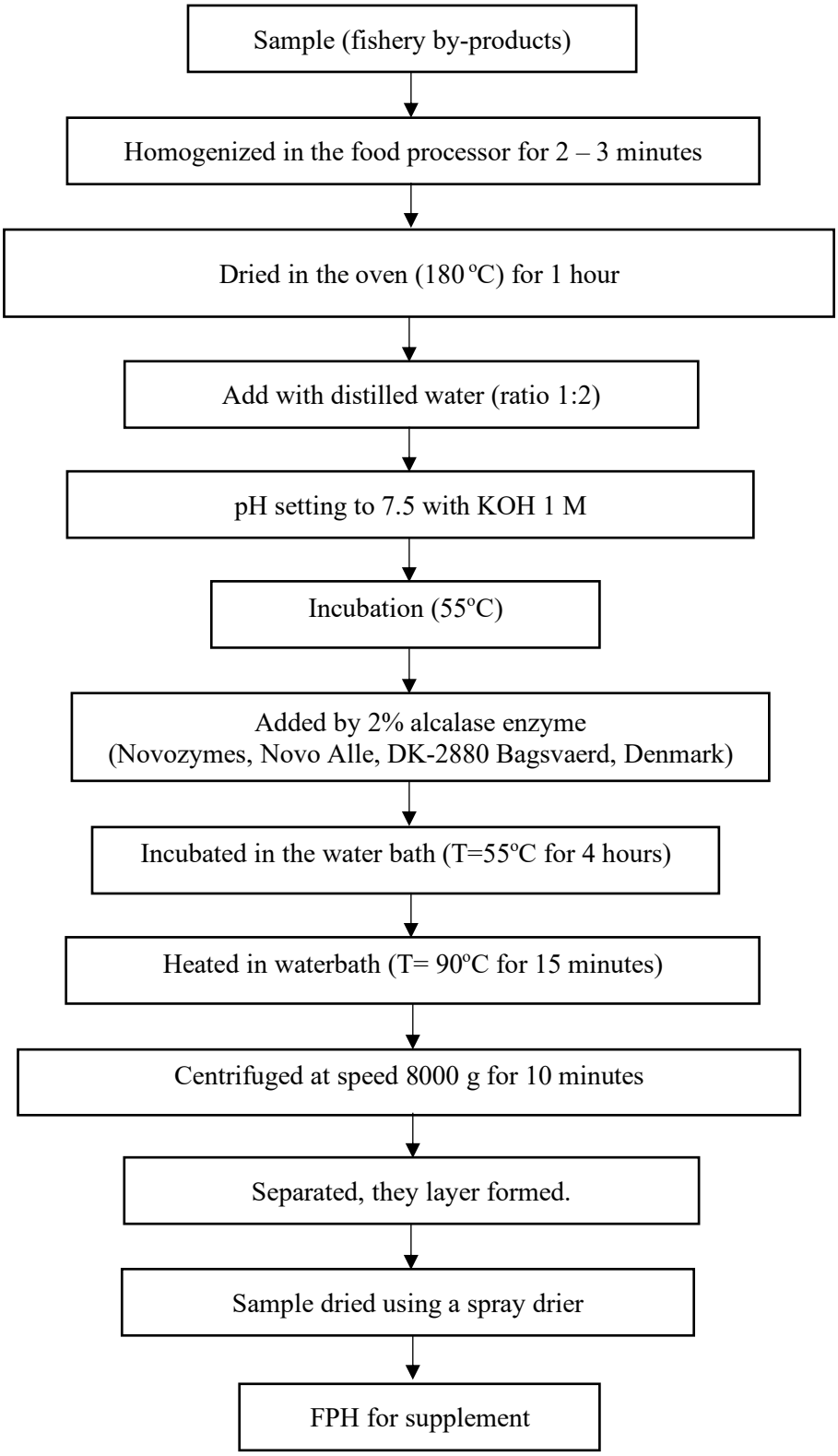

Figure 3.5 The best production method of FPH derived from fishery by-products as human consumption

3.6 Potential of FPH Derived from Fishery By-Product for PEM in Children

3.6.1 Application of FPH for Protein-Energy Malnutrition in Children

Research on applying FPH to address protein-energy malnutrition has been extensively conducted. Some previous studies include the utilization of hydrolysates from catfish *(Clarias gariepinus)* meat (Aprilia and Hati, 2016), *Micromesistius poutassou* meat (Nobile et al., 2016), Salmon salar meat (Nesse et al., 2011), and Salmon salar (Nesse et al., 2014). According to Aprilia and Hati (2016), nutritional deficiencies remain a problem in Indonesia, and one of the conditions is protein-energy malnutrition. In Indonesia, addressing this issue often involves providing infants with complementary foods known as "CFBI" (complementary foods for breastfeeding infants). CFBI plays a crucial role in addressing such issues in children. Typically, CFBI is enriched with plant-based proteins, although animal proteins offer better amino acid profiles and digestibility. However, animal proteins increase the risk of allergies in infants. Catfish *(Clarias gariepinus)* can serve as an alternative source of animal protein due to its abundant availability and affordability. This study employed a formulation of infant CFBI porridge enriched with hydrolyzed enzyme protein from catfish using crude papain enzyme (sample A) and commercial papain enzyme (sample B). Overall and partial preference tests were conducted involving 23 mother panellists, followed by sensory acceptability tests by 9 infants using facial expression scales. The nutritional values of sample A were 2.73% protein, 4.87% fat, and 89.28% carbohydrates, while sample B had values of 2.57% protein, 3.49% fat, and 76.08% carbohydrates. The relatively low protein content and high carbohydrates were attributed to CFBI being prepared with a mixture of skim milk (75%) and hydrolysate (25%) to remove the bitter taste from the papain enzyme in the hydrolysate. Sensory evaluation results indicated that mother panelists and infants accepted the formulated sample B product, while the sample A product did not. Infant porridge with 25% added sample B could be developed as an alternative CFBI product with promising protein sources. However, further research is required to optimize its content for higher protein content and consumer acceptability.

Another study by Nesse et al. (2011) focused on applying fish protein hydrolysates using salmon *(Salmon salar)* as the raw material. This study utilized "Amizate," a commercial fish protein hydrolysate derived from salmon. This FPH exhibited advantages such as high di- and tri-peptide content and essential and non-essential amino acids and micronutrients. The fish protein hydrolysate is a valuable nutritional supplement due to its readily absorbable bioactive compounds that support various metabolic activities. Amizate was evaluated in children with protein-energy malnutrition (438 children aged 6-8 years) categorized into Class I and Class II. Immunoglobulin levels, the CD4/CD8 ratio, and hemoglobin were measured as parameters. Over a 4-month research period, adding Amizate showed no significant impact on the immunological parameters of protein-energy malnourished subjects.

In another investigation conducted by Nobile et al. (2016), the objective was to evaluate the impact of Slimpro, a commercial fish protein hydrolysate derived from Micromesistius poutassou, on the stimulation of cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) secretion in individuals dealing with protein-energy malnutrition. The selection of fish protein hydrolysate as a protein source was based on its well-balanced amino acid composition and

favorable gastrointestinal absorption effects. This clinical study involved 120 participants, 25% males and 75% females. Fish protein hydrolysate was administered in food supplements at two different dosages (1.4 g and 2.8 g). Various parameters were assessed, including body weight, fat mass, body water mass, body circumference measurements (thigh, waist, hip), and the levels of CCK and GLP-1 in the bloodstream. Measurements were taken both before and after the administration of FPH at two-time points (45 and 90 days). Monitoring CCK and GLP-1 levels was particularly relevant due to their pivotal role in appetite regulation. The results demonstrated enhancements in body composition, elevated concentrations of CCK and GLP-1 in the blood, and noteworthy improvements in preventing malnutrition, with both the 1.4 g and 2.8 g FPH doses proving effective in achieving these outcomes.

Nesse et al. (2014) extended their previous research (Nesse et al., 2011) using Amizate, a high-quality fish protein hydrolysate derived from Atlantic salmon *(Salmo salar)* and hydrolyzed using endogenous enzymes. This FPH contained a majority of amino acids and short-chain peptides. A clinical trial was conducted to study the effects of supplementing Amizate in 438 randomly chosen malnourished children. The children were subjected to three treatments: chocolate drink (control) and chocolate drink with added Amizate (3 g and 6 g). Results showed that the growth indicators (weight gain, height, and body mass index) increased without adverse effects due to Amizate supplementation. Routine biochemical analyses involving blood and urine samples revealed no abnormalities attributed to Amizate supplementation. This study demonstrated that consuming 3 g or 6 g of fish protein hydrolysate daily is safe and suitable for enhancing nutritional content in the diets of malnourished children. Earlier studies on the application of FPH to prevent protein-energy malnutrition can be seen in Table 3.7.

Table 3.7 Previous research on the application of FPH for PEM

Species	Product	Application	Parameters	References
Clarias gariepinus	FPH from catfish meat	Porridge	Oraganoleptic (liking and palatability) and nutritional value of the product	Aprilia dan Hati, 2016
Salmo salar	Amizate (Commercial FPH from salmon)	Chocolate	Immunoglobulin, ratio CD 4/CD8, and hemoglobin	Nesse *et al.,* 2011
Micromesistius poutassou	Slimpro (Commercial FPH from Blue Whiting)	Supplement	CCK and GLP-1	Nobile *et al.,* 2016
Salmo salar	Amizate (Commercial FPH from salmon)	Chocolate	Growth (weight gain, height, ınd body mass index), routine biochemicals analysis (blood ıd urine) and immunoglobulin, ratio CD 4/ CD8, and hemoglobin	Nesse *et al.,* 2014

Protein hydrolysate is a composite mixture containing intricate amino acids and peptides. It finds utility as an alternative protein source in food formulations tailored to fulfill specific nutritional requirements. For instance, hydrolyzed protein has been incorporated into protein supplements and formulations for infant and elderly nutrition. Moreover, supplementing with hydrolyzed protein proves advantageous in cases of malnutrition. Evidence suggests that hydrolyzed protein can enhance nitrogen absorption, particularly in individuals with gastrointestinal disorders and clinical conditions like malnutrition. Recent studies indicate hydrolyzed protein, enriched with di- and tri-peptides, is more readily digestible and absorbable than intact native protein. Amino acid absorption is also more efficient when derived from hydrolyzed protein than non-hydrolyzed protein, owing to the lower osmolarity of hydrolyzed protein (Nesse et al., 2011). Peptides originating from fish protein hydrolysates have garnered considerable attention in nutrition and pharmaceuticals. Recent research underscores the suitability of fish protein hydrolysates as protein sources for human food, given their well-balanced amino acid composition and favorable impact on gastrointestinal absorption. Consequently, they promise to enable malnourished individuals to meet nutritional requirements and avert clinical conditions (Irianto and Fawzya, 2018).

Protein-energy malnutrition remains a significant health burden in low-income and developing countries. Children are particularly vulnerable to undernutrition, as they undergo rapid growth and have high calorie and nutritional demands. Inadequate nutrition during childhood can have detrimental effects on growth and development. One approach to preventing malnutrition is supplementary feeding interventions that include balanced protein, amino acids, and energy supplementation (Nesse et al., 2011). Recent studies demonstrate that therapeutic foods designed for protein-energy malnutrition serve as effective protein and energy sources for patients. However, the protein content ranges from 10% to 16%, with the highest value obtained from a combination of tempeh protein with vegetable oil and skim milk, resulting in 16.9% protein (Komari and Lamid, 2012). The protein content of FPH derived from fishery by-products is outlined in Table 3.5. FPH content is considerably higher compared to plant-based protein sources. Fishery by-product protein content ranges from 15% to 80%, depending on fish species, fish part, enzyme type used, and hydrolysis conditions. The highest content was achieved through hydrolysis of red meat, skin, skeleton, and fins of *Oreochromis niloticus* fish using alcalase enzyme, resulting in 80.6% protein (Alvares et al., 2018), significantly higher than tempeh therapeutic food.

Considering these findings, applying FPH as a nutritional supplement is highly feasible due to its high nutritional content. Previously analyzed fish waste with protein and amino acid content offers excellent potential to address protein-energy malnutrition. A comparison of nutritional content between fishery by-product protein from tilapia fish *(Oreochromis niloticus)* (Alvares et al., 2018) and commercial FPH amizate, which has been studied for its effects on protein-energy malnutrition (Nesse et al., 2014), is provided. Nutritional content comparison between fishery by-product FPH and amizate can be seen in Table 3.8. Results show that protein and amino acid content in fishery by-product tilapia *(Oreochromis niloticus)* (Alvares et al., 2018) are significantly higher than in amizate FPH. Fishery by-product FPH contains 80.6% protein, whereas amizate includes 75%. For essential amino acids, fishery by-product FPH outperforms amizate, with the former containing 4.63% isoleucine, 7.50% leucine, 6.61%

methionine, 9.17% phenylalanine, 12.20% threonine, 8.28% valine, and 7.79% lysine. Essential non-essential amino acids, critical during child growth, include 17.87% histidine and 7.40% arginine. These values meet the daily protein and amino acid intake standards outlined in the WHO 2018 guidelines (refer to Table 3.9). For instance, the potential of fishery by-product protein and essential amino acids for children suffering from protein-energy malnutrition is illustrated in Table 3.9. This approach is based on daily protein and amino acid needs and the z-score values for 2-year-old children according to the 2010 Indonesian Ministry of Health of the Republic of Indonesia. As per Table 3.9, to meet the daily protein intake for a 2-year-old child, fishery by-product FPH from tilapia *(Oreochromis niloticus)* of 15 g for an average child and 30 g for a malnourished child are required. According to Aprilia and Hati (2016), adding FPH protein to food ingredients can enhance the protein content of those foods. Thus, fishery by-products have significant potential for application as supplements, food additives, and alternative nutritional sources for children with protein-energy malnutrition, as they offer favourable physiological effects for health.

Table 3.8 Comparison protein and amino essential amino acid of FPH derived from fishery by product and amizate

Reference	Alvares *et al.,* 2018	Nesse *et al.,* 2014 fish protein hydrolysate supplementation in malnourished children
Source	Fishery by-products from *Oreochormis niloticus*	Amizate (Commercial FPH from Atlantic salmon)
Protein	80,6 %	75 %
Moisture	7,1 %	7,5 %
Amino acids (TAA%)		
Histidine	17,87 %	1,6 %
Isoleucine	4,63 %	3,1 %
Leucin	7,50 %	5,5 %
Methionine	6,61 %	1,9 %
Phenylalanine	9,17 %	2,9 %
Threonine	12,60 %	3,1 %
Valin	8,28 %	4,1 %
Lysine	7,79 %	5,3 %
Tryptophan	5,72 %	0,8 %

Table 3.9 The potential of fishery by-products as a source of protein and essential amino acid

	Daily Nutrient Requirements for Normal Children (Normal)	Daily Nutrient Requirements for Normal Children (Aged 2 years and average weight 12,2 kg)	Nutritional Content of Nile Tilapia by Product Per 15 grams	Daily Nutrient Requirements for Malnourished Children	Daily Nutrient Requirements for Malnourished Children with Z-score SD -3 (Aged 2 years and average weight maximum 8,3 kg)	Nutritional Content of Nile Tilapia by Product Per 30 grams
Reference	(WHO, 2018)	(Ministry of Health RI, 2010)	(Alvares *et al.,* 2018)	(WHO, 2018)	(Ministry of Health RI, 2010)	(Alvares *et al.,* 2018)
Protein	0,79-0,95 g protein/ kg BW/ day	11,59 g/ hari	12,09 grams	2,82 g protein / kg BB/ day	23,40 g	24,18 grams
Amino Acids (TAA%)						
Histidine	27 mg/ kg BW/day	329,4 mg/day	2150 mg	66 mg/ kg BW/day	547,8 mg/day	4320 mg
Isoleucine	35 mg/ kg BW/day	427 mg/day	559 mg	95 mg/ kg BW/day	788,5 mg/day	1119 mg
Leucin	75 mg/ kg BW/day	910 mg/day	910 mg	198 mg/ kg BW/day	1.643,4 mg/day	1813 mg
Methionine	35 mg/ kg BW/day	427 mg/day	799 mg	88 mg/ kg BW/day	730,4 mg/day	1598 mg
Phenylalanine	73 mg/ kg BW/day	890,6 mg/day	1108 mg	177 mg/ kg BW/day	1.469,1 mg/day	2217 mg
Threonine	42 mg/ kg BW/day	512,4 mg/day	1108 mg	103 mg/ kg BW/day	854,9 mg/day	3046 mg
Valin	49 mg/ kg BW/day	597,8 mg/day	1523 mg	139 mg/ kg BW/day	1.153,7 mg/day	2002 mg
Lysine	73 mg/ kg BW/day	890,6 mg/day	941 mg	183 mg/ kg BW/day	1.518,9 mg/day	1883 mg
Tryptophan	12 mg/ kg BW/day	146,4 mg/day	691 mg	29 mg/ kg BW/day	240 mg/day	1383 mg

3.6.2 The Treatment of PEM Using FPH Derived from Fishery By-Products

Protein-energy malnutrition is characterized by insufficient energy and protein intake from external sources. According to Hoffer et al. (2001), weight loss and muscle mass reduction occur in patients with protein-energy malnutrition, partly due to decreased blood albumin levels (hypoalbuminemia). Individuals suffering from protein-energy malnutrition typically have albumin levels below 35 g/L of blood plasma—the failure to synthesize albumin results from insufficient amino acids and energy required for prealbumin formation. The study by Hassan and Hassan (2017) demonstrated that supplementing with whey protein containing 75% protein at a dosage of 1.2 g/kg body weight per day for 12 weeks increased albumin levels in hypoalbuminemia patients. The average albumin level rose from 34.1 ± 1.4 g/L to 40.8 ± 1.5 g/L of blood plasma. A similar outcome was also observed in the study by Nesse et al. (2014). This study used enzymatically hydrolyzed protein from Atlantic salmon (Salmon salar), which contained 70% protein content, primarily short peptides, and 60% free amino acids. The study involved 488 children with protein-energy malnutrition aged 6 to 8 years. The subjects were given chocolate drinks mixed with Atlantic salmon hydrolysate at 0 g (control), 3 g, and 6 g daily for 16 weeks. Albumin level reduction in the 3 g/day treatment (46.6 to 46 g/L of blood plasma) was significantly lower than in the control group (46.998 to 45.48 g/L of blood plasma). The research indicates that fish protein hydrolysate is highly suitable as a supplement for malnutrition patients.

Besides affecting blood albumin levels, malnutrition patients often experience reduced leptin and insulin synthesis, negatively impacting growth. According to Hawkes and Grimberg (2015), patients with chronic protein-energy malnutrition encounter reduced leptin secretion by adipose cells. Decreased leptin levels directly affect a decline in insulin-like growth factor-1 (IGF-1), a crucial growth regulator in humans. Lee et al. (2011) investigated the effects of hydrolysate supplementation on leptin secretion in embryonic rat fibroblast cells (3T3-L1 cells). The study revealed that rat cells given 1 mg/ml of hydrolyzed protein significantly increased leptin production by 175.9 ± 11.1%. This is attributed to the simplicity of peptides in hydrolyzed protein, containing numerous free amino acids, making it easier for cellular digestion. According to Nobile et al. (2011), supplementing with fish protein hydrolysate *(Micromesistius poutassou)* at 1.4 grams per day for 12 weeks in adults resulted in a higher Glucagon-like peptide-1 (GLP-1) value (26.8 ± 2.1 pmol/l) compared to isolated wheat protein supplementation (25.8 ± 2.1 pmol/l). Kaku (2020) suggests that Glucagon-like peptide-1 (GLP-1) plays a pivotal role in triggering insulin secretion by pancreatic beta cells. Increased GLP-1 secretion can stimulate insulin secretion, positively linked to growth.

Furthermore, the fishery by-product protein hydrolysate administration is believed to influence enhanced immune status and disease resistance in protein-energy malnutrition patients. Nesse et al. (2011) revealed that providing enzymatic hydrolysate from Atlantic salmon *(Salmon salar)* at 3 grams daily for 16 weeks increased the CD4/CD8 ratio from 1.41 ± 0.51 to 1.50 ± 0.55. A standard CD4/CD8 ratio is 1, where higher values indicate better immune status. Gerriet and Maclver (2014) suggest that decreased CD4 levels in the blood are indirectly caused by reduced leptin secretion in adipose tissue, leading to decreased metabolism in CD4-producing T cells. Additionally, decreased CD4 levels can result from reduced intracellular ATP in thymic

cells, leading to low viability and proliferation of CD4-producing T cells. A direct reduction in CD4 levels leads to decreased immune status in mammals.

3.6.3 Fishery By-Product Protein Hydrolysate as an Alternative Therapeutic Food for Protein-energy Malnutrition in Children

Nutritional management for malnourished toddlers often involves therapeutic foods, including those known as Ready to Use Therapeutic Food (RUTF). RUTF is typically lipid-based or in the form of dense paste. These medicinal foods are widely used in several African and Asian countries for in-hospital care, Therapeutic Feeding Centers (TFCs), Nutrition Rehabilitation Centers (NRCs), Community Therapeutic Centers (CTCs), and home-based recovery. However, RUTF is not currently utilized in Indonesia to treat severe malnutrition in hospitals and community health centers (Fadjarwati, 2012). RUTF can be produced on an industrial or household scale using peanuts as a base ingredient. It is a high-energy food specially designed for treating malnourished toddlers, available in various forms such as spreads and biscuits. RUTF products in spreads are smooth-textured, palatable, enriched with vitamins and minerals, and can be consumed anywhere, anytime, without cooking (Komari and Lamid, 2012).

A study conducted by Kustiani et al. (2017) compared crackers fortified with by-product protein from catfish heads and moringa leaf powder, which serves as a natural protein source. Moringa leaves are a plant-based protein source that has recently gained attention. Since 1988, the World Health Organization (WHO) has introduced moringa as an alternative food source to address nutritional problems (malnutrition). One approach to enhance the utilization of moringa leaves is to convert them into moringa leaf powder, which offers extended shelf life and can be easily incorporated into other food items. In this case, it's added to crackers to increase their nutritional content. The nutritional content of moringa leaf crackers and catfish head by-products can be observed in Table 3.10.

Table 3.10 The nutritional composition of crackers from moringa leaf and head of catfish waste

Composition	Crackers Moringa Leaf	Crackers Head of Catfish
Moisture	1,70 %	1,86 %
Ash	2,76 %	3,89 %
Fat	18,34 %	17,90 %
Protein	9,04 %	11,16 %
Carbohydrate	68,16 %	65,20 %
Protein digestibility	78,34%	77,48 %

Source: Kustiani *et al.*, 2017

From the research findings of Kustiani et al. (2017), the therapeutic food product derived from catfish head by-products applied to crackers presents advantages and disadvantages compared to moringa leaf crackers as a protein source. The protein content of catfish head by-products significantly influences the increase in protein content of the crackers. However, moringa leaf powder does not have a significant effect. Consequently, catfish head by-products

hold great potential as a natural protein source for therapeutic food products. The strengths and weaknesses of catfish head by-products in medicinal food products are outlined in Table 3.11.

Table 3.11 Advantages and disadvantages of therapeutic food products from catfish by-products

Advantages	Disadvantages
• Significantly lower cost • Abundant availability • Easily accessible • Higher protein content • Protein digestibility similar to that of moringa leaves	• Low fat and carbohydrate content • Requires combination with other ingredients to complete its nutritional profile • The resulting product can be somewhat hard

Source: Kustiani *et al.*, 2017

Therapeutic food products are typically made from main ingredients such as rice or cereals enriched with plant-based rather than animal protein. However, animal protein contains higher levels of protein and amino acids, more comprehensive vitamins and minerals, and saturated fatty acids and cholesterol, which are essential for children's physical and cognitive development. Animal protein sources can come from fish due to their affordability and accessibility. Animal protein is also reported to be more easily synthesized within the body and capable of increasing muscle mass (Aprilia and Hati, 2016).

Fish by-products, including residual meat, heads, skeletons, entrails, and fins, serve as economical and abundant animal protein sources. They can potentially be considered as candidate raw materials for therapeutic food for malnutrition, as they are affordable and accessible. The research by Kustiani et al. (2017) demonstrated that by-products from the heads of catfish can be utilized for food production due to their high protein content. Derivative products from catfish have the potential to be used in the production of crackers to enhance the utilization value of catfish and the nutritional content of the crackers. According to Alvares et al. (2018), fishery by-products can be used as a safe and promising source of human protein and amino acids. However, their utilization is not yet maximized.

A hydrolyzed fish protein, derived from fishery by-products, can be used as an additional ingredient to enhance the nutritional content of therapeutic food, thereby preventing and treating energy-protein malnutrition. However, hydrolyzed protein from these by-products must be combined with other raw materials to meet the UNICEF 2012 standards for quality Ready-to-Use Therapeutic Food (RUTF). The selection of raw materials in developing local RUTF products is based on energy sources, protein, availability, and price of ingredients, as stated by Komari and Lamid (2012). Ingredients for RUTF formulation can be combined with legumes, vegetable oils, skim milk, sugar, and a mix of vitamins and minerals. The UNICEF 2012 RUTF standards can be seen in Table 3.12.

In the process of producing RUTF products, there is a possibility of contamination by microbes and heavy metals originating from raw materials. This can pose direct hazards to consumers. Metal contamination poses an accumulative risk, while microbial contamination has acute impacts. Therefore, before human consumption, products using fishery by-products, especially fish entrails, must undergo testing for heavy metal levels and microbial contamination. The standards for metal and microbial contamination contained in RUTF are shown in Table 3.12 and Table 3.13, respectively.

Table 3.12 Macronutrients and micronutrient standards for ready-to-use therapeutic food

Macronutrients	**100 grams RUTF**
1. Energy	520-550 cal
2. Protein	13-16 g
3. Fat	26-36 g
Micronutrients	
1. Vitamin A	800-1200 mcg/RE
2. Vitamin B1	>0,5 mg
3. Vitamin B2	>1,6 mg
4. Vitamin B6	>0,6 mg
5. Vitamin B12	1,6 mcg
6. Vitamin D 1	12-20 mcg
7. Vitamin E	20 mg
8. Vitamin K	15-30 mcg
9. Vitamin C	50 mg
10. Folic acid	200 mcg
11. Pantothenic acid	3 mg
12. Iodine	70-140 mcg
13. Iron	10-14 mg
14. Zinc	11-14 mg
15. Selenium	20-40 mcg
16. Magnesium	80-140 mg
17. Potassium	1100-1400 mg
18. Sodium	230 mg
19. Calcium	300-600 mg
20. Biotin	60 mg
21. Niacin	5 mg
22. Phosphorus	300-600 mg
23. Copper	1,4-1,8 mg

Table 3.13 Standards for metal and microbial contamination in RUTF

Metals	Content
Lead	maximum 0,1 ppm
Mercury	maximum 0,02 ppm
Cadmium	maximum 0,03 ppm
Tin	maximum 40 ppm
Microbes	
Total Plate Count	maximum 1 X 10^4 cfu/ml
Mold/Yeast	maximum 6 X 10^1 cfu/ml
Coliform	Negative

3.6.4 Safety of Hydrolyzed Fishery By-Product Protein as a Candidate for Ready-to-Use Therapeutic Food (RUTF)

Fishery by-products are potential sources of protein that are currently underutilized. One way to enhance the quality of protein from fishery by-products is by processing them into hydrolysates. Hydrolyzation eliminates non-protein components from fishery by-products, resulting in a simple, protein-rich material that is easier to consume. As a candidate alternative source for RUTF, the safety of the raw materials derived from these by-products must be ensured for consumption (Vidanarachchi et al., 2014).

Hydrolyzed protein from fishery by-products has been used and applied in human consumption. Alvares et al. (2018) conducted a study involving hydrolyzed protein from Nile tilapia *(Oreochromis niloticus)* products as a vascular supplement for healthy individuals. No health issues were found in individuals consuming hydrolysates from these by-products. Before application, the fish by-product hydrolysate was tested for proximate content, amino acids, total antioxidant capacity (TAC), and Biogenic amines (BAs).

Fish is highly perishable due to its easy spoilage characteristics. Fish by-products contain high water content. Hence, their freshness needs to be considered before further processing for consumption. Several parameters, such as pH value, Thiobarbituric acid-reactive substances (TBARS), and Biogenic amines (BAs), should be monitored when using fish by-products as raw materials for human consumption (Palmeira et al., 2016).

pH Value (Potential Hydrogen)

The pH value of fish is a parameter that reflects the overall quality of its freshness. Changes in pH value occur due to enzymatic activity and bacterial processes during decomposition. Generally, live fish have a pH ranging from 6.0 to 7.1. This value decreases as glycogen converts to lactic acid during the pre-rigor phase. Rigor mortis is characterized by low glycogen levels in fish muscle, making the flesh stiff. The pH value of the flesh increases towards neutrality due to enzymatic and bacterial activities. When fresh, the pH value of fish by-products does not exhibit significant differences (Palmeira et al., 2016).

Thiobarbituric Acid-Reactive Substances (TBARS)

TBARS measurement aims to assess the freshness level of fishery by-products based on their lipid conditions. TBARS measurement quantifies the content of malondialdehyde (MDA), a low molecular weight compound and a primary and secondary end-product of lipid peroxidation. TBARS value indicates the extent of oxidation that has occurred in fish fat. Oxidized fats can deteriorate sensory quality in fish (Surasani, 2018). Fish entrails, the most significant waste in fish processing, have a relatively high-fat content, usually ranging from 19-21%. Oxidized fats can compromise the quality of the product (Vidanarachchi et al., 2014). Nurhayati et al. (2014) revealed that fats constitute the most significant component in fish entrails or viscera. The white snapper fish viscera contain about 22.33±1.22% fat, higher than its protein content (11.34±0.03%) and carbohydrates (2.18±1.51%). This trend is also seen in catla fish, with a higher fat content (12.46%) compared to its protein (8.52%) and carbohydrates (0.27%).

The high-fat content in fish by-products underscores the importance of TBARS assessment before using them as food materials. Fresh fish generally have low TBARS values; for instance, the TBARS value of new Brazilian catfish flesh is about 0.035 mg MDA/kg (Palmeira et al., 2016). This value increases over time and with storage temperature. The TBARS value for fresh silver catfish *(Rhamdia quelen)* is 0.04±0.04 mg MDA/kg, which continues to rise with extended storage time (Piccolo et al., 2014). According to Antonio et al., a safe TBARS concentration for human consumption is approximately 7-8 mg MDA/kg.

Biogenic Amines (BAs)

BAs measurement aims to quantify low molecular weight nitrogen compounds in food products. These compounds are naturally produced through three mechanisms: transaminase of aldehydes and ketones, nitrogen compound hydrolysis, and amino acid decomposition or decarboxylation (the most common mechanism). The major BAs compounds in fish products are histamine and tyramine. Consuming these compounds in high quantities can lead to allergic reactions such as headaches, dizziness, rashes, itching, nausea, vomiting, diarrhea, edema, local inflammation, hypotension, blood vessel constriction, and even death (Palmeira et al., 2016).

BAs measurement in fish products serves two primary purposes: assessing fish freshness and determining the quality level based on their potential to cause food poisoning. The major BAs compounds in fish products are histamine and tyramine, derived from histidine, tyrosine, ornithine, and lysine decarboxylation. There are no established standard limits for BAs in food in the European Union. However, according to European Commission Regulation No. 1019/2013, histamine content should be below 40 mg/kg (Sørensen et al., 2018). According to Palmeira et al. (2016), the Food and Drug Administration (FDA) recommends that histamine in fish raw materials be below 50 mg/kg, tyramine below 100 mg/kg, and total BAs below 1000 mg/kg. The safety of hydrolyzed protein from fishery by-products can be observed in Table 3.14.

Table 3.14 The safety of FPH derived from fishery by-products as an RUTF candidate

No.	Parameter	Source	Standard	Value	Reference
1	pH	FPH Raw Material	Fresh Fish	6,0 – 7,1	Manteiro *et al.,* 2014
2.	TBARS	FPH Raw Material	Malondialdehyde (MDA)	7-8 mg MDA/ kg	Antonio *et al.,* 2017
3.	Biogenic Amines	FPH Raw Material	1. Histamine	<40 mg/ kg	European Commission No. 1019/ 2013
			1. Histamine	<50 mg/ kg	FDA, 2016
			2. Tyramine	<100 mg/ kg	
			3. Total Biogenic Amines	<1000 mg/ kg	

3.7 Conclusion

This chapter explains the potential of fishery by-products as alternative raw materials for therapeutic food for children suffering from protein-energy malnutrition. Based on the data obtained from previous research, it can be concluded that fish by-products, including leftover meat, entrails, heads, skeletons, fins, and red meat, have a high protein content with a relatively complete composition of amino acids. Moreover, their abundant availability and untapped potential make these fishery by-products a viable candidate for an alternative and economically sustainable raw material for therapeutic food for malnourished children. Since fishery by-products cannot be consumed directly, processing must ensure that humans can absorb and utilize their nutritional content. The hydrolysis process aims to produce concentrated protein compounds that are easily digestible by humans. However, up to this point, the commercial application of protein hydrolysates from fishery by-products remains limited. Therefore, further research on the practical application of protein hydrolysates from fishery by-products as therapeutic food for malnutrition patients is crucial to enhance our understanding of the technology and its implementation.

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