Extraction of Collagen, Gelatin and its Derivatives from Fish Wastes

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Processing of fish generate enormous amount of by-products (wastes). Fish processing waste is defined as secondary fish material generated during primary processing of the fish. The secondary fish material is in the form of scales, skin, head, viscera, bones, frames, fins etc. and comprised of around 50-80 % of the whole fish. Such waste generated in fish processing plant can become raw material for auxiliary industries or other industries. Some of the high value products which can be obtained from the solid waste of fish are oils, pigments, meals, minerals, enzymes and protein concentrate etc. Other possible utilization of processing waste would be manufacturing of valuable biomolecules such as collagen and gelatin. It is estimated that about 30% of the wastes generated in fish processing consists of skin and bone which are rich in collagen content. The global collagen market is anticipated to reach USD 6.63 billion by 2025. Collagen, gelatin and their hydrolyzed products are the key product segments in this market. The market is growing due to increasing demand for collagen-based products in healthcare applications (such as wound healing, tissue engineering, bone reconstruction etc), food & beverages and cosmetics industries.

Collagen and gelatin

Collagen is a structural protein having a characteristics triple helix structure. Collagen is insoluble in water and fibrous in nature. Approximate molecular weight of a collagen molecule is 300KDa. There are 19 genetically distinct collagen types which are characterized by considerable complexity and diversity in their structure, their splice variants, and the presence of additional, non-helical domains, their assembly and their function. Each of the three α-chains within the molecule forms an extended left-handed helix with a pitch of 18 amino acids per turn. The proper folding of each of these chains requires a glycine residue to be present in every third position in the polypeptide chain. For example, each α-chain is composed of multiple triplet sequences of repeating Gly-X-Y units where Gly is glycine, Y positions are mostly occupied by the imino acid proline and hydroxyproline and X is any of the other amino acids, account for 2% of the molecule and plays a vital role in fibril formation. The three chains are supercoiled around a central axis in a right-handed manner to form the triple helix known as tropocollagen. The triple-helix is approximately 300 nm in length and 1.5 nm in diameter, followed by short extra helical telopeptides. The telopeptides do not adopt triple-helical conformations due to the absence of repeating Gly-X-Y units. The triple helices are stabilized by inter-chain hydrogen bonds. β dimmers and γ trimmers are also found in collagen. The β component is due to the intermolecular crosslinking while that of γ component indicates intramolecular crosslinking. Collagen is rich in nonpolar amino acids such as glycine, proline and hydroxyproline. The stability of the collagen triple helix is related to the total content of

proline and hydroxyproline. Collagen derived from fish is generally of Type I and Type III. Type I and Type III collagen are the building blocks for connective tissues, bones and skins.

Gelatin is soluble protein produced by controlled hydrolysis of fibrous insoluble collagen. Warm-water extraction process is generally used for hydrolysis of collagen into gelatin. Heat treatment cleaves the hydrogen and covalent bonds to destabilize the triple-helix, resulting in helix-to-coil transition and conversion into soluble gelatin.

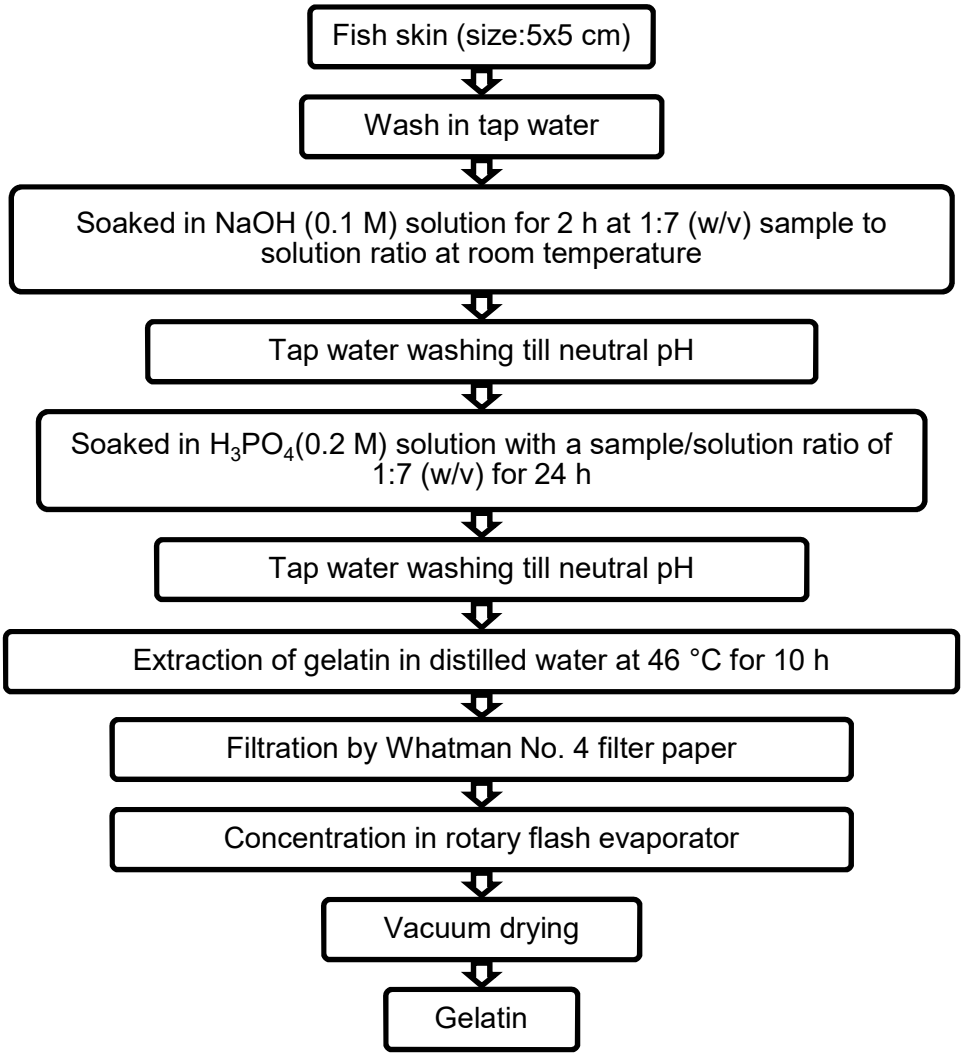

Figure 1: General steps for isolation of gelatin from fish skin. (Source: Hanjabam *et al*., 2015)

Some of the functional properties of collagen and gelatin are discussed here. Gel strength/Bloom value is the test to measure the strength of gel. The gelation process for both collagen and gelatin is thermo-reversible, but in opposite directions: collagen gels melt by lowering the temperature, while gelatin gels melt by raising the temperature. The commercial value of gelatin is determined by its bloom value. Fish gelatin typically has a gel strength ranging from as low as 0 to 426 g. warm-water fish gelatin have been reported to exhibit high gel strengths than cold water fish gelatin.

The setting and melting points of gelatin are also considered important indices of the quality of gelatin preparations. Being thermo-reversible, gelatin gels will start melting when the temperature is increased above a specific point, the melting point, which is usually lower than the temperature of the human body. For gelatins from fish species, setting temperatures are in the range of 8–25°C, while the range of melting temperatures is 11–28°C. The melting and gelling temperatures of gelatin have been shown to correlate with the proportion of Proline and Hydroxyproline in the original collagen.

Collagen is not soluble in water. However, fish type I collagen is unique in its extremely high solubility in dilute acid compared to avian and mammalian collagen. Gelatin is only partially soluble in cold water; however dry gelatin swells or hydrates when stirred in water. On warming to about 40°C gelatin that has been allowed to hydrate for 30 minutes melts to give a uniform solution. The solubility of collagen is affected by the pH and NaCl concentration of the solution.

Collagen and gelatin hydrolysates

Although collagen/gelatin has several functional properties, its bioactivity is lower due to its high molecular weight. Hydrolyzing will enhance the bioactivities of the collagen/gelatin. Collagen or gelatin hydrolysates are produced by controlled hydrolysis of collagen or gelatin. Acid, alkali, enzyme or heat may be used for hydrolysis. During hydrolysis the peptide bonds are broken down producing low molecular weight peptides. The molecular weight of hydrolysate is generally in the range of 5.0-25 kDa. In case of gelatin, hydrolysate can be produced using two different processes. In the first process, hydrolysate could be manufactured after gelatin extraction from the source by enzymatic hydrolysis. In the second process, the hydrolysates derived peptides can be prepared without prior extraction of gelatin. The second process could shorten the processing time and production costs by eliminating the gelatin extraction step. Commonly used proteases and their reported optimal hydrolysis conditions (pH and temperature) for the production of hydrolysate protease are alcalase (8.0–9.5; 50–60), pepsin (2.0; 37-50), papain (6.5–7.0; 40–70), trypsin (7.0–8.0; 37–50), pancreatin (7.0–8.0; 37–50), bromelain (7.0; 40-50), flavourzyme (6.0–7.0; 50–60), protamex (7.0; 50), neutrase (6.5–7.0; 50–60) etc. Figure 2 shows the overall process for production of gelatin hydrolysate from fish skin using thermal and enzymatic hydrolysis. Thus hydrolysis of collagen or gelatin yields bioactive peptides that have great potential in processing industries as natural preservatives. Collagen and gelatin peptides are known to have excellent antioxidant properties unlike its parent molecules. Recently gelatin hydrolysate has been explored as plastisizer in protein film, identified as antihypertensive, cryoprotectant in additions to its wide known antioxidant activity.

The recovery of chemical components from seafood waste materials, which can be used in other segments of the food industry, is a promising area of research and development for the utilization of seafood by-products.

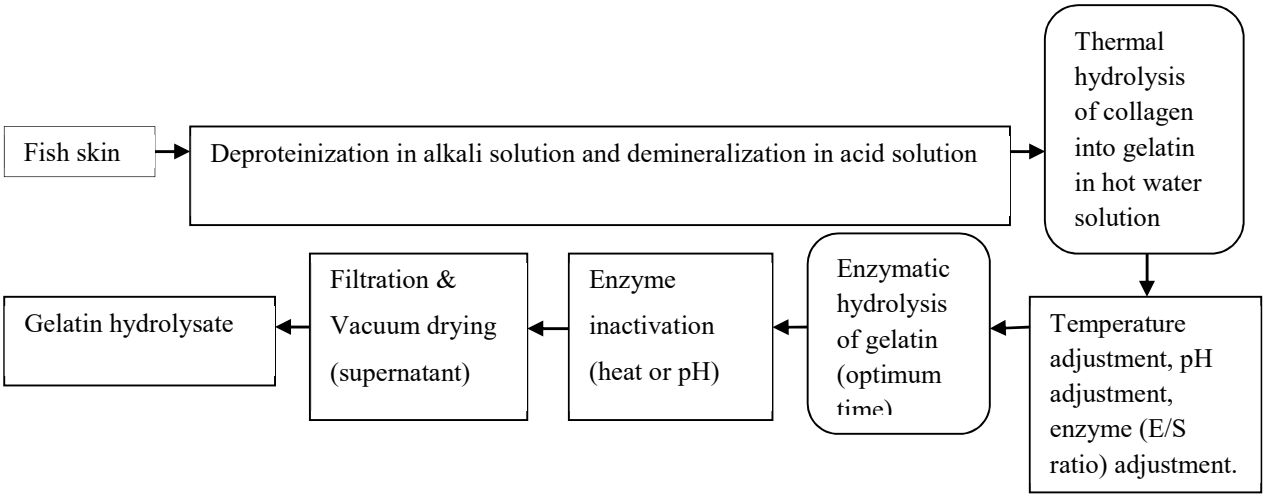

Fig 2: Process layout for enzymatic hydrolysis of fish skin gelatin

Suggested Readings

- Hanjabam, M. D., Kannaiyan, S. K., Kamei, G., Jakhar, J. K., Chouksey, M. K., & Gudipati, V. (2015). Optimisation of gelatin extraction from Unicorn leatherjacket (*Aluterus monoceros)* skin waste: response surface approach. *Journal of food science and technology*, *52*(2), 976-983.