Obtaining of Collagen Extracts Used as Biomaterials with Applications in the Medical Field

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Abstract

Collagen is the core protein of connective tissues: skin, bone, tendon, base membrane, etc. Collagen is actually a family of several different genetic types. Currently they are known, in vertebrates, at least 27 different types of collagens, which shows a remarkable diversity in molecular and supramolecular organization of the tissue distribution and function, discovered and developed over 45 years. They were studied, in detail, 12 main types. Collagen-based bioproducts can be produced in a variety of molecular structures (micro and nano structures) in powder form, hydrogels and injectable solutions, films, membranes and matrices, etc. This paper presents the drying processes that are selected depending on the nature of the extract (undenatured or denatured) and morphological structure bioproduct or sponge, fibers or membranes. The most frequently used procedures for drying are freeze-drying and free drying at a temperature of approx. 25 ° C. Both processes produce no distorts to the extracts They are presented bioproducts derived from collagen which are used in medicine.

Keywords: Collagen, bioproducts, molecular structures, hydrogels

1. Introduction

Collagen is a unique in its ability to form insoluble fibers that have a high tensile strength and right-handed triple superhelical rod consisting of three polypeptide chains and is found in connective tissues, including tendons, bones and skins (e.g. type I collagen) [1]. It is classified into a number of structurally and genetically distinct types. Type I ncollagen is a heterodimer composed of two alpha1(I) chains and one alpha 2(I) chain that spontaneously forms a triple helix scaffold at neutral pH and 37 C.

Collagen type I is an excellent substrate for the culture of hepatocytes, fibroblasts, spinal ganglion, muscle cells, Schwann cells, embryonic lung cells, epithelial cells, and a number of other cell lines. It has also been used in the study of growth, differentiation, migration of cell lines, and tissue morphogenesis during development.

Although fish-derived collagen does not form high-viscosity gels, it is extremely convenient for some applications, such as micro-packaging or obtaining photosensitive coatings. Films and porous matrixes can be obtained from collagen-based gels, just as from mammal-derived ones. They can be successfully used in dental medicine for treating oral diseases – they form bio-absorbable membranes and matrixes and they can incorporate different active ingredients which can be subsequently released in order to obtain the intended therapeutic effects [3]

Collagen has been, traditionally, isolated from the skins of land-based animals, such as cow and pig. Non-denatured collagens from these sources find applications in food, cosmetics, biomedical,and pharmaceutical industries. Denatured collagen, known as gelatin, finds applications in the food and biomedical industries. Biomedical and pharmaceutical applications of collagen include the treatment of hypertension, urinary incontinence and pain associated with osteoarthritis, use in tissue engineering for implants in humans, inhibition of angiogenic diseases, such as diabetes complications, obesity, and arthritis [4].

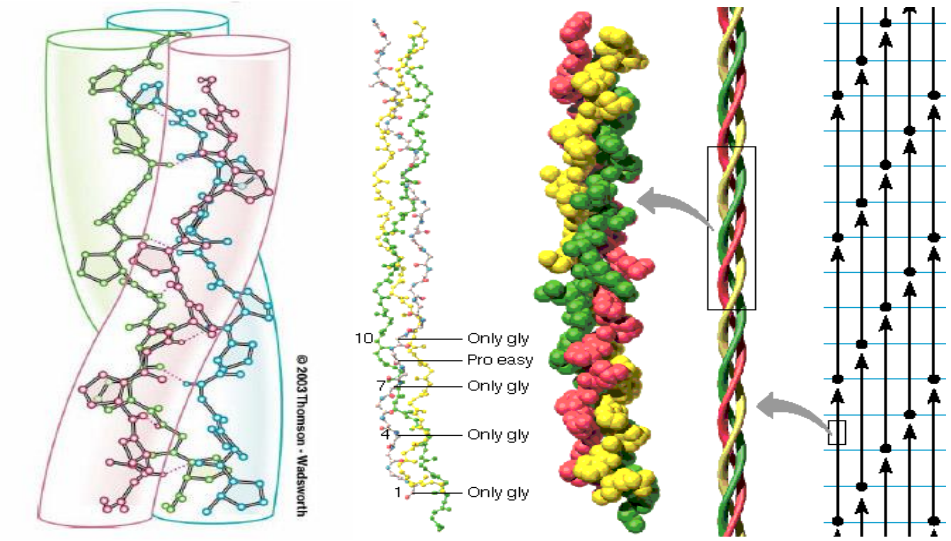

Fig. 1 Overview of the collagen triple helix. [2]

a) Triple helix.

b) The basic structure of the 3 helix collagen

The alternative sources of collagen, especially from aquatic animals including freshwater and marine fish and moof fish by-products as a source of collagen can

beneficially impact waste management. Collagen molecules in solution denature close to the upper limit of the physiological temperature or the maximum body temperature of the animal species from which the collagen is extracted [5]. Many researchers have focused on the practical utilization of marine animals to produce collagen [6]. Some concerned collagens from freshwater fish, such as carp [7], [8] and grass carp [9]. However, relative lower denaturation temperatures, i.e., lower thermostability, have become one of the main limiting factors for the application of fish collagens, especially for those from marine fish.

Gelatin is a multifunctional ingredient that has long been used in the food industry as a gelling, thickening, and film-forming agent, as well as an emulsifier and stabilizer [10]. The source of collagen and the manufacturing process significantly affect the physicochemical and functional properties of gelatin [10]. Traditional gelatin production involves the pretreatment of raw material followed by extraction and purification steps [10]. Acid or alkali is usually used in the pretreatment steps to remove impurities and cleave the collagen crosslinks, then gelatin is a produced by a partial thermal denaturation of collagen.

This paper aims at presenting two comparative biotechnology used to obtain collagen-based gels from grey mullet skin : Cold-treatment with 0.5M acetic acid, and treating with hydrochloric acid . Obtaining the biomaterial was prosigns by freeze-drying the hydrogel .

2. Materials and Methods

Fresh "grey mullet (Mugil cephalus)" fish were collected from market. The skin were washed thorough- ly with distilled water, and stored at –25°C until used. All reagents used were of analytical grade.

A. The Biotechnology used to Obtain Collagen from "grey mullet (Mugil cephalus)"

a.1. Demineralization Process

Initially, the fish scales were washed twice in 10 wt% of NaCl solutions to remove unnecessary proteins on the sur face by stirring the solution for 24 h. Demineralization was achieved with 0.4 mol/l HCl solution (dry scales: solution = 1:15) for 90 min. The demineralized scales were washed three times with distilled water for collagen extraction.

A.2 Isolation of Collagen

Collagen Extraction from *Grey Mullet* by Acid Treatments

A.2 .1 Cold-Treatment with 0.5m Acetic Acid

As room temperature enabled – in the case of bovine skin – removal of the epidermis, this pre-treatment was also applied to grey mullet Considering the fact that the

denaturation temperature of collagen extracted from fish skin is lower than that obtained from bovine skin, the 0.5M

acetic acid pre-treatment was applied both at room temperature (23-26 ^{o}C), and in the frdige (5-7 ^{o}C), without stirring, using variable skin/acid solution ratios, between 1/7.5 and 1/20, aiming – each time – at submerging the skin entirely. Shark skin quickly becomes soaked in the 0.5M acetic acid solution,significantly increasing its volume and weight. Thus, at 27 ^{o}C it gains – in about 1 hour – circa 74% in weight, it thickens and gets a jelly-like aspect. Horny epidermis can be cleaned (it was cleaned) and the solution will be clear and of low viscosity, so under these circumstances collagen was not extracted completely (losses are negligible). After cleaning, the skin was washed twice in tap water and twice in distilled water to remove any remains of horny layer, then it was finely chopped and divided into two batches in view of extracting collagen: one batch was still treated with acetic acid of the same concentration, while the other was treated with pH 3.5 hydrochloric acid, in both cases the ratio between soaked and filter paper-dabbed collagen and solution being 1/7. The samples were stirred energetically for 1 hour and 40 minutes, and then they were placed into the fridge. In the meanwhile viscosity increased, appearing to be higher than in the case of the hydrochloric acid solution. On the second day they were removed, left to reach room temperature and forced through fine muslin to remove the jelly-like component, whose suspension also contains visible bits of skin. Colourless filtrates were centrifuged for 30 minutes, then placed into the fridge, while a new amount of acid was inserted over the jelly-like component to continue extraction.[3]

A. 2.2 Treating with 0.5m HCL

The other batch of skin was inserted into a pH 3.5 hydrochloric acid solution, using a wet skin/solution ratio of 1/5. After 24 hours it seems unchanged and, indeed, its weight increased during this while by only 9%. The skin was removed from the hydrochloric acid, it was washed entirely using tap and distilled water until all chlorine ions had disappeared, then the other treatment was applied, involving cold 0.5M acetic acid solution (skin/acid ratio =1/7) and storage in the fridge. After 24 hours the skin increased its volume considerably, it started peeling off, although with difficulty and incompletely, which is why it was left for another 24 h in acetic acid, just as in the case of direct treatment with acetic acid.

After 48 hours the epidermis was cleaned, the clean skin was chopped into small pieces and then inserted into 0.5M acetic acid solution. Both the vessel containing skin and acetic acid and the colourless and relatively viscous supernatant were placed into the fridge. After 5 days the viscous supernatant was separated from the undissolved skin by decanting, and the two samples were mixed.[3]

3. Results and Discussion

Some physical and chemical characteristics of collagen from Grey mullet, are presented in Table 1.

The technology used to dry is selected according to the nature of the extract (denaturated or non-denaturated) and the morphological structure of the bioproduct, that is, sponge, fibres or membranes. The most commonly used drying techniques are lyophilisation and natural air drying at temperatures of approximately 25°C. Neither of the two techniques leads to denaturation of the extracts.

Table 1 Physical and chemical characteristics of collagen

CARACTERISTICS	*COLLAGEN EXTRACTED with 0.5M acetic acid*	*COLLAGEN EXTRACTED with 0.5M HCL*
Aspect	*fibrous*	*fibrous*
Colour	*White-yellow*	*White-yellow*
Humidity, %	*0.130*	*0.162*
Minerals %	*97.95*	*99.77*
PH	*3.5*	*5.5*

Lyophilisation is a drying procedure relying on the rapid freeze of collagen solutions from – 25°C to – 70°C and sublimation of the ice directly in the water vapour phase (2 x 10-3 torri). In place of the ice crystals, pores are formed and collagen molecules are restructured into fibres and fibrils. Lyophilized collagen is represented by a sponge called matrix, with similar characteristics to the extracellular matrix.

Natural air drying of collagen extracts is performed in shelved dryers with warm air current at a temperature of 25°C and a rigours control of humidity, so that drying can be performed slowly in 48-72 hours. Under these circumstances, between the collagen molecules of the extract intermolecular bonds are made, without requiring the intervention of chemical agents.

Fig. 2 Collagen extracted: with 0.5M acetic acid with HCL 0.5M	*Fig.3 Obtaining collagen biomaterials*

Atomization drying is used for denaturated collagen extracts, especially for hydrolysates. Procedures for obtaining gel or colloidal solutions require technological filtration and condition in sterile environments so that the bioproducts are not contaminated with microorganisms.

Regarding the process of obtaining biomaterials from non-denaturated collagen (pastes and gels or collagen solutions) key-steps are represented by: restructuration, chemical alteration, compatibilization with various bioactive compounds and drying or conditioning as a finished product.[10]

Fig.4 Extraction with 0.5 M acid acetic

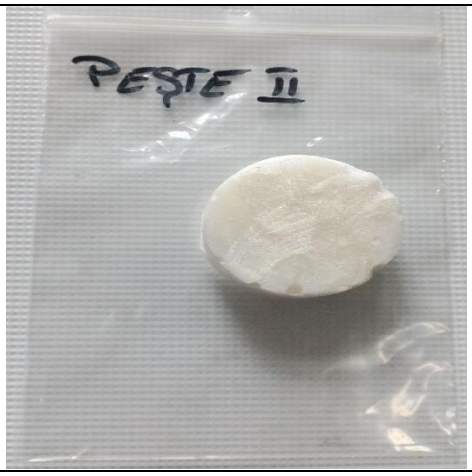

Fig.5 Extraction with 0.5 M HCl

Conclusion

In this study, acid soluble collagen (ASC)from skin of Grey Mullet were extracted with weak acid solutions of 0.1M - 0.5 M acetic acid and HCl0. 0.1 M – 0.5 M.

Better results were obtained after acid extraction with 0.5 M acetic ACTA.

The result showed that it is possible to use the fresh fish skin as an important collagen or gelatin source.

The isolated collagen may serve as an attractive alternative to mammalian collagen for biomedical and pharmaceutical applications .

At the same time , the study aims marine waste valorisation of the Black Sea, avoiding environmental pollution.

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