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Collagen entanglement influenced by the addition of acids

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ABSTRACT

Collagen represents a biopolymer widely used in tissue engineering and the food industry. However, pH and acid effects, and the function of non-helical telopeptides on the collagen fibril microstructure and overall network structure are not completely understood. Thus, native and telopeptide-poor collagen type I were examined at different concentrations in order to characterize the pH and acids effects on the mechanical properties and entanglement characteristics of collagen suspensions. Collagen suspended in phosphoric, sulfuric, hydrochloric, or perchloric acid at pH 1, 2 and 3 were examined by rheological measurements and scanning electron microscopy.

Collagen entanglement increases with increasing pH values below the isoelectric point. This was confirmed by critical overlap and entanglement concentrations, storage and loss moduli, as well as by scanning electron microscopy. Furthermore, decreased ionic strength, increased Debye screening lengths, increased intrinsic viscosities, and increased radii of gyration accompanied collagen entanglement. Phosphoric acid was the most effective acid in terms of critical overlap and entanglement concentrations. By contrast, oscillation tests assessed the highest storage modulus for perchloric acid, while sulfuric acid reinforced protein–solvent interactions according to the reversed Hofmeister series for pH values below the isoelectric point. Moreover, scanning electron microscopy visualized the acid effects for both, wherein native collagen revealed undefined, but finely meshed amorphous structures and telopeptide-poor collagen indicated crystalline-like and well-ordered structures.

We demonstrated that collagen entanglement depends strongly on the pH and acid type, thus highlighting the importance of the preparation step of collagen raw materials for subsequent processing that could offer new insights for food manufacturers.

1. Introduction

Collagens constitute a family of proteins present as major structural components of connective tissues in vertebrates [1]. Collagen serves as a suitable scaffold for tissue engineering in diverse applications based on unique physical properties, including uniformity, tensile strength, flexibility, biocompatibility, and biodegradability [2]. It can be fabricated into forms such as gel, sponge, fiber, and films for cosmetic, pharmaceutical and, in particular, for the food industry [3,4]. Thus, co-extruded collagen casings display a decent alternative, due to the relatively high cost of using natural casings and the growing demand of sausages guts [5]. Although the patent was established 30 years ago and collagen casings are used extensively in the meat industry today, there is very limited published research focused on the physicochemical properties of

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collagen formulations and its impact on the mechanical properties of the co-extruded casings [6].

The primary structure of collagen contains repetition of the proline rich tripeptide Gly-X-Y that forms the trimeric collagen triple-helices with each chain having an individual twist in the opposite direction [3]. Type I collagen consists of a heterotrimer with two identical α1-chains and one α2-chain with non-triple-helical extensions called N- and C-telopeptides located at the amino/N-terminal and carboxy/C-terminal ends of the molecule, respectively (Fig. 1) [7]. In two of the three chains, the C-telopeptide has been reported to possess a hair-pin/hook conformation, while the three N-telopeptides display a more extended structure [8]. These telopeptides are involved in enzymatical intra- and interchain crosslinking of collagen by lysyl oxidase during fibril formation [9,10]. However, deformation mechanisms of N- and C-crosslinks and functional and structural roles for the N- and C-telopeptide conformations are not yet well known [11].

Collagen assembling is based on its physicochemical properties [12,13]. Collagen type I is characterized by uninterrupted helical regions with alternating polar and nonpolar domains allowing head-to-tail, lateral alignment of molecules in a quarter staggered array [9]. In particular, hydrogen bonds between polar residues of 4-hydroxyproline and 5-hydroxylysine, as well as the formation of hydration networks, and electrostatic interactions affect collagen stability and association [1,14]. The electrostatic interactions emerge from ionizable side groups present in 15–20% of all amino acid residues either in the X or in the Y position of the Gly-X-Y triplets [15]. Freudenberg et al. [16] assumed the stabilization of collagen type I with increasing ionic strength based on superior screening of charged residues and the formation of salt bridges. Moreover, Leikin et al. [17] stated that collagen type I is not able to form fibrils in acidic conditions because of the protonated and positively charged collagen fibrils that exhibit no attractive interaction at any inter-helical distance.

Due to collagen being highly hydrated, conditions affecting the content of water or solutes in collagen matrices also modulate structure and assembling [18,19]. Chaotrope ions from the Hofmeister series, so-called "water structure breakers", for instance, have a destabilizing effect on proteins, thus salting them in and promoting protein–solvent interactions [20]. In contrast, kosmotrope ions are "water structure makers", cause proteins to salt out, support protein–protein interactions, and increase protein stability [21]. The reversed Hofmeister series is applied for pH values below the isoelectric point (*pI*) [22]. "Water structure making and breaking" effects are only valid for short distances though, since anions affect only hydrogen bonding networks in their direct vicinity, and thus, do not influence bulk water [23]. Non-electrostatic interactions gain more and more importance at and above biological salt concentrations, while electrostatic interactions are weakened due to the screening effects of ions [24]. Non-electrostatic interactions form the basis for explaining the effect of ions on proteins including hydrogen bonding, π-electron–cation interactions, dipole–dipole interactions, dipole-induced dipole interactions, interactions between induced dipoles, and hydration forces [22].

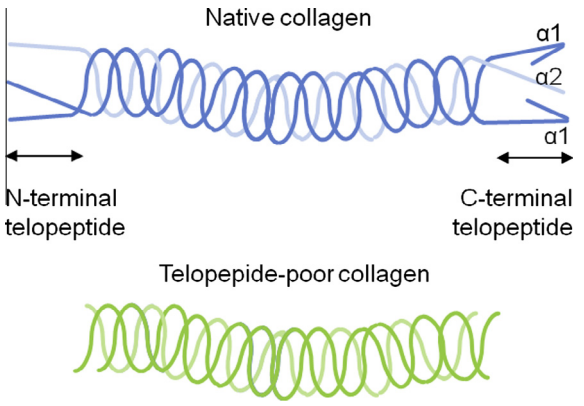

Fig. 1. Schematic illustration of native collagen characterized by the presence of non-helical N-terminal and C-terminal telopeptides and telopeptide-poor collagen.

We hypothesize that the pH and acids influence collagen entanglement and network formation. Thus, modulation of hydrogen bonds, screening effects or any other intra- and intermolecular interactions may lead to new collagen functionalities. While kosmotropic anions are expected to improve entanglement due to increased protein–protein interactions, chaotropic anions might lead to a state where protein–solvent interactions are favored. Furthermore, the ionic strength of mono- or multivalent acids is expected to influence collagen entanglement differently. Therefore, rheometry and scanning electron microscopy (SEM) was conducted to characterize the acid effect along the reversed Hofmeister (chaotrope > SO_4^{2-} > HPO_4^{2-} > Cl^- > ClO_4^- > kosmotrope) series at pH 1, 2 and 3 on native collagen and telopeptide-poor collagen. The acidic pH values were selected based on the pH conditions of collagen applied for co-extruded sausage casings. This facilitates a better understanding of non-helical telopeptides and collagen entanglement and its impact on collagen structure and functionality within the scope of the co-extrusion process.

2. Materials and methods

2.1. Preparation of collagen suspensions

Native collagen was provided by Kalle GmbH (Wiesbaden, Germany). In addition, telopeptide-poor collagen was obtained from Protein Consulting (Singhofen, Germany) by splitting off telopeptides and intermolecular crosslinks from native collagen to obtain single collagen triple helices [25].

Native collagen and telopeptide-poor collagen were diluted in phosphoric acid (Th. Geyer AG, Renningen, Germany), sulfuric acid (Carl Roth, Germany), hydrochloric acid (Carl Roth, Essen, Germany), or perchloric acid (VWR, Fontenay-sous-Bois, France). The pH was adjusted to pH 1, 2 and 3, respectively. The suspensions were prepared using a Stomacher Circulator 400 from Seward (West Sussex, UK) for 5 min (300 rpm) and the collagen concentration was set regarding the connective content determined via chemical characterization.