JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

EXTRACTION OF COLLAGEN FROM FISHRESOURCES AND ITS APPLICATIONS

[1] Zalak Mehta, [2] Pushkar Bhojane
[1] Undergraduation student, [2] Lecturer
[1] Department of Biotechnology
[1] Patkar Varde College of Arts & Science, Mumbai, India

Abstract: Collagens are the foremost abundant high relative molecular mass proteins in both invertebrate and vertebrate organisms, including mammals, and possess mainly a structural role, existing differing types according with their specific organization in distinct tissues. In the last 20 years, marine collagen has attracted great scientific and industrial interest as a 'blue resource', with potential to be used in various health-related sectors, like food, medicine, pharmaceutics and cosmetics. Consequently, tremendously diverse marine organisms became an excellent source of various biological macromolecules which will be wont to develop tissue-engineered substitutes with wound healing properties. The marine collagen market is additionally briefly discussed to spotlight the opportunities and therefore the most profitable areas of interest. The collagen is extracted using fish skin, scales, bone, etc. After addressing the extraction of collagen from aquatic sources and its physicochemical properties, this review focuses on the utilization of marine collagen and its derivatives.

IndexTerms: **Collagen, application, marine collagen, extraction methods, characterization.**

I. INTRODUCTION

India is one of the countries endowed with good diversity and heritage. India's inland water resources have a great diversity, as they are abundant reservoirs that contribute the single biggest inland fishery resources in terms of size and production. Fish fauna of a reservoir basically represents the diversity of fish and their abundance in environment. Available marine fishes in India consists of ribbon fish, eel fish, horse mackerel, Katti fish, leather jacket, and Mahi. comprehensive utiliz ation of marine fish, especially the production of value-added products, has both environmental and economic importance.

Collagen is the abundant & important structural proteins in an organism. In extracellular matrix, of the many connective tissues. Collagen is a group of naturally occurring proteins. Collagen is the complex microprotein which has almost 20-30% of the total protein found in humans. Collagen is found in terrestrial organisms along with marine species. It has many properties, collagen is well-known as a structural support for biomedical devices, dermal implants and health applications, as well as being largely used in nutricosmetic, food and beverages.

Collagen is an important component of the wound-healing process; it acts as a natural structural substrate for tissue growth and plays an essential role in all phases of wound healing. Collagen is good surface-active agent. It also has many interesting properties regarding to surface behaviour, which includes emulsion, foam formation, adhesion & collision. The substantial own family of collagens with about 90% of the overall collagen is represented through the fibril forming collagens. In fibrous tissues encompassing of skin, tendons, and ligaments its miles as elongated fibrils. It constitutes 1 up to 2% of muscle tissue in which it is an important thing. The distribution of molecular weight, structure and composition, and subsequent functional features and properties of collagen, depend on processing conditions of raw materials from which is derived and the specificity of the enzyme used in extraction process. There is demand in the food industry for collag en and gelatin because of their high protein content & functional properties. Collagen has become very useful in both bio medical and non-bio medical industries in this modern era, with an extended range of usage. Many studies examined collagen in order to get bioactive compounds with antimicrobial, antioxidant and anti-hypertensive properties.

Collagen are about 280 nm long, with a molar mass of 360,000 Da; they are stabilized by hydrogen bonds, which are composed of three helical polypeptide chains, each with about 1000 amino acids, called an α chain. The chains become entangled, a stable triple helix which is varied in size. The triple helix molecules have terminal globular domains and they are called procollagen. The structural organization of collagen molecules can be lost by a process called denaturation, an irreversible kinetic process, resulting in random coiled polymeric chains, termed gelatin. The coiling of the three left-handed helices into the right-handed triple helix requires that every third amino acid is a glycine, while many of the remaining positions in the chain are filled by proline and Hyp. The sequence may be a repeating pattern of X-Y-Gly, where X and Y could also be attributed to the other aminoalkanoic acid residues.

At least, 29 collagen types have been identified, and each differs considerably in their sequence, structure and function. Different types of collagens have been reported, which are classified according to their structure into: fibrous, non-fibrous, microfibrillar and those which are associated with fibril. Described with types I, II, III, and IV to represent over 90% of the collagen in the body. Type I is found in many tissues like skin, liver, bones, aorta and cornea and it's the foremost abundant collagen type in vertebrates' tissues. It consists of 3 polypeptide chains, two are identical, which are called chain α1 and α2 and which are composed of different amino acids. Type-II collagen is that the basis for articular and cartilage. Type II collagen occurs almost exclusively in cartilage tissue and it's believed that the α1 (II) subunit is analogous to the α1 (I) subunit. Type III is quite similar with type I. It represents the 5-20% of the total collagen in mammalian tissues such as skin, bones and aorta. Type III collagen is dependent on age: young skin can contain up to 50%, but with the passage of time that percentage can reduced to 5-10%. Type II and III are formed by three a-chains of an equivalent type, a1(II)3 and a1(III)3 respectively. Type-IV collagen chain formation is a1(IV)]2a2(IV) and is found primarily within the basal lamina. Other sorts of collagen are only present in very small quantities, mainly in specific organs like the basement membranes, cornea, cardiac muscle, lungs and intestinal mucosa.

Structure of Collagen [4]:

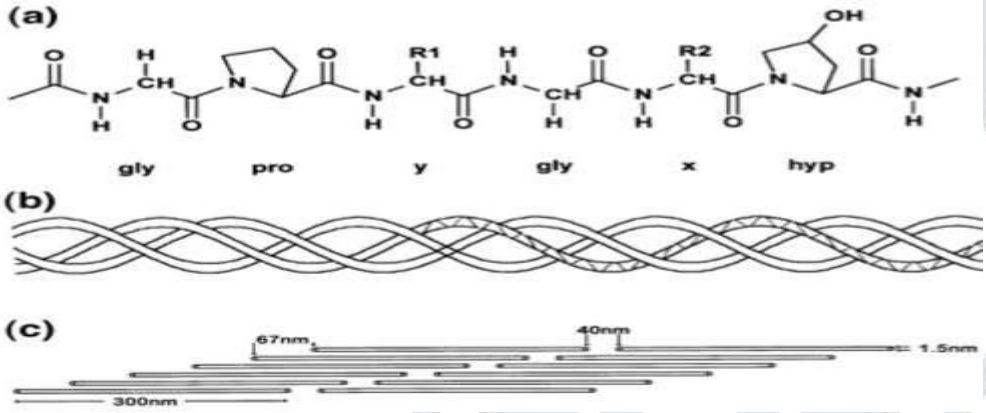

Figure 1

Chemical structure of collagen type i. A] primary amino acid sequence b] secondary left handed helix and tertiary right handed triple-helix structure and c] staggered quanternary structure

II. Marine collagen

Collagen can be extracted from many different organisms. Preferential sources of collagen are bovine skin and tendon also as porcine skin [5]. Marine collagens such as fish skin, bone, cartilage, and scales, including both marine vertebrates and invertebrates, are more bioavailable compared to bovine or porcine collagen and have high absorption capability and rapid bloodstream circulation due to low molecular weight and small size. About 75% of fish waste is discarded, in form of skins, bones, fins, heads, guts and scales. It is obtained predominantly type I collagen from skin, tendon, bone and muscle, which is most abundant type of collagen. Nevertheless, type II collagen also be obtained if cartilage is the selected source. Marine collagens are fibrillar and nonfibrillar, have lower gelling and melting temperatures than the mammalian collagen. Fish collagen is heat sensitive thanks to labile cross links as compared to mammals. These chain variants have approximately an equivalent relative molecular mass. Solubility of collagen is age dependent: collagenous tissues of older animals have a better number of crosslinkers, which makes them harder to solubilize than collagenous tissues from young animals. Fish collagen end in an outsized amount of waste—50% to 70% of original raw materials, which is generated from fish shops and processing factories. To make better use of this fish, the present study was conducted to extract and characterise pepsin-solubilised collagens (PSC] from bighead carp fins, scales, skins, bones and swim bladders and to provide a simultaneous comparison of five different sources from one species at one time [6].

III. Marine resources

The marine resources used in extraction of collagen includes:

3.1 Fish Skin

Fish skin typically contains type I collagen with a good degree of purity. Collagen from skin demonstrates a superb capacity to retain and exhibits no irritant potential, thus being suitable for dermal applications, collagen from the skin of catfish, pomfret, and mackerel requires a low extraction temperature, long extraction time, and generates yields of 2.27%. Isolation of the collagen was done by using 0.1MNaOH in 12 h, and it had been hydrolyzed using 0.5 M ethanoic acid [Acetic acid]. Another approach to extract collagen from fish skin is that the utilization of water acidified with CO2 that was wont to isolate collagen from Atlantic cod. This approach is widely used since the methodology can be easily applied in an industrial extraction of collagen [16].

3.2 Fish Scales

Fish scales constitute a substantial amount of waste from the fish processing industries. collagen obtained from scales has properties same as of type I collagen consisting of two α1 chains and one α2 chain. A study on collagen from the scales of tilapia, showed a high denaturation temperature (57.9–79.0 ºC) that was possibly due to its high amino acid content and higher intra/inter bonds. Collagen from the scale of tilapia, catfish, pomfret, and mackerel requires a higher extraction temperature and more extraction time with lower extraction yields compared to fish skin. Collagen from scales has also shown to possess proper water absorption and retention properties, which make it suitable for medical and therapeutic applications. Collagen-based wound dressing from the scales of fish has shown excellent antimicrobial activity through a disk diffusion method. Moreover, the wound dressing exhibited high wound closure capacity indicating the role of scale collagen within the speed up of re-epithelialization. Fish scales contain an outsized content of useful materials including organic components about 40% to 55% (collagen, fat, lecithin, various vitamins, etc.) and inorganic components about 7% to 25% (hydroxyapatite, calcium phosphate, etc.). Especially, collagens extracted from fish skin or fish scale waste are type I collagen, the most plentiful protein in the organisms and humans [6].

3.3 Fish Bones

Collagen from fishbone shows properties of type I collagen consisting of two A1 and one A2 Chain. Collagen from fish bones of tilapia, catfish, pomfret, and mackerel requires a high extraction temperature and shorter extraction duration compared to fish scales and skin. High-intensity pulsed electric fields (PEF) is one among the most approaches to extract collagen from fishbone. Extraction method of semi-bionic extraction (SBE) and PEF treatments were applied for the isolation of calcium, chondroitin, and collagen from waste fish bones. In the SBE method, the digestion and absorption process of the human alimentary canal is simulated through a repetitive acid and alkaline extraction. Desalting is additionally suggested as an important process in bone collagen extraction thanks to its high hydroxyapatite and calcium content, which are removed by EDTA or HCl during the pretreatment; however, using HCl can degrade the collagen [6].

3.4 Fish Cartilage

Fish cartilage consists predominantly of type II collagen, and some other types of collagen in minor quantities such as type IX and XI found in the nasal cartilage of Hoki. Type I collagen is found in cartilage of few species. The physicochemical and antioxidant properties of collagen isolated from silvertip shark were evaluated. Type II acid-soluble collagen, pepsin-solubilized collagen. The denaturation temperature of type II gelatin was higher than that of two other collagens. The collagens isolated from the silvertip shark are often an appropriate candidate for biomedical applications thanks to its higher antioxidant activity. Fish cartilage collagen shows a lower denaturation temperature than bovine collagen, which is attributed to the habitat of the species. This temperature instability limits the appliance of some collagen-derived biomaterials in human medical applications. Therefore, further investigation is required to find a sustainable alternative to fish collagen with a higher denaturation temperature, which is more convenient for biomedical applications and guarantee better performance in terms of thermal and mechanical stability [6].

3.5 Other Marine Sources

Fish waste is abundant worldwide and several studies, projects. The local and international authorities should focus on how to use this valuable waste. Fish processing industries produce a lot of amounts of fish waste every year. The waste mostly consists of bones, scales, fins etc. They are useful as the most of the collagen is found in this part only [7]. Discarded fish were obtained from a trawler vessel which operates in the West coast of the Iberian Peninsula [16]. Jellyfish is a prominent source for marine collagen extraction. Jellyfish has the potential to become a source of collagen because its collagen content is almost 60%. Some of the jellyfish collagens are comparable to vertebrate collagen IV or V [5].

IV. Role Of Collagen

Collagen is a crucial natural organic material and has been notably studied as a polymer for use in synthetic materials. Leather is chemically handled or treated animal product, whilst gelatin is that the animal animal tissue that's denatured and degraded through warmth and chemicals. Both consist largely of collagen however are very different in form. Collagen is element in cosmetics, resin-based composites, skin regeneration templates, biodegradable matrices and collagen shields in the discipline of ophthalmology. It is also used as strong-assist microcarrier within the production of enzymes. Collagens are powerful in penetrating growing old pores and skin. It also shows property of such as high tensile strength, low antigenicity and good biocompatibility [2]. The collagen degradation results in wrinkles, sagging skin, stiff joints, and dry skin [10].

V. Extraction methods

5.1 Pre-treatment

Due to the character of the cross-linked collagen that's present within the animal tissue of animals, it dissolves very slowly, even in boiling water. As a result, a light chemical treatment is important to interrupt these cross-links before extraction. In the acidic sort of pre-treatment the staple is immersed in acidic solution until the answer penetrates throughout the fabric. As the solution penetrates the structure of the skin at a controlled temperature it swells to 2 or 3 times its initial volume and therefore the cleavage of the non-covalent inter- and intra-molecular bonds occurs. The acidic process is more suitable for more fragile raw materials with less intertwined collagen, like porcine and fish skins. The alkaline process consists of treating the staple with a basic solution, typically caustic soda (NaOH), for a period which will take from a couple of days to many weeks. NaOH and $Ca(OH)_2$ are often used for pre-treatment, but caustic soda is best for pre-treating skins because it causes significant swelling, which facilitates the extraction of collagen by increasing the transfer rate of the mass within the tissue matrix. Concentrations of NaOH from 0.05 to 0.1 M were effective in removing non-collagenous proteins without losing the ASC and structural modifications at different temperatures. In addition to the utilization of acids and bases, enzymes or chemicals can also be wont to cleave the cross-linked bonds to get products with different characteristics [14].

5.2 Acid Extraction Procedure

ASC is understood when collagen is extracted by use of acid. Acids (such as HCl and Acetic acid) hydrolyze the triple helix of collagen and solubilize its single chains in solution, where the depolymerization of heavy weight proteins into shorter peptides. The interaction between the acid and therefore the collagen molecules break the crosslinks present within the collagen helix and increases the extraction efficiency. Acetic acid is one of the most common compounds through which collagen extraction from animal and marine sources is done. The range of concentrations for the acid extraction solution is between 0.5 and 1 M, which allows the cleavage of intra and inter-molecular crosslink without affecting the structure of the collagen. The yield of collagen increased gradually with increasing of the Acetic acid concentration so that a maximum yield was obtained. However, beyond 0.6 M, the collagen yield was reduced. The rheological behavior of the collagen solution as a function of Acetic acid concentration was investigated to understand the interaction between collagen and the solvent [10].

5.3 Enzymatic hydrolysis

For the extraction by enzymatic hydrolysis, raw material, which can be the residue of acidic extraction, is added to 0.5 M acetic acid solution containing selected enzymes such as pepsin, Alcalase and Flavourzyme. The mixture is continuously stirred for about 2 days at 4°C followed by filtration. The filtrate is subjected to precipitation and dialysis under an equivalent condition as for obtaining acid-soluble collagen. Pre-treatment was performed with NaOH (0.5 N) at 9°C for 12 to 36 hours for the removal of non-collagenous protein. The optimal extraction conditions were obtained with a pre-treatment of 0.9 N NaOH for 24 hours and digestion with pepsin at a concentration of 0.98% (w/v) for 23.5 hours. Initially, the skin was pre-treated with NaCl and Tris-HCI and then the saline-soluble collagen was extracted in 0.45 M NaCl at pH 7.5 for 1 day with continuous stirring; this was performed six times. After the extraction with salt, the residue was suspended in 0.5 M ethanoic acid for the extraction of acid-soluble collagen (ASC); the procedure was administered for twenty-four hours, twice. Pre-treatment was performed using NaOH and EDTA. Thereafter, the residue that wasn't dissolved by the acidic extraction was extracted with porcine pepsin in ethanoic acid for 48 hours at 4°C. The method of extraction can influence the length of the polypeptide chains and therefore the functional properties of collagen, like viscosity, solubility also as water retention and emulsification capacity [6].

VI. Characterization methods

6.1 SDS Page

One of the methods that are wont to characterize collagen purity and breakdown is that the Sodium dodecyl sulfate polyacrylamide gel electrophoresis [SDS-PAGE]. SDS-PAGE of freeze-dried collagen was carried out according to the methods [6;13]. With gel electrophoresis, proteins and their fragments are often separated, supported their size [16]. It is possible to watch the protein fragments by loading protein samples within the small wells of the gel, which, under an applied electrical field, travel through the gel matrix counting on their size: the littlest go further than the massive ones, which stay trapped within the gel net. In the case of collagens from an equivalent type but from different species, where the aminoalkanoic acid sequence is modified although an equivalent chain and types are present [for instance, α1, α2 and β chains in type I collagen], slightly shifts within the position of the bands could also be observed. Following electrophoresis, the gels were stained with 0.04% Coomassie Blue in 25% v/v ethanol and eight v/v ethanoic acid for 30 min at 60°C. Excess stain was removed with numerous washes of destaining solvent. 4% stacking gel and a 5% resolving gel made in the lab is used [11; 8]. Electrophoresis was performed on gels in 0.1M phosphate buffer [12].

6.2 FT-IR (Fourier transform infrared spectroscopy)

Using FT-IR, the functional groups and the interactions between the bonds of the extracted collagen were analysed. The sample was prepared using KBr (Potassium bromide) pellet method and scanned between 650 and 4000 cm−1 wave number, using FT-IR

instrument. The absorption intensity of the peak was calculated by the baseline method [9].

6.3 Scanning electron microscopy (SEM)

SEM (Scanning Electron Microscope) was wont to analyze the structure of extracted collagen. Some pieces of lyophilized collagen samples is first placed on the clean SEM plate then were coated with gold [1]. The structure was viewed under scanning electron microscope using 20 kV as the accelerating voltage [16]. The synthesized collagen was observed under naked eye and SEM to analyze its morphology [2].

6.4 Ultraviolet-visible spectroscopy (UV-Vis)

UV-Visible spectrum of collagen solution was carried out on a UV Spectrophotometer in the range of 200 to 400 nm [8]. Prior to measurement, a base line was set with 0.5 M acetic acid solution [9].

6.5 Differential scanning calorimetry (DSC) analysis

Freeze-dried samples were solubilized in 50 mM ethanoic acid. Thermostability of ASC solutions were measured in a microcalorimeter. All samples were introduced within the calorimeter at 278.15ºK and left for 1 h to stabilize. After, temperature increase was set to 1ºK/min. Two parameters were determined: the utmost denaturation temperature and transition temperature by the onset method. a lower thermal denaturation temperature is related to lower degree of proline hydroxylation in fish collagens [16]. The instrument used is calibrated for temperature and enthalpy using indium as the standard and the measurements were done while samples were constantly purged with ultrahigh-purity nitrogen at 50 ml/min [11].

6.6 Amino acid analysis

The amino acid composition of the collagens was expressed as amino acid residues per 1000 total amino acid residues [8]. Amino acids were analyzed in ASC after acid hydrolysis using 6.0 N HCl. It also contained 0.1% w/v under inert atmosphere and 110ºC for a day. After the hydrolysis, HCl was removed from the ASC hydrolyzate using vaccum [16]. Inc. Amino acid analysis was done using pre-column derivatisation with o-phthalaldehyde and fluorenylmethyl chloroformate. The individual amino acid content was based on the area of the corresponding peak as determined by the software on the elution curves of the sample and standard [5].

6.7 Peptide hydrolysis patterns

PSC samples (0.2 mg) were dissolved in 0.1 ml of 0.1 M sodium phosphate buffer (pH 7.2) containing 0.5% (w/v) SDS, and then 10 µl of the same buffer containing 5 ug of *S. aureus* V8 protease was added to the protein solution. Peptides generated by the protease digestion were separated using the SDS–PAGE method. The Precision Plus Protein All Blue Standards were used as markers [11].

6.8 Determination of denaturation temperature

The denaturation temperature of purified collagen was measured according to the method [6]. Freeze-dried collagen was dissolved in CH3COOH 0.5 M solution for viscosity measurement by using an Ostwald viscometer. The thermal determination curve was achieved by measuring viscosity of the collagen solution at different temperatures from 20°C to 50°C. The temperature was increased in stepwise manner and kept for 30 minutes at each point. The denaturation temperature, Td, was determined as the temperature that the change in viscosity was half completed [12; 8]. The collagen in this work having high denaturation temperature has promising applicability in food and pharmaceutical industry [6].

VII. Applications of collagen

7.1 Biomaterials Application

Due to water solubility, protection, biocompatibility, biodegradability, and easy extractability, as well as low immunogenicity, marine collagen has attracted scientific consideration for biomaterial applications. One of the main aims of tissue engineering is the regeneration of damaged, diseased organs and tissues using porous, biocompatible, and biodegradable scaffolds. Trauma infection, skeletal abnormalities, tumor resection, vascular necrosis, and osteoporosis are the agents that cause defects and damage in bones. Osteogenic activity of scaffold fabricated by marine collagen has been studied for bone tissue engineering applications. As cartilage does not have the ability to self-regenerate, cartilage tissue engineering attempts to repair or regenerate injured or diseased articular cartilage. Skin defects can be reconstructed via infection, trauma, genetic defects, and burns, as well as other diseases. To accelerate the wound-healing process, two main approaches can be used: skin regeneration engineering and wound dressing. Collagen derived from marine species could also be used in other tissue engineering areas such as dental, vascular, and corneal [10, 5].

7.2 Drug Delivery System

Researchers try to deliver drugs to specific body tissues or organs, thereby eliminating significant challenges such as poor bioavailability, stability, solubility, and absorption. To improve the bioavailability of allopurinol for treating gout and high levels of uric acid in the human body. Prepared a pH-sensitive hydrogel based on collagen from fish scales and carrageenan as a drug carrier. Marine collagen could be used as a wound dressing agent and drug carrier simultaneously [10].

7.3 Biomedical and Pharmaceutical Applications of Collagen

Numerous attempts have recently been made to use collagen as a biomaterial. Collagen is that the most promising natural biomaterial as scaffold in tissue engineering, because it is abundant, biocompatible, biodegradable, resembles the components present within the extracellular matrix and supports the connective tissue including skin, tendon, bone, cartilage, blood vessel, and ligaments collagen from fish is less crosslinked and its mechanical strength is poor compared to bovine collagen. Marine collagen is also widely used in dentistry generally as membrane, bone graft materials, an agent for local delivery and a haemostatic agent. The collagen which is used for local drug delivery is generally in the form of membranes. Membranes consist of two components: chlorhexidine and collagen. Collagen is also used to control bleeding. Different products are available commercially. The use of collagen-based materials in drug delivery involves the study of different aspects, such as in vivo instability, bioavailability, solubility with target-specific delivery and tonic effectiveness [7].

7.4 Food Additives and Packaging

Collagen can find wide application within the food industry, as an artificial additive or packaging [7]. Collagen has become a needed ingredient toward the healthy food development. The production of collagen within the body decreases with age and with an unhealthy diet. Collagen-based edible films and coatings have already been proposed to guard, maintain and extend the time period of food products. The film, during this case, as a barrier layer against migration of oxygen, moisture and solutes, providing structural integrity and vapor permeability to the foodstuff. Prevents fat oxidation, discoloration, microbial growth. collagen casings can be also coextruded around sausage meat batter obtaining a process that is continuous & well-controlled. as a serious limitation to widespread use, they need a robust sensitivity to moisture, which is liable for a drastic reduction in barrier and as thermomechanical properties. However, new methods and formulations for the production of marine collagen-based films with improved final properties and potential applications require further exploration [5].

7.5 Tissue Engineering and Regeneration

Marine fish collagens are utilized in various biomedical applications. Bone tissue engineering is the gold aim, with studies in this field. Apart from having mechanical elastic properties, it exhibited good absorption characteristics with interconnectivity between pores, which allowed human Mesenchymal Stem cells to adhere and proliferate. More studies have pointed that marine collagen proved to be good base for bone tissue engineering scaffolds. Jellyfish collagen is out there and relevant source to use as a matrix component for tissue engineering, since it exhibits low amount of impurity. Although marine collagen is considered to possess low antigenicity when considering its use in Tissue Engineering and Regeneration applications [15].

7.6 Cosmetic, Skin Care and Other Medical Applications

Collagens have an excellent potential to be utilized in the cosmetic field, where new generations bring new targets, toward beauty and maintenance of young appearance. The look for safe and cheap ingredients are constant. Marine proteins, and marine collagens especially, nowadays, are being presented as excellent functional ingredients for the cosmetic industry. Its properties take to the development of creams and gels with high moisturizing action, other activities are also foreseen, as anti-aging, anti-wrinkling or UV radiation protectors [5]. Collagens has been found to have applications in the healing of wounds resulting from different traumas, with collagen-based materials being used mainly to prevent moisture and heat loss from the wounded tissue, while providing also a microbial infiltration barrier. Besides, they need also been utilized in drug delivery systems. Collagen shields in ophthalmology, tablets for protein delivery, gel formulation together with liposomes as controlling material for transdermal delivery, and nanoparticles for gene delivery [15].

Researchers and developers are focusing on different sources to avoid the use of bovine collagen this due, as mentioned priorly, to the protein misfolding and allergenicity. For instance, marine collagen [type I] is being presented as excellent functional ingredients, also because its source is reasonable and avoids bovine spongiform encephalopathy [BSE] 40 making it an appealing option for product developer. Now in cosmetics, its properties fancy the event of creams and gels with high moisturizing action, but other activities also are foreseen, like anti-aging, anti-wrinkling, UV radiation protectors, and healing of wounds among other applications [3].

VIII. Conclusion

The standard types of collagens, including type I collagen which is widely used in healthcare products and foods, have been isolated and purified from several mammal and non-mammal marine sources, at a high purity and with pleasing yields, by using conventional collagen purification techniques. Contemporary societies across the world are facing an urgent need to find alternative, sustainable and eco-friendly resources due to the overexploitation of terrestrial resources and the problem of waste disposal. Till date, the sources of collagen mainly relied on terrestrial organisms, but they are becoming limited due to the spread of diseases and increasing alternative dietary choices of humans. Collagen has several biomaterials and the food industry. Among the methods of extraction that were discussed, acidic extraction can be efficient, but enzymatic extraction has some advantages, such as specificity, degree of

control of hydrolysis, moderate action conditions and less waste; for those reasons it is extensively used. The re-use of industrial by-products is of great importance in the search for cleaner and more sustainable production. Marine collagens should really be a true alternative source of collagens. Marine species presents a distinct advantage as a lower known risk of transmission to humans of infection-causing agents and are thought to be far less associated with cultural and religious issues concerning the human use of marine derived product. Its also anticipated that it may be requested an additional structural stabilization of such marine collagens by chemical derivatizations, resulting in higher denaturation temperatures and increased resistance to enzymatic degradation. Collagen-based biomaterials are very important for tissue engineering and regenerative medicine because of its superior biocompatibility and low immunogenicity due to the sources where collagen is taken from. It is important to say that because of all the types of collagens now exist, all cosmetic and pharmaceutical products should indicate which type they are using in their formula and why. It is expected that it will expand more and more in the coming years, opening in these way, new strong and interesting opportunities in the research field applicable to the cosmetic industry. Great amount of freshwater fish scales were dumped as a waste, but the result showed that it is possible to use the fresh fish scales as an important collagen or gelatin source. As a whole, valuable product recovery was achieved from the underutilized material, which may overcome the limitations for use of collagen in industries such as availability, purity and high cost. Thus, there is a possibility of using the fish processing waste as an alternative source of collagen; which otherwise may cause serious environmental pollution. FT-IR analysis indicated presence of helical arrangement of collagen. The SEM analysis of collagen confirms the presence and structuring of collagen fibrils. However, more investigation on the safety and efficacy should be done more to establish the use of collagen in cosmetic products and others.

REFERENCE

[1] Aminudin, A., Muhammad, S., Hamdan, R. H., Shaari, R., Nordin, M. F. M., Saufi, R. A., & Mei, S. J. (2015). Characterization of collagen extract from the skins of commercial freshwater fish. Jurnal Teknologi, 77(33).

[2] Arumugam, G. K. S., Sharma, D., Balakrishnan, R. M., & Ettiyappan, J. B. P. (2018). Extraction, optimization and characterization of collagen from sole fish skin. Sustainable Chemistry and Pharmacy, 9, 19-26.

[3] Avila Rodríguez, M. I., Rodriguez Barroso, L. G., & Sánchez, M. L. (2018). Collagen: A review on its sources and potential cosmetic applications. Journal of Cosmetic Dermatology, 17(1), 20-26.

[4] Berhie A. & Pandita A. (2019). Extraction, Characterization and utilization of collagen from Fish waste. International Journal of Scientific and Engineering Research, 10, 7.

[5] Berillis, P. (2015). Marine collagen: Extraction and applications. Research trends in biochemistry, molecular biology and microbiology, 1-13.

[6] Chinh, N. T., Manh, V. Q., Trung, V. Q., Lam, T. D., Huynh, M. D., Tung, N. Q., ... & Hoang, T. (2019). Characterization of collagen derived from tropical freshwater carp fish scale wastes and its amino acid sequence. Natural Product Communications, 14(7), 1934578X19866288.

[7] Coppola, D., Oliviero, M., Vitale, G. A., Lauritano, C., D'Ambra, I., Iannace, S., & de Pascale, D. (2020). Marine collagen from alternative and sustainable sources: Extraction, processing and applications. Marine drugs, 18(4), 214.

[8] Fengxiang, Z., Anning, W., Zhihua, L., Shengwen, H., & Lijun, S. (2011). Preparation and characterisation of collagen from freshwater fish scales. Food and Nutrition Sciences, 2011.

[9] Iswariya, S., Velswamy, P., & Uma, T. S. (2018). Isolation and characterization of biocompatible collagen from the skin of puffer fish (Lagocephalus inermis). Journal of Polymers and the Environment, 26(5), 2086-2095.

[10] Jafari, H., Lista, A., Siekapen, M. M., Ghaffari-Bohlouli, P., Nie, L., Alimoradi, H., & Shavandi, A. (2020). Fish Collagen: Extraction, Characterization, and Applications for Biomaterials Engineering. Polymers, 12(10), 2230.

[11] Liu, D., Liang, L., Regenstein, J. M., & Zhou, P. (2012). Extraction and characterisation of pepsin-solubilised collagen from fins, scales, skins, bones and swim bladders of bighead carp (Hypophthalmichthys nobilis). Food Chemistry, 133(4), 1441-1448.

[12] Nagai, T., & Suzuki, N. (2000). Isolation of collagen from fish waste material—skin, bone and fins. Food Chemistry, 68(3), 277-281.

[13] Pati, F., Adhikari, B., & Dhara, S. (2010). Isolation and characterization of fish scale collagen of higher thermal stability. Bioresource technology, 101(10), 3737-3742.

[14] Schmidt, M. M., Dornelles, R. C. P., Mello, R. O., Kubota, E. H., Mazutti, M. A., Kempka, A. P., & Demiate, I. M. (2016). Collagen extraction process. International Food Research Journal, 23(3).

[15] Silva, T. H., Moreira-Silva, J., Marques, A. L., Domingues, A., Bayon, Y., & Reis, R. L. (2014). Marine origin collagens and its potential applications. Marine drugs, 12(12), 5881-5901.

[16] Sotelo, C. G., Comesaña, M. B., Ariza, P. R., & Pérez-Martín, R. I. (2016). Characterization of collagen from different discarded fish species of the west coast of the Iberian Peninsula. Journal of aquatic food product technology, 25(3), 388-399.