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Microstructure and physical–chemical properties of chicken collagen

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ABSTRACT

The global consumption of sausages has increased immensely over the last few years, while bovine collagen has become scarce. Thus, collagen from alternative sources is being considered for application in the food industry. Therefore, chicken skin and bone collagen were characterized and compared with the bovine telopeptide-poor collagen aiming at the feasibility of producing pure, co-extruded chicken sausages. Hence, the chemical composition, microstructure and rheological properties of the different collagen samples were examined and SDS-PAGE, mass spectroscopy, and ζ-potential analysis were conducted.

Weak bands in the SDS-PAGE gel indicated only partial maceration of chicken bone collagen, whereas chicken skin and telopeptide-poor collagen revealed distinct bands indicating collagen type I and III. This was also verified by mass spectroscopy. Large fragments were visible in optical microscopy for chicken bone collagen, whereas chicken skin collagen revealed a delicate network. Moreover, the highest dynamic consistency index, at 14146 Pa s^{n^*}, was determined for chicken bone collagen, followed by chicken skin and telopeptide-poor collagen at 606 and 320 Pa s^{n^*}, respectively. By contrast, the intrinsic viscosity was the highest for telopeptide-poor collagen (3.16–3.26 L/g), whereas chicken bone collagen exhibited the lowest value at 0.13 L/g, suggesting poor swelling behavior. Moreover, telopeptide-poor collagen featured the highest dynamic power law factor, suggesting the least crosslinks, serving a negative standard rather than an actual chicken collagen analog.

Finally, chicken skin collagen displayed the most suitable source of collagen for the co-extrusion process compared to the well established bovine hide split collagen.

1. Introduction

Collagen represents a family of proteins that serve as major structural components of connective tissues in vertebrates (Lee, Singla, & Lee, 2001). It has unique physical properties, including uniformity, tensile strength, flexibility, biocompatibility, and biodegradability. Therefore, it is used for a wide range of applications, e.g., as scaffold in tissue engineering, for implantations or wound dressing in surgical operations, as a capsule matrix material or binder in pharmaceutical applications, and for the production of gels and films in food (Osburn, 2002). A promising use of collagen in the food industry is the manufacture of co-extruded sausage casings, due to the rapidly rising cost of natural, intestinal-derived casings (Barbut, 2010). Moreover, continuous processes allow the hygienic production of sausages in large quantities with consistent end product quality attributes (Irmscher et al., 2013; Irmscher et al., 2015; Osburn, 2002). Currently, bovine hide split collagen is used in the co-extrusion process (Bueker, Bueker, & Grolig, 2009). However, the global consumption of meat products, in particular sausages, is rising and supplies of bovine collagen are getting low. Moreover, the need of kosher and halal products is increasing constantly. As a result, chicken collagen could supply the need, since it might be easily gained as a by-product from skins, cartilages, bones, and feet from the poultry manufacturing industry. In addition to good availability, fibril-forming collagens are predestined for the production of edible sausage casings, as the stable network enables shrinking and stretching to accommodate the contraction and expansion of meat batter during processing (Osburn, 2002). Bovine and chicken skins predominantly contain the fibril-forming collagen types I and III (Abedin & Riemschneider, 1984; Bräumer, 1974; Gelse, Pöschl, &

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Aigner, 2003; Ramshaw, 1986). On a molecular basis, fibril-forming collagens feature an uninterrupted helical region with alternating polar and nonpolar domains leading to lateral alignment of molecules in a quarter staggered array (Reiser, McCormick, & Rucker, 1992). Collagen type I is a heterodimer composed of two identical α1-chains and one α_2-chain (Bräumer, 1974; Gelse et al., 2003; Ramshaw, 1986), whereas collagen type III is a homotrimer of three α_1(III)-chains and usually occurs in the same fibril with type I collagen (Kadler, Holmes, Trotter, & Chapman, 1996). Collagen stability and structure is based on hydrogen bonds between polar residues of 4-hydroxyprolin and 5-hydroxylysin, the formation of hydration networks, and electrostatic interactions (Gelse et al., 2003). The latter ones emerge between 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 (Chan, Ramshaw, Kirkpatrick, Beck, & Brodsky, 1997).

Chicken collagen is less crosslinked due to the degree of covalent crosslinks increasing with advancing age due to lysyl oxidase-initiated crosslinks (Bailey & Shimokomaki, 1971). Thus, chickens are slaughtered after approximately 6–7 weeks (Maurer, 2003), while the average age of beef is 18–36 months (Joseph, 2003). The performance of telopeptide-poor collagen as a model system for less crosslinked collagen types, such as chicken collagen, was investigated in former studies of the author. We investigated the influence of acids, Hofmeister salts, and co-gelling proteins on native and telopeptide-poor bovine collagen rheology and microstructure (Oechsle, Wittmann, Gibis, Kohlus, & Weiss, 2014; Oechsle, Häupler et al., 2015; Oechsle, Landenberger et al., 2015).

Thus, the aim of this study was to characterize chicken bone and skin collagen, which was provided by the industry partners due to their low-cost availability, and to evaluate their suitability for the extrusion process compared to a telopeptide-poor collagen. Therefore, results obtained from chemical analysis, microscopy, ζ-potential, mass spectrometry, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analyses were compared. In this context, we hypothesized that chicken collagen behaves according to the telopeptide-poor collagen in terms of rheological and microstructural characteristics. Furthermore, we postulated that chicken skin collagen fibers stay intact, whereas the demineralization of bone leads to a break down of the collagen fibers, leading to weaker gels than telopeptide-poor collagen.

2. Materials and methods

2.1. Materials

The raw material of the bovine telopeptide-poor collagen was kindly provided by the Kalle (Wiesbaden, Germany) and Protein Consulting (Singhofen, Germany) prepared the telopeptide-poor collagen by splitting off telopeptides and intermolecular crosslinks from native collagen to obtain single collagen triple helices (Bueker et al., 2009). Chicken collagen was extracted using a procedure similar to that used for bovine collagen using chicken bone and skin as raw materials (Protein Consulting, Singhofen, Germany). For each samples one batch was provided by the industry partners that comprised the extraction product of several extraction procedures.

2.2. Chemical characterization

The standard analysis methods according to German law were used for the collagen characterization, according to § 64 Lebensmittel- und Futtermittelgesetzbuch (BVL, 2011). Dry matter content was determined according to L 06.00-3 (BVL, 2011). The ash (minerals) determination was conducted according to L 06.00-4 (BVL, 2011). The protein content in collagen samples was quantified applying the Kjeldahl method L 06.00-7, whereas a combination of two methods was used (L 07.00-41 and L 07.00-57) for non-protein nitrogenous substances in the samples (BVL, 2011). The amount of collagen degradation products as well as hydroxyproline and connective tissue content in the collagen samples were determined according to L 06.00-8 (BVL, 2011). The lipid content was assessed by the method L 06.00-6 (BVL, 2011).

2.3. Sample preparation

The collagen samples were diluted with 0.001 M phosphoric acid to a concentration of 6 g/100 g based on their protein content and mixed with an Artisan 5KSM 150 equipped with a flex edge beater 5KFE5T (KitchenAid, St. Joseph, Minnesota, USA) for 5 min at the highest setting. Samples were subsequently adjusted to pH 3 with phosphoric acid (Th. Geyer AG, Renningen, Germany) prior to storage over night to enable the hydration of the collagen molecules. Phosphoric acid at pH 3 was found in a previous study to yield the lowest critical overlap concentration by promoting collagen interactions (Oechsle et al., 2014). Before measurement, the 6 g/100 g suspensions were diluted to concentration needed for the analysis as indicated in Sections 2.4–2.7, homogenized for 5 min (300 rpm) using a Stomacher Circulator 400 from Seward (West Sussex, UK), and the pH was adjusted if necessary.

2.4. SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970). Solubilized collagen samples were mixed in a ratio of 1:1 (v/v) with the sample buffer containing 0.5 M tris(hydroxymethyl) aminomethane-HCl, pH 6.8 (Applichem, Darmstadt, Germany), 20 g/L SDS, 250 mL/L glycerol (VWR, Fontenay-sous-Bois, France), 50 mL/L β-mercaptoethanol (Sigma Aldrich, Munich, Germany), and 0.1 g/L Bromphenol Blue (Merck, Darmstadt, Germany). Consequently, the

Table 1
Chemical composition of bovine telopeptide-poor collagen, chicken skin collagen and chicken bone collagen.

Composition	Telopeptide-poor collagen (g/100 g)	Chicken skin collagen (g/100 g)	Chicken bone collagen (g/100 g)
Dry matter content	9.88 ± 0.40	34.26 ± 1.28	41.29 ± 1.46
Ash content	4.14 ± 0.32	3.86 ± 0.35	2.77 ± 0.08
Protein content*	6.47 ± 0.37	25.23 ± 2.59	31.25 ± 1.23
Non-protein nitrogenous content	n. d.	0.06 ± 0.01	n. d.
Hydroxyprolin content	0.90 ± 0.02	2.56 ± 0.22	4.28 ± 0.17
Connective tissue content	7.22 ± 0.13	20.89 ± 1.49	34.70 ± 0.85
Connective tissue degradation products	0.01 ± 0.01	n. d.	n. d.
Lipid content	0.08 ± 0.07	0.91 ± 0.08	0.74 ± 0.06

Not detectable (n. d.), *Factor 5.5 was applied to calculate the protein from the nitrogen content.