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Modification of extruded chicken collagen films by addition of co-gelling protein and sodium chloride

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ABSTRACT

The properties of extruded chicken collagen sausage casings can be tailored by the addition of sodium chloride (NaCl) or proteins prior to extrusion in order to gain films with modified mechanical properties. In this study, 4% (w/w) chicken skin collagen and telopeptide-poor collagen from bovine hide were modified by the addition of 0.05 mol/kg NaCl and/or partial substitution of collagen by soy protein isolate, at 1.25% (w/w). The collagen formulations were extruded to films and characterized in terms of their microstructure (light microscopy and scanning electron microscopy) and rheological properties (storage and loss modulus and phase angle). Moreover, the tensile strength and film thickness were examined. The addition of NaCl to the gel allowed for telopeptide-poor and chicken collagen films with high tensile strengths and elasticities to be formed. In contrast, a substitution of collagens with soy proteins decreased gel and film strengths. The soy protein induced weakening of collagen networks could be compensated by again adding NaCl leading to more homogeneous gels yielding films with higher storage moduli upon extrusion. The compensating effect of NaCl was more pronounced for chicken skin than for telopeptide-poor collagen in the film state suggesting differences in molecular interactions and network formation between the two different collagen types. Overall, the modulation of chicken collagen interactions by NaCl and soy protein addition enables the production of functional chicken collagen films, in turn providing the food and pharmaceutical industry with a viable alternative to the increasingly scarce beef collagen.

1. Introduction

Collagen is a structural protein and major component of connective tissue in vertebrates (Oechsle et al., 2014). Facilitating the formation of a fibrillar network of the extracellular matrix, it features unique properties such as high tensile strength, flexibility, biocompatibility and biodegradability. This makes the biopolymer very useful in many applications such as in the fields of tissue engineering, cosmetics and the food industry (Caliari and Harley, 2011; Campbell et al., 2009; Oechsle et al., 2014). In the meat industry, the utilization of collagen to manufacture sausage casings has gained in particular interest since demand and cost of natural casings have risen (Oechsle et al., 2014). Currently, bovine hides are the predominant source for collagen extraction, and there are increasingly shortages thereof. However, pork skin, poultry skin and fish skin have also been reported to be potential sources (Büker et al., 2010; Cliche et al., 2003; Eckmayer et al., 2002; Liu et al., 2015). Since diseases like bovine spongiform encephalopathy and concerns over carbon footprints of meat have increased demand for poultry products, an increasing amount of byproducts, such as skin, bones and cartilages are available. The use of these raw materials manufacture for collagen production could therefore be interesting both from a consumer acceptance and an economical point of view (Cliche et al., 2003). However, to this date, collagen products

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produced from chicken have not had the necessary technofunctionalities to manufacture sausage casing due to insufficient tensile strengths and elasticities. Generally, boiled sausage casings need to be able to withstand a certain pressure exerted by the raw sausage masses during filling, and they need to be able to expand and shrink upon heating and cooling.

Collagen extracted from homogenized and heated chicken skin contains about 75% of type I collagen (Cliche et al., 2003). Collagen type I is a heterotrimer consisting of two identical $\alpha 1$ chains and one $\alpha 2$ chain (Zeugolis and Raghunath, 2011). Based on the repeating Gly-X-Y amino acid sequence the three helical chains form triple helical monomers with non-helical C- and N-terminal extensions, called telopeptides, are responsible for collagen-collagen crosslinking (Caliari and Harley, 2011; Oechsle et al., 2014). Further, the monomers spontaneously assemble into fibrils and fibers (Prockop, 2013). It has been reported that the orientation of the fibrils within a sausage casing is crucial to the strength of the film (Hoogenkamp et al., 2015). A homogenous distribution with random orientation of fibrils could overcome possible weak spots along the orientation direction. This often occurs when fibrils are arranged in their natural, parallel quarter staggered conformation (Lieberman, 1964).

The aim of this study was to assess the effect of two different approaches to modify extruded collagen films, i.e. by addition of low concentrations of NaCl and by partially substituting collagen with soy protein isolate. Based on previous studies, we hypothesized that low amounts of NaCl may promote the formation of larger collagen assemblies that could, in effect, lead to strengthened collagen films having increased elasticities (Oechsle et al., 2015b). Furthermore, it was postulated that a partial substitution of collagen by soy protein isolate while possibly slightly weakening collagen gel strengths might still result in formation of stable films due to protein-protein interactions. There, addition of NaCl may allow for a strength compensation. In our studies, a telopeptide-poor collagen – established in previous studies - served as a model system for less crosslinked samples (Oechsle et al., 2016a; Oechsle et al., 2015a; Oechsle et al., 2016b; Oechsle et al., 2015b; Oechsle et al., 2014). In addition, a skin collagen extract was used to investigate animal specific alterations in collagen functionality.

2. Materials and methods

2.1. Preparation of collagen gels

Telopeptide-poor collagen was kindly provided by Kalle GmbH (Wiesbaden, Germany). It was produced by cleaving off telopeptides severing intermolecular cross-links from native bovine collagen to obtain single triple helices (Oechsle et al., 2015b). A chicken skin collagen extract was generously supplied by Protein Consulting (Singhofen, Germany), with manufacture procedures having recently been published in form of a patent (Büker et al., 2010). Soy protein isolate PRO FAM 974 was obtained from ADM (Chicago, IL, USA). Commercially available NaCl (Bad Reichenhaller, Bad Reichenhall, Germany) with a purity of >99% was used. Gels with a total protein content of 4% (w/w) containing either no salt or 0.05 mol/kg of NaCl were produced. For some samples, 1.25% (w/w) of the collagen was replaced by soy protein isolate. In addition, samples containing 4% collagen and 1.25% soy protein were prepared for comparison purposes.

All formulations were prepared in an Artisan 5KSM150 stand mixer equipped with a flex edge beater 5KFE5T (KitchenAid, St. Joseph, Minnesota, USA). Mixing of all components was done for a total of 20 min at room temperature at a low to medium speed setting. NaCl was added as a concentrated solution with water content in the mixture being adjusted accordingly. Soy protein isolate was added after the first 5 min of mixing. The pH was adjusted to 2.7 in 1 min intervals, using 20% phosphoric acid during the last 10 min of mixing. Gels were stored airtight over night at 1 °C before homogenizing for 5 min at a low speed and evacuating in a chamber machine (C400, Mulitvac, Wolfertschwenden, Germany) to remove entrapped air prior to use.

2.2. Preparation of collagen films

Collagen gels were extruded onto stainless steel rods (24 mm diameter, 1 m length) acting as a receiving template to facilitate the production of closed casings. Non-thermoplastic extrusion was carried out on a QX Fresh System (Marel Townsend Further Processing B.V., Boxmeer, Netherlands) with access kindly provided by Kalle GmbH. The settings of the counter rotating extrusion cones were set to their lowest speed as preliminary studies had shown that otherwise film rupture due to shear thinning would occur (data not shown). The ring nozzle measured gap width of 25 μm. Steel rods supporting extruded collagen films were placed into a saturated NaCl solution (5 °C) immediately after extrusion to facilitate precipitation and fixation. Films were then carefully cut, removed from the rod, and stored airtight at 1 °C with sponge cloths having been soaked in saturated NaCl solution acting as separators (Kalle GmbH, Wiesbaden, Germany).

2.3. Imaging and microscopy

Photographic images of gels and films were taken with a digital single-lens reflex camera (Type K-5, Pentax Ricoh Imaging, Hamburg, Germany). Contrast and brightness of all collagen gel images were enhanced using ImageJ (1.48v, Wayne Rasband, National Institutes of Health, USA).

Light microscopic images were taken using a Scope.A1 Axio transmission microscope combined with an AxioCam ICc3 camera (Zeiss, Oberkochen, Germany). Images were taken with a total magnification of 100× (10× objective lens, 10× ocular lens).

Scanning electron microscopy was conducted with a DSM 940 (Zeiss, Hamburg, Germany) at a high vacuum and with an accelerating voltage of 5000 kV. Pieces of the collagen films, 0.5 mm × 0.5 mm, were placed on a microscope slide and freeze-dried in liquid nitrogen. The slides were sputtered with a gold/palladium alloy (20:80) prior to microscopy.

2.4. Rheological measurements

Experiments were performed in a modular compact rheometer, Physica MCR 502 (Anton Paar, Ostfildern, Germany), at 5 °C using a plate-eplate geometry PP25/S with 24.98 mm diameter and a sandblasted surface to avoid wall-slip (Anton Paar, Ostfildern, Germany). The phase angle δ, the storage G' (Pa) and loss modulus G'' (Pa) of collagen gels and films were determined via amplitude sweeps (1 Hz, 0.1–100% strain) and frequency sweeps in the linear viscoelastic region (1% strain for gels and 0.2% strain for films, 0.1–100 Hz). Gels were measured at a plate-plate measurement system gap of 1 mm, while gaps for film samples were adjusted individually to accommodate for their respective thickness. There, the upper plate was automatically stopped when a force of 0.1 N was registered. Films were cut into 25 mm diameter circles prior to measurements.

2.5. Film thickness and tensile strength

Film thickness was determined during rheological measurements as described in a previous study (Oechsle et al., 2016b). The plate-plate geometry was lowered until a force of 0.1 N was

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