

Potential Of Solid Waste Conversion Into Gelatin In The Fisheries Industry Of Indonesia Priyosetyoko¹, Widayat^{2*}, Meiny Suzery³, Tri Winarni Agustini⁴

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Abstract

There has been an increase in aquaculture production in Indonesia from 2015 to 2019, which is 62.31%. Statistics Indonesia showed an increase in commodities for capture fisheries and aquaculture, namely 7.94% and 28.87%, while there was a decrease in fresh or cold fillet fish commodities, namely -6.89%, referring to data from January to June 2021 compared to the previous quarter of 2020. The development of fishery processing industries such as fish fillets, leaving waste in the form of skin, bones, fins, scales, heads, offal, and liquid. The remaining waste, if not managed properly, can have negative effects on the environment. One of the researches on the utilization of fish meat processing industrial waste is to make gelatin. Gelatin is a product of the hydrolysis of collagen from animal skin or bones. Gelatin from fish needs to be developed because it is a halal product. Gelatin hydrolysis can be carried out under acidic, alkaline, and enzymatic conditions. In the filtration process of making gelatin sheets, there is a by-product in the form of liquid gelatin. Research on the purification of polypeptides from gelatin from fish has been widely carried out. Polypeptides have benefits in the fields of cosmetics and medical health. In general, glycine in fish gelatin is higher than in mammals, while proline is the opposite. Research related to the purification of glycine from fish gelatin is interesting because it is viewed from the point of view of the benefits of biomolecular science and aspects of Indonesia's natural resources.

Keywords: Waste; Gelatin; Polypeptides; Glycine

INTRODUCTION

Global food fish consumption increased at an average of 3.1 percent per year from 1961 to 2017, food fish consumption per capita grew from 9.0 kg (live weight equivalent) in 1961 to 20.5 kg in 2018, approx. 1.5 percent per year (FAO. 2020). Indonesia's fishery production in 2019 is targeted at 38.30 million tons (The Ministry of Marine Affairs and Fisheries Republic of Indonesia. 2019). Data from Statistics Indonesia shows an increase in the commodity of capture fisheries and aquaculture, namely 7.94% and 28.87%. There was a decrease in the commodity of fresh or cold fillet fish, which is -6.89%, referring to data from January to June 2021 compared to the quarter of 2020 (Statistics Indonesia 2021). Among the industries covered by the Program for Pollution Control, Evaluation and Rating (PROPER), the fish processing industry performs at the lowest level in terms of environmental indicators. Therefore, efforts are being made to adopt and implement appropriate wastewater treatment methods. Many of these efforts can help achieve water quality and climate change goals while contributing to sustainable development goals (Gómez et al., 2020). Realizing the potential of fishery waste in Indonesia, in this study, we will discuss a little some common techniques of processing fishery waste and information on how to produce fish gelatin from researchers interested in food, especially gelatin extraction which is explained from a little chemical side, and some of its applications. Almost all of the research data collected in this study is still on a laboratory scale, although it is hoped that it can be taken into consideration on an industrial scale.

Fish processing industry waste

The problem of fishery waste continues to increase to become a global concern that has an impact on several aspects of biological, technical, and operational factors such as socio-economic. It is important in the future to relate the environmental impacts of fish waste (Coppola et al., 2021). Amasuomo et al., (2016) explained that physical waste is divided into 3, namely solid, liquid and gas. Based on the source, waste is divided into 6, namely domestic waste, industrial waste, agricultural waste, commercial waste, demolition and construction waste, and mining waste.

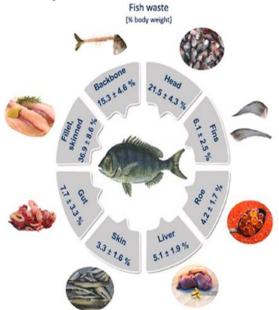


Figure 1. Products and side streams of fish (Thirukumaran et al., 2022)

Characteristically, fishery industry waste is divided into two, namely solid waste and liquid waste. Solid waste is fish parts such as the head, tail, body, offal, and skin (Dubey et al., 2021). The liquid waste of fish is blood and water (Caldeira et al., 2018). Solid waste in the fish processing industry is generated during the skinning and packaging process.

a) Solid waste from fish processing

Fish is rich in high-quality protein nutrients which include essential amino acids, essential fats, omega 3, fatty acids, vitamins such as vitamins A, B, and D, as well as minerals containing calcium, iodine, zinc, selenium, potassium, iron, phosphorus (Araujo et al., 2021; Coppola et al., 2021). Fish waste also contains abundant amino acid organic compounds, namely glycine and alanine (Kang et al., 2004) with high protein and lipid content in scales, bones, and fins (Naylor et al., 1999). They such as skin, bones, and scale contains collagen which is dominated by type I collagen, while cartilage is dominated by type II collagen (Jafari et al., 2020).

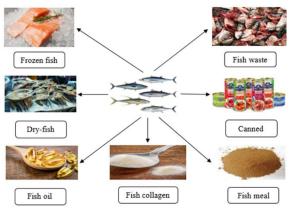


Figure 2. Fish processing industry (modified scheme Shaik & Sarbon. 2020)

b) Liquid waste from fish processing

Processing industrial wastewater contains suspended solids, pH, nitrogen, and phosphate (Dubey et al., 2021). Various by-products of fish processing production are blood, heads, eyes, tongues, livers, testes, roe, cut-offs, skin, bones, backs, guts, swim bladder cuts, skin, bones, backs, guts, and swim bladders. The waste such as blood and testes of fish processing may be used in the development of functional feed for fish and warm-blooded animals. The course can potentially reduce the cost of producing animal feed ingredients (Ottesen et al., 2016).

c) Fish Waste Treatment Methods

The following are some of the methods commonly used to treat fish waste. Each method has different techniques and principles. The above method will be shortly described as follows:

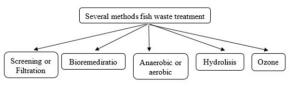


Figure 3. Several methods fish waste treatment (modified scheme Arvanitoyannis & Kassaveti. 2008, Arvanitoyannis & Tserkezou. 2014).

Screening/filtration treatment

Filtration and screening have the same characteristics, namely the separation of particles based on differences in size. The opening (screen) and pores (filtration). There is a potential for the use of

ultra-low pressure membranes for aquaculture wastewater treatment. Fish wastewater samples after being filtered using sand filtration are then passed using a polyethersulfone membrane with wet or dry inversion techniques. The technique can remove ammonium and phosphorus up to 85.70 and 96.49% at pressures of 0.4-0.8 MPa (Arvanitoyannis & Tserkezou. 2014). The filtration process simultaneously removes solid waste and antibiotic resistance genes from flow-through fish farm waste. With a filter sized 25 μ m, the total number of particles in the effluent is reduced to about 40.3% (Kim et al., 2018).

Bioremediation treatment

Bioremediation is the process of treating hazardous materials contaminants by relying on microorganisms and has the advantage of being particularly cost-effective (Sharma et al., 2022). Bioremediation is defined as the use of organisms capable of degrading contaminants through their metabolic activity, to solve environmental problems, such as those generated by pollution. There are several concepts for applying bioremediation treatment to aquaculture waste, including bioremediation for organic compounds, nitrogenous compounds, and Hydrogen Sulphide (H2S). Bioremediation of organic compounds using decomposing microbes requires organic matter to be oxidized. Decomposing microbes carry genetic information to oxidize organic matter, decompose aquaculture wastes with their metabolic capabilities and convert them, either into less reduced organic forms or into inorganic compounds CO2 and H2O. The end product of the process is either a complete oxidation product or mineralization, thereby recovering the contaminated site and controlling pollution. Bioremediation of nitrogenous compounds is used to control ammonia, nitrite, and nitrate compounds. There are several stages or phases. The first is the accumulation of ammonia, the second is the removal of ammonia compounds by autotrophic nitrification or by heterotrophic nitrification. The last phase is denitrification. Bioremediation of H2S can be carried out under aerobic and anaerobic conditions. Under aerobic conditions, organic sulfur decomposes into sulfides, which can be oxidized to sulfates. It is highly soluble in water, due to which it gradually disperses from the sediment. It process is carried out by different microorganisms. Under anaerobic conditions, sulfate can be used as a terminal electron receptor of microbial metabolism, leading to the production of hydrogen sulfide gas, which can be metabolized anaerobically (Musyoka. 2016).

Anaerobic treatment

The principle of anaerobic treatment refers to the biological treatment of dissolved and colloidal wastewater in the absence of oxygen at a low redox potential (EH < 200 mV) (Pavlostathis et al., 2011). Anaerobic treatment is one of the most effective and economical methods of wastewater treatment. In wastewater treatment, anaerobic biology treatment appears to be a promising technology because methane gas is produced from anaerobic digestion (AD) and can be used as renewable energy (Zeb et al., 2013). Anaerobic treatment is very sensitive to pH. The process thrives well in the pH range of 6.5-7.8. with an optimum pH near neutral (Bhunia. 2014). The anaerobic treatment uses AD which produces biogas and methane (Bücker et al., 2020; Ivanovs et al., 2018). AD is a series of biochemical processes involving various species of microorganisms and is considered an engineered ecosystem in which organic molecules undergo biodegradation. Fish waste and fish crude oil extraction waste are converted into biogas and methane using AD. Biogas processing is capable of producing a very abundant yield of methane. Fish waste is a promising alternative for biogas production in a mono-digestion process (Bücker et al., 2020).

Aerobic treatment

Aerobic treatment is a biological process by which dissolved oxygen is used by microorganisms (aerobes) for organic waste degradation (Mondal et al., 2018). The autothermal thermophilic aerobic digestion technique is part of aerobic processing and is still being developed since the 1960s. It is part of the treatment of biological waste treatment using various types of specific microorganisms. The autothermal thermophilic aerobic digestion has high biodegradation efficiency and is simple to operate in its processing (Zhang et al., 2022). Some of the systems of aerobic treatments include; aerobic granulation, biofilm reactor, and activated sludge process. The first is aerobic granulation. This system used a bio-granulation approach to produce granular sludge. In aerobic systems, 88% of ammonia can be oxidized. Short-time aerobic digestion achieves better mud flocculability. Bio-granulation can produce two types of granular sludge, which are aerobic granular sludge (AGS) and anaerobic granular sludge. AGS was successfully cultured by high-strength pyridine wastewater treatment, using a single bacterial strain. The second is a biofilm reactor. It's a community or group of microorganisms that are attached to a surface. In cell biofilms, there may be unfavorable survival situations. This situation is caused by the matrix acting as a barrier and protecting the cells within it from environmental distress. It can minimize the impact of modification of pH, temperature, and concentration of toxic substances. There are several biofilm reactor methods, namely, integrated anaerobic-aerobic fluidized bed reactor, anaerobicaerobic fixed-film bioreactor, rotating biological anaerobic-aerobic contactor. granular biofilm bioreactor, aerobic membrane bioreactor, and movingbed. biofilm reactor. The last method of aerobic treatment is the activated sludge process. In this process, there are several methods, namely; microbubble aerator, contact stabilization, and trickling filter (Mondal et al., 2018).

Hydrolysis treatment

The enzymatic hydrolysis method is a processing treatment. It takes place under controlled conditions and has specificity in waste treatment. The advantage of this treatment is that it does not leave toxic chemical residues or organic solvent residues on the products produced after the treatment (Tacias-Pascacio et al., 2021). Enzymatic hydrolysis of fish waste can produce high-value products. It uses the Alcalase enzyme which produces products in the form of oil recovery, hydrolyzed protein recovery, and collagen recovery. The factors that affect the product of enzymatic hydrolysis are the enzyme concentration factor, the protein concentration in the waste, and the ratio of the enzyme to the substrate. (Araujo et al., 2021). Enzymatic hydrolysis of fish waste protein using fruit waste Ananas comosus comosus and Carica papaya can increase the available nitrogen content and enrich organic liquid fertilizer for the growth of Basella alba (Ranasinghe et al., 2021).

Ozone treatment

Ozone (O3) can be used in wastewater treatment. Ozone treatment in the organic waste can be considered an effective and faster treatment compared to other treatments such as chlorine or hydrogen peroxide. Ozone is the allotropic form of oxygen (O2). It is composed of the same atoms, but they are combined in the form of three oxygen atoms and have a low molecular weight (MW = 48), which is, that three oxygen atoms are chemically arranged in chains (Gonçalves. 2009). The application of ozone is categorized as a non-thermal technology generated in fish products targeting the preservation of product shelves. The chemical and physical properties of ozone greatly affect its efficacy. How ozone is produced today, whether it is corona discharge or the ultraviolet method, is not much to deter researchers from trying its application to water treatment, sewage treatment, food odor, and so on. In addition, ozone is considered not to damage the biochemical properties of fish meat and is also considered to be the most powerful oxidizing agent commercially available (Okpala. 2017).

d) Potential Utilization of Fish Solid Waste

Fish waste has no commercial or low value. This is important considering the environmental impact that fish waste can have on ecosystems because the release of organic waste can significantly change community structure and biodiversity. Therefore, fish waste management involves various aspects to reduce this source of pollution and find the best way to solve this problem. There are several studies on processing solid waste from the fish processing industry into useful products (O'Donnell et al., 2021). Fish waste has the potential as gelatin (Usman et al., 2021), fish meal (Santos et al., 2017), animal feed mixture (Shabani et al., 2019), protein hydrolysates (Henriques et al., 2021), and handicrafts (Mariz et al., 2020). All these efforts have been made as a step to improve the quality of the environment as well as to increase the selling value of fishery waste.

e) Fish gelatin

Gelatin is a product of the thermal destruction of acid, alkaline, or enzymatic fibrillar protein present in the skin, connective tissue, bones, and organs of mammals and fish (Derkach et al., 2019) It is an important protein present in the skin, bone, and connective tissue of animals, formed by partial hydrolysis of collagen (Fawale et al., 2021). It is an amorphous mixture consisting of different chains, which are α -chain, β chain, and γ -chain, which are stabilized by soluble hydrogen. During the gelatin extraction process, both intramolecular and intermolecular hydrogen bonds in the collagen molecule are disrupted. Intramolecular hydrogen bonds (aldol condensation and Schiff base), intermolecular, and peptide main chains are hydrolyzed, causing the triple helix to unravel (Ohairul et al., 2021). Fish bones and scales can be produced as the more desirable source of gelatin extraction in the market (Alipal et al., 2019). There are factors to be considered in the production of gelatin from fish skin, such as the triple helical structure of the collagen molecule, the and susceptibility of collagen material to degradation (Alfaro et al., 2015).

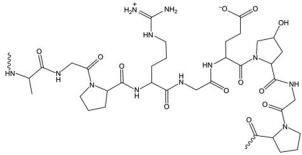


Figure 4. Gelatin structure

Fish gelatin has physical and chemical characteristics that have the potential as an alternative to mammalian gelatin (Mahmood et al., 2016). The gel strength, viscosity, gelling, melting points (Karim & Bhat. 2009, Nitsuwat et al., 2021), viscosity, and melting point (Alipal et al., 2019), color, moisture (Alfaro et al., 2015) of gelatin are important physical parameters in the commercial gelatin industry. The standard of gel strength as measured by bloom is classified into 3, namely high bloom, 200–300 g, medium bloom, 100–200 g, and low bloom, 50–100 g. The physical

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properties of warm water fish gelatin with a bloom of 200 grams have solubility, melting temperature, and gelling temperature >35°C, 28–29°C, and 21–22°C (Haug and Draget. 2011). The chemical characteristics such as the average molecular weight and molecular weight distribution, gel maturation time, gel maturation temperature, pH, salt content (Karim & Bhat. 2009), amino acid composition, heavy metal, and moisture (Alfaro et al., 2015).

f) Extraction of gelatin from waste fish

In general, there are three methods of extracting gelatin, namely acid extraction, alkaline extraction, and enzymatic extraction (Mahmood et al., 2016). This study tried to show the yield of gelatin from various extraction methods because it is considered important to be applied in industry.

The yield of gelatin can be calculated from the percentage of the dry weight of extracted gelatin compared to the dry weight of fish waste used (Aliet al., 2018, Liao et al., 2021, Bedis et al., 2022). The acid and alkaline processes of gelatin extraction as shown in Figure 6 and some of the research are presented in Table 3:

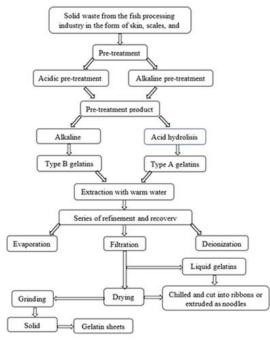


Figure 6. Preparation method of gelatin from collagen (modified scheme Al-Nimry et al., 2021)

| Treatment method | Advantages | Disadvantages |
|----------------------|---|---|
| Screening/filtration | Low cost, easy installation, and simple operation | Clogging of filters and clogging of screens |
| Bioremediation | Low cost, chemical hazard degradation, and more naturally microorganisms to dedegradeolid waste | Not rapid, contaminants degradation, only for biodegradable compounds, and constant monitoring for effectiveness assurance |
| Anaerobic | Low cost, biogas production, low energy consumption, and less waste sludge generation | Odor problems and high maintenance |
| Aerobic | The stability of mature compost, low cost, biogas production | Odor and color problems, harmful effects to plants and aquatic, and it can be producing leachate which pollution |
| Hydrolysis | Prospective recovery of | High operation cost |
| | unique value-added and valuable compound from waste | |
| Ozone | Rapid, simple operating, and oxidation higher environmental quality human demand | High cost and energy consumption |

Table 1. The methods commonly used to treat solid waste

(Arvanitoyannis & Kassaveti. 2008, Musyoka. 2016, Kumar et al., 2022, Guo et al., 2022, Liu et al., 2019, Gopikumar et al., 2020, Korkmaz & Tokur. 2022, Rosen et al., 2022, Wang et al., (2022)

| Kind of waste | Treatment | Product results |
|---|---|---|
| Fish waste (FW) fish heads, skins, viscera, mangled muscles of Salmon fish, small fishes, as well as mollusks such as squid and mussels | Enzymatic hydrolysis | Protein hydrolysates, collagen, and fish oil |
| FW and fish crude oil extraction waste | Anaerobic mono digestion | Biogas and methane |
| FW of fish farm sediments | Bioremediation | Decreased biochemical composition of organic matter |
| FW silage resh sardine wastes containing heads, skins, fins, tail, viscera, and bones | Biological fermentation by <i>Bacillus subtilis</i> | Improved nutritional properties of FW |
| FW <i>Onchoryncus mykiss</i> (skeleton, fin, head, skin, and viscera) | Hydrolysis | Protein hydrolysates |
| FW fish species namely, <i>Trigla spp.</i>), <i>Trachurus trachurus</i>), <i>Micromesistius</i> <i>poutassou</i> , <i>Scorpaena scrofa</i> , <i>Trisoreptus luscus</i> , and, <i>Lepidorhombus boscii</i> . | Enzymatic hydrolysis | Protein hydrolysates |
| FW (skin, bone, and other materials. | Biopolymer extraction | Bioplastic |
| Solid waste aquaculture | Filtration used polyethylene | Reducted antibiotic resistance genes (ARGs) |
| Fish processing wastewater and fish blood | Aerobic biodegradation | There was an inhibitory effect on biomass growth and substrate removal from a salt concentration of 3.0% |
| FW Abrótea filleting (head and carcass abdomen), (<i>Urophycis brasilienis</i> ,) and (cephalothorax and tail) (<i>Farfantepenaeus subtilis</i>) | Oven and microwave oven | Protein and minerals that can be used in animal feed |
| FW from market | Anaerobic | Biogas and methane |
| FW Yellowfin tuna heads | Ultra-high pressure pre-treatment to enzymatic hydrolysis | Fish oil |

Table 2. Fish waste, treatments, and product results

(Araujo et al., 2021, Bücker et al., 2020, Ape et al., 2019, Shabani et al., 2019, Korkmaz & Tokur. 2022, Henriques et al., 2021, Araújo et al., 2018, Kim et al., 2018, Ching and Redzwan 2017, Santos et al., 2017, Kafle and Kim. 2012, Zhang et al., 2021)

| Table 3. Extra | Table 3. Extraction methods and products of fish gelatin | | |
|---|--|---------------------------|--|
| Kind of material fish | Extraction methods | Product gelatin yield (%) | |
| Fish (Sparus aurata) skin | Acid | 14.38 ± 0.82 | |
| Fish silver carp spine bone | Enzymatic | 13.3 | |
| Nile tilapia skin | Acid with fermentation pretreatment | 49.99 | |
| Catfish (<i>Leiocassis longirostris</i> <i>Günther</i>) skin | Acid | 21.8 ± 1.1 | |
| Silver carp (Hypophthalmichthys molitrix) skin | Acid | 22.9 ± 1.1 | |
| Milkfish scale | Acid | 6.67 | |

| | Table 3. Continue | |
|---|--|------------------|
| Tilapia fish skin | Acid | 41.91 |
| Kalamtra Sturgeon (Huso dauricus $	imes$ | Alkaline | 7.25 |
| Acipenser scherenkii × Acipenser transmontanus) head | Acid | 5.01 |
| Starry triggerfish (<i>Abalistes stellaris</i>) skin | Acid | 13.84 ± 0.86 |
| Tilapia skin | Acid | 21.1 ± 1.5 |
| | Hot water | 18.4 ± 1.0 |
| | Enzymatic | 22.0 ± 0.8 |
| Fish Saithe (Pollachius virens) skin | Alkaline | 5.8 |
| | Acid | 8.3 |
| Dogfish skin | Acid | 8.67 |
| Skipjack Tuna bones | Acid | 6.37 ± 0.64 |
| Unicorn leatherjacket (Aluterus monoceros) skin | Acid | 12.2 |
| Golden carp skin | Two Acids assisted ultrasound | 62.12 |
| Carp (Cyprinus carpio) skin | Combined alkaline, organic and inorganic acids | 12.00 |
| Tuna skin | Alkaline and acid-assisted ultrasound | 22.60 |
| Nile tilapia skin | Acid | 74.37 ± 1.90 |
| Red tilapia skin | Acid | 69.62 ± 3.61 |
| Sea Bass skin | Acid | 65.61 ± 4.30 |
| Nile tilapia bone | Acid | 60.08 ± 2.42 |
| Red tilapia bone | Acid | 54.74 ± 2.74 |
| Sea Bass bone | Acid | 51.71 ± 0.92 |
| Yak skin | Enzymatic | 26.34 ± 0.23 |
| Squid (Loligo vulgaris) skin | Enzymatic | 6.82 |
| Giant grouper (<i>Epinephelus</i> <i>Lanceolatus</i>) skin | Acid | 20.27 |
| Thornback ray skin | Combine enzyme and acid | 17.03 ± 0.36 |
| Seabass (Lates calcarifer) skin | Acid | 57.30 |
| Mackerel (Scomber scombrus) heads | Acid | 3.7 ± 0.1 |
| Catfish skin | Acid | 20.10 |
| Blue shark (Prionace glauca) skin | Acid | 5.20 |

(Bedis et al., 2022, Wu et al., 2022, Chen et al., 2022, Yang et al., 2022, Rafael et al., 2021, Liao et al., 2021, Islam et al., 2020, Muyasyaroh and Jaziri. 2020, Zhang et al., 2020, Casanova et al., 2020, Salem et al., 2020, Yang et al., 2019, Renuka et al., 2019, Ali et al., 2018, Tkaczewska et al., 2018, Nhat. 2018, Tinrat & Sila-Asna. 2017, Xu et al., 2017, Abdelmalek et al., 2016, Lin et al., 2015, Lassoued et al., 2014, Sinthusamran et al., 2014, Khiari et al., 2011, Jongjareonrak et al., 2010, Limpisopho et al., 2009)

Acid extraction

Gelatin can be called type A if the acid compounds are applied to the tissue at the end of the pretreatment. Extraction of gelatin using hydrochloric acid (HCl) 0.05 M at a temperature of 65 °C for (3-3.5 hours) on the sturgeon head can be done by initial treatment to make soft tissue. The use of HCl at the end of the pretreatment followed by extraction in a water bath is categorized as a conventional type of gelatin extraction method (Huang et al., 2017). It can function as a reactant for decalcification as well as a reactant for hydrolysis (Islam et al., 2020). The use of acids such as sulfuric acid, citric acid, (Valcarcel et al., 2021), hydrochloric acid (Charoenchokpanich et acetic acid (Carvajal et al., 2022), al., 2021), phosphoric acid (Valcarcel et al., 2021), and lactic acid (Giménez et al., 2005) can be used for acid extraction. Two different types of acids can be combined as catalysts for hydrolysis. For example, Ali et al., (2018) researched the extraction of the skin of golden carp (Probarbus Jullieni) gelatin using sulfuric acid and acetic acid for 6 hours resulting in a gelatin yield of $35.54 \pm 0.85\%$. It was lower than acetic acid without sulfuric acid with the same extraction duration, which was 52.38 ± 2.34%. However, conventional gelatin extraction is not eco-friendly, because of the use of chemicals that are high in solvents, difficult to degrade, and have low yields (Qhairul et al., 2021). Table 3. shows that the yield of gelatin extraction is that the gelatin extraction of Nile Tilapia skin is greater than that of Nile Tilapia bone by Tinrat & Sila-Asna. (2017) were 74.37 ± 1.90% and 60.08 ± 2.42 respectively. The difference in % yield of gelatin will vary between fish species, it is influenced by protein content (collagen), skin/bone composition, extraction pre-treatment, and extraction method (Tinrat & Sila-Asna. 2017)

Alkaline extraction

Two types of alkali commonly can be used, such as NaOH and Ca(OH)2 (Milovanovic & Hayes. 2018). The final treatment of tissue that uses alkaline materials for the gelatin extraction process is called type B gelatin. Gelatin extraction under alkaline conditions can be carried out with 0.1 M sodium hydroxide (1 hour) at a temperature of 50 oC. The combination of acid and base as pretreatment extraction can also be carried out by initial alkaline treatment followed by acid treatment (Islam et al., 2020). Both alkaline and acid methods are expensive and take a long time to process into gelatin Qhairul et al., 2021). An example of the disadvantage of the conventional method of low gelatin yield, such as the results of research that has been carried out by Islam. It is recommended to do re-extraction if applied in the industry (Islam et al., 2020).

Enzymatic extraction

There is another type of gelatin that has not been mentioned previously, which is called type E gelatin. It is because it is hydrolyzed using specific enzymes. Protease enzymes can be used to hydrolyze collagen into gelatin (Ma et al., 2020). it's still categorized as one of the green methods (Islam et al., 2020). The enzymes used for hydrolysis are from animals, plants, and microbes. Enzymes of microbes have catalytic activity for hydrolysis. Microbial enzymes have higher levels of catalytic activity and a wider range of pH and temperature operations in which the enzymes remain stable. For example, Alcalase can speed up the gelatin extraction process significantly compared to the use of neutral or acidic enzymes. (Derkach et al., 2022). To produce gelatin by enzymatic extraction by means of powdered fins and scales mixed with distilled water and the pH was adjusted to 8.6 then added alkaline protease per gram of powdered fins and scales. Hydrolysis was carried out for 48 hours at room temperature with slow stirring. The suspension was rinsed with distilled water three times, followed by heating in 60 °C distilled water to extract the gelatin. The yield is 52.28 \pm 0.87% (Mirzapour et al., 2018), but the yield is calculated based on the hydroxyproline content, it is different from the yield calculation method that has been discussed. An interesting study that has been investigated by Ma et al., (2019) namely stating that the extraction of eco-friendly gelatin with pepsin and HCl enzymes produces type E gelatin which is called the one-step method. Several enzymes such as papain, neutrase, bromelain, pepsin, proctase, crude protease, protosubtilin, and hepatopancreatin were used as pretreatment for the enzymatic extraction of gelatin (Ahmad et al., 2017, Kolotova et al., 2020).

Ultrasound-assisted extraction

Ultrasonic applications are considered "green" for energy efficiency (Zhang et al., 2020, Xu et al., 2021, Chen et al., 2022, Feng et al., 2022). An exciting new and innovative technology for ultrasonicassisted extraction has been developed to help increase production rapid and low costs (Qhairul et al., 2021) while preserving the environment by lowering energy consumption as low as possible. Although there are other techniques such as high-pressure processing, microwave-assisted extraction, and subcritical water extraction which will not be discussed. In principle, ultrasound-assisted extraction uses ultrasound pressure waves to generate cavitation, which can damage matrix structure and improve gelatin extraction. The amount of frequency used depends on the purpose of the analytical technique; for example, between 200 to 500 kHz is the frequency required to control chemical effects and matrix structure, and improve gelatin extraction yield (Qhairul et al., 2021). Extraction of gelatin skin of golden carp (Probarbus Jullieni) with a combination of acetic acid and sulfuric acid assisted by ultrasonic can increase yield. The gel strength of the resulting gelatin varies substantially according to the pretreatment used for the extraction process (Ali et al., 2018). Gelatin properties are related to the long or short polypeptide chains. The yield of gelatin can be increased by ultrasound (Senarathna and Marapana. 2021).

Of all the gelatin extraction methods that advantages described, the have been and disadvantages of each method are not discussed. This is because each extraction method must consider costs, raw materials, and the sophistication of the equipment used. For example, the research conducted by Yang et al., (2022) comparing three methods for extracting gelatin from silver carp fin waste by acetic acid, hot water, and pepsin enzyme methods in terms of gel strength (bloom), the results are medium, low, and below low values.

Drying process

After the extraction of gelatin, a stage that determines the quality of the gelatin is the drying process. It is important to obtain gelatin with suitable properties. The relative functional properties depend on the spatial structure of the protein molecule because its association status is affected by the drying which leads to the physicochemical process, transformation of its protein. It matters due to heat and mass transfer. Convective hot air method combined with infrared radiation can be a drying option, considering they have advantages such as lower energy costs, simple equipment, easy handling, and shorter drying times. The results of the desirability function show hot air convection as the most effective method when carried out at 59.14 °C for 12.35 hours. Infrared radiation at 70 °C for 2 hours and convective drying at 70 °C for 3.5 hours is the best condition for the combined process. The obtained gelatin has a gel strength of 298.00 and 507.33 g and an emulsion activity index of 82.46 and 62.77 m2/g in combined and convective methods, respectively, and the protein content is above 90% (Silva et al., 2021). The results of the research conducted by Feng et al., (2021) showed that in the temperature range of 55 to 75 oC, the extraction of gelatin by microwave increased the stability of the structure and gelatin emulsion.

Bioactive peptides are derived from this glycine and proline-rich protein (Mirzapour et al., 2021). Bioactive peptides are fragments of specific proteins derived from plants or animals that have nutritional benefits and provide beneficial effects on health. They are inactive in their parent protein sequence but can be released by enzymatic hydrolysis. It is often extracted using several techniques such as enzymatic hydrolysis, acid-base hydrolysis, and fermentation (Abuine et al., 2019). Gelatin rich in high molecular weight polypeptide chains exhibits superior film forming capacity. Alcalase enzymes can be used to hydrolyze proteins from shark skin (spiny dogfish) (Zhang et al., 2019).

| Table 4. The amino acid content of cold water fish |
|--|
| gelatin and warm water fish. |

| Amino Acids (AA) | AA residues per 1000 residues | |
|------------------|-------------------------------|--------------|
| | Cold water | Warm water |
| | fish gelatin | fish gelatin |
| Essential AA | | |
| Histidine | 11 | 6 |
| Leucine | 21 | 23 |
| Methionine | 3 | 9 |
| Threonine | 24 | 24 |
| Phenylalanine | 13 | 13 |
| Lysine | 28 | 25 |
| Isoleucine | 11 | 8 |
| Hydroxylysine | 5 | 8 |
| Valine | 18 | 15 |
| Others AA | | |
| Alanine | 112 | 123 |
| Proline | 96 | 119 |
| 4-hydroxyproline | 60 | 79 |
| Serine | 63 | 35 |
| Tyrosine | 9 | 2 |
| Asparagine | }48 | }48 |
| Aspartic acid | | |
| Glycine | 347 | 347 |
| Glutamine | }72 | }69 |
| Glutamic acid | | |

(Phillips and Williams. 2009, Mahmood et al., 2016).

h) Fish gelatin amino acids

Fish gelatin contains amino acids that are essential for the survival of the organism. In general, glycine, proline/hydroxyproline, and alanine are the dominant amino acids (Mahmood et al., 2016). Although amino acids such as glycine, proline and hydroxyproline, and gelatin can be used as additives in the animal feed industry, these ingredients except glycine are relatively expensive. (Li et al., 2018). Amino acids are contained in the human body, 11.5% is represented by glycine and 20% of the total nitrogen of amino acids in body proteins comes from glycine (Razak et al., 2017). The following table compares the amino acids of cold-water fish and warm-water fish:

i) Applications of fish gelatin

The most important properties of gelatin that are useful for applications in a wide variety of sectors are thermoreversible gel formation, texture, thickening, emulsion formation, high water-binding capacity, stabilization, foam formation, protective colloid function, and adhesion/cohesion (Phillips and Williams. 2009). Gelatin can be applied to the fields of food, medical, pharmaceutical, and air filters (Alipal et al.,2019). Some sectors of fish gelatin application are described as follows:

Food

Fish gelatin has a unique property that is, compared to the main source of gelatin, which is obtained from pork and beef, fish gelatin consumed by the Muslim community. No disease was studied in fish compared with mad cow disease (bovine spongiform encephalitis), so fish gelatin is safe for consumption. Gelatin produced from different fish species can be mixed and prepared to present a choice of new technology in food product development (Usman et al., 2021). The properties of gelatin become important especially for gelatin-based desserts because scientists have not been able to find a gelling agent that can replace gelatin as a gelling agent for the use of food products (Sultana et al., 2018). Fish gelatin can be a coating that inhibits myofibril degradation (Feng et al., 2016). One of the roles of gelatin in an effort to reduce the impact of environmental pollution is to study material advances or biodegradable films based on gelatin (Chen et al., 2022).

Pharmaceuticals

Gelatin is used for a variety of pharmaceutical and medical applications. Fish gelatin, especially extracted from warm water fish, has the potential to be used in pharmaceutical products because it has characteristics similar to pork gelatin, so it needs to be considered as an alternative to mammalian gelatin. The low melting point of gelatin can be used in the microencapsulation of vitamins and other pharmaceutical additives such as azoxanthine (Abd et al., 2013). It is a characteristic film-forming property of gelatin that is used in the manufacture of pharmaceutical capsules (Huang et al., 2019).

Cosmetics

The characteristic properties of gelatin, which are easy to form a gel, are widely applied in cosmetic products, including face creams, body lotions, shampoos, hair sprays, sunscreens, and bath salts and bubbles. Fish gelatin hydrolysates (proteins and peptides) are used to prevent damage caused by UV radiation on the skin. They repair the damage done to the skin structure by maintaining the lipid balance of the skin due to its antioxidant properties (Al-Nimry et al., 2021).

Photographic

The properties of gelatin as a protective colloid are important in photography (Nurilmala et al., 2022). The main ingredient for modern silver bromide photography consists of an emulsion containing gelatin on a backing (paper or film). The highest demands are made for photography gelatin for the manufacture of X-ray films (Haug and Draget. 2011).

Air filters

Gelatin filters with a filter membrane can be applied in the field of air filters as part of a tool to filter SARS-Co (Alipal et al.,2019). It uses of a portable air purifier with a high-efficiency particulate air filter has been discussed as an additional way to decontaminate indoor environmental coronaviruses. The sampling method with gelatin filter shows the performance of trapping SARS-Co and MERS-Cov viruses, followed by polymerase chain reaction analysis (Rodríguez et al., 2021).

Based conductive hydrogels

Lately, many reinforcing species (such as metallic nanomaterials, a carbon-based material, and polymer) have been induced or inserted into the gelatin, and gelatin conductive hydrogels provide excellent results and improvement in various aspects. The properties of gelatin that affect the performance of the conductive hydrogel of gelatin are strength, viscoelasticity, flexibility, and swelling. Gelatin conductive hydrogel can be used as follow-up material for wearable flexible sensors (Wang et al., 2022).

CONCLUSION

Indonesia's natural resources have the potential to be used as processed products, namely fish gelatin. There are generally waste treatment methods, screening or filtration processes, such as bioremediation, anaerobic, aerobic, hydrolysis, and ozone, to process fishery waste into products such as biogas, methane, collagen, fish oil, gelatin, protein hydrolysate, bioplastics, animal feed to handicrafts which has a higher resale value. Gelatin can be produced by acid, alkaline, enzymatic, and ultrasonic extraction processes. Considering all methods of them, ultrasound-assisted extraction of gelatin is an and environmentally friendly cost-effective technology that is being developed in the future. Fish

gelatin can be applied in the fields of food, medical, pharmaceutical, photography, air filters, and the base of the conductive hydrogel.

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