Keratin – origins, properties, application

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Introduction

Keratin (*gr. Keras-horn*) belongs to the highly heterogeneous family of fibrillar proteins of compact structure. Large number of disulphide bridges in keratin's structure significantly influences its properties, in particular high mechanical and chemical resistance. Keratin is also characterized with high resilience to the activity of proteolytic enzymes, such as trypsin, pepsin or papain, is water insoluble but is highly hygroscopic.

In human epithelial cells a total of 20 keratin isoforms have been identified, with molecular weight from 40 to 70 kDa. Approx. 10 isoforms have been discovered in animal cells, i.a. in feathers, hooves, horns, talons, reptile scales and hair.

One of the main sources of keratin waste is the poultry industry. Feathers are made in 90% of keratin and constitute 5-7% of the total weight of an adult chicken [1]. During poultry processing the feathers are discarded and, as a waste product, pollute the environment [2]. The global growth of the poultry industry generates over 4 million tons of feather waste annually [3]. Half of this amount is produced by the U.S. [4]. A medium-sized farm in Poland generates approx. 7 tons of chicken feathers per day. The annual nationwide production of this waste amounts to 8,000÷9,000 tons [5].

Already at the beginning of the 20th Century studies were conducted on the use of keratin materials. Materials such as hair, hay or algae were used in the production of carpets, thermal insulations or scrubbing brushes. Later, keratin was also included in the production of cushioning layers, felt, wood preservers, technical boards, cements and filters [6].

Currently the poultry plants process the feather waste into low-nutrition forages. The nutritional value of such products is determined by the content of amino acids such as methionine and histidine. The amino acids content drops with the age of poultry [7]. Legal regulations in some countries prohibit the use of feather waste in forage production. There, the majority of feathers are stored [8, 9]. The main storage issues are odours emerging due to decomposition of substances in the biomass, as well as greenhouse gases such as methane and carbon dioxide [10]. Furthermore, the non-volatile decomposition products can be released with water into the soil and potentially contaminate ground waters.

Due to the constant rise in the volume of keratin waste, new methods are investigated to utilize those materials. Those studies aim at processing keratin waste into products with appropriate properties or recovering keratin for further use.

Characteristics of keratin

Keratin has a three-dimensional fibrillar hierarchical structure [11]. It comprises small, nanometric amino acids that polymerise in the known sequence to protein molecular weight of 10÷100 nm. The molecular weight of feather keratin is approx. 10500 Da. The cystein/cystine content in an amino acid sequence is 7%. In accordance with the amino acid sequence, keratin has in its structure approx. 40% hydrophilic and 60% hydrophobic chemical complexes. Protein molecules may gather in the structure of α-helix, β-sheet or a random, non-sequenced macrostructure. The keratin fibres composition in feathers includes 41% α-helix, 38% β-sheet and 21% random structures [8]. Figure 1 presents the α-helix and β-sheet structures.

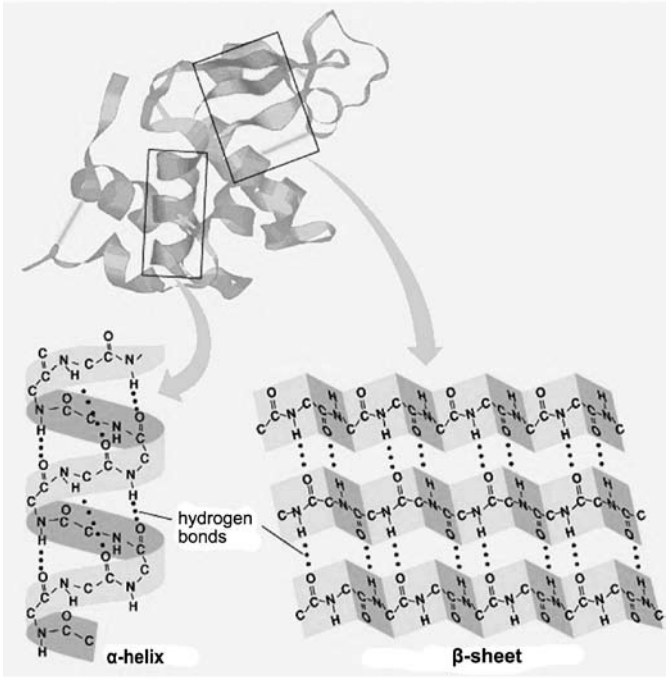


Fig. 1. Structure of α-helix and β-sheet [12]

The α-helix structure contains intramolecular hydrogen bonds between the carbonyl group of one amino acid and the amino group of another amino acid. The β-sheet structure is characterised by inter-chain hydrogen bonds between amino and carbonyl groups. The hydrogen bond can be joined with water bounded in the protein structure [8]. Keratin is characterised by high stability due to the intermolecular bonds between the polar and the non-polar amino acids and low solubility caused by the presence of S-S cystine bonds [11].

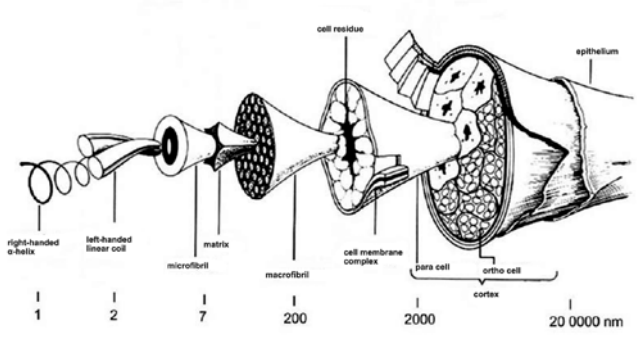


Fig. 2. Schematic structure of a wool fibre [13]

Animal hair comprises three main morphological components: epidermis made from thin layer of overlapping cells surrounding the keratinocytes, cornified layer made from oval cells arranged towards the fibre axis and membranous cells connecting the epidermis and the cornified layer. Furthermore, each cornified layer is composed of microfibres which in turn are made from many densely compressed α-helices. Keratin is a self-arranging fibre created in

the growth process, which is controlled by over 30 growth factors and cytokinesis. When the hair punctures the skin, the hair fibre is formed into a very stable structure, impermeable for environmental factors. The structure of natural fibres is very similar to synthetic polymers, as both those structures contain micromolecules, cross-link bonds, plasticizers and UV stabilizers. Figure 2 presents the schematic structure of wool fibre which is structurally similar to human hair [13].

Keratin properties

Keratin is characterised by high content of amino acids such as glycine, alanine, serine and valine. Also, keratin contains in its structure minor amounts of methionine, lysine and tryptophane. Most eminent components (7÷12%) are cystine and cysteine, which are sulphur-containing amino acids. One of the cystine carboxyl groups makes peptide bonds of a single chain, while other groups, i.e. amino and carboxyl, are included in the peptide bond of another polypeptide chain. The chemical activity of keratin is largely dependent on cystine that undergoes hydrolysis, oxidation and reduction. Table 1 presents the amino acids composition of keratins of different origins.

Table 1

The amino acids composition of keratin from wool, human hair and bird feathers, g of amino acid/ 100 g of proteins [6]

Composition		Sheep wool	Human hair	Bird feathers
Nonionic polar amino acids	Cysteine	12.72	15.90	7.80
	Histidine	0.70	1.00	0.20
	Serine	9.40	-	14.10
	Threonine	6.76	6.40	4.10
	Tyrosine	5.80	3.10	1.40
Ionic polar amino acids	Arginine	10.40	10.70	3.80
	Aspartic acid	7.27	3.00	5.60
	Glutamic acid	15.27	12.20	6.90
	Lysine	3.30	2.60	0.60
Non-polar amino acids	Alanine	4.40	-	8.70
	Phenylalanine	3.80	2.70	3.10
	Glycine	6.50	4.00	13.70
	Isoleucine	-	3.00-4.00	3.20
	Leucine	11.30	7.00-10.00	8.30
	Methionine	0.70	1.00-2.00	0.10
	Proline	6.75	9.50	9.80
	Valine	4.72	3.3-6.00	7.80

The composition varies but similarities can be found in the amino acid structure of keratin originating from different species.

Amino acids are classified as ionic or non-ionic, polar or non-polar. Cysteine and cystine (containing sulphide and disulphide bonds) are responsible for creating covalent bonds. Hydrogen bonds are created by nonionic polar amino acids, such as threonine, histidine or glutamine. Ionic polar amino acids, glutamic acid, arginine and lysine are responsible for ionic influences. In the case of non-polar amino acids (methionine, proline or phenylalanine) the influences are non-polar [6].

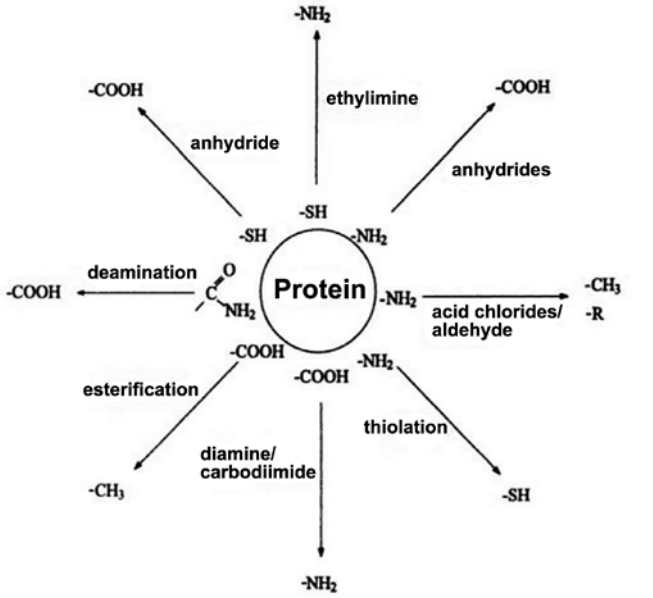


Fig. 3. Diagram of chemical modification of proteins [14]

Keratin reactivity depends on a number of factors. The main being its chemical composition, morphological structure and also the character of intermolecular bonds and active keratin groups. Keratin reactivity is also influenced by its solubility, water susceptibility, adhesion to various substrates and mechanical properties [14]. The above factors depend on the hydrogen, ionic and covalent bonds present in the protein. One of keratin's major influences is making hydrogen bonds with surrounding water molecules. The energy of hydrogen bonds is lower than that of ionic or covalent bonds, but higher than the energy of hydrophobic influences. Hydrogen bonds can be found between =NH and ≡C−OH groups. The presence of various groups in keratin structure enable it to participate in various processes. Figure 3 presents the possible chemical modifications of proteins, including keratin.

Potential applications

Tissues are a natural source of keratin. Keratins are classified as „soft" and „hard". „Soft" keratins can be found in epithelial tissue. They are relatively easy to decompose. Protective tissues found in fibrillar structures such as hair, nails, horns, hooves or feathers are made from "hard" keratins. "Hard" keratins are 2-phase structures with high sulphur content, characterised by high resistance to degradation. „Hard" keratins are the subject of biomedical studies.

Due to their water insolubility and limited methods of extraction and processing, keratin has been used for narrow applications. In the past it served as biomaterial for regenerative medicine. In the past few years interest in keratin and its modifications has increased, particularly with respect to keratin originating from wool. Yamauchi and Tachibana described the increase of the degree of distribution of fibroblasts on the keratin coating, as well as keratin-chitosan coatings. Fuji investigated the impact of proteins from human hair on rat labrocytes. Verma described the preparation of the protein structure of human hair for applications in tissue engineering. Dyke research

group investigated hydrogels and porous structures based on hair keratin, and their interactions with cells and tissues. Recently, attempts have been made to use keratin-based biomaterials in biomedical applications. The literature of the subject also mentions attempts to use keratin coatings in reconstructing ocular surfaces [15].

Environmental concerns, rising prices and depletion of oil reserves enforce looking for new solutions to replace the petrochemical products with biomaterials. Feather keratin appears promising in this respect. Feathers are a stable, renewable and natural material, produced in large quantities (an average of 5 million tons/year globally). Keratin is an inedible protein but can be transformed into low-nutrition forage. Whole feathers and their fibres have found application in textile industry. Producing raw materials with high quantity of additives containing fibres from feather keratin is possible by dissolving and then regenerating keratin [13].

Studies are in progress on the use of keratin as an additive to rubber products in order to facilitate biodegradation. The thus created protein-elastomer composites are claimed to have good mechanical parameters and, after their term of use has expired, to decompose better in natural environment [6].

Chicken feather fibres are composed of hydrophobic keratin, a protein with durability similar to nylon and diameter smaller than wood fibres. A surprising quality of chicken feather fibres is their semi-crystallinity and cross-link structure that improves the resistance of polymer-based composites to mechanical strain and ensures relatively high elastic modulus (3.4÷5 GPa). Furthermore, chicken feather fibres are characterised by high extension coefficient. The aforementioned qualities indicate the potential for successful application of chicken feather fibres as polymer reinforcement [9].

Keratin meets with high interest in the pharmaceutical, medical, cosmetic and biotechnological industries. Keratin materials obtained from wool can be transformed into porous foams in various shapes, sponges, cushioning layers, coatings, gels, microfibres and high-weight materials. Non-antigen keratins have a positive impact on healing of wounds and tissue reconstruction. Keratin implant layer, structure or biomaterial can be absorbed by surrounding tissues [11].

Obtaining keratin

Keratin proteins separated from hair can be classified into three groups: alpha-, beta- and gamma-keratin. Keratin is removed from the cortex by chemical breaking of disulphide bonds in the tissues. Alpha- and beta-keratins are transformed into non-cross-link forms by oxidation and reduction, while cystine is transformed into cysteic acid or cysteine. Next, the proteins are extracted together with denaturing solvent. The emerging solution can be purified using filtering or dialysis [13].

Schrooyen and Yanauchi described the method of obtaining keratin from bird feathers. The process involved washing bird feathers with water at 60°C. Then, feathers were dried, minced into 75÷700 μm pieces and extracted with petroleum ether for 12 h in order to remove fat residuals [16, 17].

Bird feather keratin can also be obtained through alkaline hydrolysis. This reaction reduces the disulphide groups to thiol groups using 0.05-1 M ammonium sulphide or 0.05÷0.5 M sodium sulphide. Also, the free –SH groups are partially modified using 0.1÷1.5 M mercaptoethanol. The process is conducted for 90 min at 45÷80°C. The molecular weight of obtained keratin was from 1 to 11 kDa. The product was characterised with durability of 15÷20 MPa, 10÷50% extension and elastic modulus of 100÷300 MPa [18].

Keratin degradation

High resistance of keratin to chemical and physical factors is an obstacle in the process of degradation. Keratin is degraded using acids, bases, enzymes, increased temperature or UV radiation.

Exposed to mineral acids the protein fibres undergo degradation. The peptide bonds are broken and the emerging keratin solution consists of ammonium salts and free amino acids.

Under the influence of bases keratin expands and then degrades. The increased solubility of keratin in bases is caused by the breaking of peptide and disulphide bonds, which increases its plasticity and reduces durability.

Enzymatic degradation leads to complete degradation of keratin, caused by hydrolysis and oxidation. It is triggered by microorganisms. Keratin degradation in feathers is a result of reduction of disulphide bridges. This method requires an appropriate bacterial flora, an addition of mineral nutrients, specific temperature, pH and oxygen supply. It was also observed that, aside from bacteria, the biodegradation process is also triggered by certain species of fungi (e.g. Aspergillus fumigatus), producing a keratinolytic enzyme that decomposes keratin. Enzymatic degradation is a multistage process, however, it appears beneficial from the viewpoint of industry, since it occurs at room temperature, which reduces the costs of the process.

Keratin degradation triggered by increased temperature yields oligomers, polypeptides and amino acids. Visible changes in fibre structure are observable already at 185°C, with the heating time of 30s.

Keratin is a natural polymer, which means it is photodegradable. It consists of elements absorbing the UV radiation, i.e. the photodegradation occurs according to the photochemical mechanism [6].

Summary

The paper outlines the properties, structure and application of keratin. Also methods of keratin degradation were discussed. Keratin proteins can be found in animal cells, mainly in feathers, hair and nails. Keratin is a fibrillar protein with compact structure. Its hydrogen, disulphide and covalent bonds are mainly responsible for its chemical and mechanical resistance. Therefore, it is essential to look for new methods of processing of keratin that would solve the problem of storage of keratin waste.

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The government decided on the establishment of the National Research Programme

At the meeting on 16 August the government adopted a resolution on the establishment of the National Research Programme prepared by the Ministry of Science and Higher Education. The National Research Programme will enable stronger link between scientific research and development and the needs of the Polish economy. The Government Information Centre announced in the communication sent to PAP after the government meeting that the National Research Programme "shall define strategic for the state directions of long-term research and development"."Strategic research and development programs are the basis for the determination by the National Centre for Research and Development of research programs that define the medium-term objectives" - according to the message. The objective of the National Research Programme is to enable a closer link between scientific research and development work with the development needs of the Polish economy, the choice of research areas, particularly of interdisciplinary research, which will increase the integration of dispersed scientific community, boost the sustainable economic development. According to the Government Information Centre communication, one of the major problems of the national innovation system is a weak link between science and industry and service sectors. "This is reflected in low participation of businesses in the financing of research and development (R&D), despite relatively high public expenditures. Moreover, relatively high funding of basic research is observed, and low - in relation to the needs of the economy - funding of applied research and development work. Characteristic of R&D sector is also institutional fragmentation and low level of involvement in international cooperation. It is therefore necessary to increase expenditures on R&D sector, especially from private funds, and provide mechanisms to stimulate the implementation of major research projects in cooperation with businesses" - according to the message. Dominant in the implementation of the National Research Programme will be seven priority areas of scientific research and development work: - new technologies in the field of energy; - lifestyle diseases, new drugs and regenerative medicine; - advanced information technologies, telecommunications and mechatronics; - modern material technologies; - environment, agriculture and forestry; - social and economic development of Poland in conditions of globalizing markets; - national security and defence. The Ministry of Science reported on its website that the criteria taken into account at the stage of the selection by the Scientific Policy Committee and the Ministry of strategic areas of research and development works include long-term needs of the economy, high levels of research (competitiveness at world level), development of innovative industries based on new Polish technologies; priority areas of development of research in European research programs which further the development of European research networks. An essential reference in the work on the National Research Programme, according to the Ministry of Science, was the National Environmental Programme Foresight Polska 2020. Institutions, with which the National Research Programme was consulted, were: the Polish Confederation of Private Employers, Employers RP, BCC, Main Council of Research Institutes, Polish Academy of Sciences and the Conference of Rectors of Polish Academic Schools. As noted in the Government Information Centre communication, the implementation of the National Research Programme should help increase the effectiveness of research into new technological solutions, and increase the number of patents, which in turn will stimulate the development of innovative economy and its competitiveness.