Scientific Bulletin. Series F. Biotechnologies, Vol. XXV, No. 2, 2021
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

METHODS FOR OBTAINING COLLAGEN FROM VARIOUS FISH SOURCES

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Abstract

Collagen is a natural biopolymer, widely used in various fields, including medical, pharmaceutical, cosmetic or food industry. It is obtained from terrestrial and aquatic sources. Waste from fish meat processing industry is an important source of collagen. The purpose of this paper was to show the possibility to obtain high quality collagen from the residues of the fish processing industry, the main methods of getting and characterizing it,as well as the possibilities of using it. The main methods of collagen obtaining are chemical methods using acetic acid, sodium chloride and sodium hydroxide and enzymatic methods using pepsin, trypsin or pronase. A more recent method, such as ultrasound improves the yield of collagen and its quality. In the case of hard tissues (bones, scales) it is necessary a decalcification often using EDTA or hydrochloric acid. The soft (meat, skin) and hard tissues are mainly treated with sodium hydroxide, butyl alcohol, acetone, etc. to remove non-collagenous proteins and fats. The obtained collagen is analyzed in terms of yield, hydroxyproline content, amino acids analysis, infrared spectra, molecular weight and denaturation temperature determination.

Key words*: collagen, hydroxyproline content, methods, chemical, enzymatic.*

INTRODUCTION

The agri-food industry generates a huge amount of biodegradable organic wastes, in solid and liquid form, with high humidity and biological instability, which favor microbial activity. They can disrupt the ecosystem around the place of storage, if improperly deposited (Nayaka & Bushand, 2019). The residues from fish processing industry represent 0.4% of the total waste amount. Total solid residues yielded by fish processing industry consist of the head, tail, skin, bones, scales, fins and viscera, which represent up to 75% of the fish weight, while fish skin and bones represent 30% from the fish residues (Sylvipriya et al., 2016). A series of valuable products, such as gelatin, collagen, bioactive peptides, proteins, amino acids, enzymes or biogas can be obtained by their processing and are useful for the pharmaceutical, food, cosmetic or medical industries.

Collagen is the main protein present in the extracellular matrix of connective tissues, such as skin, bones, ligaments, tendons, cartilage, internal organs and eyes. It is found in significant amounts in the vertebrates body, but also in invertebrates. In vertebrates body, it represents 30% of total protein content. Collagen molecule has a molecular weight around 300 kDa, a diameter of 14-15Å and a length of about 2800Å (Subhana et al., 2020). The polymeric chain contains repetitive sequence domains of Gly-X-Y or Gly-Y-X, in which Y is proline or hydroxyproline, the latter being the collagen-specific amino acid. Glycine is present in 30-40% and hydroxyproline/proline residues represent 25-35% of total amino acid content.

Collagen molecule consists of 3 helical polypeptide chains, twisted to the right, which have different amino acids composition. The resulting triple helix structure turns to the left (Figure 1). The triple helical structure is unique to collagen and confers its mechanical stability, elasticity and strength. The structure is stabilized by the presence of hydrogen bonds formed between constituent chains, involving the participation of amino and carboxyl groups,

water molecules and hydroxyl groups of hydroxyproline.

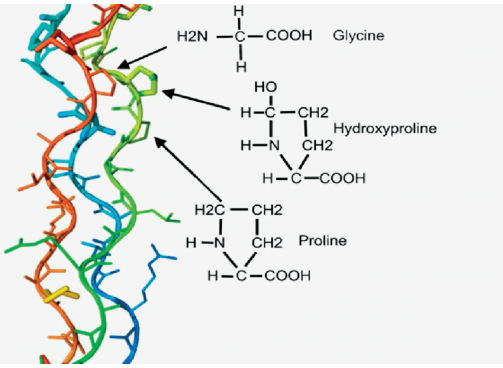

Figure 1 Collagen triple helix formation (Berillis, 2015)

Collagen present on the market has animal origin, being isolated from pigs or cattle. It is known that collagen protein of porcine origin is not accepted by population of Muslim or Jewish religion, while bovine collagen possess a certain risk of transmitting diseases to humans, for example bovine spongiform encephalopathy (Yuswana et al., 2021). Collagen of fish origin eliminates the religious restrictions and zoonosis risks (Sylvipriya et al., 2016).

Collagen can be extracted from any type of solid residue resulting from fish processing, such as bones, skin, scales and cartilage. These tissues represent a quite accessible raw material. The technological flow used for collagen extraction is presented in Figure 3. In order to obtain collagen with high purity and yield, a pretreatment of the raw material it is necessary. Depending on the used raw material, the preliminary processing stage has certain particularities. The second stage represents the effective extraction of collagen and it is performed by chemical methods (using dilute acids), enzymatic methods (using pepsin, trypsin, etc.) and methods that use ultrasound to improve the extraction yield

The aim of this work was to review recent approaches used for collagen isolation, purification and characterization from different fish tissues, in order to valorize wastes, improve environmental conditions and to establish its possible use as main ingredient of medical, food or cosmetic formulations.

COLLAGEN SOURCES AND THEIR PRETREATMENT

The raw material pretreatment is necessary to improve the collagen extraction efficiency and the final product quality. At this stage, the tissue is washed of any impurities and noncollagenous proteins are extracted (Arumugan et al., 2018; Ahmed et al., 2018; Tan et al., 2018). In some cases, the tissue is degreased, e.g. the skin, while in others tissue decalcification is required, for example in the case of bone tissue (Skierka et al., 2007).

Skin

Fish skin is rich in type I collagen, representing up to 70% of total protein content, depending on the species and the season (Chin et al., 2019). Collagen extracted from fish skin has an excellent ability to retain water, about 6% of its weight, and has no irritant potential, thus being suitable for medical applications (Nayaka & Bushand, 2019).

The pretreatment process consists of the mechanical cutting into small pieces of the skin, after removal of the adherent tissues (fat, blood, etc.), followed by extensively washing with tap water and then, with distilled water. To remove noncollagenous proteins, the skin is treated with basic solutions, generally with NaOH in concentrations ranging between 0.1-0.3 M or salts like NaCl for different time periods. Arumugan et al. (2018) treated the skin tissue with 0.3 M NaOH solution, for 4 h, while Ahmed et al. (2019) treated the skin with 0.1 M NaOH solution, for 24 h. In both cases, the ratio between the tissue and the basic solution was 1:10 (w/w) and NaOH solution was changed 3 times during the pretreatment. After NaOH treatment, the skin is washed with distilled water until reaching neutral pH. The next stage is degreasing, which is done with cold acetone, hexane or butanol. Arumugan et al. (2018) used 20% butanol solution, for 10 h, to remove fat. The ratio between the tissue and the degreasing solution is generally 1:10 (w/w). Wang et al. (2008) treated the deep-sea redfish skin with 1M NaCl solution, for 24 h to remove noncollagenous proteins and degreasing was performed with hexane.

Bones

A fairly high percentage of the fish processing industry residue is represented by bone tissue. Bones, including fish heads and backbones, are mineralized tissues with an extremely complex structure. They are rich in type I collagen, in a proportion of at least 30% (Jafari et al., 2020).

Preparation of bone tissues for collagen extraction begins with mincing and washing in common water. In order to obtain as much collagen as possible, it is necessary to decalcify the bones. This process can be done with EDTA or HCl solutions of different concentrations, in the ratio of 1:10 (w/w) between the bone tissue and the decalcification solution. The duration of the decalcification stage varies from 2 h to several days, depending on the type of solution used and the hardness of the bone tissue. Ahmed et al. (2019) decalcified the fish bones in 0.5 M EDTA solution, pH 7.5, for 3 days. Other studies have used a treatment with 0.1 M and 0.5 M EDTA solutions, pH 5, followed by 0.1 M, 0.5 M and 1 M HCl solutions treatment, at intervals of 1-4 days, using a ratio of 1:5 (w/w) between the bone tissue and the decalcification solution, in order to obtain bone decalcification (Skierka et al., 2007). It has been shown that decalcification with EDTA is less efficient, reaching up to 65%, compared with the method that uses HCl, which can reach up to 100% when using a concentration of 1 M HCl. The percentage of tissue decalcification is proportional with the concentration of the used solution and the time of incubation. Thus, incubation of bone tissue with 0.5 M EDTA solution for 96 h determined a slightly higher demineralization percentage, compared to that achieved after 72 h of treatment (Skierka E. et al., 2007). Bone tissue softened by decalcification pretreatment is then subjected to basic solution treatment to remove noncollagenous proteins and degreasing treatment, identical to that used for the skin tissue.

Scales

Fish scales contain inorganic components, such as hydroxyapatite (40-45%) (Zainol et al., 2012) and a percentage of 40-55% organic material, which mainly contains collagen (Chin et al., 2019). The decalcification of fish scales is done using a similar treatment as that performed on bone tissues. First of all, the scales are washed and then, they are treated with strong organic acids, HCl or EDTA, at pH 7.4-7.5. Chin et al. (2019) have obtained collagen from carp scales after demineralization with HCl in concentrations of 1 M and 1.25 M, and H_3PO_4 in concentrations of 0.5 M, 1 M and 5 M, or H_2SO_4 in concentrations of 0.5 M and 1 M. The highest amount of collagen was obtained after decalcification with a solution containing a mixture of 0.2 M HCl and 0.5 M H_2SO_4 (Chin et al., 2019). Ahmed et al. (2019) decalcified the scales using an identical pretreatment to that of bones decalcification using 0.5 M EDTA solution, pH 7.5. After decalcification, the process of noncollagenous proteins and fat removal takes place as described for the skin tissue.

Cartilages

Cartilages are rich in type II collagen, but also contain small amounts of types IX and XI collagen, which represent about 1-6% of the total collagen amount. The pretreatment stage consists of tissue washing and then, the minced tissue is treated with 4 M hydrochloric guanidine, in order to remove the proteoglycan components from its structure (Cumming et al., 2019).

METHODS FOR COLLAGEN EXTRACTION

Collagen fibers are found in the triple helix form with stable inter- and intra-molecular hydrogen bonds, which gives them water insolubility. Therefore, it is necessary to use specific techniques to increase the solubilization of collagen and the extraction efficiency (Jafari et al., 2020). The main methods used for collagen extraction are: chemical methods, which use dilute acids, enzymatic methods using different enzymes and methods that use ultrasound to improve the extraction yield (Kim et al., 2012).

Chemical methods

Acid extraction of collagen uses organic acids, such as acetic acid, or inorganic acids, such as HCl. Regardless of the used acid type, a low concentration is preferred. The ratio between the extraction solution and the tissue is of high importance, as well as the extraction period. The extraction is done at low temperature with

continuous stirring, necessary to increase the contact surface. The most used reagent for collagen extraction is acetic acid in a concentration ranging between 0.2 M and 1 M. Previous research has shown that the collagen extraction yield obtained after 0.5 M acetic acid treatment of tuna by-products, for 36 h has reached 13.5% (Ahmed et al., 2019). Chin et al. (2019) extracted collagen from fish scales using 0.5 M acetic acid, for 24 h, obtaining a yield of 15.33%. Wang et al. (2008) extracted collagen from deep-sea redfish skin, scales and bones using 0.5 M acetic acid, in a ratio of 100:1 (w/w) relative to wet tissue. A treatment of 24 h led to obtaining a yield of 47.5% in case of skin extraction, 6.8% in case of scales extraction and 10.3% for bone collagen extraction (Wang et al., 2008).

Collagen was extracted from the channel catfish skin using 4 different acids: acetic acid, hydrochloric acid, citric acid and lactic acid, in a ratio of 50:1 (w/w) relative to the tissue, with stirring, for 48 h. The used solutions had different pH values of 1.8, 2.1, 2.4, 2.7 and 3. The highest amount of collagen was obtained when using HCl extraction at pH 2.4 (42.36%), followed by extraction with acetic acid at pH 2.7 (39.45 %). The lowest yield results were obtained in the case of extractions with lactic acid and citric acid (Tan & Chang, 2010). In order to increase the extraction yield, the tissue residue obtained after collagen extraction is subjected to another extraction by repeated acid extraction or applying an enzymatic extraction.

Enzymatic methods

In order to extract collagen from fish tissues, a series of proteolytic enzymes, such as pepsin, pronase, trypsin, etc. are usually used. To obtain the best possible results, it is necessary to work at the optimal pH of the enzyme. The most widely used enzyme is pepsin, which is frequently dissolved in acetic acid at pH 2-3 (Jafari et al., 2020). A series of working parameters are of great importance for collagen extraction, such as pepsin concentration, the time of hydrolysis, the ratio between enzyme and substrate. Extraction of collagen from catfish skin was performed by digestion with varying pepsin concentration (0.118-23.6 KU/g) dissolved in hydrocloric acid solution pH-2.4, obtaining the highest protein recovery rate (64.19%) at a pepsin concentration of 23.6 KU/g (Tan et al., 2018). Ali et al. (2018) extracted type I collagen from golden carp skin using 1% pepsin solution in 0.5 M acetic acid, in a ratio of 1:15 (tissue: enzyme solution) with continuous stirring, for 24 h, to obtain an extract yield of 49.8%. Enzymatic extraction with pepsin is often repeated to extract collagen, if a large amount of tissue residue is obtained after the first acid extraction. Thus, Ahmed et al. (2019) subjected the remaining residue after acidic extraction to a digestion with 0.5% pepsin solution dissolved in 0.5 M acetic acid, using a ratio of 0.2 g pepsin/mg residual tissue. To inactivate pepsin in the obtained collagen solution, a dialysis stage was performed against Na_2HPO_4 at pH 7.2 (Ahmed et al., 2019). Tan & Chang (2010) extracted collagen from the channel catfish skin using different concentrations of pepsin ranging between 0.118-23.6 units (KU)/g skin, dissolved in HCl at pH 2.4, at a ratio between the tissue and the extraction solution varying between 1:5 and 1:20. It was observed that the highest protein yield was obtained at the pepsin concentration of 23.6 units (KU)/g tissue.

Previous research has evaluated the effect of bacterial collagenolytic enzymes extracted from *Bacillus cereus* FORC005 and *Bacillus cereus* FRCY9-2 on the extraction of collagen from skin of bigeye tuna (Ahmed et al., 2018). Thus, the undissolved residue obtained after acetic acid extraction was treated with bacterial collagenolytic enzyme in Tris HCl buffer containing $CaCl_2$, at 40°C, for 48 h. The yield of collagen extraction using this method reached values up to 18.8%.

Ultrasound methods

Ultrasound is a sound wave with frequencies higher than the upper limit of human hearing. Ultrasound devices operate at frequencies from 20 kHz to a few GHz. They are used in different types of extractions using various frequencies. Ultrasonic treatment generates a large amount of vibration-induced energy that increases the kinetic energy of the particles, providing energy for a reaction that can promote extraction (Kim et al., 2012).

Kim et al. (2012) have used ultrasound to isolate collagen from sea bass skin using an ultrasonic processor. After pretreatment, the

skin was incubated in 0.5 M acetic acid solution, in a ratio of 1:200 and subjected to ultrasound treatment with a frequency of 20 kHz with amplitudes of 20%, 40%, 60% and 80%, the exposure time being between 0 and 24 h. The temperature was maintained at 4°C by a circular cooling system in the water bath. In parallel, a control extraction was performed, under the same conditions, but without ultrasound. At 80% amplitude, 10.3 times more collagen was extracted than in the case of control extraction (Kim et al., 2012). Ali et al. (2018) have used ultrasound to extract collagen from the skin of the golden carp. The tissue was incubated in 0.5 M acetic acid solution, in a ratio of 1:15 and it was introduced into an ultrasonic reactor with a flat tip probe of 20 kHz, at a temperature of 4°C. The treatment was performed at different degrees of amplitude: 20%, 50%, 80% for variable periods of time of 10, 20 and 30 min, respectively, and after that, the extraction continued with stirring, for 48 h. An ultrasound-free extraction method was performed under the same conditions. The collagen concentration was proportional to the used amplitude and the period of sonication time. The highest amount of collagen was obtained in the case of sonication exposure at 80% amplitude, for 30 min. The application of ultrasonic treatment increased the amount of collagen by 81.53%, compared to the treatment without ultrasound (Ali et al., 2018). Ultrasonic collagen extraction could also be used to improve the enzymatic extraction with pepsin. Thus, the tissue was incubated in 0.5 M acetic acid, in a ratio of 1:15 between the tissue and the enzyme solution, and different pepsin concentrations were added to reach 0.1%, 0.5% and 1%. Sonication was performed at 80% amplitude, for 30 min. A parallel extraction experiment was done in identical conditions, but without sonication. The amount of extracted collagen was proportional to the concentration of pepsin and the yield was 120% higher in the case of sonication, compared to the identical method, but without sonication (Ali et al., 2018).

Other extraction methods

Another method of obtaining collagen is based on the extrusion process, mainly used to prepare food. Thus, fish powder was mixed with a solution of 1.26% citric acid, pH 2, or 9.37% acetic acid, pH 2, in a ratio of 4.7:1 (w/v) and subjected to extrusion cooking at 135 °C, than grounded. The resulting product was dried, mixed with water in a ratio of 1:10 and incubated at 25°C and 50°C, for 1 h. The mixture was centrifuged at 10200 g and the resulting supernatant was dried by lyophilization. The yield of type I collagen extraction using this protocol was 10.9% (Huang et al., 2016).

Supercritical fluids technology using CO_2 acidified water has been used to extract collagen from the skin of Atlantic cod fish. Supercritical fluid is a gas at temperatures and pressures above its critical temperature and pressure, but a pressure below the critical value is needed to compress it into a solid. The physical properties of a supercritical fluid lie between those of a gas and a liquid, and can be controlled by pressure and temperature, allowing the ability to dissolve some materials better than some gases. It was reported that, after tissue pretreatment, the mixture of cod skin and water was placed in a high pressure vessel heated to 370°C and pressurized to 50 bar, for 3 h. After depressurizing the vessel, the extract was filtered and lyophilized, and the yield of collagen obtaining process reached a value of 13.8% (Sousa et al., 2020).

METHODS FOR COLLAGEN PURIFICATION

The solution of acid- or enzyme-solubilized collagen resulting from the collagen extraction process, regardless of the performed method, is subjected to centrifugation and then, collagen present in the supernatant is purified using precipitation with salts, such as NaCl or chromatography techniques (Yata et al., 2001; Chin et al., 2019).

Salt precipitation

NaCl precipitation is the most widely used method and is based on the insolubility of collagen in NaCl solutions at different concentrations, but it depends on the used extraction method. Thus, the solution containing extracted collagen is centrifuged and NaCl is added until reaching the necessary concentration for precipitation. Then, the

solution is centrifuged, in order to collect the precipitated collagen, which is dissolved and dialyzed against dilute solutions of acetic acid. This method of precipitating collagen is convenient and can be used in industry. Ahmed et al. (2019) added a concentration of 2 M NaCl to the resulting centrifuged supernatant containing collagen, followed by centrifugation. The resulting precipitate was dissolved in 0.5 M acetic acid solution and then, it was dialyzed against 0.1 M acetic acid, for 24 h and against water, for 48 h. The resulting collagen was conditioned by lyophilization (Ahmed et al., 2019). Other studies purified collagen by precipitation with NaCl at a concentration of 0.9 M NaCl, after which it was centrifuged and the obtained precipitate was dissolved in 0.5 M acetic acid and then, dialyzed against 0.1 M acetic acid and water (Wang et al., 2008). Tan & Chang (2010) also used a concentration of 0.9 M NaCl to precipitate the chemically extracted collagen in acetic acid, hydrochloric acid, lactic acid and citric acid solutions. The same concentration was used to precipitate the enzymatically extracted collagen in pepsin/hydrochloric acid solution. The resulting precipitate was dialyzed against distilled water to remove NaCl. The obtained collagen was then lyophilized.

Chromatography technique

Purification of collagen by chromatography is frequently performed after precipitation with a salt, the most used being NaCl. Chromatography technique is used for separating the components of a mixture based on the ionic charge differences by their binding to a stationary phase and elution with a mobile phase. Chromatography performed on ion exchange columns allowed separation of type V collagen from other types of collagen, in particular types I and II. Yata et al. (2001) have extracted and separated type V collagen and type I collagen from the skin of 3 types of fish (common horse mackerel, yellow sea bream and tiger puffer). The collagen was solubilized with pepsin and precipitated with ammonium sulfate, resulting in 2 fractions. Then, the collagen extracts was purified by ion exchange chromatography using a phosphocellulose column. The fractions were separately loaded on the column and then, eluted with different NaCl concentrations. Finally, they were dialyzed against water and a solution of 20 mM sodium phosphate (Yata et al., 2001).

Yield of purified collagen

Regardless of the used extraction method, the efficiency of collagen isolation from different tissues is finally determined by extraction yield calculation. The main method used to estimate the yield is the determination of hydroxyproline content (Wang et al., 2008; Ali et al., 2018), the total protein content (Arumugan et al., 2018) or the amount of lyophilized collagen, which are reported to the initial tissue weight (Tylingo et al., 2016).

METHODS OF COLLAGEN CHARACTERIZATION

Analysis of the primary structure

This analysis has an important role for the evaluation of protein properties. To determine the amino acid composition of collagen, the acid hydrolysis of a sample is performed, followed by ion exchange liquid chromatography analysis. The amount of amino acids within samples differs depending on the source from which the collagen is extracted. Glycine represents 30% of the total amino acids and hydroxyproline is the specific amino acid in collagen molecule. It was reported following amino acids analysis that glycine was found in a proportion of 222-227 residues/1000 amino acid residues in the collagen extract obtained from bigeye tuna skin (Ahmed et al., 2018), 328-341 residues/1000 amino acid residues in the collagen extract obtained from the deep-sea redfish skin, bones and scales (Wang et al., 2018), 378-390 residues/1000 amino acid residues in collagen extracted from fish scales and 325-332 residues/1000 amino acid residues in collagen from golden carp skin (Ali et al., 2018). The presence of hydroxyproline in bigeye tuna skin collagen was 80-82 residues/1000 amino acid residues (Ahmed et al., 2018), 61-65 residues/1000 amino acid residues in the case of collagen obtained from the skin, bones and scales of deep-sea redfish (Wang et al., 2018) and 77-81 residues/1000 amino acid residues in the case of collagen extracted from the skin of golden carp (Ali et al., 2018).

Analysis of the secondary and tertiary structure

Identification of the chemical bonds and tertiary structure of isolated collagen is usually performed by FT-IR technique, which is based on an infrared spectrum of absorption or emission. Previous studies have shown that the specific absorbance of the -NH group from the amide involved in a hydrogen bond occured at frequencies less than 3330 cm^{-1} and was present in the case of collagen extracted from bigeye tuna skin (Ahmed et al., 2018), hoki hyaline cartilage (Cumming et al., 2019) and in collagen extracted from the skin, scales and bones of tuna bigeye (Ahmed et al., 2019). In the same studies, the C=O-coupled amide group had frequencies between 1600-1700 cm^{-1}. The presence of these chemical bonds confirms the existence of the triple helix.

Electrophoresis technique

The separation of proteins in polyacrylamide gels, based on their mobility, depending on the molecular weight is performed by electrophoresis technique. Typical electrophoretic patterns of type I collagen extracted from different tissues is presented in Figure 2.

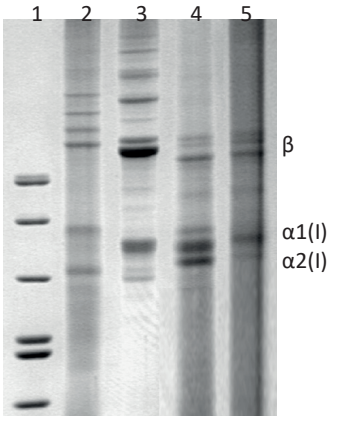

Figure 2. SDS-polyacrylamide gel electrophoresis pattern of collagen extracted from deep-sea redfish tissues: skin (3), scales (4), bones (5). A molecular weight marker (1) and type I collagen from bovine tendon (2) were migrated as controls (Wang L. et al., 2008)

Previous research has shown that the fish collagen is similar to bovine collagen, used as a control, observing well defined α1 and α2 chains, but also the presence of β dimers (Ahmed et al., 2019; Ali et al., 2018; Wang et al., 2018; Arumugan et al., 2018).

Analysis of denaturation temperature

The denaturation temperature represents the temperature at which the tertiary structure of collagen triple helix changes by the hydrogen bonds breaking, leading to variation of the viscosity degree of the collagen solution. Previous research has reported that collagen extracted from the skin, scales and bones of deep-sea redfish had denaturation temperatures of 16.1°C, 17.7°C, and 17.5°C, respectively (Wang et al., 2008), while those of collagen extracted from tuna bigeye skin, scales and bones were 33.7°C, 31.6°C and 32.3°C, respectively (Ahmed et al., 2019). The denaturation temperature is directly proportional to the amount of hydroxyproline. Thus, the redfish collagen extract presenting lower denaturation temperature had 6.1-6.5% hydroxyproline content (Wang et al., 2008), while the tuna collagen extract with higher denaturation temperature had 8.2-8.7% hydroxyproline content (Ahmed et al., 2019).

Collagen extracted from fish sources has biodegradability, low antigenicity and high biocompatibility. Its applicability in different fields, such as medical, pharmaceutical, food and cosmetics industry (Figure 4) is due to its resistant fibers, which can get additional stability by self-aggregation and cross-linking.

Collagen extract is easy to handle and it has the ability to combine with other natural or synthetic polymers to give valuable biomaterials (Moldovan et al., 2009). The main biomaterials obtained from collagen are conditioned in the form of sponges, membranes and films.

The collagen sponge (spongy foil) is obtained by freeze-drying (lyophilization) process (Figure 5), the membrane results after collagen gel drying in glass plates, in an oven, at a temperature below 35°C and the film is obtained by drying the viscous collagen solution in a thin layer, at a temperature close to room temperature.

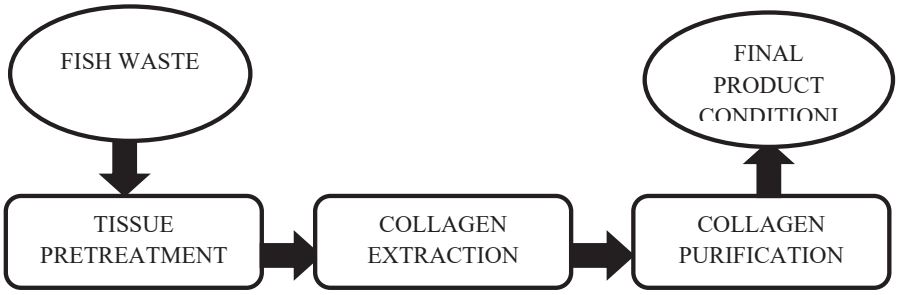

Figure 3. The technological flow chart of collagen extraction from fish residues

APPLICATIONS OF FISH ORIGIN COLLAGEN

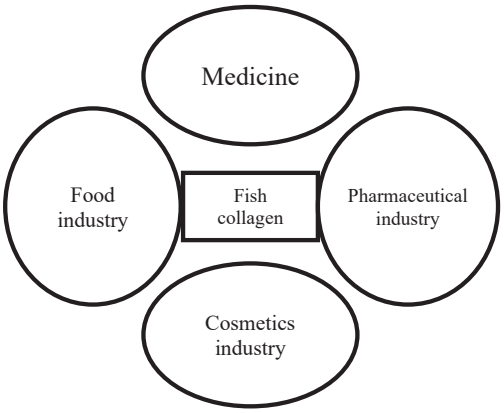

Figure 4. Applications of fish origin collagen

Figure 5. Macroscopic view of a collagen sponge (Cherim & Sarbu, 2009)

Collagen-based films can be used to replace the plastic packaging of fresh foods, such as meat (Jeevitah et al., 2013). Edible coatings developed using a thin film of collagen can prevent dehydration and oxidation of lipids in food products, thus prolonging their shelf life. In the case of fresh or frozen meat, the use of collagen layers or films helps to keep the texture, aroma, color and weight as long as possible. In combination with other compounds, such as fish bioactive peptides, the collagen films may have antioxidant and antimicrobial properties that prolong the life of products and improve their nutritional value.

In medical field collagen is widely used as a transporter of drugs to tissues or organs, eliminating the instability, solubility and poor absorption of drugs (Jafari, 2020). Collagen-based biomaterials play an essential role in repairing skin wounds, such as injuries and burns, due to the fact that this protein is the main component of the skin. Collagen membranes have high porosity, which allows the proliferation of cells at the site of application, the penetration of nutrients and oxygen, high biostability and low immunogenicity. The collagen sponge obtained from fish sources demonstrated a high capacity to stimulate cell proliferation of fibroblasts and keratinocytes, as main cells of the skin (Subhana et al., 2020).

In cosmetics, fish collagen is used to obtain skin care products with high moisturizing and softening effect. It alleviates the harmful effects of solar radiation and reduces the aging process of the skin (Jeevitah et al., 2013).

Collagen is an ideal material for tissue engineering applications due to its biological properties and the efficiency of extraction and purification processes. A disadvantage of fish collagen would be the low denaturation temperature, but after mixing with other synthetic or natural compounds, the value of the denaturation temperature could increase. Multifunctional matrices based on fish collagen have proven useful in the process of regeneration of bone tissue, dermal tissue or cartilage (Subhana et al., 2020).

CONCLUSIONS

The huge amount of residues resulted from fish industrial processing is a useful source for obtaining proteins of interest, such as collagen, a widely used biopolymer in medicine, cosmetics, the pharmaceutical industry and the food industry. Collagen can be extracted from fish tissues (skin, bone, cartilage, scale etc.) by chemical and enzymatical methods, mainly. Preliminary treatments of raw materials before obtaining collagen are of a major importance, such as washing, degrasing or decalcification, in the case of hard tissues. The evaluation of the protein properties is performed by determining the primary, secondary and tertiary structure, by determining the molecular mass, the denaturation temperature using spectrophotometry, electrophoresis, chromatography techniques.

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