Hydrolyzed Collagen

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Hydrolyzed collagen (HC) is a group of peptides with low molecular weight (3–6 KDa) that can be obtained by enzymatic action in acid or alkaline media at a specific incubation temperature. HC can be extracted from different sources such as bovine or porcine. Recently research has shown good properties of the HC found in skin, scale, and bones from marine sources. Type and source of extraction are the main factors that affect HC properties, such as molecular weight of the peptide chain, solubility, and functional activity. HC is widely used in several industries including food, pharmaceutical, cosmetic, biomedical, and leather industries.

hydrolyzed collagen peptide antioxidant activity denaturation hydrolysates

1. Introduction

Collagen is the most important protein produced by the human body, it is mainly formed by the amino acid glycine (33%), proline and hydroxyproline (22%) (primary structure) in a triplex helix which is formed by three α chains. Each alpha chain is composed for 1014 amino acids approximately with a molecular weight around 100 kDa. These chains are coiled into a left-handed helix with three amino acids per turn (secondary structure). The chains are twisted around each other into a triple helix to form a rigid structure (tertiary structure). The super helix represents the basic collagen structure (quaternary structure). This collagen structure is very stable because of the intramolecular hydrogen bonds between glycine in adjacent chains. The collagen molecule is formed for a triple helical region and two nonhelical regions at either end of the helix structure with ≈300 kDa molecular weight, 280 nm in length, and 1.4 nm in diameter [1][2][3].

2. Hydrolyzed Collagen: Extraction and Properties

2.1. Extraction and Structure of Hydrolyzed Collagen

From **Figure 1**, it can be seen that denaturation of native collagen produces three α chains in their random coiled form. It can be observed by thermal treatment of collagen above 40 °C. Once the chains are separated, the hydrolysis is carried out by the action of proteolytic enzymes (alcalase, papain, pepsin, and others). The resulting product is commonly called hydrolyzed collagen (HC). It is composed of small peptides with low molecular weight 3–6 KDa [4][5][6][7]. Its solubility and functional activity (antioxidant, antimicrobial) are related to the type and degree of hydrolysis as well as the type of enzyme used in the process [8][9][10][11]. Another type of hydrolysis is by use of chemical products in acidic [6][12][13][14] (acetic acid, hydrochloric acid, and phosphoric acid) or alkaline media [15][13]. These two types of extraction are strongly corrosive and produce a high salt concentration in the final product

after neutralization [16]. Alternative methods of extraction consist in thermal treatment [17] or applying high temperature and pressure to the protein. It includes subcritical water level (SCW) that exists at a temperature between 100 and 374 °C and a pressure of less than 22 MPa [18][19].

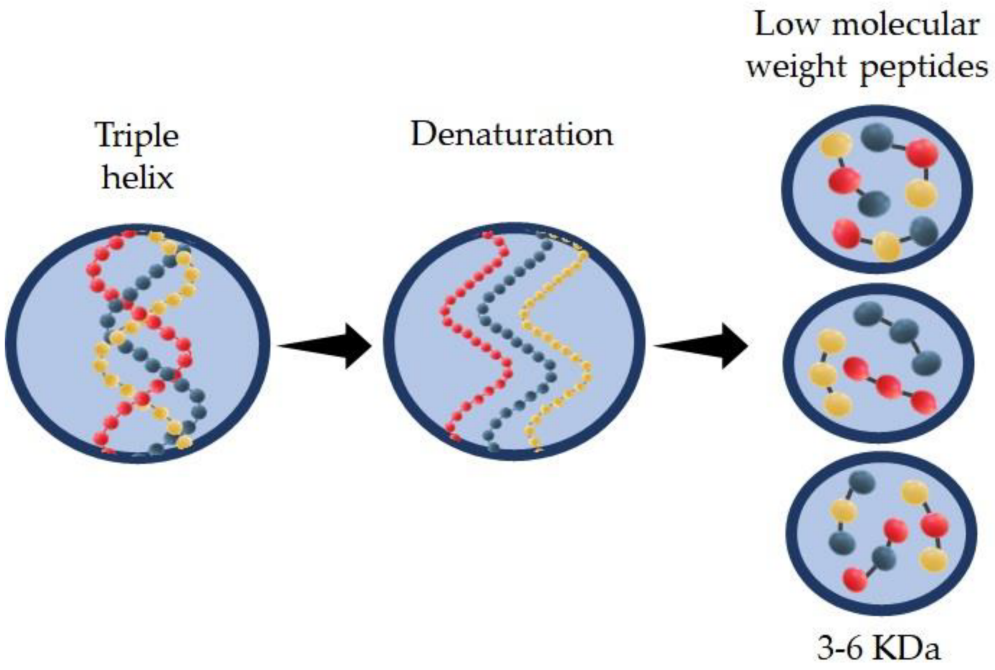

Figure 1. Denaturation of native collagen into small low-molecular-weight peptides.

2.2. Techniques for HC Molecular Weight Measurements

The determination of HC molecular weight is a difficult task because of its low molecular weight (Mw) which ranges between 3 and 6 KDa. The most common technique used is SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). It can separate proteins in the mass range of 1–100 KDa. The molecules are separated according to their charge, the moving speed is related to the charge of the molecule. This method uses polyacrylamide gels (PAGE—polyacrylamide gel electrophoresis) in the presence of the anionic detergent sodium dodecyl sulfate (SDS). The gel polymerization of acrylamide monomers produces linear chains. By including bisacrylamide, this formed a three-dimensional matrix of the gel. The size of the pores formed depends on the concentration of acrylamide and the degree of crosslinking. The first gel is the staking gel, it is a low-concentration gel (4%), and the second gel called resolving gel usually has a 10–12.5% concentration and is used to separate proteins in the range of 1–100 KDa. Thus, varying the concentration of acrylamide and bisacrylamide in the gel preparation results in different degrees of porosity and therefore different protein separation intervals [20][21][22].

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is another technique that helps to detect molecules in a large range of molecular weights. It is a technique where peptides are first mixed with a large molar excess of a matrix compound such as DHB (2,5-dihydroxybenzoic acid) to ionize low-molecular-weight peptides, next the matrix that carries the peptides is vaporized by laser radiation, and finally the mass of vaporized peptides is determined from the ionic time-of-flight. However, the limitation of this technique is that some peptide peaks fail to resolve in a single matrix [23][24][25].

HPLC-MS/MS is a powerful tool not only for the identification, but also for quantification of peptides and proteins. It is rather limited to the quantification of selected peptides of biological importance such as the quantification of collagen. The quantification of collagen types is usually carried out by amino acid analysis [26][27][28].

2.3. Hydrolyzed Collagen Properties

Native collagen properties are very different to those of hydrolyzed collagen as illustrated in **Table 1**. After denaturation, the triple-helix structure of native collagen changes to a random coil form due to the dissociation of the hydrogen bonds when collagen suffers hydrolysis. This treatment can break the bonds in the polypeptide chain to obtain a large number of peptides. The molecular weight of collagen peptides obtained from hydrolysis is very low (3–6 KDa) compared to that of its precursor native collagen (285–300 KDa). Enzymatic hydrolysis affects not only the size of the peptides but also physicochemical and biological properties [29][30]. Viscosity is one of the physicochemical properties of collagen; the native form shows higher values due to stronger electrostatic repulsion among the molecular chains even at low concentrations of collagen solution. However, its hydrolyzed form shows very low viscosity no matter the concentration because of the low molecular weight of the small chain segments [31]. Electrostatic properties of proteins such as the isoelectric point (pI) are important parameters which are related to the proportion of acid amino residues and base amino residues in protein. Collagen is an amphoteric macromolecule that possesses a pI value between 7 and 8. On the hydrolysis process, the pI value is shifted to lower values between 3.68 and 5.7. This change will depend on the amino acid sequences and distribution of amino acid residues according to the type or time of hydrolysis [27][32][33][34]. The composition and degree of hydrolysis of collagen are factors that increase functional properties such as antioxidant capacity, antimicrobial activity, and higher bioavailability. These properties are related mainly to the molecular weight value. It makes HC to act as an electron donor to produce more stable products reacting with free radicals [35][36].

Table 1. Properties of native and hydrolyzed collagen.

Properties	Type of Collagen		Reference
	Native	Hydrolyzed	
Molecular weight (Mw)	~300 KDa	3–6 KDa	[27][30]
Isoelectric point (pI)	7.0–8.3	3.68–5.7	[34][37]
Viscosity	High	Low (0 Cp)	[15][31]
Film formation	Yes	No	[38][39]

3. Hydrolyzed Collagen: Sources and Applications

3.1. Sources

3.1.1. Bovine

HC can be extracted from different sources and tissues [33]; it can be extracted from bovine Achilles tendon by using different enzymes such as alcalase, pepsin, trypsin, and collagenase produced by *Penicillium aurantiogriseum*. It shows antihypertensive, antioxidant, and antimicrobial activity [40][41]. HC from bovine lung showed antioxidant and anti-inflammatory activity [42]. HC from the nuchal ligament of bovine by papain action can be used as a promising precursor of angiotensin-I-converting enzyme (ACE)-inhibitory peptides [43].

3.1.2. Porcine

Another traditional source of HC is porcine skin. It presents a low molecular weight around 1–10 KDa. It is produced by a hydrothermal process and fractionated by ultra-filtration membranes; showing antioxidant, anti-aging, skin permeation properties [44], and ACE-inhibitory potency [45]. HC from porcine skin contains functional peptides commonly used in dietary supplements [46]. HC porcine extraction can be carried out by treatments that include high temperature (150–250 °C) and pressure (350–3900 KPa). These parameters of extraction generate peptides with lower molecular weight than 15 KDa [47].

3.1.3. Marine

HC extraction from traditional sources such as porcine and bovine involves some limitations due to health problems such as swine flu [48] and bovine spongiform encephalopathy [49]. Moreover, religious issues must be included [50]. Researchers have been focused on the development of a new source of extraction. Alternative sources have been investigated from marine sources such as fish and other invertebrates such as jellyfishes or sponges [51][52][53]. HC from *Prionace glauca* extracted with alcalase enzyme hydrolysis reported peptides with molecular weight lower than 20 KDa and nutraceutical effects [54]. Tilapia scales (*Oreochromis niloticus*) have been used to produce HC with high quality and low Mw [55]. HC extracted from pacu and rohu waste by using collagenase Type I from *Clostridium*, showed molecular weight hydrolysates of around 5 KDa. It was used as a peroxide inhibitor in lipid-based food and cytoprotective agent in cell culture [56]. Marine byproducts such as fish viscera also represent a good source for extraction of HC. This waste material has been used to produce HC with functional bioactive properties [57]. However, by changing the extraction parameters from different temperatures (150–300 °C), pressure (50–100 bar), and reaction time (5 min), it is possible to obtain HC from tuna skin with low molecular weight (<600 Da) and antioxidant and antimicrobial activity [58].

Other marine sources for preparation of HC were obtained from cod protein hydrolysate (*Gadus morhua*) [59], Alaska pollock [60], and cartilage of spotless smooth hound [61]. The extracted biomaterials results were lower molecular weights (3–5 KDa) and antioxidative activity.

3.1.4. Alternative Sources

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Some alternative sources present great functionality properties. HC extracted from chicken legs by enzymatic action (proteases) [62] and skin of *Rana chensinensis* by acid hydrolysis [63] exhibited high solubility, angiotensin-converting enzyme inhibition, and antioxidant activity. Chicken feet treated with papain enzymes at different temperatures (4, 30, and 56 °C) and different extraction times (20, 24, and 28 h) showed functional properties such as water and oil retention capacity as well as emulsifying and foaming properties [64][65].

3.2. Applications

The molecular weight and functional properties of HC depended on the source, type of extraction, and type of enzyme used during extraction. These properties could help determine the applications of HC in cosmetic, pharmaceutical, biomaterials, food and nutraceutical industries [57][66][67].

3.2.1. Oral Collagen Supplementation

Collagen loss in the body starts at 18–29 years of age; after 40 years the human body can lose around 1% per year, and at around 80 years collagen production in the body can decrease 75% overall in comparison to that of young adults [68][69]. There are other factors contributing to this such as free radicals in the organism, deficient diet, smoking, alcoholism, and disease. The role of collagen in the body is very important because it helps the development of the organs; wound and tissue healing; cornea, gums, and scalp repair. Collagen helps in bone and blood vessel reparation. In the cornea, collagen tissue gets mechanical and optical properties. It is present in biological functions of the cell such as proliferation, cell survival, and differentiation; so collagen is present in the human body as a whole in bones, tendons, ligament, hair, skin, and muscles [2][70][71].

3.2.2. Food Industry

HC presents antioxidant and antimicrobial activity, so it can be used as a functional ingredient in food supplements as well [72][73][74]. Collagen hydrolysates can attach calcium ions, improving its bioavailability, therefore, HC can be used in functional food ingredients in the management of mineral deficiencies [75][76]. HC acts as an anticoagulant because it helps to decrease the damage in cells and tissues originated by low temperatures, therefore, it could be useful in foods that require storage in cold or freezing temperatures [77]. HC has been used in the preparation of different products such as meat products, beverages, soups, and others. It helps increase and maintain their sensorial, chemical, and physical properties.

HC has been used in processed foods such as sausages to replace pork fat at 50% level of replacement. The final product results had greater water holding capacity, better stability after cooking, and improved texture such as hardness and chewiness [78]. The use of fish HC in meat products such as buffalo patties, resulted in higher protein content, lower fat content, similar sensory acceptability, and better texture as compared to the buffalo patties without HC [79]. HC from bovine skin was used in combination with modified starch and guar gum in ham elaboration. Lower syneresis with 2.0% of HC gel concentration in the product was reported as the best treatment [80].

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HC from fish can be added in beverages such as orange juice (2.5%) and the product showed an improvement in nutritional and functional properties with a higher protein content, bioavailability, and low viscosity as well as high solubility in water [9]. The development of a fermented dairy drink using ricotta cheese whey with HC added as a functional ingredient presented low syneresis and sedimentation, with good physical–chemical and microbiological properties [81]. Dairy beverages using HC, açaí pulp, and cheese showed higher sensorial acceptability, affecting positively the viscosity and presenting adequate physicochemical and microbiological parameters after 28 days of storage [82].

HC can be added in soup as well, it has an effect in its viscosity and the functional properties. It presented high 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activities [83]. Addition of HC from pigskin shavings (collagen waste) in a chrysanthemum beverage, showed an excellent clarification effect, better sensorial quality, and storage stability. However, the amount of HC added as a clarifier was lower compared to that of other commercial clarifiers [84]. HC from pig skin extracted by enzymatic action exhibited high flocculation capability under acidic and neutral conditions, this property could be caused by the synergistic effect of optimal molecular weight distribution and electric charge [85]. Additionally, HC has been used in different food products to develop their physicochemical and functional properties. This makes HC one of the most promising functional ingredients because it does not affect sensorial properties.

3.2.3. Biomaterials

Collagen presents good biocompatibility and biodegradability, hence, it is safe and effective as a biomaterial, it has been used in the last years as a safe and effective biomaterial in tissue engineering and clinical applications [39][86][87].

Compared to native collagen, HC has a major advantage—it presents higher solubility; moreover, HC extraction is simple and does not require a multistep extraction procedure [39][88][89]. However, HC cannot form scaffolds by itself because of the low molecular weight of the peptides, but it can be mixed with other biopolymers such as cellulose and chitosan.

Films prepared with a blend of cellulose–HC exhibited good transparence, good ultraviolet radiation absorption, and excellent support for cell adhesion and proliferation. High biocompatibility indicates that the films would have promising applications in the biomaterial field [88]. Collagen–HC films developed from leather waste were very transparent and had excellent barrier properties against UV light and studies such as FTIR and differential scanning calorimetry (DSC) showed total miscibility between both polymers [90].

The development of a HC collagen biomaterial could be beneficial for the management of bone and joint disorders because of HC's low molecular weight and amino acid composition. It is more bioavailable and induces a better osteointegration, promoting collagen synthesis [89].

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