



## Review

# The “sisters” $\alpha$ -helices of collagen, elastin and keratin recovered from animal by-products: Functionality, bioactivity and trends of application



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## ABSTRACT

**Background:** Huge amounts of animal by-products are generated along processing for human consumption. This material adds up to 50% of the animal weight, representing a serious economic and environmental concern.

**Scope and approach:** A shift to a molecular level of utilisation of those residues could be a viable and cost-effective option, where the biomolecules recovered would be applied outside the agricultural sector. In this regards, the structural fibrous proteins collagen, elastin and keratin, and respective hydrolysates and small peptides, can be recovered from fisheries, poultry and red meat slaughter residues and employed in high-priority fields such as food, biomedicine and pharmaceuticals, and in highly profitable areas such as cosmetics. Bones (from fish and meat), skin (from pig and fish) and fish scales are a source of collagen; ligaments (from cattle, pig and poultry) are a source of elastin while keratin could be recovered from poultry feathers and pig hair. Beside the documented relevance of collagen, elastin and keratin in cosmetic and pharmaceuticals, and of gelatine in the food and photographic industry, a number of new applications can look at them as promising ingredients.

**Key findings and conclusions:** The spontaneous self-assembly of the native  $\alpha$ -helix of collagen, elastin and keratin allows conceiving biomaterials, emulsions, foams, stable colloids, as well as micro- and nano-capsules. Alongside this, the biological activities of small peptides, such as the antihypertensive, antimicrobial, antioxidant, wound-healing, osteogenic, haemostatic and chelating capacity, give the possibility to look at animal by-products as a remarkable source of functional compounds, whose application will contribute to the agro-food industry sustainability.

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## 1. Introduction

High quantities of residues are generated throughout land and aquatic animal processing for human consumption. It is estimated that 20 and 100 million tons of discards are generated worldwide, each year, from fisheries and meat (cattle, pigs and poultry) residues respectively, where ca. 5.2 and 16.5 million tons, respectively, arise from the sole European Union (Gustavsson, Cederberg, Sonesson, van Otterdijk, & Meyback, 2011). Presently, the European Union economy loses a significant amount of potential

secondary raw material represented by this waste stream, and for which landfilling, animal feeding and incineration seem the more convenient management options (European Commission, 2014; Leoci, 2014). However, current usage of by-products as fertilizers, livestock feed and pet food, has not been totally effective. High management costs are required, ca. 45 EUR/ton (Gustavsson et al., 2011) and residues like feathers, hair, hooves, scales and bones are degraded very slowly leading to the development of very unpleasant odour (Lasekan, Abu Bakar, & Hashim, 2013). A molecular level of recycling of animal residues could be a viable and more profitable option since the biomolecules recovered could be applied outside the agricultural sector. In this regards, the structural fibrous proteins collagen, elastin and keratin, along with respective hydrolysates and small peptides, can be recovered from fisheries, poultry and red meat slaughter residues and employed in

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high-priority fields such as biomedicine, pharmaceuticals and food, and in highly profitable areas such as cosmetics.

Mammalian collagen recovered from cow hides and pig skin, and respective bones, has gained great attention as a carrier for drug delivery, as haemostatic, in wound-healing, in cosmetic, in many composed biomaterials and in edible casings for meat products (Ferraro et al., 2013; Gómez-Guillén, Giménez, López-Caballero, & Monteiro, 2011; Sing et al., 2011). The marine collagen has unique features with respect to the mammalian equivalent; however, it has been not thoroughly valorised. Indeed, only about 1% of marine residues are explored in fine chemistry (Ferraro et al., 2013). At the moment, collagen hydrolysates (gelatine and small peptides) have important commercial and biological values and it is expected to grow (Liu, Nikoo, Boran, Zhou, & Regenstein, 2015). There is an increasing number of publications reporting on the antioxidant and antimicrobial activity of collagen peptides (Di Bernardini et al., 2011; Gómez-Guillén et al., 2011; Liu et al., 2015, and references therein) but on the contrary very scarce information is available on the bioactive properties of elastin and keratin either in the whole or hydrolysed form (Fontoura et al., 2014; Hattori, Yamaji-Tsumamoto, Kumagai, Feng, & Takahashi, 1998) as also reviewed recently by Brandelli, Sala, and Kalil (2015). Also, few and quite far literature is available on the recovery of elastin from by-products. Together with collagen, elastin constitutes the connective tissue. It is currently produced through bio-synthetic ways in the form of tropoelastin, elastin-like polypeptides or hybrid with other molecules, by recombinant such as *E.coli* or by chemical synthesis. However, all these forms lack in the natural cross linker amino acid (iso)-desmosine (Daamen, Veerkamp, van Hest, & van Kuppevelt, 2007; Mecham, 2008). Natural elastin can be recovered from residues such as ligaments of cow, pork and chicken (where represents 70% of the dry weight), as well as from the skin of fish, pig and chicken (2–4% dry weight). It can and applied in biomedicine for the regeneration of skin, large arteries, ligaments, bladder and lungs (Daamen et al., 2007; Mecham, 2008), in cosmetic formulations such as face and body creams, and also in the textile industry (Rodríguez-Cabello, García-Arévalo, Martín, Santos, & Reboto, 2010). Finally, keratin can be obtained from ready available by-products such as poultry feathers, discarded wool and porcine hairs, where represents ca. 90% of the weight. It is estimated that ca. 5 million tons on keratinous materials are discarded each year in the EU (European Union, 2016; Gustavsson et al., 2011). Keratin could be recovered and used in the whole form, or hydrolysed into peptides, either in cosmetics, for the reinforcement of skin elasticity and hydration, or in biomedicine, for the production of grafts and for bone and skin regeneration, as well as in the textile industry (Barba et al., 2008; Brebu & Spiridon, 2011; Leoci, 2014; Zhang et al., 2013). Keratin is a very good candidate for the development of hard biomaterials thanks to its toughness, being chitin the only other biological matter known to approximate the mechanical properties of a keratinized tissue (Hill, Brantley, & Van Dyke, 2010; Lasekan et al., 2013; Leoci, 2014).

This review aims to focus on the trends of application of collagen, elastin and keratin recovered from animal by-products, highlighting similarities and differences among them and possibility of a combined usage. These fibrous proteins have in common a supramolecular organisation in the form of  $\alpha$ -helix which is the underlying factor for the characteristic mechanical properties and functionality, and can release very promising bioactive peptides.

## 2. Fibrous proteins

Collagen, elastin and keratin represent an important subset of fibrous proteins. They provide structural support for cells and tissues of mammals and invertebrates, being crucial for locomotion

(Brinckmann, Notbohm, & Müller, 2005; Daamen et al., 2007; Vasconcelos & Cavaco-Paulo, 2013). Fibrous proteins, also called scleroproteins, have very different structures and functions with respect to globular proteins, as reported in Table 1.

Most important characteristic is the presence of repeating motifs, which in some case are exact in length and sequence, and which are responsible for specific mechanical properties. Fibrous proteins contain, in fact, patterns of seven residues – heptad repeats – labelled as *abcdefg* or *HPPHCPC*. In these sequences H is a hydrophobic amino acid (often a leucine, isoleucine or valine), P is a polar (then hydrophilic) amino acid and C is, in general, a charged amino acid (Ushiki, 2002). Those motifs confer to collagen, elastin and  $\alpha$ -keratins a common secondary structure in the form of  $\alpha$ -helix (also called *Pauling–Corey–Branson  $\alpha$ -helix*), a coiled, or spiral, conformation in which every N-H group donates a hydrogen bond to the C=O group of the amino acid four residues earlier (Hill et al., 2010; Parry & Squire, 2005). Each  $\alpha$ -helix wraps around another and produce a rope-like structure, the unit arrangement of fibrous proteins called protofibril (triple  $\alpha$ -helix in the case of collagen and elastin, and quadruple  $\alpha$ -helix in the case of hard keratin) (Filipello Marchisio, 2000). Protofibrils, in turn, arrange to others by specific cross-linking mechanisms and give rise to the fibre. Collagen and elastin are the major constituents of the connective tissue; both are crucial for locomotion where collagen provide rigidity while elastin provides pliability and rotatability (Ushiki, 2002). In spite of the different mechanical properties, the two major mechanisms for cross-links are the same: those initiated by the enzyme lysyl oxidase and those derived from non-enzymatically glycosylated lysine and hydroxylysine residues. Lysyl oxidase, a copper metalloenzyme, is the only enzyme known to be responsible for cross-linking in collagen and elastin. It converts the amine side chain of lysine and hydroxylase into aldehydes, giving reducible aldimine condensation cross-linking or nonreducible trivalent bonds cross-linking (Liu et al., 2015). The non-enzymatically derived cross-links occur more adventitiously and are especially important in pathobiological processes (Reiser, McCormick, & Rucker, 1992). A sulphur amino acid is responsible for cross-linking in  $\alpha$ -keratins, namely cystine, which forms strong disulphide bonds between adjacent chains. For this reason, the  $\alpha$ -keratin acts like a rubber and represents an ecological problem for the high resistance to degradation in the environment (Filipello Marchisio, 2000).

The chemical composition, structure and sources of collagen, elastin and keratin, and respective current and potential application are disclosed in details in the following sections.

### 2.1. Structure and role of collagen

The use of collagen, and derived hydrolysates and small peptides, is increasing in many areas, such as food, cosmetics and pharmaceuticals, thanks to the interesting functional and bioactive properties (Gómez-Guillén et al., 2011; Ferraro et al., 2013). Collagen is the principal structural protein in the connective tissue, the most abundant structural protein in vertebrates and invertebrates, and represents about 30% of animal's and human's total proteins (Duconseille, Astruc, Quintana, Meersman, & Sante-Lhoutellier, 2015; Liu et al., 2015). To date, 29 different types of collagen have been identified, however type I is the dominant one and type III comes just after (Liu et al., 2015). A collagen molecule consists of three  $\alpha$ -chains containing approximately 1000 amino acids each, and with a molecular weight of ca. 100 kDa each. Due to the high glycine content, which represents around 30% of the total amino acids pool (Table 2), the weight of collagen is lower than many other proteins with same number of amino acids residues (Brinckmann et al., 2005). Each  $\alpha$ -chain forms a left-handed helix,

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