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DEEP PROCESSING OF COLLAGEN-RICH POULTRY PRODUCTS FOR DIFFERENT USE

(review)

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Abstract

The competitiveness of meat processing technologies requires deep processing of protein containing raw materials including low-value wastes and by-products of meat processing. The connective tissues after animal and poultry meat processing can reach 16 % of initial carcass weight and hence the reasonable utilization of these resources is reasonable. Low-value by-products can be transformed to protein products via hydrolysis resulting in the preparations of isolated collagen-rich high-purity proteins with key functional and technological properties for food, feed, medical, and cosmetic industries. Chicken skin (J. Stachowiac et al., 2004), necks and bones (M.I. Kremnevskaya et al., 2016; P.F. De Almeida et al., 2013), trachea of chickens, ducks and ostriches (T. Jaroenviriyapap et al., 2009) were studied as secondary collagen-rich raw materials. The most common techniques of collagen extraction are acidic and high-temperature hydrolysis (K.A. Munasinghe et al., 2014), papain and pepsin hydrolysis (P. Hashim et al., 2014), alkalase and trypsin hydrolysis (Z. Khiari et al., 2014) and microbial fermentation (A.Yu. Poletaev et al., 2011; O.V. Zinina et al., 2013). Deep processing of secondary collagen-rich raw materials in the meat industry will reduce the existing deficit of food and feed protein, expand the assortment and increase the output of meat products and low cost digestible feeds, and improve the ecological situation. For pharmacology, short peptides are of interest, the regulatory function of which has been known for a long time and is used in medicine (A.D. Neklyudov et al., 2007) which could be produced by deep processing of animal and poultry carcasses. The importance of dietary collagen is also associated with imino acids with –NH groups (proline, hydroxyproline) which are necessary for tissue growth and development (S. Busche, 2011). Different techniques of processing collagen-containing raw materials allow to manufacture protein products with specified properties for use in food, feed and other industries.

Keywords: collagen-containing animal wastes and by-products, enzymatic hydrolysis, short-run high-temperature hydrolysis

Effective utilization of resources is one of the basic concepts of modern global agriculture. In this regard, opportunities for conversion of non-digestible animal-derived protein components such as collagen, elastin, and reticulin are being sought. Massive yield of connective tissue from animal carcasses may reach 16 %. Processing of collagen-containing substrates is predicted to save 10-20 % of the main raw material in manufacturing full-value meat products and up to 70-100 % of the main raw material in production of artificial covers and films. The use of collagen protein allows decreasing the cost of production, loss from thermal processing, and improves quality upon use of the low-grade meat for processing [1]. Most traditional technologies have already been used for a long time for production of protein stabilizer from the pork skin and beef vein, liver and sub-product sausages, sulze, meat jelly, etc. [1]. Skin collagen forms transparent gels and may be used as gelling agents.

This review summarizes information on properties of collagen-containing raw materials, methods of its processing and their effect on the functional properties of collagen. Particular emphasis was made on the use of protein-based by-products obtained upon processing of poultry production and on properties of protein-containing substrates for food, pharmaceutical and feed industries.

Collagen structure and functions. Specific amino acid composition and spatial structure of collagen molecules define not only their physiological functions, but also technological features, in particular, stability at extraction, an ease of separation from other components, ability of restoration from solutions with formation of supramolecular structures, which extends the sphere of application of modified collagen products.

Tropocollagen consists of three polypeptide equal size chains forming a triple helix [2, 3] based on hydrogen bonds with numerous inter-chain cross-links between the amino acid (AAs) residues (each polypeptide chain contains nearly 1000 residues). Convergence of chains is ensured by regular distribution of glycine (each third position), being the only amino acid lacking β-carbon and lateral radicals. Collagen includes 19 AAs which, depending on structure of the lateral radicals, are divided into three groups: non-polar chemically inert AAs (glycine, valine, alanine, isoleucine, phenylalanine and proline), AAs having reactive groups (serine, threonine, tyrosine, methionine, oxyproline), and polar AAs dissociating as bases (lysine, oxylysine, arginine, histidine) or as acids (glutamine and asparagic acid).

Collagen in wastes of skin is rich in proline and oxyproline (nearly 20 %), glycine and alanine (over 50 %), practically lacking (or containing in small quantities, if insufficiently purified from other proteins) any aromatic, heterocyclic, and sulfur-containing amino acids [1]. Collagen is one of few proteins not only containing oxyproline, but also oxylysine. Thermal stability of tropocollagen units and fibrils increases with the increase of share of proline and hydroxyproline amino acids [4, 5]. The content of proline and hydroxyproline is significantly lower in fish, in particular cold-water fish, than in mammals [5].

The distinguishing features of the collagen are its unique mechanical properties, chemical inertness, amino acid composition, and ability to transform into low-molecule protein product, gelatin, upon prolonged heating in water, and in water-based solutions of acids and alkali. Various factors affect physical and chemical properties of collagen [6].

By its physiological effect, proteins of the connective tissue, including collagens, are similar to dietary fiber displaying clear cytoprotective activity, normalizing microbiocenosis of intestines, preventing dystrophic changes in epithelium and permeability of colon mucosa cell barrier, thus, reducing the likelihood of penetration of bacteria, toxins, and polymeric residues with allergizing effect. Collagen breakdown products (gluten, gelatin, etc.) own properties of the dietary fiber stimulating secretion of the digestive juice and intestinal motility, and favorably affecting good microflora [7]. Uncombined charged AAs groups in the collagen molecule, which are localized in polar zones having interlaced with hydrophobic areas, may link ions of heavy metals in the intestinal duct with further formation of the insoluble complexes, which can not penetrate, and are excreted from the body [5]. The ion absorption by hydrolyzed collagen in acidic (pH = 1.2) and alkali (pH = 7.8) environment comprises 71.00 and 82.00 %, respectively, for Pb^{2+}, 68.00 and 74.67 % for Cd^{2+}, 25.33 and 50.00 % for Cu^{2+}, and 81.2 and 91.00 % for Hg^{2+}. Absorptive ability increases with the increase of the serial number of element (and, accordingly, ion radius) with the maximum for mercury ions. Linking mechanism has not yet been clearly identified, but it had been established that all proteins are characterized by its apparent ability for non-specific linking with metals by guanidine group of arginine, etc. Possibly, peptide chains break down upon fermentative processing of vein waste, due to which functional groups become easier accessible for reaction with metals.

By-products of collagen-containing raw material in poultry farming. Traditional technology of use of the connective-tissue proteins in meat product formulations had not led to the desired results (http://www.pandia.ru/text/category/vovlechenie/), since native components of the connective tissues have low organoleptic value and are functionally incomplete. Besides, because of bovine spongiform encephalopathy (BSE) and influence of Muslim traditions, the need for more safe and acceptable collagen sources is raised.

In Russia, nearly 20 % of poultry production is subjected to deep processing, thus yielding nearly 650 thousand tons of by-products per annum. Legs, skin, trachea, and bones are used for extraction of the collagen proteins in poultry, whereas product yield is lower than in case of cattle and pigs. No tryptophan and small amount of methionine was found in the connective tissue (tendons, cartilage, and skin) mainly consisting of the collagen; these proteins are characterized as incomplete and are recommended as dietary fiber for improvement of the intestinal motility and functioning of the digestive duct. Meat without visual connective tissue has lower biological value than meat with natural content of the connective tissue. This confirms feasibility of use of poultry skin in production of meat products.

By-products of poultry, in particular skin, are rarely processed and transformed into valuable products. Extraction of collagen, whether containing or not containing telopeptides, from the chicken skin had been described [8]. However, chicken skin may be used for production of the collagen substrates, which are highly valued due to its unique functional properties. Chicken skin contains 75 % of I type collagen (the strongest archetypical form is fibrillous triple helical protein) and 15 % of III type collagen (embryo derma collagen). Upon collagen extraction by pepsin or ethylenedyamine (after removal of fat by heating of the chopped chicken skin at temperature of up to 40-60 °C), the output product is rich in telopeptides. Therefore, poultry skin serves good alternative source of high-quality collagen.

Upon mechanical dissection of the bird's carcass or its parts, 27-40 % of meat and bone residue is produced, with bone percentage of 15-20 % and ash to protein ratio of 0.7 in the chicken bones. Interestingly, 25-30 % is attributed to complete proteins, at that, protein and mineral substances are mainly contained in bone tissue, and liquid and fat — in meat tissue [9].

Due to complex structure of chicken tracheas, which are also characterized by high content of muscle and connective tissues, separation of meat from the bones is practically impossible. Technology of 8-hour lasting hydrolysis of trachea and bone residues at temperature of 100 °C in presence of inorganic catalyzer had been developed. Maximum density of the produced collagen product is 123 g/cm^3. Advanced production technology of ready-cooked and smoked whole-muscle products allow for addition of brine in the raw material. Herewith, retention rate and duration of processing mostly depend on diffusion rate of the brining agents. To decrease such rate, mixtures of collagen and muscle fractions of protein products of meat and bone residues of the poultry carcass may be used in various proportions. Addition of the protein mixture allows obtaining higher yield and nutritional value of ready-to-use meat products, and allows improving its organoleptic characteristics and rheological properties [10].

Chicken, duck, and ostrich tracheas may be used as sources of collagen and chondroitic sulphate [11]: it was noted that chondroproteins comprise from 49 to 73 % in the output products, chondroitic sulphate (mainly in form of chondroitine-4-sulphate) was identified by high-efficiency liquid chromatography (HELC) at 0.574-6.37 % level in terms of dry matter (mainly in ostrich, lower in duck, and the least in chicken). Hydroxyproline in collagen and chondroitic sulphate makes 66.19 and 84.38 %, respectively, or 4.04 and 7.40 % per dry matter. Collagen extracted from chicken buttock was compared with collagen extracted from the Achilles tendons in cows [12]. Jelly produced based on chicken leg collagen had been highly valued, thus highlighting the opportunity for production of high-quality gelatin from such type of raw material [13].

Processing methods and properties of protein products. Hydrothermal, acid, alkaline, and fermentative hydrolysis are used for extraction of the collagen and production of protein-containing substrates with high accessibility of the collagen from low-value raw materials. Each of the above-listed methods allows for production of the collagen both in solvable and in denaturized forms. Hydrothermal processing results in collagen denaturation with breaking of the specific configuration of polypeptide chains, and bonds within and between molecules. As a result, sizes and structure of the collagen fibers are changed. Full collagen hydrolysis takes place at temperature of 126 °C and lasts 3 hours. Strong acid hydrolysis results in complete destruction of tryptophan and in destruction and racemization of oxyacids, dicarbonic acids, and proline with production of D-isomers of several known amino acids, which are not metabolised by cell and may act as cell growth inhibitors. Alkaline hydrolysis causes racemization of the majority of amino acids and destruction of arginine, lysine, cystine, and cysteine. It results in production of the complex of defective toxic substrates. Fermentative hydrolysis with the use of proteolytical ferments lacks the drawbacks of acid and alkaline hydrolysis. Although such type of hydrolysis can be no more than 70-80 % complete, components produced due to the cleavage are physiological, easily penetrating in cell, and are engaged in metabolic process. Fermentative hydrolysis may also be performed with the use of live cultures of bacterial producers.

In USA, poultry processing industry is one of the fastest growing food industries [14]. In examining the contents of collagen in chicken bones and skin, the authors of the research had extracted collagen by **acetic acid**, lemon acid, alkali, with the use of two-stage hydrolysis by **acetic acid** and pepsin. Research results had shown high potential of chicken by-products as an alternative source of collagen for production of highly-valued products [14].

A multiple stage procedure has been developed for collagen extraction from chicken bones, allowing for removal of 87.5 % of mineral substances and 57.1 % of fat, provided loss of protein of nearly 18.6 % and hydroxyproline of 14.9 %. Collagen yield had comprised nearly 85 % of the initial volume, with of its quality and functional properties assessed [15].

The extraction procedure of the collagen from chicken legs by acetic acid at 4 °C within 24 hours with the use of papain and pepsin yielding 18.16 and 22.94 % collagen, accordingly, had been described [16)]. Produced collagen is rich in glycine, glutamine acid, proline, and hydroxyproline. Electrophoretogram of samples had shown presence of two α-chains (α_1 and α_2) and β-chain, i.e. main component of chicken leg collagen is collagen of I type. In both cases, samples were resistant to thermal denaturation at 48.40 and 53.35 °C, respectively.

One of perspective processing techniques of secondary protein-containing raw material is fermentative hydrolysis with the use of live microorganisms. Fermentative processing allows for practically complete preservation of all essential amino acids. Herewith, use of ready-to-use fermentative preparations in the industrial scale may be expensive and costly, whereas introduction of live microorganisms allows decreasing the processing cost of collagen raw material [17, 18]. Effect of microbial cultures tissues is assessed by alteration of functional, technical, physical, chemical, and morphological properties, as well as by nutritional and biological values of the product. The effect of processing by microbial ferments on certain structural tissue elements, in particular collagen fibers had been confirmed by histological micro-structural studies [19].

Collagen is known for its high swelling ability, on which its functional and technical properties are based. Collagen proteins have unchallengeable features as compared to vegetable origin structure-forming agents, for instance carageenans and vegetable gums. Unlike vegetable origin hydrocolloids, properties of which are effected by concentration of culinary salt, food-grade phosphates, defrosting process, etc., collagens excellently preserve and display its functional properties in meat systems [20-22].

Areas of interest for pharmacology are short peptides (two amino acids and more, with molecule weight less than 10 kDa) which are the components resulted from meat processing. Their regulatory function is known for a long time and is used in medicine [23]. Dietary collagen significance is also associated with amino acids with –NH groups (proline, hydroxyproline) which are necessary for tissue growth and development [24]. Upon fermentative hydrolysis of collagen, low-molecule biologically active peptides could be extracted from secondary products of turkey processing. Protein-containing substrates and solid biomass may be produced from turkey heads followed by fermentative hydrolysis during various periods. Molecular weight distribution studied in turkey head hydrolysates by exclusive chromatography had shown that mixture of proteolytic ferments could produce great amount of low molecular weight peptides ranging from 555.26 to 2093.74 Da. These collagen peptides showed excellent solubility over a wide pH range (pH 2-pH 8) and were able to bind cholic acids. Enzyme cocktails for hydrolysis represent a potential new way to produce low molecular weight bioactive collagen peptides from poultry by-products [25].

Another modern technology for processing collagen-containing raw material, which is considered to be among the most perspective methods, is short-term hydrothermal hydrolysis. Dry collagen-containing proteins produced by this method from meat-and-bone residues and chicken legs are completely water-soluble. Digestibility of these products exceeds 95 % indirectly indicating the high protein assimilation. By physical and chemical properties, the collagen-containing proteins from chicken legs are favorably comparable to animal proteins from meat-and-bone residues due to an increase in weight proportion of collagen in dry protein by 13.8 % and fat decrease by 40.0 % [26]. Such results had shown possibility of domestic production of collagen-containing extracts in lieu of the imported additives of the animal proteins from pig and beef skins. Among the advantages of short-term high-temperature processing is a 100-fold decrease in time, double increase of protein concentration in broth, triple increase of the total output of protein and dry substances and increase of the protein yield from the chopped chicken legs by 24.3 % as compared to meat-and-bone residues at equal conditions (26).

Traditionally, no-waste technologies are aimed at processing of trash products for protein feed production. As we know, AAs are divided into essential and non-essential. It had previously been thought that animal body may independently synthesize non-essential amino acids, but this is clearly not confirmed. Non-essential amino acids (for instance, glutamine, proline, glycine, and arginine) play an important role in regulation of gene expression, cell signaling, antioxidant activity, neurotransmission and immunity. Besides, glutamine and aspartate participate in maintenance of the integrity of small intestine and its digestive function [27]. Accordingly, animal feed formulations shall be balanced by essential and all non-essential amino acids, which shall be accounted for upon revision of "a perfect" protein conception [27)]. It had been shown that feeding of broiler chickens with low-protein diets lacking glycine and serine may decrease the rate of their growth and cause depression [28].

Not only feed additives, but also veterinary preparations may be produced based on protein-peptide compounds from low-value animal raw materials. Thus, Kolimak and Dinormin are designated for treatment of the immune deficit and gastrointestinal diseases in piglets [29, 30]. By effectiveness and economic indicators, such preparations outperform the imported analogues. Kolimak contains liophilic extracts of pig stomach, duodenal and pancreas, and has clear protective and medicinal properties at gastrointestinal disorders, such as antibiotic-associated diarrhea and gastroenteritis. The developed technology preserves active substances of protein origin in the extracts [31]. Low-value raw material can also be used for creation of functional products with rehabilitation, preventative, and medicinal effects. In addition to compounds responsible for regulatory functions, slaughter by-products contain antimicrobial peptides participating in protection from external factors. Dinormin is a preventative and medicinal preparation from extracts of immune competent organs in pigs (thymus, spleen, and mesenteric lymph nodes) containing significant amount of neuromediating amino acids (aspartic, glutamine acids, glycine). It is highly effective at treatment of immunodeficient diseases in animals due to the essential regulation of the immune system, allows increasing weight gains and improving survival rates of the livestock [32].

Feed additives and meat properties. It is evident that animal rising technologies affect quality of the meat and by-products. Detection and identification of proteins and peptides in animal and vegetable raw materials extend opportunities for their use [33]. Obviously, development of feed additive production based on deep processing of protein-containing raw material, as well as effective use of such additives to pre-form future properties of the meat products during animal's life requires deep knowledge of relationship between meat consumer and technological qualities, meat protein composition and how it is influenced by stress and free-radical oxidation. All these must be accounted in developing feed formulations.

A large number of polypeptides of two to 30 amino acids ensuring effective meat processing and quality of finished products are extracted from muscle proteins of cattle, chicken, and pigs. Compounds associated with meat tenderness and moisture-retaining property are well studied [34, 35]. Relationship between the heat shock proteins and meat quality (tenderness and moisture-retaining property) was established [36, 37]. Heat shock proteins (HSP) are highly-conservative and expressed in all cells of a body as a response to physical, chemical or biological stressing factors [38, 39]. Absolute majority of the pathological changes in functions of cells, tissues, and organs is followed by deviation from the normal protein profile of healthy body [40].

Slow-down in lipid peroxidation promotes better taste, texture, and nutritional value of meat products [41]. Consumer preferences most of all depend on color of chicken skin and meat [42, 43]. Broiler body may not synthesize pigments [44], and standard commercial diets based on maize and soybean grains do not ensure sufficient quantity thereof [45]. Therefore, pigments are usually added in feeds [46]. Majority of pigments used for a long time in poultry farming industry in many countries are synthetic. They are more stable and cheaper than natural ones, but their safety is often questioned [42], and choice is often made in favor of healthy products rich in natural carotenoids [47-49]. Food-grade extract of calendula can improve growth, pigmentation, antioxidant ability and quality of chicken broiler meat [50]. Calendula extract contains mixture of xanthophylls with active components luthein and several zeaxanthins (xanthophylls of carotenoid group) which are considered safe since are present in edible plants. Amongst natural pigments, extracts of marigolds are mostly often used in poultry feeding [45]. Luthein may not only improve the color of chicken skin, but has very strong antioxidant properties [42, 51] which are important given the fact that antioxidant status is closely related to meat quality. However extract of dietary marigold in poultry feeding is mainly used for changing color of egg yolks. At the same time study of the effect of marigold extract on meat quality in broiler chicken had shown that natural carotenoid improves moisture-retaining property of meat. That is, more tender texture may be due to the increase of antioxidant activity or changing in moisture-retaining ability [52]. Intra vitam, antioxidant status affects animal and poultry health, and antioxidant potential of muscle tissues after slaughter significantly determines the meat quality. Lipid peroxidation by free radicals causes stress and increases contents of malonic dialdehyde, being the finished product of lipid peroxidation [53], which also deteriorates meat quality and changes its color. Accumulation of antioxidants, on the contrary, stabilizes color and increases meat shelf life [54]. Dietary antioxidants, such as vitamin E, may compensate unfavorable effect of oxidation stress on growth and broiler meat quality [55] and, accordingly, may improve economic effect of feed additives based on deep processing of protein-containing by-products.

Pale soft exudative (PSE) meat remains a serious problem for poultry processing industry. This defect is directly due to protein denaturation caused by rapid decrease of pH in early post-slaughter period at high temperatures [56, 57]. It had also been shown that stressing agents (increased stocking density, heating stress, and transportation stress) may result in PSE [58, 59]. This example indicate the necessity for accounting all factors of the economical effectiveness of feeding additives derived from deep-processed poultry by-products, which are intended for intra vitam formation of the technological properties of meat.

Thus, secondary by-products in poultry farming are safe and available sources of collagen and protein for food, pharmacology, and feed production industries. In Russia, deep annual processing of poultry yields nearly 650 thousand tons of by-products. Hydrothermal, oxidation, alkaline, and fermentative hydrolysis are used for extraction of the collagen from low-quality raw material. Dry collagen-containing proteins derived from short-term hydrothermal hydrolysis of meat-and-bone residues and chicken legs are completely water soluble. Over 95 % proteolysis indirectly indicates high digestibility of the produced proteins. To develop technologies for production of feed additives by deep processing of protein-containing raw material, as well as their effective use, requires proteomic study of poultry farming products focused on intra vitam formation of consumer and technological properties of poultry.

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