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Fish gelatin (definition, manufacture, analysis of quality characteristics, and application): A review

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Abstract
Gelatin is a derivative product from the hydrolysis of collagen contained in the bones and skin of animals. Gelatin is a polypeptide consisted of covalent bonds and peptide bonds between the amino acids that made it up. Gelatin is obtained by carrying out the hydrolysis process using acidic or alkaline solutions. Skin raw material is the largest raw material used by the gelatin industry because it has a higher collagen content, available in large quantities, and can be continuous. Quality characteristics of gelatin could be seen from several measurement results such as yield, ash content, fat content, protein levels, pH, viscosity, gel strength, isoelectric points, white degrees, amino acid content, and heavy metals content. Nowadays, the utilize of gelatine as the raw material, used in the food and non-food industries.

Keywords: gelatin, hydrolysis, collagen, quality, utilization

1. Introduction
Currently, the used of animal waste as an alternative source of gelatin has been widely studied, one of which is the utilization of fish waste, which are skin and bones of fish. Fish skin and bone waste is one alternative source as raw material for making gelatin. Utilization of gelatin by using waste from fish skin as the raw material can increase the economic value of fish skin waste which hasn't been maximally utilized and also it can overcome the halal problem of gelatin products which cause many problems for the civilians who in their religion, forbid to consume some types of animals, such as pigs and cows. Besides, utilizing gelatin from fishery waste can avoid the disease of Bovine Spongiform Encephalopathy (BSE) [8].

Gelatin is a type of conversion protein obtained through the hydrolysis of collagen from the skin, bones, and white fibrous tissue of animals. Gelatin was obtained from collagen through an acid pre-treatment process (gelatin type A) with an isoelectric point at pH 7–9 or base (gelatin type B) with an isoelectric point at pH 4.7–5.4. The gelatin of fish skin was generally obtained from acid pre-treatment since the collagen tissue in the skin is softer. The acid treatment caused the bonding to be cut off between the carboxyl group and the amide group in the collagen molecule. The triple helix collagen molecular structure was split into the α-helix chain so that the molecular weight of gelatin has a wide range of 90-300 kD [35].

Gelatin has the characteristics of bright yellow or transparent close to white. In the form of sheets, powder, or as if flour, stems, or as if leaves. Soluble in hot water, glycerol, citric acid, and other organic solvents. Gelatin can expand and absorb water 5-10 times its original weight. Gelatin widely utilized for various industrial purposes, both food and non-food industries, for its specific characters. The industry that utilizes gelatin the most is the food industry. Gelatin application in foodstuff can be used as a gelling agent, thickener, emulsifier, foam maker, edible film, and stabilizer. In the pharmaceutical department, gelatin is widely used for the manufacture of soft and hard capsules [33].

2. Collagen
Collagen is the main protein of insoluble fibers found in additional cellular matrices and connective tissues. Collagen is obtained from the scales and fish skin through enzymatic digestive methods. Collagen can also be applied in some sectors such as cosmetics, biomedical, pharmaceutical, and food industry [42].

The main structural component of white connective tissue in vertebrates and invertebrates is

called collagen. Collagen fulfills nearly 30% of the total protein in vertebrate and invertebrate tissues and organs. The amino acids glycine, proline, and hydroxyproline are the main constituent ingredients of collagen and are included in the protein group of fibrils. Therefore, collagen is the main ingredient as a constituent of gelatin [30].
The protein molecule in collagen consists of several elongated polypeptide chains that form stable fibers since they are connected one to another by several cross bonds. The structural unit that forms collagen is tropocollagen. The stem structure of the tropocollagen with 300,000 BM together forms a triple helical structure because the three polypeptide chains of the same length [22]. Heating or treating with substances such as acids, bases, urea, and potassium permanganate can cause the tropocollagen to degrade. Collagen fibers will shrink when it heated above the shrinkage temperature (Ts). The shrinkage temperature (Ts) of fish collagen is 450C. If the collagen is heated at T>Ts, then the triple-helical fibers are broken down into longer lengths. The breakdown of these structures into random water-soluble windings is called gelatin [20].
The essential transformation in forming gelatin is to convert water-soluble collagen into water-soluble gelatin. Converting collagen into a suitable form means that collagen must be given a pretreatment so the collagen can be extracted. Extraction is the process of separating a substance based on the difference in solubility. Both of which are soluble liquids of different organic solvents, commonly water and other [39]. This extraction can cause the breaking of hydrogen bonds between the three free chains, two bonded chains, one free chain, and three bonded chains. Collagen fibers wouldn't dissolve during immersed in an alkaline solution or a neutral and non-electrolyte salt solution yet will expand properly. Collagen breakdown can occur as it exposed to acid or base, then will undergo a transformation from soluble and undigested strand to water-soluble gelatin [25].
Collagen in fish skin has the ability to form a gel after got heated and is higher than the collagen of fish bones, However, the differences in fish species also affect the ability of the gel-forming [3].
The process of converting collagen to gelatin will involve three changes [12], including:

1. The breaking of a limited number of peptide bonds to shorten the chain.
2. The breaking or a number of side bonds diversion between chains.
3. The occurrence of chain configuration changes.

3. Gelatin

Gelatin (gelatos) comes from Latin which means freezing. Gelatin is a derivative protein produced from the hydrolysis of collagen in skin, bones, and cartilage. Gelatin can be obtained by heat denaturation of collagen. Heating the collagen will gradually causing the damaged structure and the separated chains. Molecular weight, shape, and conformation of collagen solutions are sensitive to temperature change which can destroy the macro molecule [51]. The gelatin chemical process would be obtained through a set of collagen hydrolysis processes contained in the skin and bones. The collagen protein is scientifically "captured" and then converted to gelatin [1]. The reactions that occur are [38]:

$C_{102}H_{149}N_{31}O_{38} + H_2O \Longrightarrow C_{102}H_{151}N_{31}O_{39}$

Gelatin Collagen

Gelatin compound is a linear polymer of amino acids whose polymer chain is generally a repetition of amino acid glycin-proline-proline or glycine-proline-hydroxyproline in which the amino acid composition is almost similar to collagen, means glycine as the main amino acid and as the 2/3 of all the amino acids that make it up. The rest 1/3 amino acid is filled with proline and hydroxyproline. These amino acids are bound together through peptide bonds and form gelatin. The composition of the amino acid gelatin is Gly-X-Y, where X is generally the amino acid proline, and Y is generally the amino acid hydroxyproline. Proline and hydroxyproline levels in fish gelatin are known as lower than mammalian gelatin [23].

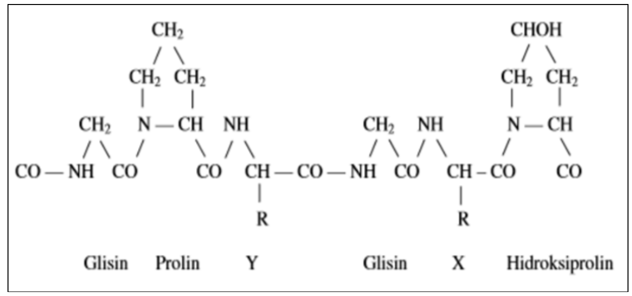

Fig 1: Chemical structure of Gelatin [45].

4. Physical properties and chemical Gelatin

The quality of gelatin is largely determined by the physical, chemical and functional who made gelatin has a unique character [14]. The physical and chemical characteristics of gelatin are affected by the raw material, animal age, collagen type, manufacturing method, tissue type, species, and collagen characteristics [44]. The older the animal's age could increase the yield, ash content and gelatin fat obtained [29]. While the use of high temperature and long extraction causing a decrease in viscosity value, the ability of gel-forming, and the gelatin physical character [19].
The physical characters of gelatin are solid, dry, tasteless, odorless, transparent, and dim yellow to amber [15]. Generally, gelatin has BM 80,000 gr/mol. Gelatin isn't soluble in alcohol, acetone, carbon tetrachloride, benzene, petroleum ether, and other organic solvents but will dissolve in water, acids, and alcohol solvents such as glycerol, propylene glycol, sorbitol, and mannitol. Under certain conditions, it also dissolves in the acetone-water and alcohol-water mixture [46]. Gelatin can expand in cold water, can form films, affect the viscosity of a substance, and can protect the colloid system. The density of gelatin is 1.35 gr/cm, and gelatin is denatured above 80^0C [47].
Gelatin is a solid colloidal system (protein) in a liquid (water) so that at high temperatures and water content, gelatin has the liquid ability, which is called the sol or hydrosol phase, on the opposite, at low temperature and water content, gelatin has a rougher or more concentrated structure, which is called the gel phase [40].
Gelatin came from collagen, which is a protein. Like most proteins, collagen has a primary, secondary, and tertiary structure. The form of collagen protein is linear as if a fiber. The composition of amino acids in gelatin is almost similar to collagen, where 2/3 of the amino acid formers are dominated by glycine. Meanwhile, the rest of the 1/3 amino acids are composed of proline and hydroxyproline. In gelatin, amino acids are chained together through peptide bonds.
Glutamic acid and aspartic acid, which made up collagen are mostly in the form of amide (about 35%), called glutamine and asparagine. In gelatin type B (bases), asparagine and glutamine are almost all converted into aspartic acid and

glutamic acid. Meanwhile, the composition of collagen and gelatin of type A (acid) of amino acids is very different. This can be explained by the difference of isoelectric points (IEP) in the two types of gelatin. The isoelectric point is the pH value when the gelatin molecule is neutral. The isoelectric point of gelatin type A at pH 8-9, and type B at pH 4.8-5.5 [31].

The functional characteristics of gelatin are very important in the application of a product because it will affect the product. The functional qualities are physical and chemical characteristics that influence the gelatin role in food during the process, storage, preparation, and consumption. The physical aspects of gelatin include the gel point, the general point, the melting point, gel strength, viscosity, emulsion activity, and stability and the degree of white, while the chemical properties of gelatin include water content, ash content, fat content, protein content, amino acid content, heavy metal content and pH [20].

Table 1: Gelatin Quality Standard

Characteristics	Terms
Color	Colorless pale-yellowish
Smell, taste	Normal
Moisture content	Maximum 16%
Ash content	Maximum 3.25%
Heavy Metals	Maximum 50 mg / kg
Arsenic	Maximum 2 mg / kg
Copper	Maximum 30 mg / kg
Zinc	Maximum 100 mg / kg
Sulfite	Maximum 1000 mg / kg

Source: SNI 06-3735-1995

Table 2: Properties of Gelatin is based on how it is making

Characteristics	Gelatin Type A	Gelatin Type B
Viscosity (Mps)	15-75	20-75
pH	3.8-5.5	5.0-7.5
Gel strength (bloom)	50-300	50-300

Source: GMIA (2012)

5. Gelatin manufacture

Gelatin manufacture divided into two types according to the gelatin classification itself, which was the process of acid and base. The difference between these two processes was the process of immersion (demineralization). Based on the strength of the cross covalent bond and the type of material hydrolyzed, the application of organic acids and bases and other hydrolysis methods such as hydrolysis time, pH, and temperature will vary. The main process of gelatin manufactured is divided into three stages, first was the pretreatment stage, the second was the conversion of collagen into gelatin (hydrolysis), and the third was purifying and drying [15].

Preparation is done by the skin and bones washing. The skin and bones cleaned from the remaining flesh and other impurities that contain high-fat deposits. To help cleaning, previously heating water is done for 1-2 minutes. The process of removing fat from bone or skin tissue called degreasing. Effective fat removal is done at a temperature between the melting point of fat and the temperature of bone albumin coagulation among 32-380 C so that the optimum fat solution produced [30]. Demineralization is an immersion process in acidic solutions that aims to remove the calcium salts and other salts so that ossein (melt bone) obtained. If the acid concentration used is too high, the protein contained in collagen can't be turned into gelatin [15]. Soaking time will also affect the quality of the resulting gelatin, where if the soaking is too long, then the protein content in the gelatin will be lower. In the process of immersion (demineralization) also results in swelling that can dispose of unnecessary material, such as fat and non-collagen proteins with minimum collagen loss [28].

The soaking stage must be done with the right time and concentration, so that collagen solubility does not occur in the solution, causing a decrease in yield produced [22]. The yield value can be the indicator to determine the effectiveness of the method applied in a study. The higher the yield value, means the more effective treatment applied. The conversion of collagen to gelatin usually based on the extraction temperature setting, soaking time, and also the concentration of acid or base used, to prevent protein damage at high temperatures [3].

6. Analysis of Gelatin

6.1 Ash content

Ash content analysis aimed to determine the mineral content contained in the gelatin test results. Ash content indicated the amount of inorganic material contained in organic matter. The high-low level of gelatin ash test results caused by the presence of mineral components that are bound to collagen which hadn't been released during the demineralization and washing process, so it was extracted and carried into the resulting gelatin [6]. Ash content was carried out using the 1995 AOAC method. Porcelain cups were dried in an oven at 100 °C, then cooled in a desiccator and weighed. One gram of sample was weighed and put in a porcelain cup. Further samples were carried out in an electric furnace at 550 °C for 5-6 hours or until ash was formed. The sample was then cooled in a desiccator and weighed. The calculation of ash content was done by calculating the weight ratio before and after the furnace process.

6.2 Water content

Water content is the water contained in the ingredients that can be expressed based on the wet and dry weight. The content of water contained in food ingredients determines acceptability, freshness, appearance, texture, taste, and food quality ingredients and durability of ingredients, therefore water content is an influential parameter in gelatin [4]. The test on the water content of gelatin aimed to determine the water contained in gelatin. Gelatin water content affects the storability because it was closely related to the microbial activity that occurs during the gelatin was stored [31].

Analysis of water content used the 1995 AOAC method. The aluminum cup dried in an oven 15 minutes with a temperature of 100 °C. Then cool it in a desiccator for 10 minutes and measured. About 1-2 gr gelatin samples in a measuring cup and dry in an oven at 105 °C for 5 hours until a constant weight reached. Cooled the next cup in a desiccator for 10 minutes and measured. The calculation of water content was to compare the before and after treatment weight in percent units.

6.3 Fat content

Fat content affected the quality changed during storage, so the analysis of fat content aimed to determine the possibility of product storability. Fat known as related to quality, where the damaged fat reduced the nutritional value and caused distortion of taste and odor [50]. Analysis of fat content carried out using the Soxhlet Extraction Method (AOAC, 1995). The fat flask that used, dried in an oven at 105 °C, then cooled in a desiccator and measured. Before measuring the fat content, the sample that had been weighed 1 gr got hydrolyzed first. The hydrolysis results were then filtered and wrapped with a sleeve and then corked with cotton and put into an extraction machine (soxhlet), which connected to the condenser and fat flask. Furthermore, it extracted for 4 hours. The fat flask contains extracted fat, dried in an oven at 105 °C for up to 5 hours, then cooled in a desiccator and weighed. Fat weight was calculated by using the formula for the ratio of initial and final weight in percent units.

6.4 Protein levels

The measurement of protein content aimed to determine the amount of nitrogen contained by an ingredient and analyzed by Kjeldahl. The sample measured about 0.5 g and put it into a 30 ml Kjeldahl flask. Then added about 0.9 g K2SO4, 40 mg HgO, and 2 ml H2SO4. If the sample weight was more than 15 mg, added 0.1 ml H2SO4 for every 10 mg of organic material above 15 mg. The sample was boiled for 1–1.5 hours until the liquid is clear. Then the solution put into a distillation device, rinsed with distilled water, and 10 ml of 30% NaOH solution was added. The NH3 gas produced from the reaction in the distillation apparatus, captured by H3BO3 in an Erlenmeyer, which had been added 3 indicator drops (a mixture of 2 parts of methyl red 0.2% in alcohol and 1 part of methylene blue 0.2% in alcohol). The condensate then titrated with HCl 0, 1 N that had been standardized until the condensate changes color to gray. The determination of the blank was done by the same method as determining the sample. Protein content calculated by calculating the difference in the volume of the titration and blank normality times of HCl and 0.014, the gelatin conversion factor (ie 5.55), the dilution factor divided by the sample weight multiplied by 100%.

6.5 pH

pH measurements carried out to determine the condition and load type found in gelatin. Gelatin is a polypeptide chain consisting of various amino acids. Amino acids have zwitterion or dipolar characteristics because, in their chemical structure, they have a negative functional group (COO-) and a positive function group (NH3 +). Amino acids were also amphoteric, which was acidic, neutral, or basic by environmental conditions [50].

6.6 Viscosity

Viscosity one of gelatin physical character of which remained the flowing power of molecules in a solution, both in water, simple organic liquid, suspension, and aqueous emulsion affected the gel character, especially the gel formation point and melting point, where high gelatin viscosity produced melting rate and gel formation higher than the low viscosity gelatin [26]. Viscosity partly influenced by hydrodynamic interactions between gelatin molecules, temperature, pH, and concentration [36]. Viscosity was related to the average molecular weight of gelatin and molecular distribution, whereas the molecular weight of gelatin was directly related to the length of the amino acid chain. It means, the longer the amino acid chain, the higher the viscosity value. The concentration of different acid solutions affected the weight of the resulting molecul4 [48]. Differences at the viscosity of gelatin may be the result of differences in the average molecular weight and molecular distribution. Higher molecular weights will increase viscosity [18].

6.7 Gel Strength

The gelatin strength defined as the magnitude of required energy by the probe to press the gel as high as 4 mm until the gel rupture. The unit to show the strength of the gel resulted from a certain concentration called the Bloom degree. The strength of gelatin gel obtained by the method carried out by GMIA. About 6.67 g of gelatin weighed and dissolved in 100 ml of aqua at a temperature of 60 ºC for 30 minutes. The solution was then cooled in a refrigerator at a temperature of 7 ºC for 16-18 hours. The gel strength measured with the texture analyzer tool which is micro stable and the value of the texture analyzer expressed in g-force.

The gel strength of commercial gelatin varies between 50 - 300 gr bloom. Based on its gelatin strength is divided into three categories below [41]:

I. Gelatin with high Bloom (250 - 300 gr Bloom)
II. Medium Gelatin with Bloom (150 - 250 gr Bloom)
III. Low Bloom Gelatin (50 - 150 gr Bloom).

6.8 White degrees

The white degree is a general description of gelatin's color. The white degree expected to be as close to 100 % since high-grade gelatin is usually colorless (clear) so that the applicability could wider (Budavari 1996). The white degree of gelatin affected by the raw material, the manufacturing method, and extraction [36].

6.9 Isoelectric Point

The isoelectric point of protein (pI) was the pH at which the protein had the same amount of positive ion and negative ion. Low protein solubility at its isoelectric point occurred in protein clumping or precipitation. The gelatin isoelectric point influences the use of various products, especially to the level of gelatin solubility [9].

6.10 Amino Acid Composition

Hydrolyzed gelatin then formed a double bond with a smaller size. Gelatin bonds in smaller structures called amino acids [24]. Amino acids were important factors that affected gel strength and gelatin viscosity. The composition of these amino acids causing gelatin becomes a multipurpose material in various industries. Gelatin could not be classified as a complete protein because there were no amino acids tryptophan [22]. The complete composition of amino acid in gelatin shown in Table 3.

Table 3: Gelatin Amino acid composition [13].

Amino acids	Total (%)	Amino acids	Total (%)
Alanine	11.0	Lysine	4.5
Arginine	8.8	Methionine	0.9
Aspartic acid	6.7	Proline	16.4
Glutamic acid	11.4	Serin	4.2
Genilanine	2.2	Cystine	0.07
Glycine	27.5	Iréne Theorin	2.2
Histidine	0.78	Tyrosine	0.3
Hydroxyproline	14.1	Valine	2.6
Leucine and ISO	5.1	Phenylalanine	1.9

6.11 Heavy Metals

Heavy metal is a type of metal with high molecular weight. Heavy metals accumulated in living things' bodies, which resulted in larger levels than those in the environment and would increase as the increasing of organism position at the food chain. The heavy metal analysis aims to determine whether the gelatin is safe to use or consume, especially in pharmaceutical products (medicines) and food products [8].

7. Gelatin Application
7.1 As stabilizer
Stabilizers play a role in maintaining product stability and preventing syneresis by increasing viscosity with its thickness [10]. The addition of gelatin as a stabilizer affects the viscosity because of the ability of gelatin to bind water so that water molecules were trapped formed gel structure [37]. Gelatin molecules contain three groups of high amino acids, which were one-third consisted of amino acids glycine or alanine, almost a quarter of them were amino acids bases or acids and the other quarter, were amino acids proline and hydroxyproline. The high proportion of these three polar amino acid groups which were hydrophilic, made the gelatin molecule have a high affinity to water [26].

7.2 As an Edible Film
Currently, a large number of research studies are focused on the use of Biopolymers and natural additives to produce the edible film with a high mechanical, barrier, antimicrobial, and antioxidant properties to preserve food and extend their storability [7]. Fish gelatin films have attracted attention because of their excellent film-forming ability, good barrier properties for oxygen and lights, and accomplishes consumer health expectations and not contradicting in any belief or it can be consumed by all religions [17].

7.3 Other applications
In photography, the gelatin used to extend the storability in storing photos, named photo-resist, that can avoid (by coating) from the presence of sensitive light [32]. Moreover, the gelatin used in photography for it has a high gel strength and sensitive to light in photo applications actively coated, therefore the gelatin from fish has the direct advantage that can be used without complicated treatment in its application and can be used to the same container for several days of use.

In the food industry, gelatin applied in products that require the formation of foam (whipping agent), for example, the ice cream making. Gelatin also increased the viscosity and function as a binder (binder), also as a thickener [47]. Gelatin also used as a food ingredient (food additive) that works for the growth of muscle precursors of keratin and as an enhancer of good taste with low-fat content. So that it can decrease the energy that the body consumed without any negative effects. Therefore, it can overcome the disease caused by obesity by helping to reduce energy due to excess fat. For patients, it is also good to give food containing gelatin because gelatin can excrete nutritious food in patients.

8. Conclusion
Fishery waste like fish skin, fish bones, fish heads, and internal organs usually left wasted and polluted the environment, while the waste section, can be processed into high-value products. Bones and fish are the waste containing collagen so that it can be used as the raw material for the gelatin industry.

Gelatin has the characteristics of a bright yellow or nearly white, shaped sheet, powder, or as if flour, stems, as if leaves, soluble in hot water, glycerol, and citric acid and other organic solvents. Gelatin was widely used for various industrial purposes, both food, and non-food industries because it had distinctive properties. Gelatin was obtained by hydrolysis using acid or alkaline solutions. The characteristics that determined the quality of gelatin was capacity, air content, ash content, fat content, protein content, viscosity, gel strength, isoelectric point, amino acid content, and the heavy metal content.

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