DEVELOPMENT OF A NOVEL SAUSAGE CASING MADE OF CHITOSAN AND ITS PERFORMANCE UNDER TRADITIONAL SAUSAGE MANUFACTURING CONDITIONS

By

NOOR ZAINAH ADZALY

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ABSTRACT

DEVELOPMENT OF A NOVEL SAUSAGE CASING MADE OF CHITOSAN AND ITS PERFORMANCE UNDER TRADITIONAL SAUSAGE MANUFACTURING CONDITIONS

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The objective of this study was to develop a novel chitosan-based casing for commercial application. In order to do so, chitosan was mixed with cinnamaldehyde, glycerol and Tween 80 in various ratios, and the resulting films were compared to commercial collagen casings for physical and mechanical properties. The chitosan film exhibited different properties due to the interactions between chitosan, glycerol and cinnamaldehyde as identified by FTIR spectrophotometry. Among the different chitosan films, the film containing cinnamaldehyde (2.2%, w/v), glycerol (50%, w/w) and Tween 80 (0.2% w/w) shown the same mechanical properties asthe collagen casing, with lower water solubility, superior transparency, and better UV light barrier property. As a next step, the selected film was made into a round shape, stuffed with meat batter and compared to meat-stuffed collagen casings for commercial feasibility of the novel casings under traditional sausage manufacturing conditions. Before and after meat stuffing and cooking, both chitosan or collagen (control) casings were compared for mechanical, barrier and physical properties. The results show that, chitosan casing showed better performance as a barrier to water, oxygen, liquid smoke and UV light compared to the collagen casing. In mechanical and physical properties, chitosan casing had higher tensile strength but lower elongation and tensile energy to break, had better transparency and similar water solubility as the collagen casing. These results show the potential of the chitosan casing as an alternative or better choice than the collagen casing for the manufacture of sausages.

To
My husband, Mohd Amin Serri and my son, Muhammad Luqman Hakim
My parents, Adzaly Md Yamin and Kiah Meklah

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KEY TO ABBREVIATIONS

CA	Cinnamaldehyde
CH	Chitosan
%E	Elongation at break
FTIR	Fourier-transform infrared
GLY	Glycerol
LSP	Liquid smoke permeability
OP	Oxygen permeability
T20	Tween 20
T80	Tween 80
UV	Ultraviolet
SEM	Scanning electron microscopy
TEB	Tensile energy to break
SMC	Sausage manufacturing conditions
TS	Tensile strength
WPI	Whey protein isolate
WVP	Water vapor permeability

CHAPTER 1

INTRODUCTION

Collagen sausage casings are the most successful edible film commercially used in the meat industry. Collagen casings are made from natural collagen extracted from the corium layer of bovine hides with several processing steps. Collagen casings were developed as an alternative to natural casings because of numerous advantages in mechanical and physical properties compared to natural casings. However, collagen casings lack the gas barrier and antimicrobial properties that would enhance the quality and safety of sausages. To improve its properties, collagen casings have been laminated with chitosan, which has good barrier and antimicrobial properties (Krkic, Lazic, Petrovic, Gvozdenovic & Pejic, 2012).

Chitosan, a copolymer of β-(1→4)-2-acetamido-D-glucose and β-(1→4)-2-amino-D-glucose units (Arvanitoyannis, Nakayama & Aiba, 1998) is produced by alkaline de-acetylation of chitin, which is the second most abundant natural biopolymer in the world after cellulose(Beverlya, Janes, Prinyawlwatkula & No, 2008; Shahidi, Arachchi & Jeon, 1999). Chitosan has been proven to have natural antioxidant properties (Darmadji & Izumimoto, 1994; Lopez-Caballero, Gomez-Guillen, Perez-Mateos & Montero, 2005), as well as antibacterial and antifungal properties due to its polycationic nature (Kim, Thomas, Lee & Park, 2003). In addition, chitosan is biodegradable, biocompatible, nontoxic and edible with good film-forming capability and at low cost (Butler, Vergano, Testin, Bunn & Wiles, 1996; Jeon, Kamil & Shahidi, 2002). All these properties make chitosan attractive for research and applications in various fields such as biomedicine, biotechnology, agriculture, cosmetics, environment, food and packaging (Chung et al., 2004; Shahidi & Abuzaytoun, 2005).

Chitosan based coatings and films have been widely used to protect meat, poultry and their processed products, since these are an ideal substrate for the reduction of lipid oxidation, color changes, and growth of pathogenic and spoilage bacteria (Samelis, 2006; Yingyuad,

Ruamsin, Reekprkhon, Douglas, Pongamphai and Siripatrawan, 2006). Currently, chitosan coatings have been used to extend the quality and safety of sausages. The shelf life of pork sausages stored at refrigeration temperature increased from seven to fifteen days after coating with chitosan (Sagoo, Board & Roller, 2002). Similarly, chitosan coatings on sausages made with a beef/chicken mixture extended their shelf at 4^{o}C compared to uncoated sausages (Bostan and Mahan, 2011).

A novel sausage casing prepared from chitosan itself rather than a coating could better improve sausage safety and quality due to its natural antioxidant and antimicrobial properties and good film-forming capabilities. More interestingly, the non-animal casing hasthe potential for Halal (meaning "permitted") products for Muslim and other religious communities. Although natural and Halal collagen casings can be obtained from sheep and goats, these casings are only considered Halal if the animals are slaughtered and the films are generated following the rule of Halal method (Nakyinsige, Man & Sazili, 2012). Currently, the use of Halal meats has increased and so requests for Halal casings have subsequently increased. Moreover, the types of sausage casings are not specified on the label of Halal and the processing methods forskinless sausages are not known very well.

Therefore, the objective of this study was to develop a novel sausage casing made from chitosan and to validate its commercial feasibility under traditional sausage manufacturing conditions.

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CHAPTER 2

LITERATURE REVIEW

2.1 Biopolymers

Biopolymers are polymers obtained from renewable natural resources such as biological systems (microorganisms, plants, animals) or biological materials (sugar, starch, natural fats or oils) after being synthesized chemically or biotechnologically. They are biodegradable and non-toxic (Flieger, Kantorova, Prell, Rezanka & Votruba, 2003). Research and development on biopolymer materials has been conducted in various disciplines such as biotechnology, biomedical research, packaging, and food science. Edible films and coatings are one of the most important applications of biopolymers in the area of food science and technology and have been developed since the last 20 years (Chapman and Potter, 2004; Gennadios, 2002). Edible films and coatings are defined as thin continuous layers of films or stand-alone films placed on food surfaces as a food cover, food wrapper or as a separation layer (Krochta, 2002).

Yuba, the first edible film made from soya-milk skin, was developed during the 15th century (Park, Hettiarachchy, Juand Gennadios, 2002 ; Wu and Bates, 1972) and was followed by a coating for cut meats in order to reduce the loss of moisture and subsequent shrinkage of the meat (Kester & Fennema, 1986). Research on edible films and coatings is evolving because protection from physical, chemical and biological deterioration has a strong impact on food quality (Kester & Fennema, 1986). In addition, edible films and coatings provide other advantages such as edibility, biocompatibility, aesthetic appearance, non-toxicity and low cost (Han, 2000), in addition to having active compounds that retard oxidation and bacterial growth (Sallam, 2007).

Edible films and coatings can be developed from hydrocolloids, which include proteins and polysaccharides like starch, alginate, cellulose derivatives, chitosan and agar, lipids (waxes, acylglycerols and fatty acids) (Min and Krochta, 2005) and their composites. They can be used

either alone or in combination. The physical and chemical characteristics of biopolymers affect the properties of edible films and coatings (Sothornvit & Krochta, 2000). Edible films and coatings have been reported as having the ability to enhance the quality of meat and poultry products (Ustunol, 2009). For instance, they can improve the quality of fresh, frozen and processed meat and poultry products by reducing moisture loss, lipid oxidation, discoloration and overall appearance (Coma, 2008; Cutter, 2006; Gennadios, Hanna & Kurth, 1997). To date, collagen casings have been the most successful applications of edible films, especially for sausage casings (Chapman and Potter, 2004). Among the various edible film materials, chitosan is the most promising biopolymer, due mainly to its inherent antibacterial and antioxidant properties.

2.1.1 Chitosan

Chitosan is a copolymer of β-(1→4)-2-acetamido-D-glucose and β-(1→4)-2-amino-D-glucose units (Arvanitoyannis, Nakayama & Aiba, 1998) derived from the process of alkaline de-acetylation of chitin (β-1,4 linked 2-acetamido-D-glucose). It is the second most abundant natural biopolymer in the world after cellulose(Beverlya, Janes, Prinyawlwatkula & No, 2008; Shahidi, Arachchi & Jeon, 1999), with an annual production of approximately 1 x 10^9 metric tons (Arvanitoyannis, Nakayama & Aiba, 1998). Chitin is present in the exoskeleton of crustaceans (crab, shrimp and crawfish) and the cell walls of fungi, insects and yeast. Chitosan is biodegradable, biocompatible, nontoxic, edible, and has a good film-forming capability, at low cost (Butler, Vergano, Testin, Bunn & Wiles, 1996; Jeon, Kamil & Shahidi, 2002). In addition, chitosan has been proven to have antibacterial and antifungal activities due to its poly-cationic nature, which makes it a key player in developing antimicrobial edible films and coatings (Kim,

Thomas, Lee & Park, 2003). Moreover, chitosan has been reported to have antioxidant properties (Darmadji & Izumimoto, 1994; Lopez-Caballero, Gomez-Guillen, Perez-Mateos & Montero, 2005). All these properties made chitosan a topic of research in various fields such as biomedicine, biotechnology, agriculture, cosmetics, environment, food and packaging (Chung et al., 2004; Shahidi & Abuzaytoun, 2005). Even though chitosan films have desirable properties, plain chitosan films are too rigid and need to be made more flexible with plasticizers to reduce frictional forces between polymer chains (Olabarrieta, Forsström, Gedde & Hedenqvist, 2001; Park, Marsh & Rhim, 2002; Suyatma, Tighzert & Copinet, 2005; Ziani, Oses, Coma & Mate, 2008).

2.1.2 Plasticizers

Plasticizers are low molecular weight additives introduced during film-formation to increase the thermo-plasticity of polymers (Guilbert and Gontard, 1995; Krochta, 2002). The plasticizers increase the free volume of the polymer structure and consequently increase the polymer chain mobility (Sothornvit & Krochta, 2000) by interrupting the inter-molecular and intra-molecular hydrogen bonds in the polymer matrix (Krochta, 2002). As a result, the ratio of the crystalline region to the amorphous region as well as glass transition temperature (Tg) will decrease (Guilbert and Gontard, 1995; Krochta, 2002). Moreover, incorporation of plasticizers into the polymer matrix affects not only the mechanical properties of edible films and coatings but their permeability to water and gases (Sothornvit & Krochta, 2000, 2001). Examples of food grade plasticizers are sorbitol, glycerol, mannitol, sucrose and polyethylene glycol.

2.1.2.1 Glycerol

Glycerol is a plasticizer commonly used in hydrocolloid-based films to improve their flexibility (Daniels, 1989). Glycerol reduces the intermolecular forces between polymer chains, increasing the free volume between these chains and consequently their mobility, which reduces the rigidity of the three-dimensional film structure (Srinivasa, Ramesh & Tharanathan, 2007). The improvement in flexibility by using glycerol in chitosan and other bio-based films has been widely reported. Suyatma, Tighzert, Copinet and Coma (2005) reported that the addition of glycerol to the chitosan matrix resulted in improved film flexibility and decreased tensile strength (Suyatma, Tighzert & Copinet, 2005). Gontard, Guilbert and Cuq (1993) found similar results when glycerol was added to films made of wheat gluten (Gontard, Guilbert & Cuq, 1993). Lee, Pranata, Ustunol and Almenar (2013) reported an increased flexibility and a decreased rigidity as a function of the increase of glycerol content for egg white based bioplastics (Lee, Pranata, Ustunol & Almenar, 2013). Glycerol has widely been studied in biopolymers intended for food packaging since it can be used for food applications due to its "Generally Recognized as Safe" status (USDA, 2013).

2.1.2.2 Water

Water molecules present in the film-forming solution can also act as a plasticizer similar to glycerol. However, it can be easily lost during the drying process at low relative humidity (Guilbert and Gontard, 1995).

2.1.3 Essential oils

Essential oils are aromatic oily liquids obtained from plant material like flowers, buds, seeds, leaves, twigs, bark, herbs, woods, fruits and roots. They can be produced by several methods including expression, fermentation, enfleurage, and extraction. However, steam distillation is the most common method used for commercial production (Van de Braakand Leijten, 1999). Examples of materials used to derive essential oils are angelica, anise, carrots, cardamom, cinnamon, cloves, coriander, dill weed, fennel, fenugreek, garlic, nutmeg, oregano, parsley, rosemary, sage, thyme and vanillin (Ustunol, 2009). Generally, essential oils are added to edible films and coatings to modify flavour and aroma, and to improve antioxidant and antimicrobial properties (Ustunol, 2009). Essential oils have been shown to have antimicrobial and antioxidant properties (Balaguer, Gomez-Estaca, Gavara & Hernandez-Munoz, 2011b), antimycotic (Mari, Bertolini & Pratella, 2003) and antitoxigenic (Juglal, Govinden & Odhav, 2002) properties, and antiparasitic (Pandey, Kalra, Tandon, Mehrotra, Singh & Kumar, 2000) and insecticidal (Karpouhtsis, Pardali, Feggou, Kokkini, Scouras & Mavragani-Tsipidou, 1998) properties, depending on their composition, structure and functional groups (Holley & Patel, 2005). Among all the essential oils, the oils of clove, thyme, cinnamon, rosemary, sage and vanillin are consistently more effective against microorganisms (Ojagh, Rezaei, Razavi & Hosseini, 2010b; Seydim & Sarikus, 2006). Apart from that, essential oils of lemon, thyme and cinnamon have received greater acceptance due to their sensory evaluation and antimicrobial properties (Bagamboula, Uyttendaele & Debevere, 2004; Viuda-Martos, Ruiz-Navajas, Fernandez-Lopez & Perez-Alvarez, 2008).

2.1.3.1 Cinnamaldehyde

Cinnamomumzeylanicum (L.), commonly known as cinnamon has a predominant component called cinnamaldehyde together with β-caryophyllene, linalool and other terpenes (Tzortzakis, 2009). Cinnamaldehyde, a "Generally Recognized as Safe" substance by the U.S. Food and Drug Administration (USDA, 2013), is widely used by the food industry as a food additive and flavoring agent (Tzortzakis, 2009). This substance has been reported to successfully crosslink biopolymer matrices. The addition of cinnamon essential oil to chitosan resulted in crosslinked chitosan-based films with decreased moisture content, solubility in water, water vapor permeability, and elongation at break (Ojagh, Rezaei, Razavi & Hosseini, 2010a). Films made from wheat gliadins and cinnamaldehyde showed increased water resistance, and mechanical and barrier properties (Balaguer, Cerisuelo, Gavara & Hernandez-Munoz, 2013; Balaguer, Gomez-Estaca, Gavara & Hernandez-Munoz, 2011a, b). Furthermore, cinnamaldehyde has been proven to have good antimicrobial and antioxidant properties (Du & Li, 2008; Ojagh, Rezaei, Razavi & Hosseini, 2010a; Ouattara, Simard, Holley, Piette & Begin, 1997; Shan, Cai, Brooks & Corke, 2007; Tzortzakis, 2009)

2.1.4 Tween 80

Tween 80 is an amphiphilic surfactant agent responsible for reducing the surface tension of the water-lipid interface and the water-air interface by the modification of surface energy in order to control the adhesion and wettability of the film (Krochta, 2002). Several surfactant agents with different Hydrophile-Lipophile Balance (HLB) have been added to edible films and coating formulations such as Tween 80 (high HLB) and Span 80 (low HLB) to improve functional properties (Andreuccetti, Carvalho, Galicia-García, Martínez-Bustos & Grosso, 2011;

Rodríguez, Osés, Ziani & Maté, 2006; Villalobos, Hernandez-Munoz & Chiralt, 2006). Furthermore, Tween 80 was incorporated in the film forming solution of chitosan to better assist dissolution of cinnamon oil (Ojagh, Rezaei, Razavi & Hosseini, 2010a).

2.2 Sausages

2.2.1 Definition

Sausage, a popular processed meat product, traditionally consists of chopped meat and spices which are stuffed into natural or artificial casings prior to cooking, with or without smoke application (Whiting and Miller, 1992).

2.2.2 Sausage casings

Sausage casings are used to form sausage products and protect them from external environments such as UV light, microbial contamination, and moisture loss in order to maintain quality and safety until they are consumed or repackaged (Feiner, 2006). Sausage casings can be divided into two types - natural and artificial. Examples of artificial casings are collagen, cellulose and plastic.

2.2.2.1 Natural casings

Natural casings are obtained from the stomachs, intestines and bladders of hogs, sheep and cattle (Harper, Barbut, Lim & Marcone, 2012). They are stored at temperatures below 4.5^{o}C to avoid microbial contamination and growth. Natural casings are still used in the meat industry because they are tender and highly permeable to moisture and smoke (Savicand Savic, 2002). However, they are not popular in sausage manufacturing plants because of problems with

handling, splitting, and standardization of weight and dimensions for sausages in automated large-scale production (Essien, 2003).

2.2.2.2 Artificial casings

Artificial casings solve many of the problems associated with natural casings. Examples include collagen, cellulose and plastic casings.

2.2.2.2.1 Collagen casings

Collagen casings are traditionally made from collagen extracted from the corium layer of bovine hides before being decalcified and ground. Acid is used to swell the collagen and make the dough easily extruded. The collagen dough contains functional additives such as cross linkers, plasticizers and cellulose fibers in order to have consistent extrusion (Nakyinsige, Man & Sazili, 2012). Collagen casings can be edible or non-edible, depending on the type of sausage. For example, edible collagen casings are widely used for fresh and cooked sausages, while non-edible collagen casings, which are basically used for larger-diameter sausages, are removed prior to consumption (Feiner, 2006). The previous market share of sausage casings is more than €4.2 billion, split equally between natural and artificial casings.

The growth in artificial casings has increased steadily over the last 6 years due to improvements in productivity, food safety and cost, compared to natural casings, which are becoming more expensive due to the decline in the sheep population(Viscofan Annual Report, 2012).In 2012 alone, the market for artificial casings grew by 8% globally compared to 2011.This growth was spearheaded by collagen casings with a market volume increase over 10%.Collagen casings have numerous advantages over natural casings: 1) the diameter of the

collagen casing is more uniform than that of natural casings, which leads to consistent product net weight; 2) collagen casings work well under various processing conditions because they are flexible and strong; and 3) collagen casings are cleaner than natural casings(Kutas, 1984). In addition, collagen casings do not require soaking before stuffing and last longer compared to natural casings (Savic and Savic, 2002).

Other sausage casings have been developed using various proteins like wheat gluten, soy protein, peanut protein, corn zein and feather keratin (Mullen, 1971; Schilling and Burchill, 1972), pectin and gelatin/sodium alginate (Liu, Kerry & Kerry, 2007) and whey protein isolate (Cagri, Ustunol, Osburn & Ryser, 2003). However, sausage casings produced by proteins other than animal collagen have no commercial use.

2.2.2.2.2 Cellulose casings

Cellulose casings are made from highly purified cellulose after several processing steps, including solubilization in strong alkali, derivitization and reprecipitation. Cellulose casings are widely used in the sausage industry due to their strength and heat stability (Nicholson, 1991). Furthermore, they have good permeability to moisture and smoke in wet environment (Romans, Costello, Carlson, Greaser and Jones, 2001). Cellulose casings must be removed from the finished product after cooking because they are nonedible (Nakyinsige, Man & Sazili, 2012). There are three types of cellulose casings - small, large and fibrous. Small and large cellulose are made from cotton linters while fibrous casings are made from a special paper pulp base impregnated with cellulose. Small cellulose casings are basically used to produce skinless wieners for small-diameter products. Large cellulose casings are used for bologna and braunschweiger. Fibrous casings are used for luncheon meat specialties, summer sausage and

bologna. In addition, small cellulose casings do not need to be soaked, in contrast with large cellulose and fibrous casings (Romans, Costello, Carlson Greaserand Jones, 2001).

2.2.2.2.3 Plastic casings

Plastic casings have variety of layers ranging from one to five layers. The production of plastic casings requires sophisticated processing technology, especially for multiple layers of plastic casings. Plastic casings are good barriers against oxygen and moisture. They are easy to handle, easy to peel and have consistent diameters. However, plastic casings shrink during the cooling process and subsequently produce a wrinkled appearance that might limit consumer acceptance. The material used for monolayer plastic casings is polyamide and for multiple layers are polyamide and polyethylene (Feiner, 2006).

2.3 Sausage processing methods

There are several types of cooking methods that markedly affect the quality of finished products. Sausage cooking should be selected to bring desirable palatability, nutritional retention, safety, visual appearance, improved cooking yield, and reduced cooking time. The sausage cooking method is also selected based on types, sizes, and properties (dryness, fermentation) of the sausages. Several methods used for processed meats are the smokehouse (e.g., Alkar, Inc), water immersion (e.g., Armor Inox), hotwater shower (e.g., Serpentin), air blowing (e.g., Impingement), radio frequency, and ohmic cooking.

2.3.1 Water bath cooking

Water immersion cooking is one of conventional methods used in the meat industry (Cheng & Sun, 2007). Water temperature and end-cook temperature are critical factors in the cooking method. Basically, water cooking is done at temperatures around 160 to 170^{o}F. This temperature must be maintained throughout processing because at higher temperatures, gelatin pockets develop and sausage casings burst (Rust, 1976). The water cooking method has numerous advantages such as:1) product tenderness and yield improvement, 2) microbiological safety enhancement through rapid heat penetration, 3) rapid availability of the method, 4) uniform and easy control of the degree of doneness, and 5) low cost and less working space (Buck, Hickey & Rosenau, 1979; Cyril, Castellini & DalBosco, 1996). This method is particularly applicable for fresh types of sausages such as bratwurst, pork sausage and country-style pork sausage, which usually have collagen casings.

2.3.2 Smokehouse

Smokehouse is commonly used for frankfurters, bologna, bacon, and ham. Smoking is the process of exposing meat products to natural or liquid smoke. This processing method has been used to produce smoke flavor, aroma and color in meat products (Feiner, 2006). The purpose of smoking is to extend the shelf life of meat and their products, since smoking is able to inhibit both microbial growth and lipid oxidation due to the formation of formaldehyde, carboxylic acids and phenols (Goulas & Kontominas, 2005). Smoke flavor is attributed to improving the organoleptic quality of the products through a “smokey flavor” (Rust, 1976). Natural smoke is generated by incomplete pyrolysis of wood materials such as sawdust or woodchips while liquid smoke is obtained through burning of selected woods under controlled

conditions and is applied to meat products by atomization, dipping/showering, brine addition, or smoke-impregnated casings (Feiner, 2006).

In the smokehouse, stuffed sausage batters are inserted into places where various cooking cycles can be applied such as drying, smoking, cooking, showering, and cooling (Romans, Costello, Carlson, Greaser and Jones, 2001). Before smoking, the sausages need to be exposed to a short drying period at $100^{o}F \pm 20^{o}$ ($37^{o}C \pm 11^{o}C$) at less than 45% RH for 30 minutes or less in order to avoid a streaked appearance and pale color.

When smoke flavorings are produced under good manufacturing conditions, FDS has approved their status to be considered Generally Regarded as Safe (GRAS) under the food additive provisions of the Federal Food, Drug and Cosmetic Act (FFDCA). Basically, the smoking and cooking processes take place at the same time, whereas steam cooking at 170^{o} to $180^{o}C$ for 5 to 15 minutes is normally applied to small diameter products (Rust, 1976). After cooking, the products will be showered immediately with cold water to a temperature of $40^{o}F$ ($5^{o}C$) or less to remove salt and fat streaks from the product surface.

2.3.3 Other cooking methods

Ohmic cooking is an emerging thermal processing technology in the food industry. This involves heat generation in the food by an electrical current (McKenna, Lyng, Brunton & Shirsat, 2006; Zell, Lyng, Cronin & Morgan, 2010). This method is believed to shorten cooking times and deliver a more consistent product appearance (Laycock, Piyasena & Mittal, 2003) and better eating experience compared to conventionally processed products (Bozkurt & Icier, 2010; Piette et al., 2004; Zell, Lyng, Cronin & Morgan, 2010). Radio frequency cooking uses dielectric heating to produce heat internally but not on the surface. In addition, this cooking method is six

times faster than conventional methods. However, the meat is often too chewy and more elastic (Lycock, Piyasenaand Mittal, 2003).

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CHAPTER 3

DEVELOPMENT OF A NOVEL SAUSAGE CASING MADE OF CHITOSAN

3.1. Materials and methods

3.1.1 Materials

Chitosan (CH) (100-300 kDa and 90% deacetylated) and Tween 80 (T80)were purchased from Acros Organic (NJ, USA). Glacial acetic acid, trans-cinnamaldehyde (CA) and magnesium nitrate were supplied by J.T. Baker Inc. (NJ, USA). Pure (100%) vegetable glycerol (GLY) was obtained from Starwest Botanicals, Inc. (CA, USA). Collagen casings were obtained from Devro Inc. (SC, USA).

3.1.2 Film preparation

CH solution (2% w/v) was prepared by dissolving 2 g of CH in 100 mL of glacial acetic acid solution (1% v/v) while stirring on a magnetic stirrer (Barnstead, IA, USA) at room temperature overnight. The solution was filtered through cheesecloth to remove undissolved impurities. GLY(37.5, 50 or 75 % w/w of CH) was added to the CH solution and the mixture was stirred for 30 min. CA (0, 1.0, 1.5, 2.0 or 2.2% w/v of CH solution) mixed with T80 (0.2 or 0.3% w/w of CA) was added to the solution and the mixture was homogenized for 5 min using an homogenizer (Polytron Kinamatica Inc, OH, USA). The resulting film-forming solutions were sonicated (Ultrasonic Cleaner, Model FS30D, Fisher Scientific, PA, USA) for 5 min to remove air bubbles. Each film-forming solution was casted on a glass plate, dried for 30 h at room temperature, peeled and conditioned in a desiccator at 23^{o}C and 51% relative humidity (RH) for 72 h. A saturated magnesium nitrate solution was used to meet the required RH. The formulations of the developed chitosan films are shown in Table 3.1.Collagen casings were cut open and conditioned in the same way as the chitosan films.

3.1.3 Film characterization

3.1.3.1 Water solubility

Chitosan and collagen films were cut into pieces of 1 x 3 cm each. These pieces were weighed to the nearest 0.0001 g using an analytical balance (Model V11140, Ohaus Corporation, NJ, USA) and dried in an oven (Precision Scientific Co. IL, USA) at 110^{o}C to a constant weight (value recorded as initial dry weight). Each piece was immersed into an 80-mL beaker with 50 mL of distilled water and agitated using a water bath shaker (Gyrotory, Model G76, New Brunswick Scientific Co., Inc, Edison, NJ, USA) for 6 h at 23^{o}C. Each piece was taken out from the beaker and dried in the aforementioned oven at 110^{o}C to a constant weight (value recorded as final dry weight). The solubility of the films in water (%) was calculated using the following equation:

$$\text{Water solubility (\%)} = \frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}} \times 100$$

Three collagen films and three chitosan films of each treatment resulting each film from a new film-forming solution were used to determine water solubility.

3.1.3.2 Thickness

The thicknesses of the chitosan and collagen films were determined using a digital micrometer (Testing Machines Inc., Ronkonkoma, NY, USA). Five thickness measurements were taken randomly for each film and averaged. Three collagen films and three chitosan films of each treatment resulting each film from a new film-forming solution were used to determine thickness

3.1.3.3 Moisture content

Each of the chitosan and collagen films was cut into pieces of 1 x 3 cm, weighed to the nearest 0.0001 g (value recorded as initial weight) using an analytical balance (Model V11140, Ohaus Corporation, NJ, USA) and dried in an oven (Precision Scientific Co, IL, USA) at 110^{o}C to a constant weight (value recorded as final weight).The moisture content of the films was calculated using the following equation:

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

Three collagen films and three chitosan films of each treatment resulting each film from a new film-forming solution were used to determine moisture content.

3.1.3.4 Transmittance

Chitosan and collagen films were cut into pieces of 2.0 x 2.0 cm, transmittance (%) of which was measured using a spectrophotometer (Lambda 25 UV/VIS Spectrometer; PerkinElmer Instruments, Waltham, MA, USA) equipped with an integrating sphere in the spectral range from 280 to 850 nm and with a scan speed of 480 nm per minute. The transmittance values at the wavelengths of 280, 320, 590, and 600 nm are recorded. Three collagen films and three chitosan films of each treatment resulting each film from a new film-forming solution were used to determine light transmittance.

3.1.3.5 Fourier-transform infrared (FTIR) analysis

The infrared spectra of the chitosan films and of the pure film compounds (CH, CA, GLY, T80) were obtained by Fourier-transform infrared spectrophotometer (Model IRPrestige-

21, Shimadzu Scientific Instruments Inc, MD, USA) using an attenuated total reflectance accessory (ATR MAX II)with a standard zinc selenide crystal. Results from 40 scans in the range of 500-4000 cm^{-1} with a resolution of 4 cm^{-1} were averaged. Prior to the measurement of each sample, a background spectrum without sample was measured. Three collagen films and three chitosan films of each treatment resulting each film from a new film-forming solution were used.

3.1.3.6 Mechanical properties

The tensile strength (TS), elongation at break (%E) and tensile energy to break (TEB) of the chitosan and collagen films were measured using an Instron Universal Testing Machine (Model 5565P6021, Instron Engineering Corporation, MA, USA) with a 50N load cell in accordance with the ASTM Standard Method D 882-12 (ASTM, 2012). The grip separation and crosshead speed were set at 50 mm and 25.4 mm/min, respectively. The films were cut into rectangular shapes (2.54-cm width and 15-cm length) using a double-blade cutter. Testing was conducted in a controlled environment of 23 ºC and 55 % RH. Three collagen films and three chitosan films of each treatment resulting each film from a new film-forming solution were used to determine light transmittance.

3.1.4. Statistical analysis

The statistical software Minitab 16 (Minitab Inc., State College, PA, USA) was used to perform a one-way analysis of variance (ANOVA; Tukey test; $p \leq 0.05$). Three collagen films and three chitosan films of each treatment resulting each film from a different film-forming solution were tested.

3.2. Results and discussions

3.2.1 Chemical structure

The chemical structures of CH films differing in GLY, CA and T80 contents as well as of pure film components were obtained using FTIR spectrophotometry. Then, the results were compared to determine possible interactions between CH, GLY, CA and T80 that could lead to different film properties. The FTIR spectra of CH films without and with GLY (films 20000 and 23000, respectively) and the spectrum of the pure GLY are presented in Figure 3.1. The spectrum of the neat CH film (film 20000) exhibited most of the characteristic absorption bands of CH. The peaks observed at 1650.9 cm^{-1}, 1529.5 cm^{-1} and 1062.8 cm^{-1} have been attributed to C=O stretching (amide I), N-H bending (amide II) and C-O stretching, respectively (Leceta, Guerrero & de la Caba, 2013; Ziani, Oses, Coma & Mate, 2008). The broad band ranging between 2800 and 3100 cm^{-1} has been assigned to C-H stretching vibration while the band between 3000 and 3500 cm^{-1} has been attributed to the stretching vibration of free hydroxyl (O-H) and N-H in amino group due to the formation of hydrogen bonds (Martins, Cerqueira & Vicente, 2012; Xu, Kim, Hanna & Nag, 2005). Therefore, the later band indicates the bonding between CH chains. By comparing this FTIR spectrum to that of the GLY plasticized CH film (23000), chemical interactions between CH and GLY were identified. The absorption peaks at 1062.8 and 1018.4 cm^{-1} in the film 20000 were merged into a single peak at 1022.2 cm^{-1} in the film 23000. This peak has been associated to interactions between O-H groups of CH and GLY by hydrogen bonding (Leceta, Guerrero & de la Caba, 2013). Bonding between CH and GLY also occurred due to the interactions between N-H groups of CH and O-H groups of GLY as shown by the shift of the absorption peak at 1529.5cm^{-1} in film 20000 to 1546.9cm^{-1}in film 23000.This shift indicates a change from asymmetric NH_3^+ bending vibrations to NH_2 bending vibrations in the

CH molecules (Lewis & McElhaney, 1996) which has been attributed to a displacement of bound acetic acid from the CH molecules by GLY during film drying(Brown et al., 2001). The FTIR spectrum of GLY shows no peak at either 1022.2 cm $^{-1}$ or 1546.9 cm^{-1}, which supports the formation of bonding between CH and GLY in the GLY-plasticized CH film.

Chemical interactions between CH and CA were identified by comparing the FTIR spectra of GLY-plasticized CH film without CA (23000) and with CA (23102, 23152, and 23202) (Figure 3.1). A peak at 1633.3 cm^{-1}was observed in the GLY plasticized CH film when containing CA. This peak has been attributed to the C = N vibration characteristic of imines (Tirkistani, 1998) which suggests the formation of Schiff bases since these present an imine group in their structures (dos Santos, Dockal & Cavalheiro, 2005). The Schiff base was formed from the condensation of an amine group present in the CH chain with the carbonyl group of the aldehyde present in CA. The formation of Schiff bases is also supported by two other changes that occurred in the CH matrix. The absence, in the GLY-plasticized CH films with CA, of the peaks at 1624 cm^{-1} and 1672 cm^{-1} observed in the FTIR spectrum of CA (Figure 3.1) indicates the loss of carbonyl groups due to the formation of Schiff bases since peaks at 1624 cm^{-1} and 1672 cm^{-1} have been attributed to the C=C stretch and C=O stretch in carbonyl, respectively (Sirichote, Hansongnern, Yaochuang & Jantaraprim, 1996). The peak at 1546.9cm^{-1} in film 23000 corresponded to interactions between N-H groups of CH and O-H groups of GLY showed a decreased intensity in films 23102, 23152, and 23202 which indicates a reduced number of interactions between CH and GLY caused by the use of the N-H groups during the formation of Schiff bases. Interactions between the aromatic rings of CA molecules are also suggested. The peaks at 686 and 748 cm^{-1} corresponded to C-H out of plane bend monosubstitution benzene (Sirichote, Hansongnern, Yaochuang & Jantaraprim, 1996) showed lower intensity in GLY-

plasticized CH films with CA than in the pure CA. This may be indicative of non-covalent attractive interactions (aromatic stacking) between aromatic rings of CA molecules.

Upon comparing the spectra of the CH films containing CA, it was observed that the higher the amount of CA in the film, the higher the intensity of the peak at 1633.3 cm^{-1}. This result indicates the formation of more Schiff bases, which reveals an increased interaction between CH and CA with the increase of CA. Another change occurred in the CH matrix was the decreased peak intensity at 1546.9cm^{-1}as a function of the increase of CA, which supported the formation of more Schiff bases with the increase of CA. This decrease in intensity resulted from the formation of bonding between the amine groups of CH with the carbonyl groups of CA molecules instead of with the O-H groups of GLY when more CA was present. Comparing the spectra of the CH films containing CA also reveals a shoulder in the peak 1022.2 cm^{-1} which increases in intensity with the increase of CA content. This shoulder at 998.6 cm^{-1}could be the sign of a starting splitting of the peak 1022.2 cm^{-1} formed by the joining of two peaks and therefore, the reduced hydrogen bonding between CH and GLY.

The spectrum of Tween 80 shows peaks at 2921 cm^{-1} and 2858 cm^{-1}, which represent the asymmetric and symmetric stretching vibrations of the oxy ethylene unit (CH_2), respectively, and peaks at 1736 cm^{-1}, 1458 cm^{-1} and 1346.3 cm^{-1}which have been attributed to C=O stretching of the ester group, CH_2 scissoring and O-H stretching vibrations, respectively (Yu, Suyambrakasam, Wu & Chavali, 2012). Ziani, Oses, Coma & Mate (2008) reported that the chemical structure of the CH matrix can be considered unchanged when Tween 20 is added to this since the presence of Tween 20 in CH formulations did not have any effect on FTIR spectra of the resulting CH films(Ziani, Oses, Coma & Mate, 2008). This is consistent with our results.

3.2.2 Water solubility

The high solubility of chitosan-based films when exposed to an aqueous environment is one of their major drawbacks (Elsabee & Abdou, 2013; Leceta, Guerrero & de la Caba, 2013). Since sausage casings are exposed to water and wet environments during sausage processing, the parameter water solubility was used to select film-forming solutions that resulted in CH films with a similar or a better resistance to water than that of the collagen casings. The selected films were used for further comparison with the collagen casing.

Figure 3.2 presents the water solubility at 23^{o}C for 6 h of CH films differing in GLY, CA and T80 contents compared to the collagen films. The neat CH film (film 20000) showed water solubility similar to that of the collagen film (~14%). However, the GLY-plasticized CH films had higher water solubility than that of the collagen films. For example, film 25000 had a water solubility of 18%. This increase in water solubility was caused by the hydrophilic nature of the GLY which allows to absorb more water, swell, and consequently, to lose more solid matter. The high water solubility of GLY-plasticized CH films has previously been reported (Hosseini, Razavi & Mousavi, 2009; Ojagh, Rezaei, Razavi & Hosseini, 2010; Leceta, Guerrero & dela Caba, 2013). The addition of CA to the chitosan matrix significantly ($p \leq 0.05$) decreased the water solubility of the GLY-plasticized chitosan films and led to chitosan films with lower water solubility than that of the collagen film. For example, the water solubility of the chitosan film plasticized with 37.5% GLY (23000) was decreased ($p \leq 0.05$) from 17% to 12-6% with the addition of 1.0-2.2% CA (23102, 23152, 23202, 23222). This decrease in water solubility can be attributed to the cross-linking effects of CA. The cross-linking resulted from the following two phenomena: 1) the formation of Schiff bases between the amines in the CH chain and the carbonyl groups in the CA molecules, and 2) the subsequent non-covalent interactions (aromatic

stacking) between the aromatic rings of the CA molecules as supported by the FTIR results (Figure 3.1). The formation of Schiff bases and the interaction of aromatic groups are shown by the presence of a new peak at 1633.3 $cm^{-1}$and the reduced intensity of the peaks at 686 and 748 cm^{-1}, respectively, in GLY-plasticized CH films containing CA (Figure 3.1). The higher the amount of CA the more the formation of Schiff bases as shown by the increased intensity of the peak at 1633.3 cm^{-1}(Figure. 3.1). Therefore, the more the CA, the more the cross-linking and the less the water solubility. At higher GLY contents (50%), larger amounts of CA (2.2%) were needed to significantly ($p \leq 0.05$) reduce the water solubility of the chitosan films. A high number of GLY molecules in the polymer matrix likely induced more interactions between the GLY and the chitosan, which reduced the number of free N-H groups for CA to interact with. The high degree of interaction between the N-H groups of CH and O-H groups of GLY is shown in the FTIR by the shifting of the absorption peak from 1529.5to 1546.9cm^{-1} (Figure 3.1). In contrast, large amounts of CA were not needed to reduce the water solubility of the CH films when these contained higher GLY contents (75%) (27202). Most likely the GLY molecules interacted with each other instead of with the CH chains due to their large number which facilitated interactions. Lee & Timasheff (1975, 1977) reported that GLY molecules exert a self-association when present in large amounts (Lee & Timasheff, 1975, 1977). Therefore, intramolecular hydrogen bonds rather than intermolecular hydrogen bonds were formed, resulting in a phase separation between GLY and CH. The less interactions between GLY and CH resulted in more free amine groups and consequently, more interactions between CA and CH, which led to more cross-linking. The interactions between the GLY molecules reduced the available number of OH groups, and therefore, the hydrophilic nature of the GLY. This resulted in less water absorption, less polymer swelling, and consequently, less loss of solid matter. There

was no significant ($p > 0.05$) effect of T80 on the water solubility of the chitosan films although this was expected since T80 was used to produce a homogeneous dispersion of CA in the CH matrix in order to ensure crosslinking between CA and CH chains.

Although the plain chitosan film (film 20000) showed a similar solubility in water to that of the collagen film, this film was not selected for further testing due its brittleness under dry conditions (Olabarrieta, Forsström, Gedde & Hedenqvist, 2001; Park, Marsh & Rhim, 2002; Suyatma, Tighzert & Copinet, 2005). The films 23000 and 25000 were not selected due to their high water solubility. All GLY-plasticized chitosan films containing CA had water solubility similar or lower than that of collagen film. As a result, they may have the potential to perform similar to the collagen film during sausage manufacture in wet and dry environments. However, only five films were chosen to investigate the effect of GLY content (23222 vs. 25222 and 25202 vs. 27202), CA content (25202 vs. 25222) and T80 content (25222 vs. 25223) on properties other than water solubility required for a sausage casing well-suited for commercial applications.

3.2.3 Thickness

All chitosan films had the same thickness as that of the collagen film ($p > 0.05$) as shown in Table 3.2. However, the chitosan films were more uniform in thickness than the collagen film. This could result in chitosan casings more uniform in diameter and more consistent in product net weight compared to the collagen casing. Based on these results, the film-forming solution formulations did not affect the chitosan film thickness. In contrast, Hosseini, Razavi & Mousavi (2009) and Ojagh, Rezaei, Razavi & Hosseini (2010) reported an increased chitosan film thickness as a function of CA content (Hosseini, Razavi & Mousavi, 2009; Ojagh, Rezaei,

Razavi & Hosseini, 2010). These different results may be due to the different types of chitosan and procedures used for the film formation.

3.2.4 Moisture content

Table 3.2 shows the moisture contents of collagen films and chitosan films differing in GLY, CA and T80 contents. All chitosan films had higher moisture contents compared to the collagen film. The reason was the presence of GLY in the CH films as shown by the increased ($p \leq 0.05$) moisture content of these as a function of the GLY increase. A 23% moisture content was observed for the chitosan films with 37.5% GLY (film 23222) while 30 and 37% moisture contents were obtained for the films with 50 and 75% GLY (films 25222 and 27202). The increased film moisture content caused by GLY was due to its hydrophilic nature which led to more water absorption. The effect of GLY/chitosan ratio on the moisture content of CH films has been reported previously (Leceta, Guerrero & de la Caba, 2013; Ziani, Oses, Coma & Mate, 2008). The increase of either CA or T80 did not affect the moisture content. Films 25202, 25222 and 25223 had the same moisture content regardless ofthe CA and T80 contents. Ojagh, Rezaei, Razavi & Hosseini (2010) reported an effect of the CA amount on the moisture content of the chitosan film in a range from 0.4 to-1.5% but not more than 1.5% (Ojagh, Rezaei, Razavi & Hosseini, 2010). Therefore, it seems that a CA concentration above 1.5% does not reduce moisture content any more, which supports our results of no moisture change between 2.0 and 2.2% CA.

3.2.5 Transmittance

The transmittance values (%) of collagen films were compared with those of chitosan films differing in GLY, CA and T80 contents (Table 3.2). All chitosan films were highly transparent (Figure 3.3) with a transmittance ranging between 90.1-91.3% in the 590-600 nm wavelength interval of the visible light. These transmittance values were higher than those of the collagen films (88.2-88.4%), which show higher transparency of the chitosan films. No significant differences ($p > 0.05$) were found for chitosan films with different GLY, CA and T80 contents. All the chitosan films showed excellent UV barrier properties with a transmittance ranging between from 0.1-2.6% at a wavelength of 320 nm (near ultraviolet) to -1.1-3.7% at a wavelength of 280 nm (middle ultraviolet). These UV barrier properties of the chitosan films were not affected by the GLY, CA or T80 contents. These results are consistent with those of Leceta, Guerrero & de la Caba (2013) who reported excellent barrier properties to UV light for chitosan films and no effect of GLY content (0, 15, and 30%) on their UV barrier properties (Leceta, Guerrero & de la Caba, 2013). All the chitosan films showed a superior barrier to UV light compared to the collagen film. Therefore, a sausage casing made from CH would have the capability for better sausage quality and shelf life due to less oxidation processes.

3.2.6 Mechanical properties

Both flexibility and strength are required for sausage casings because they must be pliable enough during stuffing and strong enough during cooking, chilling, and additional handling (Osburn, 2002). The flexibility and strength of chitosan films differing in GLY, CA and T80 contents were determined and compared to those of the collagen film using the mechanical properties TS, %E and TEB. These properties were chosen because TS, E%, and TEB represent

the maximum tension, stretch, and energy absorption, respectively, before a film is broken or ruptured. As shown in Figure 3.4, the TS of the CH films ranged between 22.7-41.4 MPa. This variability resulted from the effect of both, GLY and CA, on the film TS. An increase of GLY content from 37.5 to 50% GLY decreased by almost half the TS of the chitosan film (film 23222 vs. film 25222). This reduction resulted from the increased number of hydrogen bonds between CH and GLY, which lowered the interactions between the chitosan chains. The favorable degree of interaction between the two molecules is shown in the FTIR results by the joining of the two peaks at 1062.8 and 1018.4 cm^{-1} into one at 1022.2 cm^{-1} and by the shift of the absorption peak from 1529.5$cm^{-1}$to 1546.9cm^{-1} (Figure 3.1). GLY contents of more than 50% did not have an effect on the film TS (film 25202 vs. film 27202). This was most likely due to the self-interaction of GLY molecules rather thanthe interaction with CH chains. The increase of CA content increased the TS value of the GLY-plasticized chitosan films (film 25222 vs. film 25202). This phenomenon was due to the increased degree of cross-linking caused by the increase of CA, as supported by the FTIR results (Figure 3.1).These results are consistent with those of Ojagh, Rezaei, Razavi & Hosseini (2010) who reported an increase of TS in GLY-plasticized chitosan films as a function of CA increase (Ojagh, Rezaei, Razavi & Hosseini, 2010). All the CH films had the same TS as that of the collagen films ($p>0.05$) except for the film 23222 which had a much higher TS (41.4 MPa).

The CH films 25202, 25222 and 25223 had the same %E ($p>0.05$) as the collagen film (~ 17%) while the films 23222 and 27202 had a much lower (6%) and higher %E (30%) values, respectively. Therefore, higher GLY contents were correlated with smaller intramolecular forces between the CH chains. This resulted in an increased polymer matrix mobility which facilitates film elongation, which is consistent with Caner, Vergano & Wiles (1998) who reported an

increased flexibility of the chitosan film with the increase of GLY(Caner, Vergano & Wiles, 1998. Neither CA nor T80 had an effect on the flexibility of the chitosan film since there were no differences between the %E of films 25202, 25222 and 25223. In contrast, Ziani, Oses, Coma & Mate (2008) reported an increased %E of the CH films in the presence of Tween20. The difference may be due to the different molecular weight of the Tweens (T20 vs. T80) (Ziani, Oses, Coma & Mate, 2008).

All CH films had the same TEB as that of the collagen films ($p>0.05$) except for film 27202which had a much higher TEB (0.13 MPa).The increased TEB of 27202was most likely due to the clusters of GLY trapped within the crosslinked polymer matrix which increased the unit volume of the film for absorbing more energy before its rupture. Based on these results, a amount of GLY of 50% is necessary to obtain a CH film with the same TS, %E and TEB as the collagen casing. The use of higher amounts of GLY resulted in a film (film 27202) with enhanced mechanical properties compared to the collagen casing. However, this film cannot be used as a sausage casing because of its "greasy" surface (data not shown).

Acknowledgements

The authors thank the Meat Laboratory at Michigan State University for providing the collagen casings used as controls.

APPENDIX

APPENDIX

Code	CH (% w/v of glacial acetic acid solution)	GLY (% w/w of CH)	CA (% w/v of CH solution)	T80 (% w/w of CA)
20000	2	0.0	0.0	0.0
23000	2	37.5	0.0	0.0
23102	2	37.5	1.0	0.2
23152	2	37.5	1.5	0.2
23202	2	37.5	2.0	0.2
23222	2	37.5	2.2	0.2
25000	2	50.0	0.0	0.0
25202	2	50.0	2.0	0.2
25222	2	50.0	2.2	0.2
25223	2	50.0	2.2	0.3
27202	2	75.0	2.0	0.2

Table 3.1. Chitosan films with different concentrations of GLY, CA and T80.

Film	Thickness (μm)	Moisture content (%)	Transmittance (%)			
			280 nm	320 nm	590 nm	600 nm
Collagen	52 ± 10 A	13 ± 1 D	12.1 ± 3.4 A	38.1 ± 5.1 A	88.2 ± 2.1 A	88.4 ± 2.0 A
23222	51 ± 4 A	23 ± 1 C	2.6 ± 2.4 BC	2.6 ± 2.2 B	91.1 ± 0.3 B	91.3 ± 0.3 B
25202	53 ± 9 A	30 ± 2 B	0.2 ± 1.8 BC	0.3 ± 2.0 B	90.1 ± 0.5 B	90.3 ± 0.5 B
25222	51 ± 7 A	30 ± 1 B	1.1 ± 2.1 BC	0.1 ± 1.3 B	91.1 ± 0.3 B	91.2 ± 0.3 B
25223	51 ± 5 A	30 ± 3 B	-1.1 ± 3.4 C	0.1 ± 2.1 B	91.1 ± 0.4 B	91.2 ± 0.4 B
27202	52 ± 5 A	37 ± 1 A	3.7 ± 2.9 B	1.8 ± 1.7 B	90.3 ± 0.9 B	90.4 ± 0.9 B

Table 3.2. Thickness (μm), moisture content (%) and transmittance (%) of collagen films and chitosan films differing in GLY, CA and T80 contents.

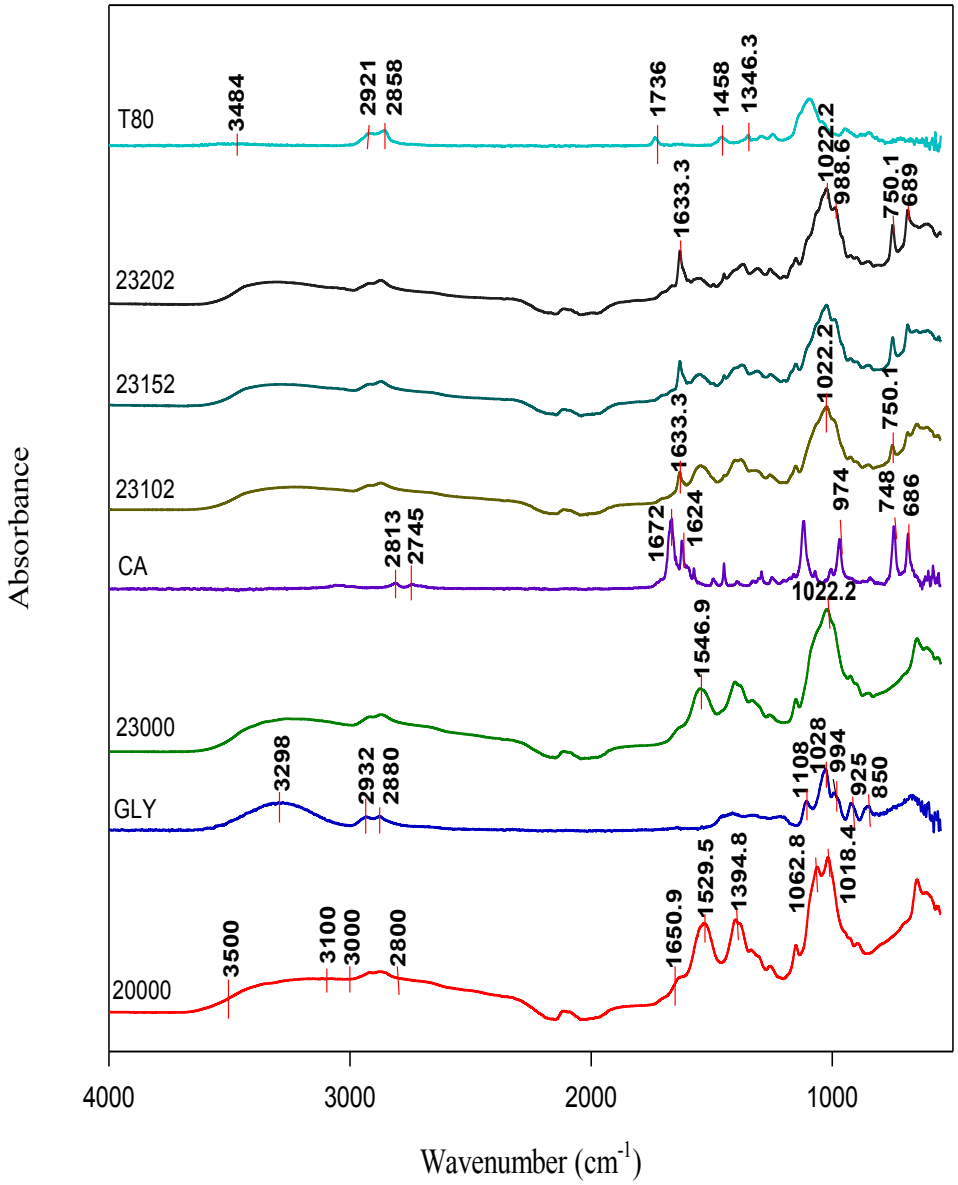

Figure 3.1. FTIR spectra of CH films differing in GLY, CA and T80 contents as well as of pure film components.

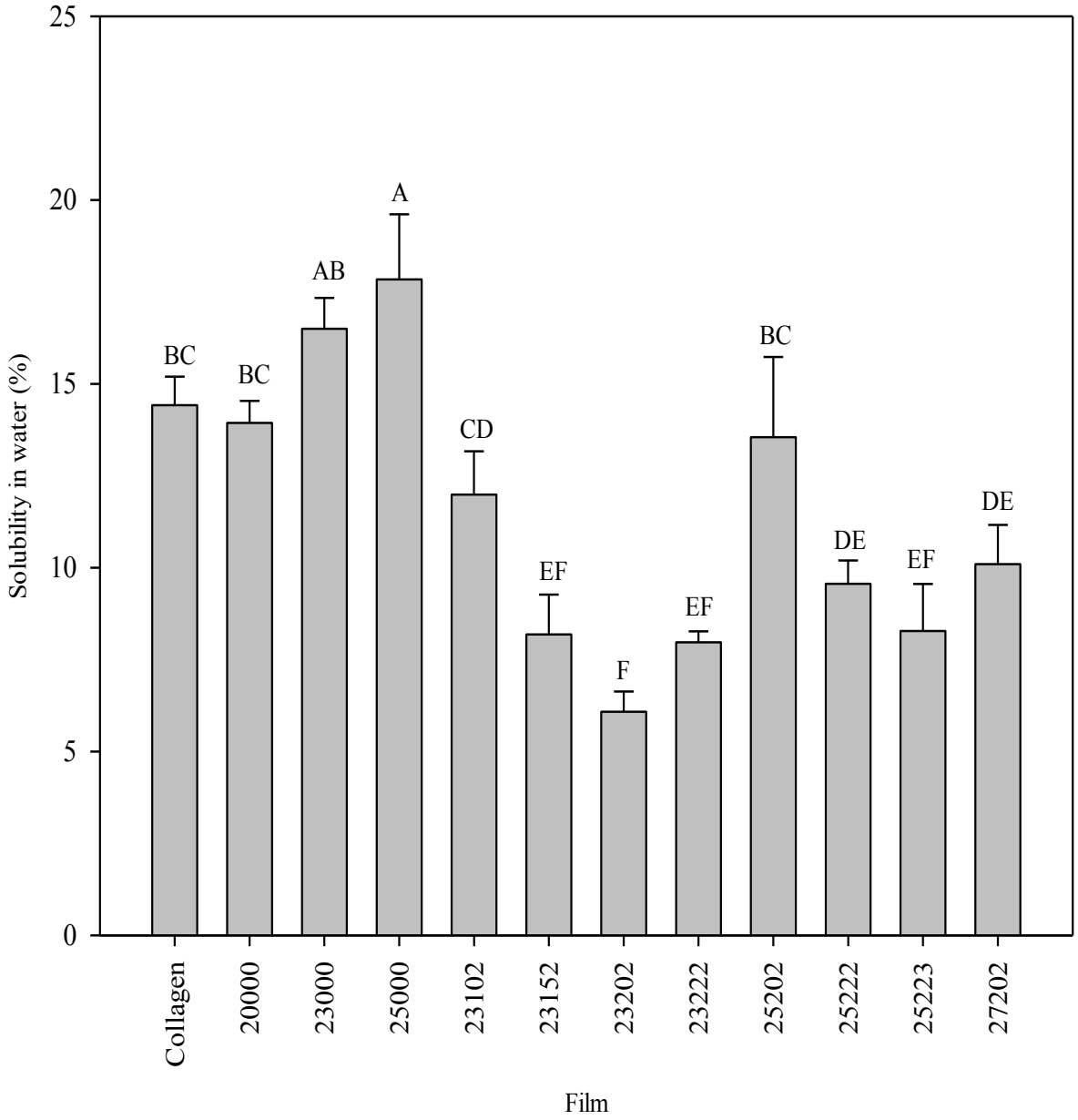

Figure 3.2. Water solubility of collagen films and chitosan films differing in GLY, CA and T80 contents at 23°C for 6 h.

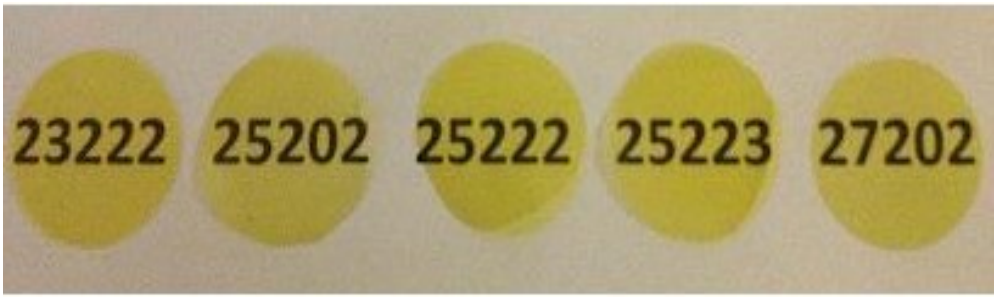

Figure 3.3. Chitosan films differing in GLY, CA and T80 contents.

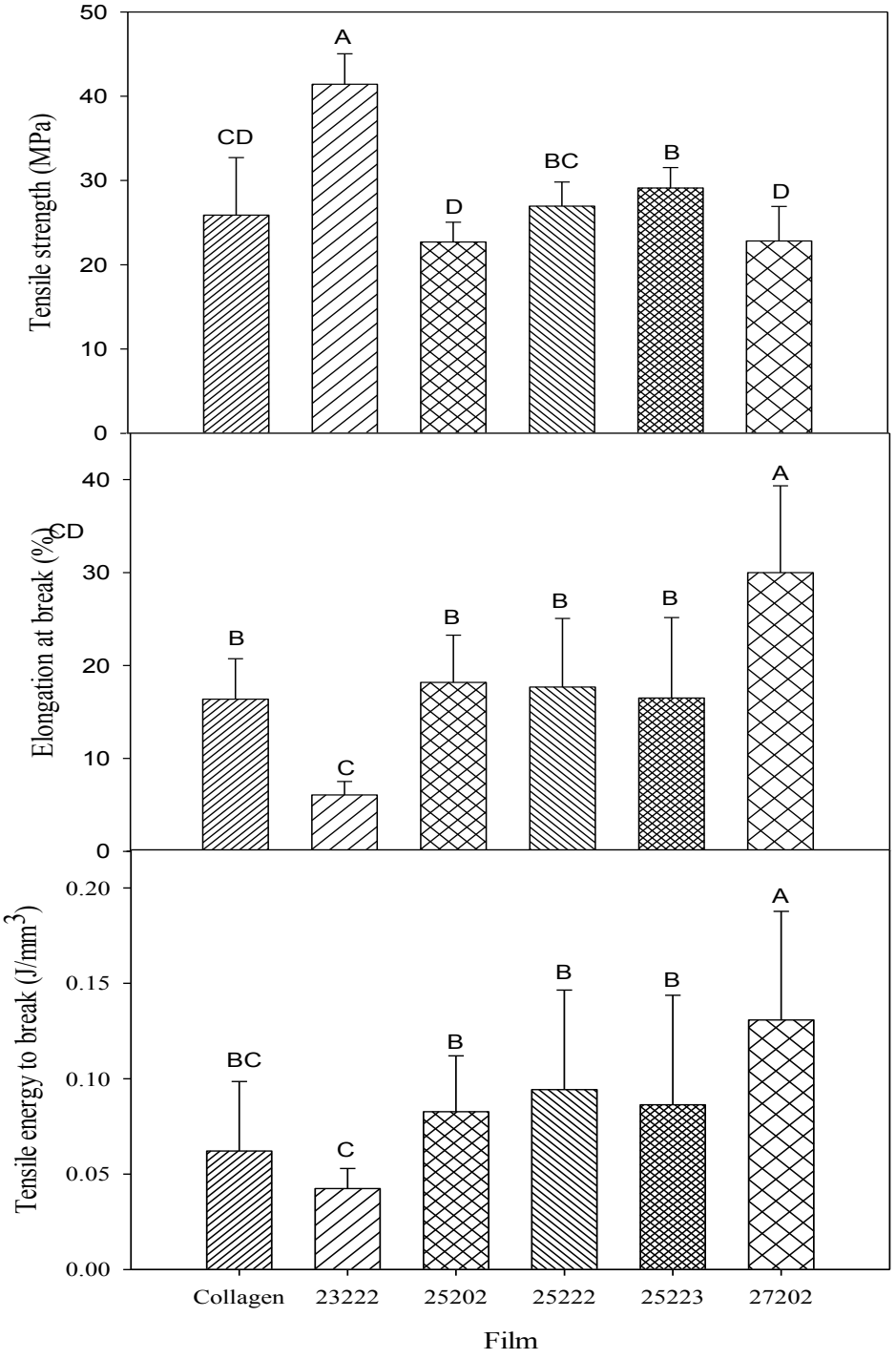

Figure 3.4. Mechanical properties of collagen films and chitosan films differing in GLY, CA and T80 contents.

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CHAPTER 4

PERFORMANCE OF A NOVEL CASING MADE OF CHITOSAN UNDER TRADITIONAL SAUSAGE MANUFACTURING CONDITIONS

4.1 Materials and methods

4.1.1 Materials

Chitosan (CH) (molecular weight = 100-300 kDa and 90% deacetylation) and Tween 80 (T80) were purchased from Acros Organic (NJ, USA). Glacial acetic acid, trans-cinnamaldehyde (CA) and magnesium nitrate were supplied by J.T. Baker Inc. (NJ, USA). Pure (100%) vegetable glycerol (GLY) was obtained from Starwest Botanicals Inc. (CA, USA). Collagen casings were obtained from Devro Inc. (SC, USA) and Philly Cheese Brat All Beef was obtained from the Meat Lab of Michigan State University.

4.1.2 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, IA, USA) at room temperature overnight. The solution was filtered through cheesecloth to remove undissolved 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 solution and the mixture was homogenized for 5 min using a homogenizer (Polytron Kinamatica Inc, OH, USA). The resulting film-forming solution was sonicated (Ultrasonic Cleaner, Model FS30D, Fisher Scientific, PA, USA) for 5 min to remove air bubbles, and 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 (0.02 x 0.15 m) while other pieces were maintained as films to investigate the effect of the batter on the chitosan matrix during cooking. Collagen casings (0.04 x 0.15 m) were used as controls since they are the most widely used edible casings. Casings and

films were conditioned in a desiccator at 23 ºC and 51% relative humidity (RH) for 72 h before testing. A saturated magnesium nitrate solution was used to create the required RH.

4.1.3 Sausages processing

Philly Cheese Brat All Beef type batter was stuffed into the collagen and chitosan casings using a hand stuffer (VOGT-deal, Chicago, IL, USA). After stuffing (Figure 4.1a), the resulting sausages were cooked using the conventional water immersion cooking method (Figure 4.1b). Briefly, the sausages were cooked in a water bath (Precision Scientific Model 25, Chicago, IL, USA) at 85^{o}C until reaching an internal temperature of 72^{o}C, which was measured using a thermocouple (Super Scientific, Ltd, AZ, USA). Subsequently, the sausages were covered with ice cubes inside a plastic container to bring down their internal temperature to 5^{o}C. The CH films were exposed to the same conditions as the sausages. Finally, the casings were peeled off, and these and the films were individually wrapped in aluminum foil and then vacuum-sealed. This was done to maintain the conditions of the casings and films until testing within the following 48h.

4.1.4 Casing characterization

CH and collagen casings as well as CH films were characterized in terms of thickness, moisture content, water solubility, transmittance, permeability(water vapor, oxygen and liquid smoke), mechanical properties and morphology before and after exposure to sausage manufacturing conditions. CH casings and films resulting from three different film-forming solutions (three batches) as well as three collagen casings were used for the characterization. The procedures used are described below.

4.1.4.1 Thickness

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

4.1.4.2 Moisture content

CH casings and films as well as collagen casings were cut into pieces of 1 x 3 cm each. These pieces were weighed to the nearest 0.0001 g (value recorded as initial weight) using an analytical balance (Model V11140, Ohaus Corporation, NJ, USA) before being dried in an oven (Precision Scientific Co, IL, USA) at 110^{o}C to a constant weight (value recorded as final weight). The moisture content of the casing was calculated using the following equation:

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

4.1.4.3 Water solubility

CH and collagen casings and CH films were cut into pieces of 1 x 3 cm each. These pieces were weighed to the nearest 0.0001 g using an analytical balance (Model V11140, Ohaus Corporation, NJ, USA) and dried in an oven (Precision Scientific Co. IL, USA) at 110 ^{o}C to a constant weight (value recorded as initial dry weight). Each piece was immersed into an 80 mL beaker with 50 mL of distilled water while stirring on a magnetic stirrer (Barnstead, IA, USA) for 20 min at 85 ^{o}C (temperature used to cook the sausages). Each piece was taken out from the beaker and dried in the aforementioned oven at 110 ^{o}C to a constant weight (value recorded as final dry weight). The solubility of the films in water (%) was calculated using the following equation:

$$\text{Water solubility (\%)} = \frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}} \times 100$$

4.1.4.4 Transmittance

CH casings and films as well as collagen casings were cut into pieces of 2.0 x 2.0 cm. The transmittance (%) of the CH and collagen pieces was measured using a spectrophotometer (Lambda 25 UV/VIS Spectrometer; PerkinElmer Instruments, Waltham, MA, USA) equipped with an integrating sphere in the spectral range from 280 to 850 nm and with a scan speed of 480 nm per minute. The transmittance values obtained at a wavelength of 280, 320, 590 and 600 nm were reported.

4.1.4.5 Water vapor permeability

Water vapor transmission rates (WVTR) of CH casings and films as well as collagen casings were measured in accordance with the ASTM Standard Method F1249-11 (ASTM, 2011) using a PERMATRAN W Model 3/33 Water Permeability Analyzer (MOCON, MN, USA). Casings and films were cut into pieces of 2 x 2 cm and then placed between two aluminum masks (MCMaster-Carr, Ohio, USA) with an exposure area of 0.38 cm^2. The test conditions were set at 23 ºC and 100% RH. Water vapor permeability was calculated using the following equation:

$$\text{WVP} = \frac{\text{WVTR} \times \text{t}}{\Delta P}$$

Where WVP is water vapor permeability (kg m/m^2sec Pa), WVTR is water vapor transmission rate (kg/ m^2sec), t is the thickness of the casing or film (m) and ΔP is the partial pressure different (Pa).

4.1.4.6 Oxygen permeability

Oxygen transmission rates (OTR) of CH casings and films as well as collagen casings were measured in accordance with the ASTM Standard Method D3985-10 (ASTM, 2010) using an Ox-Tran 2/21 Module ML (MOCON, MN, US). Casings and films were cut into pieces of 2 x 2 cm and then placed between two aluminum masks (MCMaster-Carr, Ohio, USA) with an exposure area of 0.38 cm^2. The test conditions will set at 23^0C and 0% RH. Oxygen permeability was calculated using the following equation:

$$OP = \frac{OTR \times t}{\Delta P}$$

Where OP is water vapor permeability (kg m/m^2sec Pa), OTR is water vapor transmission rate (kg/ m^2sec), t is the thickness of the casing or film (m) and ΔP is the partial pressure different (Pa).

4.1.4.7 Liquid smoke permeability

Permeation cells made from stainless steel were used to determine gravimetrically the liquid smoke transmission rate (LSTR) of the collagen casings and CH casings and films using a modification of the ASTM Method E 96-80 (ASTM, 1980). Cells were filled with 5 mL of liquid smoke and placed in an environmental chamber set at 23 ºC and 55% RH. The weights of the cells were recorded daily. Linear regression-derived slopes of the steady state (linear) portion of weight loss versus time curves were used to estimate LSTR. Liquid smoke permeability was calculated using the following equation:

$$LSP = \frac{LSTR \times t}{\Delta P}$$

Where LSP is liquid smoke permeability (kg m/m^2sec Pa), LSTR is liquid smoke transmission rate (kg/ m^2sec), t is the thickness of the casing or film (m) and ΔP is the partial pressure different (Pa).

4.1.4.8 Mechanical properties

The tensile strength (TS), elongation at break (%E) and tensile energy to break (TEB) of the collagen casings and CH casings and films were measured using an Instron Universal Testing Machine (Model 5565P6021, Instron Engineering Corporation, Norwood, MA, USA) with a 50N load cell in accordance with the ASTM Standard Method D 882-12(ASTM, 2012).The grip separation and crosshead speed were set at 25.4 mm and 12.7 mm/min, respectively. The films were cut into rectangular shapes (1 cm wide and 5 cm long) using a double-blade cutter. Testing was performed in a controlled environment of 23 ºC and 55 % RH.

4.1.4.9 Scanning electron microscopy (SEM)

Collagen casings and CH casings and films were submerged in liquid nitrogen for 30 seconds and then cut with a double-edged razor blade to produce a cross section. The pieces were mounted on aluminum stubs using high vacuum carbon tabs (SPI Supplies, West Chester, PA, USA) and then coated with osmium (≈15 nm thickness) using an NEOC-AT osmium coater (Meiwafosis Co., Ltd., Osaka, Japan). All the samples were examined using a JEOL 6610LV scanning electron microscope (JEOL Ltd., Tokyo, Japan) with an accelerating voltage of 10 kV and a working distance of 25 mm at 5000x and 850x magnification for the surface and the cross-section of the casings, respectively. For each piece, a surface of 1000 μm x 1000 μm was visualized.

4.1.5 Statistical analysis

The statistical software Minitab 16 (Minitab Inc., PA, USA) was used to perform a one-way analysis of variance (ANOVA; Tukey test; $p \leq 0.05$). At least three casings of each type and three CH films were tested before and after exposure to sausage manufacturing conditions.

4.2 Results and discussions

4.2.1 Thickness, moisture content and water solubility

The thickness of the CH and collagen casings and CH films before and after exposure to sausage manufacturing conditions are shown in Table 4.1. There were no statistically significant differences ($p > 0.05$) between the thicknesses of both types of casings and neither between the thicknesses of the casings and films before processing. The thickness of both casings increased significantly ($p \leq 0.05$) (from 60 μm to 121 μm and from 54 μm to 71 μm for the collagen and CH casings, respectively) while the thickness of films remained unchanged ($p > 0.05$) during the cooking process. The greater increase in thickness of the collagen casings was due to their greater absorption of water during the cooking process as supported by the significant increase in moisture content of this type of casing after cooking (from 13 to 24 % ($p \leq 0.05$); Table 4.1). The lower water absorption of the CH casings and films can be attributed to a low interaction between CH and water caused by the covalent bonds formed between CA and the functional groups in the CH chains which decreased the number of hydroxyl and amino groups in the CH available to interact with water (Park & Zhao, 2004). These results suggest that the CH casings possess a superior barrier to water compared to the collagen casings. To have a good barrier to water is considered a crucial property for films intended to be used as a sausage casing (Ockerman, 1989). The difference in thickness between CH casings and films can be ascribed to

the absorption of water from the meat batter by the CH casings during cooking. This is supported by the higher ($p \leq 0.05$) moisture content of the CH casings compared to the CH films after cooking (Table 4.1). These results are consistent with those of Lui, Kerry and Kerry (2007) who reported the capability of extruded edible casings manufactured from pectin and gelatin/sodium alginate blends to absorb water from pork batter when studying breakfast pork sausages(Liu, Kerry & Kerry, 2007).

The moisture content of the CH casings and films was higher than that of the collagen casings before cooking (29% vs. 13%) but lower after cooking (8-9% vs. 24%) (Table 4.1). This is because the moisture content went down for the CH casings and films and up for the collagen casings during processing. Most likely a change in the GLY content in the CH casings and films during sausage manufacturing conditions was the reason for their decreased moisture content. GLY is well known to be hydrophilic. The loss of GLY during the cooking process led to CH casings and films with a lower capability to interact with water and therefore, to CH casings and films with lower moisture content. This loss of GLY is supported by the solubility results which show a 20% loss of total soluble matter for the CH casings and films under simulated sausage cooking conditions (water at 85 °C; Table 4.1). This value is higher than the 10.4% reported by Ojagh, Rezaei, Razavi, & Hosseini (2010) for films containing 2% cinnamon essential oil. The reason for our higher value is the use of cinnamaldehyde instead of cinnamon essential oil, which contains many other components. There were no statistically significant differences ($p > 0.05$) between the losses of matter from both types of casings. Since the water solubility of the CH casings was the same as that of collagen casings, the former have the potential to perform as well as the collagen casings during the sausage manufacturing processing (maintenance of integrity during cooking and other processes that involve water exposure).

4.2.2 Transmittance

Table 4.2 presents the transmittance (%) of the collagen casings and CH casings and films before and after exposure to sausage manufacturing conditions at different wavelengths in the visible and UV range of the electromagnetic radiation spectrum. Prior and after processing, both casings were highly transparent as shown by the transmittance values between 88.26 % - 88.86 % obtained in the 590-600 nm wavelength interval of the visible light. In this wavelength interval, both casings showed the same transmittance ($p > 0.05$) prior to processing but not after processing. The collagen casings were less transparent ($p \leq 0.05$) than the CH casings after exposure to sausage manufacturing conditions (85.69 % vs. 88.32 % and 86.08 % vs. 88.67 % at wavelengths of 590 nm and 600 nm, respectively). The higher transparency of the CH casing after processing is an advantage as transparent casings are preferred by the meat industry (Feiner, 2006). The CH casings showed a superior barrier to UV light compared to the collagen casings. In fact, the CH casings showed excellent UV barrier properties since they had a transmittance zero at wavelengths of 320 nm (near ultraviolet) and 280 nm (middle ultraviolet). The UV-light barrier of both casings decreased with processing. However, this change was minimal for the CH casings, which still maintained a transmittance value near zero while the transmittance values of the collagen casings drastically increased from 38.09 % to 58.87 % and from 12.11 % to 14.14 % at wavelengths of 320 nm and 280 nm, respectively. Based on these results, the superior UV barrier properties of the CH casings compared to the collagen casing after sausage manufacturing conditions indicate the better capability of the CH casing to prevent lipid oxidation, a factor that can significantly affect sausage quality and shelf life. The transmittances at different wavelengths in the visible and UV lights of the CH casing and films were the same ($p > 0.05$) before and after sausage manufacturing conditions. This indicates that there was no interaction

between the batter and the casing that affected the fraction of light that passed through the casing at the wavelengths stated above.

4.2.3 Oxygen, water vapor and liquid smoke permeability

The WVP, OP and LSP of the CH casings and films and the collagen casings before and after exposure to sausage manufacturing conditions are shown in Table 4.3. WVP and OP were studied since water and oxygen are two main factors affecting the shelf life of sausages. LSP was studied due to the use of smoke prior to the cooking of some types of sausages to develop a specific flavor, aroma and color (Feiner, 2006). In addition, smoke is used to extend the shelf life of meat products due to its antimicrobial and antioxidant properties (Goulas & Kontominas, 2005). The OP of the CH casings was lower ($p \leq 0.05$) than that of the collagen casings before and after exposure to sausage manufacturing conditions. The lower OP of the CH casings after processing indicates their better capability of protecting sausages from lipid oxidation during the commercialization period. CH films are known to be a good barrier to oxygen (Hosokawa, Nishiyama, Yoshihara & Kubo, 1990). The OP of both types of casings and of the films was significantly increased when exposed to sausage manufacturing conditions. The increase in permeability of the collagen casings can be attributed to the absorption of water during processing as supported by the moisture content results (Table 4.1) and the SEM cross-section micrographs (Figure 4.2g-h). The water molecules weakened the collagen chain bonds since they can form hydrogen bonds with amide groups of proteins, thereby increasing permeability. The plasticizing capability of water has widely been discussed in the literature (Guilbert & Gontard, 1995). The increase in permeability of the CH casings and films was due to the interaction

between the CH molecules near to the surface with the water which changed the surface of the CH casing as shown in the SEM cross-section micrographs (Figure 4.2f).

The WVP of the CH casings was lower ($p \leq 0.05$) than that of the collagen casings before and after exposure to sausage manufacturing conditions. This lower WVP of the CH casings after processing indicates their higher capability of protecting sausages from dry out during the commercialization period. The WVP of the CH casing was unchanged (6.1×10^{-13} kg m m^{-2} $sec^{-1}Pa^{-1}$) when exposed to sausage manufacturing conditions, while the WVP of the collagen casings increased significantly (13.3×10^{-13} kg m m^{-2} $sec^{-1}Pa^{-1}$ vs. 12.1×10^{-12} kg m m^{-2} $sec^{-1}Pa^{-1}$). This shows the greater stability of the CH casings during sausage manufacturing conditions. The increase in permeability of the collagen casings can be explained by the absorption of water during processing that led to the weakening of the collagen chain bonds, thereby increasing permeability. This is supported by the moisture content results (Table 4.1) and the SEM cross-section micrographs (Figure 4.2g-h). The WVP of the CH casing was maintained most likely due to the presence of CA in the CH matrix. CH film containing CA has been reported to have low WVP due to the hydrophobic nature of the CA which changes the hydrophilic nature of the CH films (Rezaei, Ojagh, Razavi & Hosseini, 2010). The WVP of the CH casings and films was 6.0×10^{-13} kg m m^{-2} $sec^{-1}Pa^{-1}$. This result is consistent with that of Hosseini et al. (2009) who reported a WVP of 9.0×10^{-13} kg m m^{-2} $sec^{-1}Pa^{-1}$for CH films containing 1.5% of cinnamon essential oil (Hosseini, Razavi & Mousavi, 2009). Our WVP results are lower due to the higher amount of CA present in the CH matrix compared to that of the films developed by Hosseini et al. (2009). Adzaly et al. (2014) reported a higher level of cross-linking in the CH matrix with the increase of CA content. The increase of cross-linking in a polymer matrix reduces its permeability. The WVP of the collagen casings was 13.3×10^{-13} kg m m^{-2} $sec^{-1}Pa^{-1}$. This value is

higher than that reported by Krkic et al. (2012) (1.7 x 10^{-13} kg m m^{-2} $sec^{-1}Pa^{-1}$) and the reason is most likely the different source of the collagen casings.

The LSP of the CH casings was lower ($p \leq 0.05$) than that of the collagen casings (2.8 x 10^{-14} kg m m^{-2} $sec^{-1}Pa^{-1}$ vs. 10.3 x 10^{-14} kg m m^{-2} $sec^{-1}Pa^{-1}$).This value was only measured before the cooking of the sausages since the smoking step occurs before cooking during sausage manufacturing. Based on these results, the collagen casing may be a better choice for sausages exposed to smoke during their processing.

4.2.4 Mechanical properties

Flexibility and strength are characteristics required for sausage casings. The flexibility and strength of CH and collagen casings and CH films were determined and compared before and after sausage manufacturing conditions using the mechanical properties TS, %E and TEB (Figure 4.3). Prior to processing, the TS of the CH casings were lower than that of the collagen casings. This indicates that the CH casings require less extra care than the collagen casings to avoid breakage during stuffing. The TS of the CH casings increased from 25 MPa to 50 MPa while the TS of the collagen casings decreased from 49 to 19 MPa during the exposure to sausage manufacturing conditions. Anyway, the CH was strong enough to hold the meat batter during cooking. The collagen casing results are consistent with those of Cagri, Ustunol, Osburn and Ryser (2003) who reported that the TS of collagen casings decreased significantly from 35 MPa to 10 MPa after cooking and smoking hot dogs (Cagri, Ustunol, Osburn & Ryser, 2003). The decreased TS of the collagen casings can be attributed to the absorption of water during processing as supported by the moisture content results (Table 4.1). The water molecules weakened the collagen chain bonds since they can form hydrogen bonds with amide groups of

proteins, thereby reducing TS. The increase in TS of the CH casings can be attributed to the release of GLY during sausage manufacturing conditions. The decrease in GLY resulted in a decreased number of hydrogen bonds between the CH chains and GLY, which increased the interactions between CA and the chitosan chains (increased degree of cross-linking), and this resulted in an increased TS. The CH films showed the same trend as the CH casings, although the TS value of the CH films increased 20 MPa more compared to the CH casings (70 MPa vs. 50 MPa). The lower increase in TS of the CH casing compared to the CH film was due to an effect of the meat batter on the stability of the film. The CH casings absorbed moisture from the meat batter as showed by the higher moisture content of the CH casing compared to the CH film after exposure to sausage manufacturing conditions (Table 4.1). The absorbed water molecules formed hydrogen bonds with the amide groups of the CH chains, which reduced the interaction between the CH chains and CA, thereby decreasing TS.

Initially, the %E of the CH casings was lower than that of the collagen casings (15% vs. 25%). The difference was greater after sausage processing since the %E of the collagen casings remained unchanged while that of the CH casings decreased to 5% ($p \leq 0.05$). In contrast, Cagri, Ustunol, Osburn and Ryser (2003) reported that the %E of collagen casings decreased significantly from 42% to 25 % after hot dog cooking and smoking (Cagri, Ustunol, Osburn & Ryser, 2003). The authors also reported that casings made from whey protein isolate (WPI) showed an increase in %E from 12% to 24 % during hot dog cooking and smoking while their %E was unchanged when containing p-aminobenzoic acid. An increased %E was expected for the collagen casings due to the reduced interactions between the protein chains caused by the absorbed water molecules, which should have led to increased chain movement, thereby increasing flexibility. It seems that the absorbed water was not sufficient to change the flexibility

of the collagen casing. The decreased %E of the CH casings can be attributed to the release of GLY during sausage manufacturing. High GLY contents have been correlated to small intramolecular forces between the CH chains. This results in an increased polymer matrix mobility, which facilitates film elongation (Adzaly, Jackson, Villalobos-Carvajal, Kang & Almenar, 2014). Caner, Vergano & Wiles (1998) also reported an increased flexibility of the chitosan film with the increase of GLY (Caner, Vergano & Wiles, 1998). The CH films showed the same behavior as the CH casings during processing. Less %E reduction was expected for the CH casings compared to the CH films due to the water molecules absorbed from the batter. However, it seems that the amount of water absorbed from the meat batter was not sufficient to change the flexibility of the CH casing. In contrast, Cagri, Ustunol & Ryser (2002) reported an increase in %E of WPI casings containing p-aminobenzoic acid or sorbic acid when in contact with bologna and summer sausages Cagri, Ustunol & Ryser, 2002).

The total energy absorbed per unit volume of the casing until its rupture (TEB) was higher for the collagen casings than for the CH casings. Both casings become less tough after exposure to sausage manufacturing conditions as shown by the significant ($p \leq 0.05$) reduction of their TEB values (from 0.07 to 0.03 J/mm^3 and from 0.17 to 0.08 J/mm^3 for the CH and collagen casings, respectively). The TEB of the CH films was reduced less than that of the CH casings (0.04 J/mm^3 vs. 0.04 J/mm^3), which could be attributed to the less molecules of water absorbed and therefore, the more interactions between the CH chains.

4.2.5 Scanning electron microscopy (SEM)

Figure 4.2 shows the SEM micrographs of the surfaces and cross-sections of CH and collagen casings before and after exposure to sausage manufacturing conditions. The chitosan

casing had a surface with embedded microcapsules ranging between 1-2 μm and a few pores of less than 1 μm (Figure 4.2a). The pores may have resulted from no eliminated air bubbles during sonication. The presence of pores on the surface of CH films has been reported previously (Pereda, Ponce, Marcovich, Ruseckaite & Martucci, 2011). Microcapsules embedded in the CH matrix have also been observed in chitosan films with incorporated carvacrol (1.5% (v/v)) (López-Mata, Ruiz-Cruz, Silva-Beltrán, Ornelas-Paz, Zamudio-Flores & Burruel-Ibarra, 2013). These microcapsules have been explained as droplets caused by the adsorption of chitosan (positively charged) on the surface of Tween 80 (negatively charged) (Klinkesorn & Namatsila, 2009). Neither the microcapsules nor the pores were observed after the exposure of the CH casing to sausage manufacturing conditions as shown in Figure 4.2b. The surface of the CH casing after sausage manufacturing conditions showed irregularly shaped folds which can be attributed to the result of the interaction of the CH matrix with the water during the cooking process. Figure 4.2c shows the surface of the collagen casings which was characterized by the presence of collagen fibrils. These collagen fibrils were not observed after sausage manufacturing conditions (Figure 4.2d) due to the absorption of water during processing which weakened the collagen chain bonds since water can form hydrogen bonds with amide groups of proteins.

The SEM micrographs of the cross-sections of the CH and collagen casings are displayed in Figures 4.2e-h. The cross-section of the CH casing before exposure to sausage manufacturing conditions had a continuous and compact structure as shown in Figure 4.2e. Similar results were observed by Ojagh, Rezaei, Razavi and Hosseni (2010) for chitosan films containing 1.5 % (v/v) cinnamon essential oil (Ojagh, Rezaei, Razavi & Hosseini, 2010). This compact structure of the CH casings is the reason for their low TS, E%, WVP, OP and LSP before sausage manufacturing

conditions. This compact structure was damaged during sausage manufacturing conditions as illustrated in Figure 4.2f. This is supported by the water solubility results, which show a 20% loss of total soluble matter for the CH casings (Table 4.1). The cross-sections of the collagen casing before and after exposure to sausage manufacturing conditions show a discontinuous and non-compact structure due to the presence of random voids (Figure 4.2g-h). This may be one of the reasons for the weaker barrier to oxygen, water and liquid smoke of the collagen casing compared to the CH casing. The difference between cross-section areas of the collagen casings in Figures 4.2g and h supports the aforementioned swelling of the collagen casing caused by water absorption (Table 4.1).

Acknowledgments

The authors thank the Meat Laboratory at Michigan State University for providing the collagen casings used as controls.

APPENDIX

APPENDIX

Casing/Film	Thickness (µm)		Moisture content (%)		Solubility (%)
	Before SMC	After SMC	Before SMC	After SMC	
Collagen casing	60 ± 10 Aa	121 ± 16 Ba	13 ± 1 Aa	24 ± 2 Ba	17.3 ± 2.7a
Chitosan casing	54 ± 11 Aax	71 ± 12 Bbx	29 ± 1 Abx	9 ± 1 Bbx	20.5 ± 1.9ax
Chitosan film	54 ± 11 Ax	55 ± 4 Ay	29 ± 1 Ax	8 ± 1 By	20.5 ± 1.9x

Table 4.1. Thickness (µm), moisture content (%) of collagen casings and CH casings and films before and after exposure to sausage manufacturing conditions (SMC). Solubility (%) at 85°C for 20 min. of collagen casings and CH casings and films before SMC.

A-B indicate significant differences ($p \leq 0.05$) caused by SMC; a-b indicate significant differences ($p \leq 0.05$) between casings; x-y; indicate significant differences ($p \leq 0.05$) between casing and film.

Wavelength (nm)	Transmittance (%)					
	Collagen casing		Chitosan casing		Chitosan film	
	Before SMC	After SMC	Before SMC	After SMC	Before SMC	After SMC
280	12.11 ± 3.37 Aa	14.14 ± 5.93 Aa	0.00 ± 0.00 Abx	1.61 ± 1.24 Bbx	0.00 ± 0.00 Ax	1.71 ± 1.54 Bx
320	38.09 ± 5.06 Aa	58.87 ± 8.66 Ba	0.00 ± 0.00 Abx	1.61 ± 1.88 Bbx	0.00 ± 0.00 Ax	0.98 ± 1.61 Bx
590	88.26 ± 2.09 Aa	85.69 ± 3.97 Aa	88.64 ± 1.35 Aax	88.32 ± 1.49 Abx	88.64 ± 1.35 Ax	88.39 ± 2.27 Ax
600	88.43 ± 2.03 Aa	86.08 ± 3.93 Aa	88.86 ± 1.29 Aax	88.67 ± 1.45 Abx	88.86 ± 1.29 Ax	88.69 ± 2.23 Ax

Table 4.2. Transmittance (%) of collagen casings and CH casings and films before and after exposure to sausage manufacturing conditions (SMC).

A-B indicate significant differences ($p \leq 0.05$) caused by SMC; a-b indicate significant differences ($p \leq 0.05$) between casings; x-y; indicate significant differences ($p \leq 0.05$) between casing and film.

	WVP x 10^{-13} (kg m/m^2 sec Pa)		OP x 10^{-10} (cm^3 m/m^2 sec Pa)		LSP x 10^{-14} (kgm/m^2secPa)
	Before SMC	After SMC	Before SMC	After SMC	Before SMC
Collagen casing	13.3 ± 1.7 Aa	12.1 ± 2.8 Aa	12.1 ± 6.1 Aa	279.8 ± 110.5Ba	10.3 ± 1.5 a
Chitosan casing	6.0 ± 0.8 Abx	6.1 ± 3.8 Abx	0.01 ± 0.003 Abx	7.6 ± 4.4 Bbx	2.8 ± 0.4 bx
Chitosan film	6.0 ± 0.8 Ax	1.1 ± 0.3 By	0.01 ± 0.003 Ax	22.2 ± 22.6 Ax	2.8 ± 0.4 Ax

Table 4.3. Water vapor permeability (WVP) and oxygen permeability (OP) of collagen casings and CH casings and films before and after exposure to sausage manufacturing conditions (SMC).Liquid smoke permeability (LSP) of collagen casings and CH casings and films before SMC.

A-B indicate significant differences ($p \leq 0.05$) caused by SMC; a-b indicate significant differences ($p \leq 0.05$) between casings; x-y; indicate significant differences ($p \leq 0.05$) between casing and film.

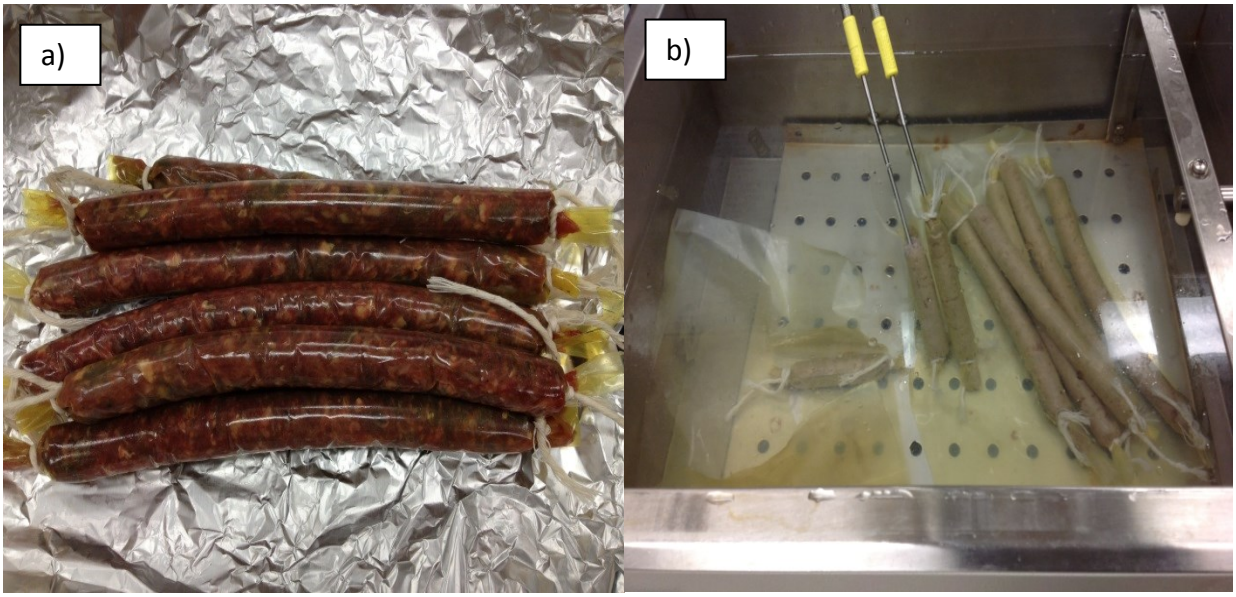


Figure 4.1. Sausages stuffed CH casing before cooking a) and b) during cooking in a water bath

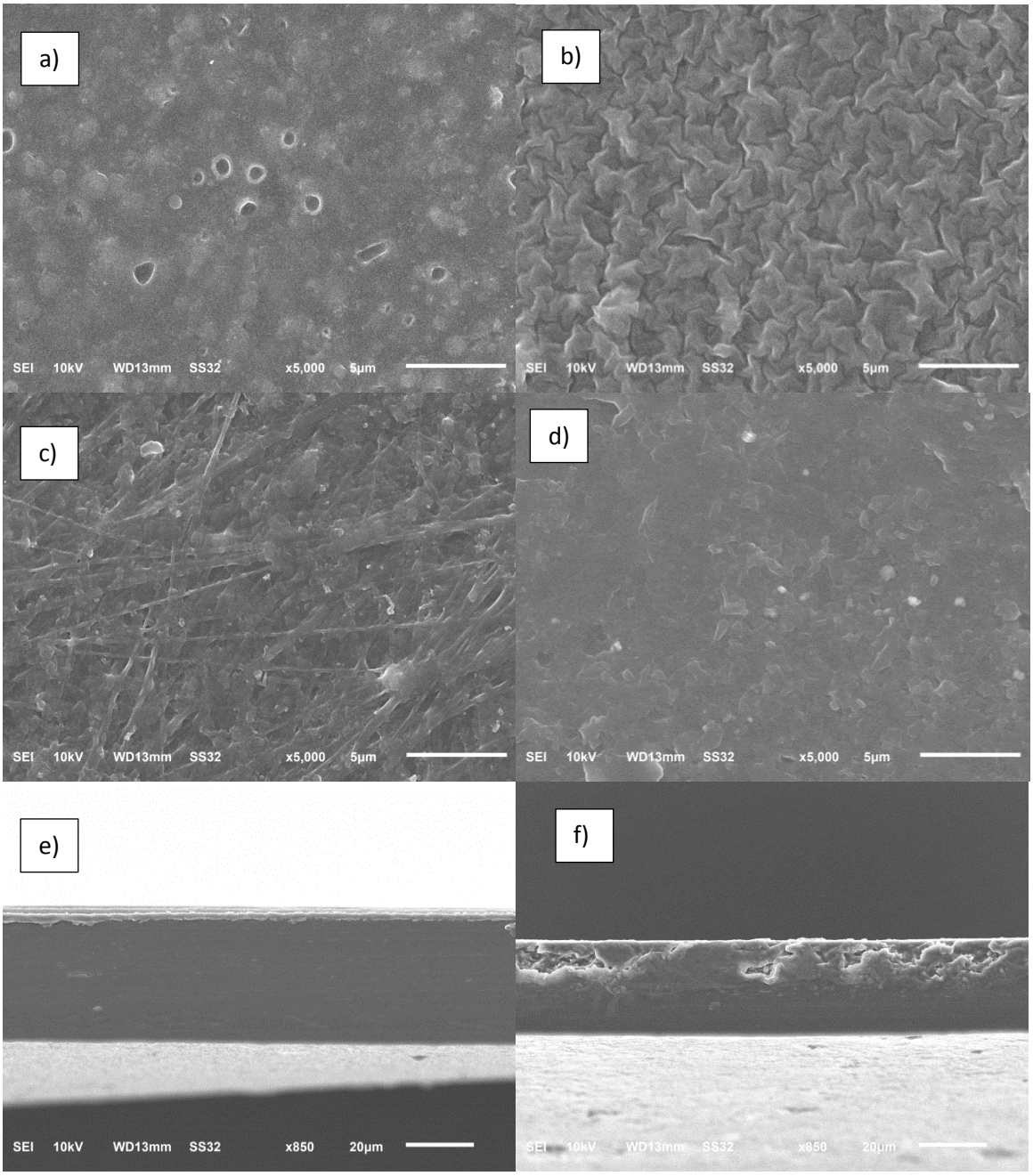
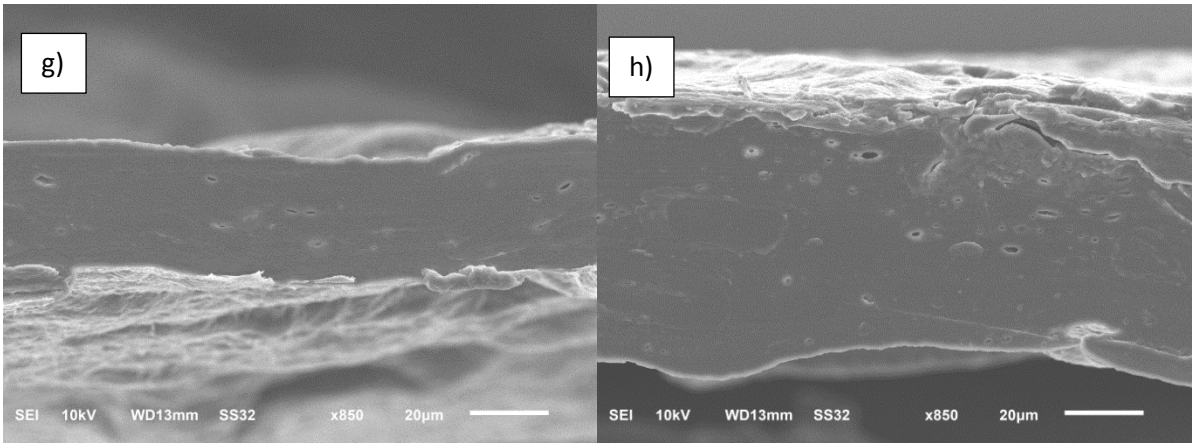


Figure 4.2. SEM micrograph of the surfaces and cross-sections of the CH and collagen casings before and after exposure to sausage manufacturing conditions: a) surface CH casing before, b) surface CH casing after, c) surface collagen casing before, d) surface collagen casing after, e) cross-section CH casing before, f) cross-section CH casing after, g) cross-section collagen casing before and h) cross-section collagen casing after.

Figure 4.2. (cont'd)



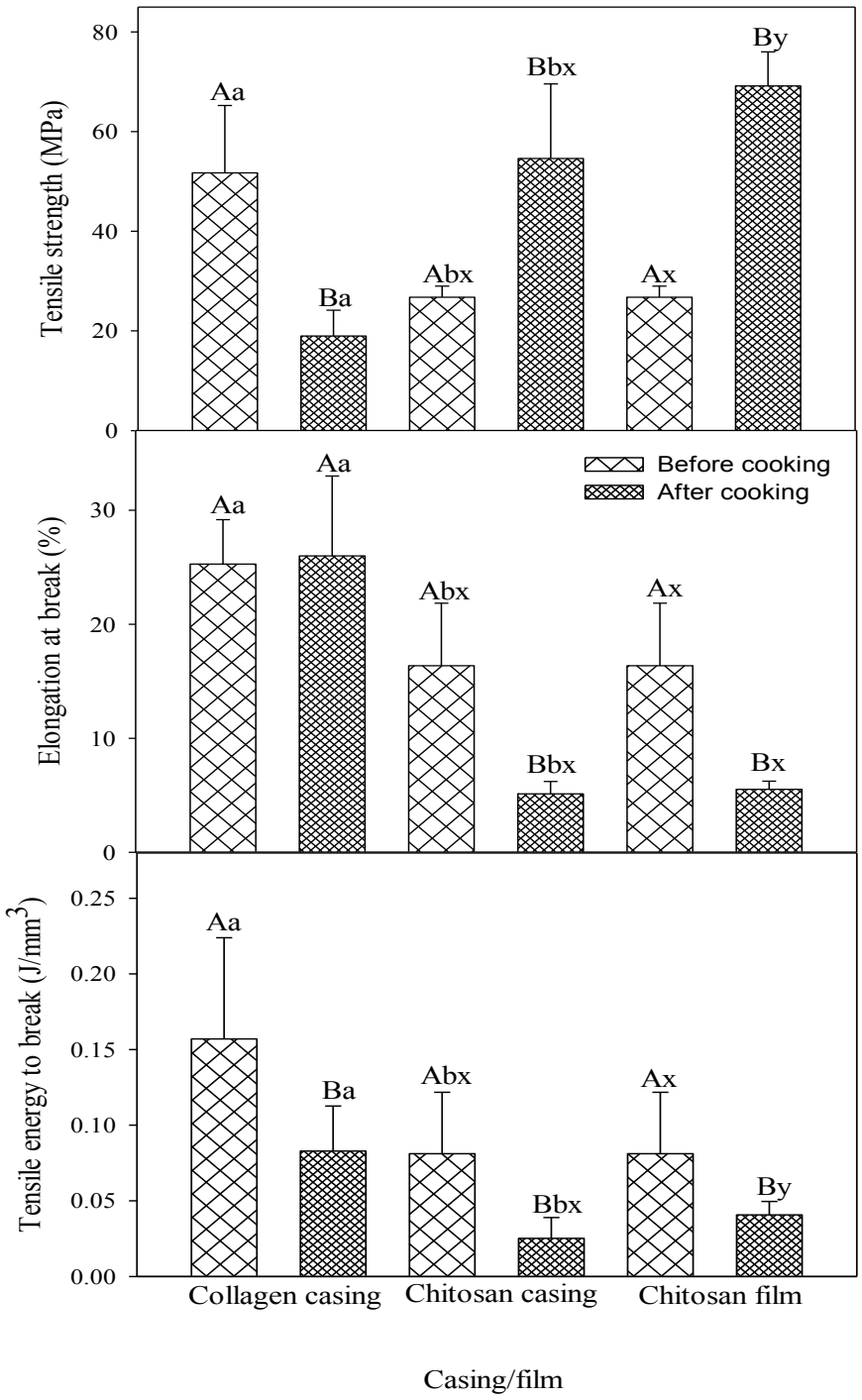


Figure 4.3. TS, %E and TEB of collagen and CH chitosan casings and CH films.

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CHAPTER 5

CONCLUSIONS AND CONSIDERATIONS FOR FUTURE RESEARCH

5.1 Conclusions

The results of Chapter 3 show that, CH films differed in physical and mechanical properties depending on the GLY and CA contents but not the T80 content. The differences were caused by the interactions between CH, GLY, and CA as identified by FTIR spectrophotometry. Among the different CH films, CH film containing 50% GLY and 2.2% CA and 0.2% Tween 80 showed lower water solubility and the same mechanical properties as the collagen casing. This film also had superior transparency and UV light-barrier compared to the collagen casing. Therefore, tailoring CH with adequate amounts of CA, GLY and T80results in a film with physical and mechanical properties similar to or better than those of the commercial collagen casing. However, further studies are needed to shape the films into casings and to validate the commercial feasibility of these casings under traditional sausage manufacturing conditions.

The results of Chapter 4 demonstrated that, the overall properties of the chitosan casing were less affected than those of the collagen casing during the sausage manufacturing conditions and have a better protection for raw meat and cooked product. Compared to the collagen casing, the chitosan casing showed a better barrier to water, oxygen, liquid smoke and UV light, which would extend shelf life due to the less lipid oxidation and water loss. In mechanical and other properties, the chitosan casing had higher tensile strength, lower elongation at break and tensile energy to break, and better transparency while the value of water solubility was similar to that of the collagen casing. These results suggest the potential of chitosan casing as an alternative or better choice than current collagen casings for the manufacture of sausages. Further studies are needed to improve the mechanical properties of the chitosan casing and generate additional information for product quality improvement.

5.2 Considerations for future research

There are several approaches that can be conducted to improve the properties of chitosan-based casings and to validate if the casings are suitable to be produced and used at industrial scale:

1. The mechanical properties of the chitosan-based casing, especially its elongation at break (%E), need to be similar to or better than those of commercial collagen casings in order to make sure that the chitosan-based casing can be used at industrial scale.
2. Shelf life studies of sausages encased with chitosan-based casings including antimicrobial (both pathogen and spoilage microorganism), antioxidant and sensory evaluations need to be conducted.
3. Studies on the migration of both, glycerol (plasticizer) and cinnamaldehyde, under sausage manufacturing conditions need to be conducted.
4. Processing of sausages encased with chitosan-based casings in the smokehouse according to commercial sausage processing method.
5. Development of a chitosan casing processing line for sausage manufacturing.