Review Article

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Poultry spent wastes: an emerging trend in collagen mining

Abstract

Collagen, the principle structural protein of multicellular organisms, is now growing as a vital ingredient in the food, pharmaceutical, and biomedical industries. Owing to its versatile properties, collagen products have gained a significant market value, forecasting an increasing demand (a rise in CAGR of 5.05% in next few years) in the global collagen industry. This pressurising demand cannot be satisfied with the conventional (bovine, porcine, and marine) sources, amid their shortcomings. Studies have shown that a novel and practically suitable source of collagen can be obtained using slaughter wastes from the poultry industry as a substitute for the traditional collagen sources. Products of the poultry industry (consumables- meat and eggs; discards- feather, skin, offal, and droppings) exhibit their utility in diverse applications, as poultry are rich in high quality protein and desirable fatty acids reports. During the past decades, the avian derived collagen is observed to be used in industries including food, tissue engineering and cosmetic applications. Hence, the poultry spent wastes are taken as the element of discussion, focussing on aspects like extraction, characterization methods, and applications.

Keywords: collagen, offal, extraction, tissue engineering, hydroxyproline

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Ragavy Radhakrishnan,[1] Puja Ghosh,[2] Thanganadar Appapalam Selvakumar,[1] Muthiah Shanmugavel,[2] Arumugam Gnanamani[2]

[1]Department of Biotechnology, Rajalakshmi Engineering College, India

[2]Microbiology Division, CSIR-Central Leather Research Institute, Adyar, Chennai 20, Tamil nadu, India

Correspondence: Muthiah Shanmugavel, Department of Microbiology, CSIR-Central Leather Research Institute, Chennai, 600020, India, Tel +91 44 2443 7135, Fax +91 44 2491 2150, Email ghosh.uja.2013@gmail.com

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Introduction

In the living species collagen plays a very pivotal role as it is the major structural and fibrillary protein existing at 25% of the overall available protein in the vertebrates and invertebrates.[1] The term "Collagen" is from a Greek word meaning Kolla (glue) and gennao (produce) -"Glue of the body." Collagen is the ubiquitously occurring structural protein, referred to as glue because it holds together the components of the connective tissue, and also of the glue-like product obtained on boiling it. It is the principle protein of the skin, bones, cartilage, tendons, the dentin of teeth, cornea, connective tissue, blood vessels, and gut.[2] Besides giving shape, it also helps in blood clotting, reducing hypertension, has anti-microbial activity, and many more functions. Ageing causes the production of collagen to decrease, and hence, the skin loses its texture, and the cartilage becomes loose and weakens. Because of its significance, collagen has attained recognition as health supplements and as an ingredient in shampoos and body lotions, lately. Livestock's are a natural form of animal protein, constituting essential aminoacids, obtained from sources like fish, birds, eggs, dairy products. The poultry sector is a profitable business, domesticating avian like chicken, duck, turkey, mallards, ostrich and many more. The poultry meat is a healthy source of many proteins, vitamin B12, minerals like iron and zinc, and desirable good fats like omega-3-fatty acids. Besides marketing the meat of high nutritional value, poultry processing industries discard by-products such as skin, feathers, feet, gut, which all are rich in proteins. If managed, these wastes can be of value for other purposes, for example, the bird droppings are of rich nitrogen content which can be applied as fertilizer.[3] Similarly, the above mentioned parts are under-utilized, but are promising source of extraction of a valuable protein named 'Collagen'. Increasing production scale of poultry sector causes increase in accumulation of the biological discards, paving way for environmental pollution. Hence, the usage of these wastes can be in another way to eliminate the environmental problem as well.

Collagen market and trending application

Collagen has become a vital ingredient in the food, pharmaceutical, and biomedical industries, because of the changing lifestyle, growing demand in personal care and cosmetic industry owing to its benefits for the consumer's health and satisfaction. Moreover, consumers in modern days have started to welcome products if there are more variety and novelty available.[4] Some of the top manufacturers of native collagen include Gelita AG, Nitta Gelatin, Weishardt Group, Darling Ingredients, Nippi, and many more. Launches containing collagen are found rising in the past decade (as per the 2007-2016 report shown in Figure 1), with a CAGR (Compound Annual Growth Rate) of 33%, and is forecasted to grow with a CAGR of 5.05% over the period from 2019-20243. In volume propositions by 2025, collagen production is expected to have a quantum of 622 kilotons at a compound annual growth rate of 5.9% from 2018. Collagen world market revenue in 2018 was figured to be US$ 4.27 billion and expected to reach US$ 6.63 billion in the nearby future by 2025.[5] Cosmetic industry is very much on the raise in Latin America, Asia and currently now African region, primarily due to aggressive corporate promotions wherein cosmetic grade collagen constituting the fashion and health wellness products showing an expected CAGR growth of 31% and 28%. Consumers are very concerned about their health issues (osteoporosis, bone, and joint health) and appearance. Hence, the new trend of "Healthy ageing" is supporting the escalation of the growing market. Not only cosmetic industry but also individual well-being nutritional supplement industry also use and utilize collagen at different process levels of these nutritional products thereby a huge demand is created for collagen. The Collagen Peptides market will account for a 4.4% CAGR in terms of revenue over the next five years.[6] There are many number of collagen products that are commercially available for end user application and can categorised in the form of personal, healthcare or medical products6, as illustrated in Figure 2. All these mathematical figures, demonstrate that each type of collagen products (food,

nutraceuticals, personal, medical and others) do have a prominent market share and achieve to express a surge in the trade demand. Because of its versatile properties, collagen has found applications in various fields. As a biomaterial, with less immunogenicity and higher biocompatibility, collagen has been employed in the form of scaffolds or cross-linked film carrying antimicrobial agents for wound healing,[7] sponge for burns and wounds, controlled drug delivery system, as nanoparticle for gene delivery, bone substitute when mixed with hyaluronic acid, ophthalmic drug applications and in plastic surgery.[8] Because of his gelling and surface modifying behaviour like emulsion, foam formation, adhesion and cohesion, collagen is used in food industries for preparing supplements like nutricosmetics; food additives in the form of emulsifiers, thickeners, preservatives; as edible films and coatings for sausages and as drinks with probiotics and collagen infusions.[9] A derivative of collagen- gelatine was used in the manufacturing of photographic films by the renowned photography industry- Fujifilm, which later started to implement the usage of collagen in cosmetic production.

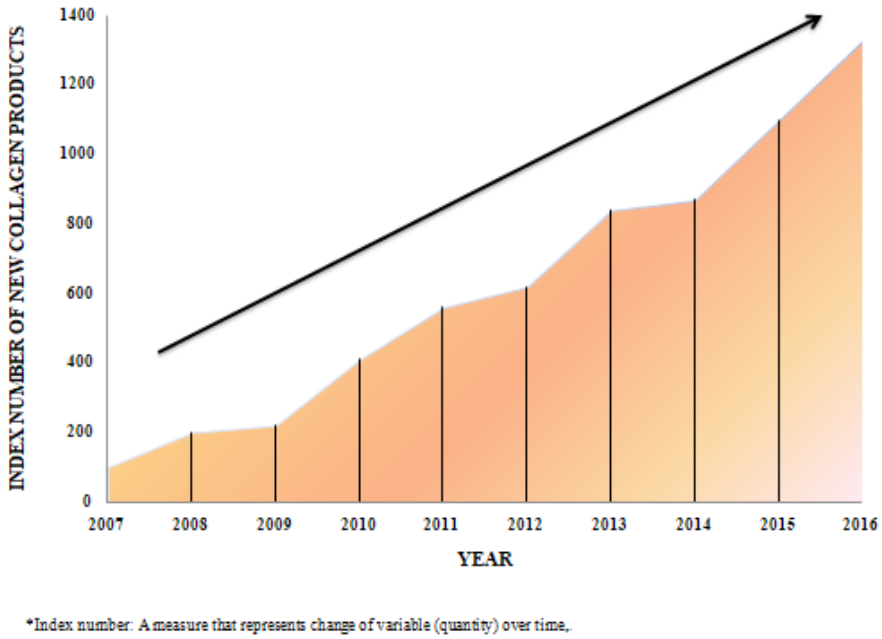

Figure 1 Exponential growth of new collagen based products released into market.

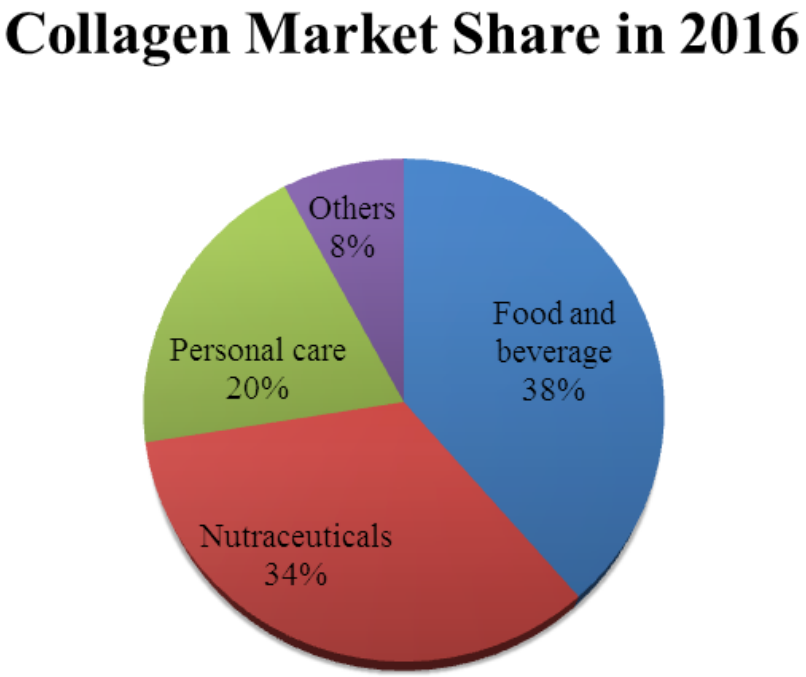

Figure 2 Collagen market share in 2016.

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Structure of collagen

Collagen is a right-handed triple superhelical rod possessing three identical polypeptide chains; the protein can form insoluble fibres that exhibit high tensile strength. There are around 28 types of collagen found to date. Collagen has a molecular weight of 300kDa, with an average diameter of 2nm and a length of 300nm. The helical structure will form a fibril, which is 1cm long and has a diameter of 500nm.[10] Fibroblast cells of the connective tissue and a variety of epithelial cells produce collagen, as separate polypeptide chains. Individual monomers of self-assemble through a process termed fibrillogenesis, which plays a significant role in stabilization of the triple helix. These polypeptide chains contain around 1000 amino acids, bundled together by posttranslational modifications, and are dispatched to the extracellular matrix, by exocytosis.[11] The intertwining of 3 polypeptide chains forms the tertiary structure of collagen named as tropocollagen into a right-handed helix. The individual polypeptide chain of collagen has repeating units of X-Y- Glycine, with Proline- Hydroxyproline-Glycine being the most common triplet (around 10.5%) as shown in the Figure 3A. Glycine, Proline, and Hydroxyproline account to 33%, >20%, and 14% of the total amino acid content in collagen.[12,13] The presence of Glycine at every third position promotes the close packing of the triple helix. The polypeptide chains fold into a left-handed α helix, with 3.3 residues per turn on an average. The recurrent N–H(Glycine)···O=C(X) hydrogen bonds that form within the triple helix Figure 3C & 3D is known to be the most abundant amide-amide hydrogen bond in the kingdom Animalia. The substitution of Glycine residues in the ladder of hydrogen bonds is due to mutations and might cause several health ailments.[14] With a degradation rate of 1-2% per year after the age of 30, the major enzymes that degrade collage belong to matrix metalloproteinases (MMPs)[15] and shed dases.[16] The effect of these enzymes triggers the process of aging, through intrinsic and extrinsic factors, and resulting in the degradation of collagen in photo-aging process.[17]

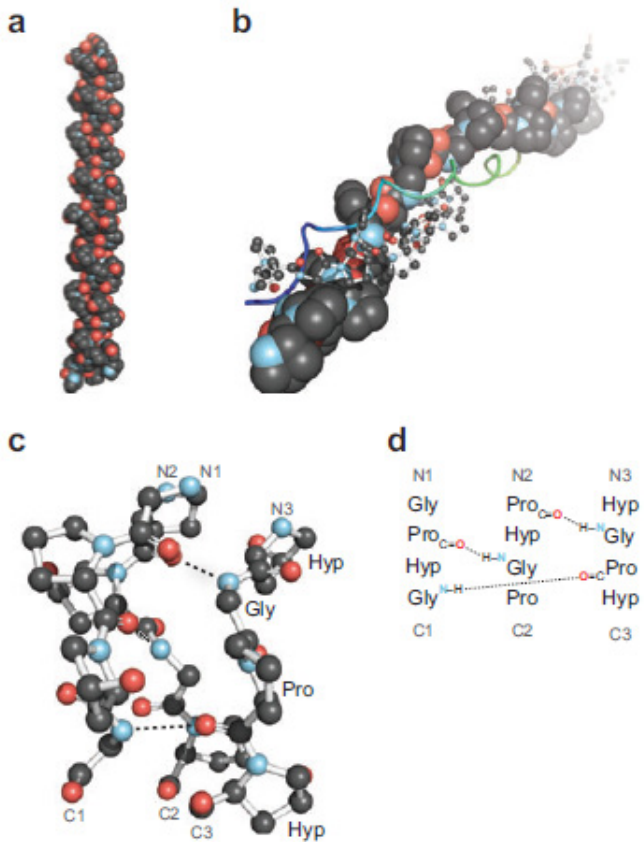

Figure 3 (Source: *Shoulders and Raines, 2009*).

A. Crystal structure of collagen triple helix from (ProHypGly)-(ProHypAla)-(ProHypGly)
B. Ball-and-stick and ribbon representation of three strands
C. Ball and stick representation of a segment of collagen triple helix highlighting the ladder of interstrand hydrogen bonds Stagger of the three strands in the segment in panel

Functions of collagen

The major functions of collagen vary concerning their presence in different tissues, most of which have been discussed by several authors.[11,18,19] The primary functions involve offering support to cells, providing strength, resilience and elasticity. It also aids the skin to defend against pathogens and toxin. Further, the triple helical protein also plays a significant role in molecular architecture, shape, skin integrity, dermal-epidermal adhesion, and mechanical properties (such as the tensile strength in the skin and the resistance to traction in ligaments). The binding of platelet to collagen or thrombin helps in inducing blood clot formation.[20] Physiological and biocompatibility of collagen is very diversified in orthopaedics and neurology where collagen gets involved in ossification, calcification[21] osteoblast differentiation and bone formation[22,23] especially in chondro-osseous junction,[24] hippocampal synapses formation[25–27] and also protection of neurons against Aβ toxicity.[28]

Sources of collagen

Bovine, porcine, marine, and poultry are the four primary sources involved in the production of collagen. Traditionally, the primary sources used for collagen extraction are by-products from the slaughter of pork and beef. Bovine source collagen contributes for 35% of the total collagen production in 2018 from all different sources hence collagen production cost is comparatively low.[29] Despite consumers, raising concerns on the diseases such as bovine spongiform encephalopathy (BSE), Foot and Mouth Disease, and other zoonoses, has left uncertainty in the growth of bovine based collagen products. Porcine based collagens are strictly prohibited (haram) for Muslims, while cattle that are halal-slaughtered are permitted. Recently, halal authenticity is an issue of significant concern in the food industry. Recent research has examined for the extraction of collagen, with particular emphasis on fish by-products.[30] Among all sources of collagen it has been worked out that marine-based has a great prospect with an annual growth of 7.6% (CAGR) due to its greater absorption rate and higher biocompatibility.[29] Bottlenecks due arises from marine collagen namely production cost and symptomatic allergic reactions which may confine the growth. Consequently, the offal's or wastes generated in the slaughterhouse are looked upon, as they serve as a good bio-resource for proteins. Avian sources collagen production units produce enormous waste components (feathers and organs) at the raw material processing units and also post production as waste water scums.[31] As per 2018 November report of FAO, the weight of poultry carcass was found to be increased by 2.6million tonnes between 2017-2018, which was more when compared with the bovine and pig meat carcass.[32] Metamorphic lineage rather than on the version of creator in the factual realism of Charles Darwin was followed through and put forth in the metamorphic pyramid wherein avian sources were closest to the mammalian counterparts.[33] Avian sources production units has reduced raw material life cycle in comparison with bovine sources cutting down the time from 6-7 weeks from 18-36 months, making the poultry-based wastes as undemanding raw material for purchase, in a short period. The structure and biochemical properties of chicken collagen were found to be very similar to mammalian collagen, which is suitable for application in the biomedical industry.[34] The by-products from the poultry house acts as source of valuable nutrients if utilized properly. However, they are also considered as a potential concern to both environmental and human health by encouraging themselves as vectors for insects and vermin, and pathogenic microorganisms. In terms of air pollution collagen production units emanates nauseating odour leading to uneasiness to the nearby people.[31] People are also concerned regarding the transmission of avian influenza virus, which acts as a break from the utilization of the potential source of valuable products. The major organs that are involved in poultry processing include skin, intestines, liver, heart, horns, gizzard, and feet, which are all discarded without processing. If managed properly, these wastes provide excellent market value. As stated earlier, collagen is a ubiquitous protein in all these tissues.

Extraction

Despite the predominant existence of collagen in multicellular organisms and cell types, the extraction process for each of the sources majorly influences the purity and applications of the protein. Furthermore, it is the cross-linked nature of collagen, which is the primary factor concerning its dissolution. Numerous methods such as ultrasonic irradiation;[35] pepsin solubilisation;[36] using green solvents like water and carbon dioxide for extraction[37] temperature denaturation38; solvent-mediated extraction;[37,39] hydro-extraction;[40] acid-pepsin-solubilisation[41] etc were experimented. Most of the abovementioned methods mandate the need for pre-treatment processes that aid in the removal of undesired compounds such as fat and non-collagen proteins from the tissues and cause partial hydrolysis, without hindering the structure and activity of the protein. This initial treatment process is by purification steps. Among the different methods stated in Table 1, the standard and most straightforward process of extracting collagen from poultry involve the use of acetic acid, as it enables extraction without causing denaturation of the protein.[42,43] Bonifer & Froning[44] compared the effect of aqueous washing on the concentration of protein in chicken skin, concerning the removal of fat from the chicken skin. The experiment concluded that the salt extractable proteins and collagen were found to be increased, with a decrease in fat content. Avian skin samples were treated using 0.1MNaCl and 0.5%$NaHCO_3$. Comparative protein quantum amount estimation was carried out between treated and untreated skin samples. Lin et al.[34] compared the extraction of collagen using 3 different organic acid systems (0.5M of lactic acid, acetic acid or citric acid) along with pepsin. They found that the acetic acid and pepsin combination showed the highest yield (3.68±0.27%), purity (72.19±18.16%), and recovery (78.26±5.73%), compared to other systems used. A comparative study was carried out by Munasinghe et al.45 using different solvent systems organic solvents like citric and acetic acid, alkali base, single step acid : enzyme and multi steps acid; enzyme by phasic systems for collagen extraction. Collagen production yield differs widely with the use of organic acids and enzymatic extraction processes namely using acetic acid: 6.1%, citric acid: 6.2%, pepsin: 38.7% and acetic acid – pepsin combination: 40.4% proving mixture of mild organic acid and enzymes gives a better yield, proving the convergence technology of chemical engineering and biotechnology. Pepsin extraction technique, after pre-swelling of chicken skin with acetic acid, provided the highest yields at the end of the study.

Characterization studies

Characterization study is an important step to ascertain the presence of certain specific characteristics in the material under the survey. It helps to assess the materials' surface, physical, and chemical properties. Depending on the need or the purpose of the study, the characterization tests are selected and performed.

Histochemical methods

The histochemical test is used to observe the organization of the biological materials under a microscopic examination. The collagen fibres appear bright red coloured when stained with Van Gieson's stain. Staining methods use different dyes as imaging agents. In this work by Etherington & Sims[12] picric acid plays a very pivotal role in tissue histopathological studies, ponceau S acts as a displacing agent wherein this agent displaces the picric acid which has been held in the skinny rough surface of the collagen fibers and facilitating the entry of larger molecules.[13] Collagen forms are distinguished using Periodic acid Schiff's reaction which works on the principle of Victoria Blue R dye with phosphomolybdic acid or phosphotungsticacid staining histochemical technique.[46]

Uv-vis spectrophotometry

The UV spectrophotometry is used to detect the chemical constituent present in the material under study, based on the principle of absorption of near UV (180-390nm) and visible (390-780nm). Residual content as impurities like polymeric monomer, inhibiting agents, antioxidants were quantified using UV-Vis spectroscopy due to

its very high sensitive lower level even at 10-5 molar concentration.[47] Conventionally elucidation of the UV scanning absorption spectra in the visible range particularly in the narrow range of 200 – 400 nm is tested for the obtained collagen extracts using chemical or biochemical extractive processes. It is observed that all the collagen types show an absorbance between the 200-230nm region, with the silky fowl feet collagen demonstrating compliance with the range as mentioned above.[48] The collagen from the emu skin showed absorbance at 231.5nm.[49] The UV spectra of chicken skin collagen displayed maximum absorbance, ranging from 230-240nm.[50] A similar UV spectrum result of the collagen extracted from goose bone showed absorbance at 232 nm, and no absorbance was seen above that, as reported by Wang et al[51] Utilization of near UV absorption spectroscopy was carried out for the thermal lability principally the denaturation process on the quantum measurement of tyrosine content for the tertiary structural conformation of non-helical telopeptides.[52] But, Lin & Liu[53] observed that the birds' feet collagen showed a broader range of absorption spectrum with a range of 190-340 nm.

Table 1 Details on methods followed in the extraction of collagen from different organs of Poultry wastes

Source	Organ	Extraction method	Reference
Silky fowl	Feet	Pepsin and acetic acid digestion	Cheng et al[28]
Ostrich	Trachea	Pepsin and acetic acid digestion	Jaroenviriyapap & Vittayanont et al[57]
Egg	Shell membrane	Pepsin and acetic acid solubilisation	Zhao et al.
Quail Muscle	Muscles	HCl treatment	Maiorano et al.
Duck	Feet	Lactic acid digestion	Huda et al[68]
Chicken	Skin	Acid digestion	Lin et al[34]
Turkey	Muscles	Enzyme treatment	Khiari et al.[64]
Emu	Skin	Acetic acid treatment	Nagai et al[49]
Duck	Feet	Citric acid treatment	Kim et al[58]
Duck	bone, feet, tendon	Pepsin digestion	Kim et al[80]
Goose	Bone	Acetic acid extraction	Wang et al[51]
Chicken	Skin	Pepsin and acetic acid solubilisation	Arunmozhivarman et al.[50]
Duck	Feet	Citric acid treatment	Kook et al[82]
Chicken	Meat	HCl extraction	Offengenden et al[83]
Chicken	Feet	Acid solubilisation	Potti et al.
Chicken	Feet	Pepsin and acetic acid solubilisation	Silva et al[62]
Duck	Feet	Lactic acid treatment	Theng et al[61]

Sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS- PAGE)

Sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) is a discontinuous electrophoresis system used to separate the protein fragments according to the molecular mass. SDS-PAGE is performed not only to assess the molecular mass of the collagen, but also the purity and reveal information of the chain composition. Collagen is a polypeptide chain that is a right-handed helix and has a molecular weight of 300kDa. In contrast, the collagen peptides or hydrolysates are relatively small molecular weight molecules obtained on treatment with certain enzymes. The study requires collagen samples of nano gram to microgram only. 4-20% of polyacrylamide gel is used commonly.[54] The protein bands are observed by staining with Coomassie Blue and compared with a standard molecular marker, which helps to identify the type of collagen extracted. A drawback of the method is that the peptide fragments with lesser affinity to the dye cannot be visualized.[55] Avian sources, especially chicken source collagen constitutes α-chain and β chain (as shown in Figure 4) of which the raw material namely the collagen obtained.[50] From the chicken dermal portions containing the unalike alpha (α)-chains (alpha-1 and alpha-2) with a unique ubiquitous single beta chain depicting the presence of collagen of Type I. This was proved by experimentation using acetic acid as the extracting solvent. Obtained fragments were all above 116kDa clearly proving the presence of type I collagen. Alikeness observations were found for collagen obtained from the avian source namely chicken (skin) with double distinct alpha chains (alpha-1 and alpha-2) with a single individual cross-linked β chain,[34,56] emu skin,[49] chicken, duck and ostrich trachea[57] ascertaining on the presence of type I collagen. The collagen extracted from the feet of different birds, on contrast were found to possess other types of collagen other than type I, like two main monomers (α1 and α2 chains) between 100 and 140kDa and β chain (dimer) were observed by Kim et al[58] in duck feet, more than 2 types of collagen from silky fowl feet[48] and types I and II collagen in the chicken feet[59] unlike the presence of type I collagen in skin.

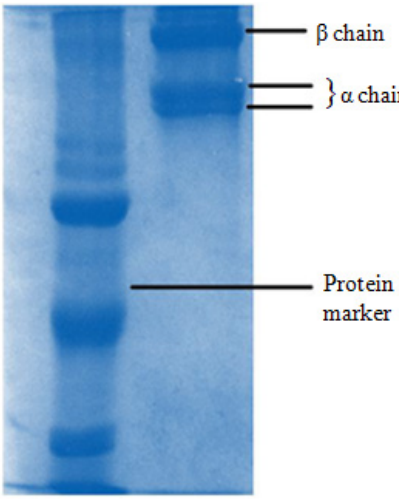

Figure 4 (Source: *Arunmozhivarman, 2017*).

Fourier-transform infrared spectroscopy (FTIR)

FT-IR works on the principle of assessing the vibration and stretching of covalent bonds with IR radiation. It is a powerful method to identify the bonding types, molecular level composition, and the functional group investigation, understudy.[60] Scanning of FTIR spectra for the collagen samples will be in the range of 4000-400 cm-1. The spectral bands in the region of 1200 to 1300cm^{-1} portrays the single specific collagen blueprint in terms of the tertiary molecular structural conformation ascribed for the tripeptide of (Gly-Pro-Hyp) n.[50] Distinguishing finger print region spectra was obtained for amide A (3305.19cm^{-1}), B (2922.52cm^{-1}), I (1633.98cm^{-1}), II (1549.08cm^{-1}) and III (1238.07cm^{-1}) respectively by Arunmozhivarman et al.[50] using FTIR studies and that of duck feet collagen[57] showed 3,309cm^{-1}, 2,854 and 2,924cm^{-1}, 1,630cm^{-1}, 1,454 and 1,549cm^{-1}, and 1,238cm^{-1}, and that of emu skin[49] showed 3309cm^{-1}, 2925cm^{-1}, 1,633cm^{-1}, 1,541cm^{-1}, and 1,237cm^{-1} displaying similarity between the collagens obtained from different bird sources. Hong et al.[56] did a comparative study on chicken skin extraction between heat soluble and pepsin soluble collagen. They observed that the pepsin soluble collagen showed the characteristic peak of amide I bond at 1660cm^{-1}, while the heat soluble collagen showed a peak at 1635cm^{-1}, which was in agreement with heat soluble collagen extracted from rat tail.

Circular dichorism

Circular dichroism (CD) is a very sensitive technique to characterize the secondary structure and to detect the conformational changes of protein chains, with the help of polarized light. Characterization of tertiary structural conformation of collagen immaterial of the sources is normally carried out using chronic dichroism (CD) depicting perfectly arranged in an orderly fashion of α helices and β sheets whereby giving a very specific optical activity because of its orderly orientation.[54]

Aminoacid analysis

Determination of amino acid content was carried out for samples which have protein content. The amino acid analysis involves hydrolyzing the peptide bonds with NaOH (for tryptophan), a combination of HCl and performic acid (for cystine+cysteine), and HCl for of rest of the amino acids.[61] High-performance liquid chromatography (HPLC) or amino acid analyser was utilized for the determination of the liberated amino acids. Avian sources collagen from emu showed Glycine (331 residues) rather than proline (121 residues) to be abundant followed by hydroxyproline (97 residues) and also the complete absence of Tryptophan and Cysteine proving the presence of Type I collagen. Nagai et al[49] & Araújo et al.[62] similarly observed that the iminoacid content was found to be around 14%, which is in agreement with the iminoacid profile of collagen stated by Etherington & Sims[13] and the glycine content being the highest of around 15%, among the rest of the amino acids. Liu et al. 59 compared the extraction of collagen using different acids and observed differences in the amount of glycine present in chicken feet, with the highest amount obtained from citric acid followed by acetic acid and lactic acid (36,32.6 and 28.2%) respectively. Observations stated that glycine and proline accounted for about 30% and 11.7%, respectively.

Hydroxyproline assay

Collagen is a helical polypeptide chain that consists of the Pro-Hyp-Gly sequence being the most common triplet. Glycine accounts for about 33% and is present in the third position favouring the compactness of the helix. In terms of the amino acid composition and composition, proline is a major component comprising of more than 20% of the summate of the other amino acids. Nearly half of proline gets hydroxylated and forms 4-hydroxyproline.[13] The presence of this amino acid helps in the quantification and characterization of collagen. In the assay, the tissue is first hydrolyzed using strong acid like HCl and perchloric acid. Then the hydrolysate is added with chloramine-T and is oxidized with Ehrlich's reagent (7-dimethyl-amino-benzaldehyde) to form red-brown chromophore that is read colourimetrically at 550nm.[63] The hydroxyproline content determination for silky fowl feet with different acids systems found maximum for acetic acid (516.6 mg/g) and HCl (516.9 mg/g), and minimum with citric (353.3 mg/g) and lactic acids (434.4 mg/g).[46] In another work, estimation of collagen comparing turkey head insoluble biomass and protein isolates observed that the former contained more hydroxyproline than the latter (4.95 and 0.05%, respectively).[64]

Applications

Food industry

Sausages, an excellent source of high-quality protein, were developed to preserve meat economically. Biphasic emulsion comprising of "oil in water' system facilitates sausage production process- fat enveloped by protein layer and stuffing meat into casing. Globally, the need for the kosher and halal based meat products is growing, and the poultry source can be an agreeable alternative to meet consumer demand. Sausages traditionally come with natural intestinal casings. However, bovine based casings have several disadvantages as problems of high cost, hygiene, preservation, bacterial loads, and handling problems. The production of collagen-based casings overcomes these inconveniences. Food grade biofilms made of collagen possesses greater advantages over food grade synthetic polymeric biofilms in terms of uniformness, durability, elasticity and consistency.[65] Viscoelastic properties of collagen from avian sources facilitate the manufacturing of food grade collagen especially to be used as sausage casings catering to the intrinsic and extrinsic properties during extrusion and in resulting biopolymeric fixation reasoning out that avian sources overcome the short comings of the mainstream and customarily used bovine collagen sources.[66]

. Meat products are rich in fat, which contribute to the texture, palatability, and physicochemical parameters. People consuming these high-fat products are prone to an increased risk of obesity and heart ailments. Attempts to reduce the level of fat proved to reduce the water retention capacity and textural properties drastically. Hence,

the alternative to replace the fat content should ensure that the typical characteristics of the meat. A study was conducted with the usage of chicken feet collagen powder as a replacement for fats in meat products.[67] The study reported that the collagen powder substituted sausages, known as 'light sausages,' showed a 29.02% decrease in fat profile compared to standard sausages. They also exhibited the lowest hardness, the lowest chewiness, and gumminess, high water retention capacity, and tenderness. The chroma appeared normal, which is comparable to that of the standard sausages. The knowledge of emulsified sausage, with the correct proportion of fat and moisture, decides the tenderness, juiciness, and overall the quality of the sausage. Viscoelasticity and emulsification competency of avian sources collagen obtained from breast and thigh is far superior to other parts like gizzard and skin. Collagen extracted from avian sources in this case duck feet raw material for its physiochemical characteristic attributes when this collagen was used a composite component to surimi and the results proved to show significant improvement in the properties of surimi. Besides improving the gel strength and hardness, the collagen scored an increase in the folding test and the colour lightness. The study concluded by recommending collagen as an alternative protein additive for surimi68. Attempt to produce jelly was made with the addition of chicken feet collagen, with pineapple and white chocolate flavours. The report showed more acceptances from the consumers.[69]

Tissue engineering

Tissue engineering is an upcoming field that provides promising solutions to the deformed host tissues, either due to congenital or traumatic conditions, by replacing or supporting the tissues during the healing period. The biomaterials used for tissue engineering purposes can be natural or synthetic and should promote host health without any adverse effects. The biomaterials may be of metallic, ceramic, or polymeric origin. Of the various options available, natural sources are always the most preferred option for biomaterial construction. After blood, the bone is the second most common transplant tissue. A statistical report by Gururaj[70] stated that the number of people hospitalized due to bone injury was found to be 2.5 million, and a continuing trend will witness the number to rise to 3.5 million by 2015. Autogenous and allogenous osteo-replacements prove inadequate and inefficient, respectively, for the bone-related treatment. Hence, graft substitutes with a combination of scaffolding properties are relatively constructive in osteo- associated management. Collagen is in as a matrix for building scaffolds for bone reconstruction, because of their supportive nature and bio-degradative properties. Collagen is a natural polypeptide chain that exists in the extracellular matrix of multicellular animals. Not only cosmetic industry but also individual well-being nutritional supplement industry also use and utilize collagen at different process levels of these nutritional products thereby a huge demand is created for collagen.[71] The biocompatibility property and degradation with the help of the collagenase enzyme makes it a healthy choice for bone trauma management. Engineering the natural polymer (collagen) with chemical compounds such as di-carboxylic acids,[72] sebacic acid,[73] succinic acid,[74] have proved to overcome the drawbacks faced for their application in the field of biomedical industry. It is also quoted as due to the lower denaturation temperature refrain the use of collagen from clinical applications, as a result of which addition of other biopolymers like alginic acid,[75] has shown to help in improving the drawback. As in case of avian source, the collagen derived is found to have additional support for being used as a biomaterial for human use as the birds and mammals have similarity in the denaturation temperature of I&II type collagen (37-39°C)[76] since type I and II are the most versatile collagen types used in bone and joint treatments, by engaging chondrocyte interaction with scaffold.[77]

Bone regeneration and restoration involve a combined effect of osteoblasts, fibroblasts, growth factors, bone morphological proteins (BMPs), and signalling cascades. The vital component of bone is collagen, which forms most of the organic part of the bones. The collagen can thus be a healthy choice for scaffold formation and bone regeneration. Lin & Liu,[78] developed and compared sponge materials made by combining hyaluronic acid and collagen obtained from bird feet and pigskin, taken at varying ratios. The work showed that by changing the ratio of collagen in the sponge could alter the properties and biodegradable nature of sponge material. The ratio of bird feet (4): pigskin (1): hyaluronic acid (0.2) was observed to be having the maximum tensile strength and biostability. This result attributed to the decrease in pigskin and an increase in bird feet collagen in the sponge material. The bio-stability of the natural polymer is found to change by blending the polymer with another element of choice, to produce a complex cross-linked content. Sailakshmi et al.[79] showed that the thermal stability and bio-degradability of collagen was found to increase by cross-linking with alginic acid. The study also showed that the cross-linked collagen showed a higher resistance to degradation by collagenase action, which is attributed to the intermolecular hydrogen bonding formed between the hydroxyl group of hydroxy proline and hydroxyl group of alginic acid, as shown in Figure 5. Kim et al.[80] introduced a new method of combining the collagen with another natural polymer- silk, because of the relatively less tensile strength when used alone in the fabrication of scaffold. The study showed that the combination of collagen with silk expressed high porosity, cell infiltration, and proliferation with additional biostability. Song et al[81] did similar work, where collagen from duck feet was used to develop sponges, by combining with hydroxyapatite with different concentrations of silymarin. Silymarin had an osteoinductive effect and with collagen, improving the porosity and better matrix for the growing cells. Kook et al[82] developed an osteogenic environment by an amalgamation of duck feet collagen, hydroxyapatite, and dexamethasone (synthetic glucocorticoid promoting cell differentiation). Later the sponge was seeded with bone-marrow-derived mesenchymal cells. The study showed that the Dexamethasone (+)-Duck feet Collagen/Hydroxyapatite proved to be enhancing bone regeneration and promoted activity of ALP, col-1, and osteocalcin.

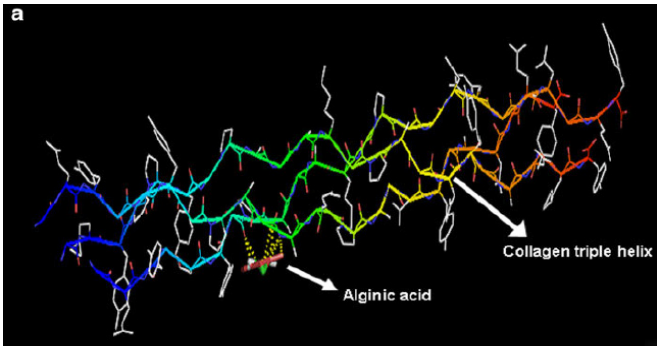

Figure 5 (Source: *Sailakshmi et al., 2012*). Bonding pattern shown between alginic acid and collagen, using bioinformatic tool.

Collagen peptides

The collagen peptides or hydrolysates are relatively small molecular weight molecules obtained on treatment with certain enzymes. Collagen peptides are now incorporated as dietary supplements and are marketed, which are consumed for their anti-ageing and health-promoting benefits.

Cosmetic and skin care applications

People are most concerned about their appearance and skin health. And collagen is essential for skin health. Collagen is responsible for the skin texture, and as ageing occurs, the collagen production in the body decreases at a rate of 1-2% every year, from the age of 30. Collagen production decreases due to various environmental reasons (UV rays, heat, moisture, etc.). Cosmetic aesthetical industry abundantly uses collagen in its various product lines because of the collagen's very unique property and ability of the moisture retaining capability.[48] Collagen and melanin rich avian sources like silky fowl possessing white silky feathers but black skin are a very prospective raw material sources in cosmetic industrial products. These components consisting of rich collagen and melanin were extracted by Cheng et al.48 using multi-steps acid systems. The collagen-containing melanin was able to absorb UV light (200-400nm), thus showcasing the UV absorbing capacity of the acid solubilised collagen. Skin loses its texture due to increased damage to the extra-cellular matrix, where collagen is an important constituent. The damage to the matrix is contributed due to the oxidative stress and external factors exposed to the tissue. Dermal fibroblasts form a vital baseline for the formation of fresh connective tissue after trauma at the site of injury. Offengenden et al.[83] work suggested that both the pH and sequential use of enzymes for collagen hydrolysis produced different types of peptides, which showcased differences in both chemical and biological profiles. The collagen peptide preparations demonstrated the presence of antioxidant role. Collagen peptides (cleaved with enzymes) also proved to have a stimulatory effect on human dermal fibroblasts, which will be supportive during wound healing conditions.

Protective roles

Collagen on enzymatic cleavage produces shorter peptide fragments that were found to contain properties like anti-inflammatory,[83] angiotensin I converting enzyme (ACE) - inhibitory activity and antihypertensive effects.

Collagen hydrolysates had a lipid-lowering effect through the regulation of hepatic lipid biosynthesis to suppress the triglyceride level. The inflammatory response causes the triglyceride concentration in plasma to increase along with inflammatory cytokines like IL-6, TNF-α. Zhang et al[33] showed that treatment with collagen hydrolysate down-regulates several pro-inflammatory cytokines associated with lipid metabolism, thereby resulted in the increment of ACE-inhibitory activity. These results concluded that the collagen hydrolysates could be of use as adjuvant therapy for the prevention and management of heart ailments. Results of Offengenden et al.[83] showed that the pH had an essential role in the production of collagen hydrolysates that showed anti-inflammatory function. The hydrolysates produced by enzyme treatment under alkaline conditions showed better anti-inflammatory activity than those treated under acidic conditions.

Conclusion

Collagen, being the principle structural protein, is indispensable for a healthy life. Hence, there is a rising trend that focuses on the development of collagen-based products that are present in routine life supplies, in the form of supplements, creams, lotions, etc. Consequently, the pressure falls on finding a conventional source as the raw material for collagen extraction, instead of traditional sources. Poultry meat is the most sought after animal protein in the world, irrespective of the diversities in traditions, cultures or income. It is to be noted that even the discards of the poultry industry including its drops, feathers, egg shells, offal make a practical utilization in other applications. Poultry industry is booming currently with respect to increasing income and growth in food service market, for the protein rich meat, resulting in masses of wastes expecting to be discovered productive. The avian spent wastes are so far, only least exploited for collagen mining, and it assures to be a better replacement for the conventional source with its promising characteristics.

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Conflicts of interest

The author declares that there is no conflict of interest.

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