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# Performance of a novel casing made of chitosan under traditional sausage manufacturing conditions

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ABSTRACT

The goal of this study was to validate the commercial feasibility of a novel casing formed from chitosan containing cinnamaldehyde (2.2%, w/v), glycerol (50%, w/w) and Tween 80 (0.2% w/w) under traditional sausage manufacturing conditions. Meat batter was stuffed into both chitosan and collagen (control) casings and cooked in a water bath. Before and after cooking, both casings were compared for mechanical, barrier, and other properties. Compared to collagen, the chitosan casing was a better ($P \le 0.05$) barrier to water, oxygen, liquid smoke, and UV light. In mechanical and other properties, the chitosan casing had higher ($P \le 0.05$) tensile strength, lower ($P \le 0.05$) elongation at break and tensile energy to break, and better ($P \le 0.05$) transparency whereas a similar ($P > 0.05$) water solubility to the collagen casing. Overall, the chitosan casing was less affected by sausage manufacturing conditions than the collagen casing, indicating that chitosan casing has potential as an alternative to the current collagen casing in the manufacture of sausages.



## 1. Introduction

Chitosan, a copolymer of β-(1 → 4)-2-acetamido-D-glucose and β-(1 → 4)-2-amino-D-glucose units, has widely been used as a substrate for edible films and coatings. Chitosan films and coatings have been proven to improve the quality of fresh, frozen, and processed food products by reducing moisture loss, enhancing product appearance, retarding lipid oxidation and discoloration, and preventing the growth of bacteria, yeasts and molds (Coma, 2008; Cutter, 2006; Gennadios, Hanna, & Kurth, 1997; Shahidi, Arachchi, & Jeon, 1999).

Chitosan based-coatings and films have widely been used to protect red meats, poultry, and their processed products since meat products are an ideal substrate for lipid oxidation, color changes, and growth of pathogenic and spoilage bacteria (Samelis, 2006; Yingyuad, Ruamsin, Reekprkhon, Douglas, Pongamphai, & Siripatrawan, 2006). Specifically, chitosan as a dip has been used to extend the quality and safety of sausages. The shelf life of pork sausages stored at refrigeration temperature increased from 7 to 15 days when dipped into chitosan solution (Sagoo, Board, & Roller, 2002). Sausages of a beef/chicken mixture dipped into different chitosan solutions showed reduced microbial growth and extended shelf life during 60 days at 4 °C compared to uncoated sausages (Bostan, & Mahan, 2011). A different approach to extend the quality and safety of sausages using chitosan has been to add a chitosan layer to commercially available sausage casings by techniques such as coating and lamination. Cellulose casings coated with chitosan using the vacuum impregnation technique resulted in casings with antimicrobial properties (Kaowkum, Boonsupthip, Thumanu, & Rachtanapun, 2012). Collagen casings laminated with chitosan showed excellent water and oxygen barrier and antimicrobial properties (Krkic, Lazic, Petrovic, Gvozdenovic, & Pejic, 2012). However, until now, very little research has been conducted to elucidate the suitability of chitosan film alone as a sausage casing. In a previous work, this research group developed a sausage casing resulting from tubular shaping of a chitosan film with physical and mechanical properties similar to or better than those of commercial collagen casings (Adzaly, Jackson, Villalobos-Carvajal, Kang, & Almenar, 2015). The reason behind developing such casing was to obtain a casing that outperforms a casing commonly used for sausages like the collagen casing in terms of properties that can help to enhance the quality and safety of sausages besides having a greater acceptance by some religious communities. Chitosan has innate antioxidant (Lopez-Caballero, Gomez-Guillen, Perez-Mateos, & Montero, 2005) and antimicrobial properties (Neethirajan & Jayas, 2011), which the collagen casing does not have. In principle, these would help to enhance the quality and safety of the sausages in slowing down oxidative processes and reducing/suppressing microbial growth (both spoilage and pathogenic microorganism). Furthermore, this non-animal casing has the potential to be used for Halal (meaning "permitted") products for Muslim and other religious communities. Collagen casings are only

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considered Halal if they are obtained from animals that are slaughtered by the Halal method and this is not specified when sausages are sold. Currently, the use of Halal meats has increased and the request for Halal casings has subsequently increased in a similar pattern (Nakyinsige, Man, & Sazili, 2012). However, this novel casing was neither stuffed with meat batter nor were the resulting sausages cooked under traditional sausage manufacturing conditions to investigate its feasibility under commercial sausage manufacturing conditions.

Therefore, the aim of this study was to validate the commercial feasibility of the developed chitosan casing under traditional sausage manufacturing conditions including stuffing, cooking, and peeling.

## 2. Materials and methods

### 2.1. Materials

Chitosan (CH) (molecular weight = 100–300 kDa and 90% deacetylation) and Tween 80 (T80) were purchased from Acros Organic (New Brunswick, NJ, USA). Glacial acetic acid, trans-cinnamaldehyde (CA), and magnesium nitrate were supplied by J.T. Baker Inc. (Phillipsburg, NJ, USA). Pure (100%) vegetable glycerol (GLY) was obtained from Starwest Botanicals Inc. (Sacramento, CA, USA). Collagen casings were obtained from Devro Inc. (Columbia, SC, USA), liquid smoke was supplied by Red Arrow (Manitowoc, WI, USA), and bratwurst meat batter was prepared in the Michigan State University Meat Laboratory.

### 2.2. Casing preparation

CH solution (2% w/v) was prepared by dissolving 2 g of CH in 100 mL of acetic acid glacial solution (1% v/v) and stirring on a magnetic stirrer (Barnstead, Dubuque, IA, USA) at room temperature overnight. The solution was filtered through cheesecloth (Regency Wraps, Dallas, Texas, USA) to remove un-dissolved impurities. GLY (50% w/w of CH) was added to the CH solution and the mixture was stirred for 30 min. CA (2.2% w/v of CH solution) mixed with T80 (0.2% w/w of CA) was added to the CH/GLY solution and the mixture was homogenized for 5 min using a homogenizer (Polytron, Kinematica Inc., Bohemia, OH, USA). The resulting film-forming solution was degassed for 5 min at room temperature to remove air bubbles using an ultrasonic bath (degasification level = 5, Ultrasonic Cleaner, Fisher Scientific, Allentown, PA, USA). The film-forming solution was then casted on a glass plate, and dried for 30 h at ambient temperature. The formed films were peeled and cut into pieces using a double-blade cutter. Some pieces had the outer edges sealed with glacial acetic acid to form tubular casings (2 × 15 cm), while other pieces were maintained as films to investigate the effect of the batter on the CH matrix during cooking. Collagen casings were used as controls since they are the most widely used edible casings. Casings and films were conditioned in a desiccator (5-gal bucket with an air-tight lid, both supplied by Century Container Corporation (New Waterford, OH, USA)) at 23 °C and 51% relative humidity (RH) for 72 h before testing. A saturated magnesium nitrate solution was used to create the required RH.

Three film-forming solutions were made in order to create three different batches of chitosan casings and chitosan films. Each of the solutions was prepared in a different week and produced twelve chitosan casings and twelve chitosan films. A total of 36 casings and 36 films were produced. Before exposure to sausage manufacturing conditions, three casings and three films from each batch were used to determine all the evaluated parameters except for mechanical properties, which were determined from three different chitosan casings and three different chitosan films from each batch. After exposure to sausage manufacturing conditions, three films and three casings from each batch were used to determine all the evaluated parameters except for mechanical properties, which were determined from the remaining three chitosan films and casings from each batch. Three large 2-cm collagen casings of the same type were used to create three batches of collagen casings. Each of these casings were cut into pieces (12 casings per batch for a total of 36 casings) and used to determine before and after exposure to sausage manufacturing conditions in the same manner as chitosan casings.

### 2.3. Sausage processing

Bratwurst meat batter was prepared using beef (80% lean/~20% fat), salt, corn syrup solids, onion, green pepper, hydrolyzed soy protein, mushroom, spices, Worcestershire sauce, soybean oil, and <2% silicon dioxide, then stuffed into the collagen and CH casings using a hand stuffer (Vogt-deal, Chicago, IL, USA). After stuffing (Image 1, left), the resulting sausages were cooked using the conventional water immersion cooking method (Image 1, right). Specifically, the sausages were submerged in a water bath (Precision Scientific, Chicago, IL, USA) at 85 °C until reaching an internal temperature of 72 °C, confirmed by a calibrated thermocouple (Super Scientific, Ltd., Scottsdale, AZ, USA) inserted into each sausage. Subsequently, the sausages were chilled to an internal temperature of 5 °C by exposing them to ice for 25 min. The CH films were exposed to the same heating and cooling conditions as the sausages. After manually peeling the casings off the sausages, casings and films were individually wrapped in aluminum foil and then vacuum-sealed for further testing within the following 48 h.

### 2.4. Casing characterization

CH and collagen casings as well as CH films were characterized in terms of thickness, moisture content, transmittance, permeability (water vapor and oxygen), mechanical properties, and morphology before and after exposure to sausage manufacturing conditions (including stuffing, tying, cooking, and chilling). Liquid smoke permeability and water solubility were only measured prior to sausage manufacturing conditions since the smoking step usually occurs before cooking and water solubility is a parameter that needs to be measured before cooking. The procedures used are described below.

#### 2.4.1. Thickness

The thicknesses of the CH and collagen casings and of the CH films were determined to the nearest 0.001 mm using a digital micrometer (Testing Machines Inc., Ronkonkoma, NY, USA). Five thickness measurements were taken randomly and averaged for each casing and film.

#### 2.4.2. Moisture content

CH and collagen casings and CH films were cut into pieces (1 × 3 cm) and then weighed to the nearest 0.0001 g (value recorded as initial weight) using an analytical balance (Ohaus Corporation, Parsippany, NJ, USA) before being dried in an oven (Precision Scientific Co, Chicago, IL, USA) at 110 °C to a constant weight (value recorded as final weight). The moisture content of the casing was calculated using the following equation:

$$\text{Moisture content}(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

#### 2.4.3. Water solubility

CH and collagen casings and CH films were cut into pieces measuring 1 × 3 cm each. The pieces were weighed to the nearest 0.0001 g using an analytical balance (Ohaus Corporation, Parsippany, NJ, USA) and dried in an oven (Precision Scientific Co., Chicago, IL, USA) at 110 °C to a constant weight (value recorded as initial dry weight). After drying, each piece was immersed into an 80 mL beaker with 50 mL of distilled water while stirring on a magnetic stir plate (Barnstead, Dubuque, IA, USA) for 20 min at 85 °C (water temperature used in sausage cooking). Each piece was then removed and dried in the aforementioned oven at