SCIENTIFIC BULLETIN OF THE TECHNICAL UNIVERSITY OF LODZ
No. 1058 Food Chemistry and Biotechnology, Vol. 73 2009

KATARZYNA DYBKA
PIOTR WALCZAK
Institute of Fermentation Technology and Microbiology
Technical University of Lodz

COLLAGEN HYDROLYSATES AS A NEW DIET SUPPLEMENT

Review: Professor Marianna Turkiewicz, Ph.D., D.Sc

Paper reviews literature data connected with properties of collagen hydrolysates applied as diet supplements. Biological and health promoting activity of collagen derived peptides has been well documented in many studies, especially for the therapeutical treatment of bones and joints diseases as well as for the improvement of skin, hair and nails conditon. High tolerance of patients for long-term ingested collagen hydrolysates make them attractive for use as health promoting diet supplement.

1. Introduction

Modern lifestyle characterized with permanent lack of time results in consumption of a highly processed food which does not have any beneficial effect on our health. Inbalanced and incomplete diets can be a reason of many diet depended diseases. If we care about healthy lifestyle, we have to include nutrient-rich food to our normal diet. Diet supplements are such kind of health beneficial substances which contain concentrated source of nutrients or other components causing positive physiological effects. Diet supplements are produced in the form of powder, capsules, powder in sachets, liquid in bottles with droppers or in other forms suitable for proper dosage. It is well recognized that diet supplements are not medicines and their use is not regulated by the pharmaceutical law [25].

2. Collagens characteristic

Collagen proteins are the most abundant in the human and animal body. They are the major proteins of connective tissue, skin, tendons, cartilage, ligaments, cornea, teeth, nails and hair [8]. Proteins of collagen family represent a group of varied extracellular matrix molecules linked by the occurrence of the collagen triple-helical domain as a common structural element [5]. In vertebrates organisms, at least 27 types of collagen with 42 distinct polypeptide chains has been reported [19]. According to similarieties in their structure and supramolecular organization, they are classified into fibril-forming, fibril-associated collagens with interruptions in triple helix (FACITs), network-forming collagens, anchoring fibrils or transmembrane collagens [22]. The different collagen types are characterized by considerable complexity and diversity in their structure, their splice variants, the presence of additional, non-helical domains and their function. All members of the collagen family have one characteristic feature – a right-handed triple helix composed of α-chains (Fig. 1). Triple-helix can be formed by three identical α chains (homotrimers) as in types II, III, VII, VIII, X, XIII, XV, XVII, XXIII, XXV collagen and by two or three different chains (heterotrimers) as in types I, IV, V, VI, IX, and XI collagen [19, 22]. Each of the three collagen α-chains coils into a left-handed helix which assemble to rope-like figure bordered by the C- and N-propeptides [8].

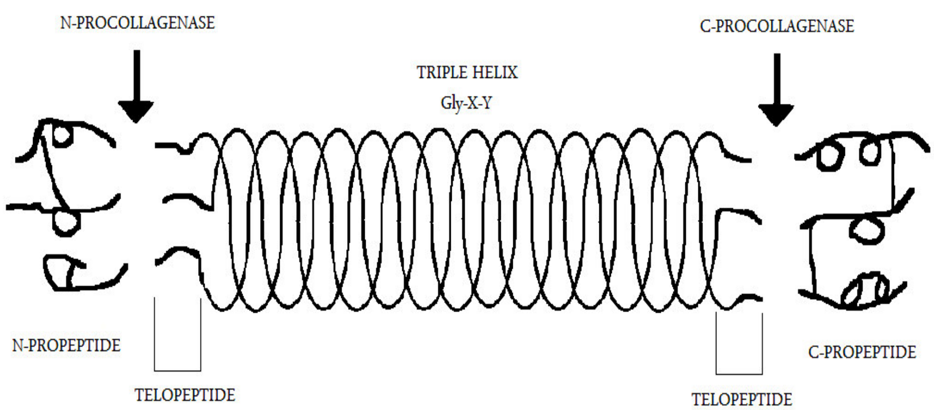

Fig. 1. Molecular structure of fibrillar collagens

Collagens consist of a high amount of glycine (about 33% amino acid residues), proline (12-14%), 4-hydroksyproline (<14%) and 4-hydroksylysine (1.5%) [17]. Tryptophan and cysteine were not noticed [21]. Collagens are known to share a repeating pattern Gly-X-Y in which the X and Y positions are frequently occupied by proline (Pro) and 4-hydroksyproline (Hyp) residues

[2, 7, 17]. Research have reported that the content of Hyp plays an especially important role in stabilizing the triple-helical conformation in collagen and in peptides with collagen-like domains [2, 4]. Hydroksyproline residues stabilize triple helical conformation by sharing direct hydrogen bonds[2]. The most common motif in fibril-forming collagens is repeating sequence Gly-X-Y resulting in triple helical domains of 300 nm in length which corresponds to about 1000 amino acids [8]. The three residues in the repeating triplet occupy distinct positions within the supercoiled helix. The central possition of Gly residues makes this residue not suitable for interacting with other residues. Interactions caused by proximity between neighboring chain are related with less solvent accessibility of Y position residues. In contrast to Gly and Y positions in triplet motif, the greatest exposure for interactions, shows the X position [5]. Bella et al. [2] suggested that the water molecules aggregate as a shell to the carbonyl and hydroxyprolyl groups resulting diverse conformation with a specific motifs of water bridges bonding oxygen atoms within a single chain or between different chains of triple helix.

3. Biosynthesis and degradation of collagen

Permanent collagens exchange processes in our body takes place during the whole human life. Old fibrils are replace by new one all the time. When we are young, collagen production and degradation are in dynamic balance, but during maturation of tissues, degradation is being more intenssive. UV radiation, smoking cigarettes, stress and unhealthy diet lead to the degradation of natural collagen structure and to earlier senility.

3.1. Biosynthesis of collagens

The biosynthesis of fibril-forming collagen is a multisteps and complicated process which takes place in intracellular and extracellular spaces. It begins with transcription of the genes and ends with assemble of a triple helix collagen fibrils into fibers with their final distinctive functions in tissues (Fig. 2). Cell type, growth factors and cytokines are considered as particularly agents in the system of transcriptional regulation during collagen biosynthesis. It is well konown that the major group of collagen genes assemble into a complex of 3 to 117 exons and introns, characterised with more than 50 exons encoding the mRNAs of fibrillar collagens. It was reported that other mRNA species could be found. They are related with mulitple initiation sites of transcription or alternative splicing of exons. The process of mRNA translation into synthesized polypeptide chains (preprocollagen) takes part in membrane-bound ribosomes.

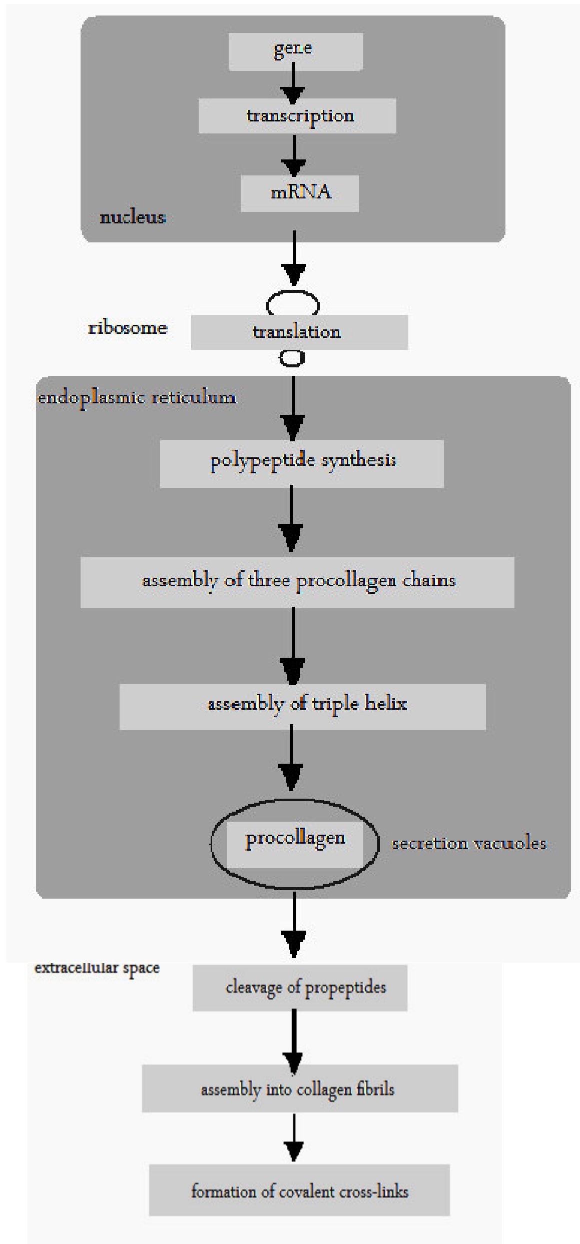

Fig. 2. The main steps in biosynthesis of fibril-forming collagens

In the endoplasmic reticulum preprocollagen is involved in several posttranslational modifications. Three vitamin C-depended enzymes, prolyl 3-hydroxylase, prolyl 4-hydroxylase and lysyl hydroxylase catalyze hydroxylation of proline and lysine residues. Presence of 4-hydroxyproline is critical for hydrogen bonding within molecule [8, 19]. Hydroksylysine residues are recognized as bonding agents within fibril chains. The 3-hydroxyproline function has not been reported, yet. Other action is glycosylation of some of hydroksylysine residues and asparagine residues in C and/or N propeptides. After the association of C propeptides and formation of disulfide bonds, three α chains form molecule called procollagen, this precursor of collagen is secreted and released into the extracellular space in transport vesicles of Golgi apparatus.

Then procollagen trimers are processed in different ways which depend on the collagen type. The C-propeptides and N-propeptides are removed by specific metalloproteases. Following the procollegen modifications, the tropocollagen fibrils are assembled. It was found that some of fibril-forming collagens (e.g. I, II, III, V, XI) associate spontaneously into fibrillar structures during in vitro test. It has been compared to the crystalization process. Several models described self-assembly structure encoded in collagens and formation mechanism of periodic fibrils. Fully formed fibers are stabilized by hydrophobic and electrostatic interactions between collagen monomers and covalent cross-links joining differently orientated fibrils in tissues [8, 28].

3.2. Degradation of collagen

Collagen is a very stable protein in normal healthy conditions. Collagen degradation may proceed in different ways, but generally it is belived that there are two possibilities – intracellular and extracellular. The main cause of intercellular degradation process are proteolytic enzymes, particularlly cathepsins. Cathepsins are various proteolytic enzymes found in animal tissue that catalyze the hydrolysis of proteins into polypeptides in acid environment. In the extracellular way there are several stages including depolymerisation which effects with deterioration of molecular structures; activity of collagen-specific enzymes – tissue collagenases; heat-disintegration at body temperature of products of collagenases degradation, which lose triple helices structure and become available for non specific proteinases. Collagenases can be synthesized by many cells of human body (e.g. fibroblasts, neutrophils, and tumor cells [28].

4. Collagen hydrolysates production

The main source of collagen peptides are bovine hide, bone, pigskin or fishbones and fish skin. Marine sources are an alternative to bovine or porcine and they are not associated with the prions related to risk of Bovine Spongiform Encefalopathy (BSE) [12]. Collagen hydrolysates are manufactured in controlled hydrolysis process to obtain soluble peptides. The raw material is washed, homogenized and demineralized with diluted mineral acid or alkaline. The raw material is extracted in several stages with warm water. Further enzymatic degradation of gelatin results in a final product which is collagen hydrolysate [18, 24, 26]. Clemente [6] has presented enzymatic hydrolysis as the most appropriate method for preparation of tailor-made peptides. Collagen hydrolysates vary from each other with respect of peptides molecular weight, mostly their molecular weight range from 2 to 6 kDa [18, 26]. Its molecular weight is less than the average molecular weight of peptones. After purification, the product is concentrated and dried. The most common post-dried procedures are related to the control of molecular size and the elimination or reduction of bitterness in the resulting hydrolysates. The most efficient procedure to remove residual high-molecular weight peptides and proteins or to reduce the antigen content of hypoallergenic formulas, is ultrafiltration [6].

Several analysis may be done for the quality control of these products: the osmolarity, analysis of the hydrolysis degree, the molecular weight distribution, the total nitrogen, amino acid composition and the presence of toxic compounds (e.g. biogenic amines or pathogens). Protein hydrolysate qualitative analysis use different techniques based on spectrophotometric, chromatographic and electrophoretic methods (UV-spectrophotometry, HPLC, SDS-PAGE) [23].

5. Properties and applications of collagen hydrolysates

Gelatin and collagen hydrolysates have been reported to have beneficial biological functions. Hydrolyzed gelatin products have been designated as generally recognized as safe (GRAS) food products or food additives by the Food and Drug Administration (FDA) [1, 18]. Despite the fact that collagen hydrolysate has been generally regarded as having a low biological value, because it does not contain all of the essential amino acids, its a reputable nutritional component often used to supplement other proteins because of its superb digestibility and high consumer tolerance [26].

5.1. Beneficial role of collagen hydrolysate in health

According to the opinion of many researchers, beneficial effects of oral administration of collagen hydrolysates results of crossing the intestinal barrier, by a dietary bioactive peptides, which reach the blood circulation and become available for metabolic processes [26]. Collagen hydrolysates are used in medical applications, such as high-energy supplements, geriatric products and enteric, therapeutic or weight-control diets. Applification of protein hydrolysates in treatment of patients with specific disorders of digestion, absorption and amino acid metabolism. Tests also included clinical cure of patients with malnutrition attached with trauma, burns, cancer and hepatic encephalopaties [6]. Collagen hydrolysates are good source of amino acids for people suffering from anorexia, anaemia and for vegetarians (because of absence of meat in their diet). Diet supplements conataining collagen hydrolysates are considered as improvement agents in tendon or joint regeneration in physically active athlets with activity-related joint pain [18, 26].

Orally consumed collagen hydrolysate has been shown to be absorbed intestinally and to accumulate in cartilage. Speciffically, collagen hydrolysate ingestion stimulates a significant increase in the synthesis of extracellular matrix macromolecules by chondrocytes [3]. According to medical data clinical investigations suggest that ingestion of collagen hydrolysates reduces pain in patients suffering from osteoarthritis and osteoporosis. It is considered that about 15% of world population suffer from joint pain-related diseases. In Poland an increasing problem become joint-related diseases connectet with other high risk disorders agents which are abundant. Increasing risks agents are senility (over 50% of elderly people suffer from rheumatism), gender (a high amount of patients are women, particularlly after menopause), body weight (huge body weight is a reason of joint overload and results in joint pain), constantly excessive sport activity, joint injury (e.g. dislocations), metabolic diseases (e.g. diabetes) [24]. Collagen hydrolysates are involved in cartilage matrix synthesis [26]. Over almost two decades scientists have studied a relationships between therapeutic trials in joint diseases and collagen, gelatin or collagen hydrolysates. In numerous studies researchers accepted dose of 10 g of collagen hydrolysates daily as a safe and well tolerated by patients. Additionaly clinical tests have proved that this level of daily ingested proteins can reduce the pain in comparison with placebo group patients [18].

Several scientific reports have presented good bioavailability of hydrolyzed collagen, after oral administration by animals and human beings. Oesser et al. [20] discovered that about 95% of orally applied collagen hydrolysate was absorbed within the first 12 h. Wu et al. [26] described the high safety of eating collagen hydrolysates in an animal model (1.66 g/kg of body weight per day). Studies related with preparations consisting gelatin derivated peptides showed good tolerance and little side effects including a sensation of unpleasant taste, a feeling of heaviness in the stomach, and a bloated feeling or pyrosis after oral administration [20].

According to opinion of Zague [26] some studies described chemotactic activity of short peptides (Pro-Hyp and Pro-Hyp-Gly) to human fibroblast, peripheral blood neutrophils and monocytes in the cell culture. Collagen-degradation peptides might attract these cells and result in repair of damaged tissue. It is believed that collagen hydrolysates can not be absorbed from skin and the basis of the skin effectiveness of collagen hydrolysate depends on a gradual improvement of water absorption to skin as a result of possitive effect of the oral administration of supplement. A beneficial effects has been also observed for skin-related organs and for hair and nail quality.

Antihypertensive and antioxidative activities of bioactive peptides isolated from collagen hydrolysates have been discovered [26]. Collagen and gelatin digests contain angiotensin-converting enzyme (ACE) inhibitory peptides. ACE play an important role in blood pressure regulation and inhibition of this enzyme can cause an antihypertensive effect [14, 15, 16]. Protein supplements (e.g. collagen hydrolysates) may be useful to enhance nitrogen retention [10].

5.2. Industrial application of collagen hydrolysates

Gelatin and hydrolyzed collagen are utilized in food industry in confectionery (to improve texture, chewiness and foam stabilization), dairy (as stabilisation and texturization agents), bakery (to provide stabilization, emulsification and gelling), low-fat spreads (to provide fat reduction, creaminess and mouthfeel), in meat-processing (to provide water-binding e.g. in reconstituted hams), in wine and fruit juices production (fining agent) [1, 12, 13, 27]. Collagen hydrolysates, like all protein hydrolysates show technological advantages such as good solubility, heat stability and relatively high resistance to precipitation by many agents, such as metal ions or pH [6]. Protein hydrolysates have an excellent solubility at high degree of hydrolysis, which is a substantially useful characteristic for many food applications and influences other functional features such as emulsifying and foaming properties [9, 15].

Collagen hydrolysate has a high water-binding capacity and can be used as an essential product low-calorie carbohydrates or low fat food production. In the pharmaceutical industry gelatin and collagen hydrolysates are used to manufacture capsules, implants and intravenous infusions [11, 12].

6. Conclusions

Collagens are the most abundant group of organic macro-molecules in human and animal body. Because of their tensile strength, they perform numerous important structural functions within the body, especially in connective tissues. Collagen proteins are essential in connective tissues of such organs as heart, intestines, lungs or parenchymal organs like liver and kidneys; as protein matrix of the skeleton and its related structures (e.g. bones, teeth, tendons, cartilage and ligaments); in fibrous matrix of skin and blood vessels [6, 7, 18, 26]. Its excellent properties are result of their amino acid composition and molecular structure. Collagens are also involved in the management of cellular mediators.

Collagen protein (in the form of collagen hydrolysate) has been shown to improve skin hydration, reduce wrinkles and decrease pain and functionality disorders in joint diseases. In addition, collagen hydrolysate seems to be a relatively inexpensive and widespread available protein source.

Collagen hydrolysate and gelatin can be used in food, cosmetics or pharmaceutical industry as a natural additive revealing an antioxidant properties with competitive foaming and emulsifying functionalities [9,15]. Finally, such properties like excellent biodegradability, low immunogenicity and the possibilities for large-scale production make them interesting compounds for a wideespread industrial use in food industry, cosmetics industry or medicine.

7. References

[1] **Baziwane D., He Q.:** Gelatin: the paramount food additive. Food Rev. Int. **19**, 423-435, (2003).

[2] **Bella J., Brodsky B., Berman H.M.:** Hydration structure of a collagen peptide. Structure. **9**, 893-906, (1995).

[3] **Bello A.E., Oesser S.:** Collagen hydrolysate for the treatment of osteoarthritis and other joint disorders: a review of the literature. Cur. Med. Res. Opin. **22(11)**, 2221-2232, (2006).

[4] **Bornstein P.:** Covalent cross-links in collagen: a personal account of their discovery. Matrix Biol. **22**, 385-391, (2003).

[5] **Brodsky B., Ramshaw J.A.M.:** The collagen triple-helix structure. Matrix Biol. **15**, 545-554, (1997).

[6] **Clemente A.:** Enzymatic protein hydrolysates in human nutrition. Trends Food Sci.Techn. **11**, 254-262, (2000).

[7] **Dioguardi F.S.:** Nutrition and skin. Collagen integrity: a dominant role for amino acids. Clin. Dermatol. **26**, 636-640, (2008).

[8] **Gelse K., Pöschl E., Aigner T.:** Collagens-structure, function, and biosynthesis. Adv. Drug Deliv. Rev. **55**, 1531-1546, (2003).

[9] **Giménez B., Alemán A., Montero P., Gómez-Guillén M.C.:** Antioxidant and functional propertioes of gelatin hydrolysates obtained from skin of sole and squid. Food Chem. **114**, 976-983, (2009).

[10] **Hays N.P., Kim H., Wells A.M., Kajkenova O., Evans W.J.:** Effects of whey and fortified collagen hydrolysate protein supplements on nitrogen balance and body composition in older women. J. Amer. Diet. Assoc. **109**, 1082-1087, (2009).

[11] **Karim A.A., Bhat R.:** Gelatin alternatives for the food industry: recent developments, challenges and prospects. Trends Food Sci. Techn. **19**, 644-656, (2008).

[12] **Karim A.A., Bhat R.:** Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydroc. **23**, 563-576, (2009).

[13] **Kim S.K., Kim Y.T., Byun H.G.:** Purification and characterization of antioxidative peptides from bovine skin. J. Bioch. Mol. Biol. **34**, 219-224, (2001).

[14] **Korhonen H., Pihlanto-Leppälä A., Rantamäki P., Tupasela T.:** Impact of processing on bioactive proteins and peptides. Trends Food Sci. Techn. **9**, 307-319, (1998).

[15] **Li B., Chen F., Wu Y., Wang X., Ji B.:** Isolation and identification of antioxidative peptides from porcine collagen hydrolysat by conescutive xhromatography and electrospray ionization-mass spectrometry. Food Chem. **102**, 1135-1143, (2007).

[16] **Mendis N. Rajapakse N., Kim S.K.:** Antioxidant properties of a radical-scavening peptide purified from enzymatically prepared fish skin gelatin hydrolysate. J. Agric. Food Chem. **53**, 581-587, (2005).
[17] **Minakowski W., Weidner S.:** Biochemia kręgowców. PWN. Warszawa 2005.
[18] **Moskowitz R.W.:** Role of collagen hydrolysate in bone and joint disease. Semin. Arthritis Rheum. **30**, 87-99, (2000).
[19] **Myllyharju J., Kivirikko K.I.:** Collagens, modifying enzymes and their mutations in humans, flies and worms. Trends Genet. **20**, 33-43, (2004).
[20] **Oesser S., Seifert J., Adam M., Babel W.:** Oral administration of ^{14}C labeled collagen hydrolysate leads to an accumulation of a radioactivity in cartilage of mice (C57/BL). J. Nutr. **129**, 1891-1895, (1999).
[21] **Pytrus-Sędłak B.:** Kosmetyka ozdobna i pielęgnacja twarzy. Medpharm Polska. Wrocław 2007.
[22] **Ricard-Blum S., Ruggiero F.:** The collagen superfamily: from the extracellular matrix to the cell membrane. Path. Biol. **53**, 430-442, (2005).
[23] **Silvestre M.P.C.:** Review of methods for the analysis of protein hydrolysates. Food Chem. **60**, 263-271, (1997).
[24] **Świderski F., Czerwonka M., Waszkiewicz-Robak B.:** Hydrolizat kolagenu nowoczesny suplement diety. Przem. Spoż. **4**, 42-44, (2009).
[25] **Wrześniewska-Wal J.:** Suplementy diety w nowej ustawie o bezpieczeństwie żywności. Przem. Spoż. **6**, 49-51, (2007).
[26] **Zague V.:** A new view concerning the effects of collagen hydrolysate intake on skin properties. Arch. Derm. Res. **300**, 479-483, (2008).
[27] **Zhang Z., Li G., Shi B.:** Physicochemical properties of collagen, gelatin and collagen hydrolysate derived from bovine limed split wastes. J. Soc. Leath. Techn. Chem. **90**, 23-28, (2005).
[28] http://www.osteoforum.org.pl/kolagen.html; 4.08.2009

HYDROLIZAT KOLAGENU JAKO NOWY SUPLEMENT DIETY

Streszczenie

W artykule dokonano przeglądu literatury dotyczącej właściwości hydrolizatów kolagenu stosowanych jako suplementy diety. Aktywność biologiczna i oddziaływanie prozdrowotne hydrolizatów białek kolagenowych zostały udowodnione naukowo, zwłaszcza w leczeniu chorób zwyrodnieniowych kości i stawów oraz poprawie kondycji skóry, włosów i paznokci. Wysoka tolerancja pacjentów na spożywane hydrolizaty kolagenu w długim czasie powoduje, iż są one atrakcyjnym, prozdrowotnym suplementem diety.

Instytut Technologii Fermentacji i Mikrobiologii
Politechnika Łódzka