PAPER

CO-EXTRUSION OF COLLAGEN CASINGS. EFFECTS OF PREPARATION, BRINING, AND HEATING ON STRENGTH, RHEOLOGY AND MICROSTRUCTURE

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ABSTRACT

Properties of five collagen preparations were investigated to enhance understanding of in-line co-extrusion casing formation. The first study revealed that 30% NaCl and 5 min brining provided maximum strength. The second study showed 100% difference in tensile strength between preparations; when adjusted to % protein, the difference was smaller but still existed. Extrusion force and elastic modulus (G') also varied; appear to be acid dependent. Denaturation temperature of raw dispersions was between 36.7 and 38.9˚C. Upon salt brining, it substantially increased to 63.3 - 65.3˚C. Polarized light microscopy revealed numerus intact segments of connective tissue fibers and some cellulose fibers.

Keywords: casings, collagen, dispersions, films, mechanical properties

1. INTRODUCTION

A sausage casing is an essential component in the transformation of comminuted meat into a finished product. In the past 100 years there have been a number of technologies that helped improve processing, handling and functional properties of casings' uniformity, hygiene, strength, flexibility and stability during storage (OSBURNE, 2000; SAVIC and SAVIC, 2016). Before the early Twentieth Century almost all sausages were produced with natural casings, derived from animal intestines. Although natural casings are still considered the 'gold standard', advances in casing technology have led to different types of engineered casing. Today, casings are produced with various materials, ranging from regenerated biopolymers (e.g., collagen, cellulose) to thermoplastic materials (e.g., polyvinyl alcohol polymers). Manipulation of these materials has made it possible to tailor casings with specific functional attributes (WANG, 1986; BARBUT, 2015).

Until recently, modern casings fabrication required specialized production facilities due to the complexity of the process (SAVIC and SAVIC, 2016; KARMAS, 1974). The fairly recent development of co-extrusion has eliminated the need to prefabricate, and store casings prior to stuffing. Co-extrusion is a continuous operation of sausage production, where a thin layer of casing material is extruded on the comminuted meat" rope" coming out of the stuffer. Immediately after formation, the casing material undergoes stabilization, through dehydration and/or cross-linking to enhance its mechanical strength. A brine application (spray, drip, immersion) dehydrates the casing and allows the material to conform to the shape of the meat. Further stabilization is commonly performed through cross-linking (e.g., employing the aldehyde in liquid smoke) and air-drying as they both help provide the mechanical strength needed during linking, cooking and packaging (MORGAN *et al*., 1988).

Co-extruded casings can be produced with a collagen gel of fibrous and soluble collagenous material or a dispersion of a hydrocolloid gum such as alginate (MORGAN *et al*., 1988; HARPER *et al*., 2013). The collagen is typically derived from cattle hides and is mainly composed of collagen (RATANAVARAPORN *et al., 2008*). Generally speaking, collagen casing production involves corium separation, decalcification and regeneration. During collagen separation, hides are washed and limed (pH 11 to 13) to remove impurities. Calcium is later removed to promote uniform swelling of collagen fibrils and then the material is chopped and ground. Following decalcification, collagen undergoes regeneration where swelling is promoted via the use of acids (SAVIC and SAVIC, 2016). In commercial products, hydrochloric acid (HCl) is the most commonly used swelling agent, but other acids such as acetic are also used (RATANAVARAPORN *et al., 2008*). Overall, this is a lengthy (few weeks) and complicated process involving quite a few steps; some are proprietary. Prior to extrusion (i.e., at the meat processing plant), the material is mixed one last time to reorient the fibers to improve film strength (SAVIC and SAVIC, 2016; BARBUT, 2010).

Although one of the first patents for collagen co-extrusion technology was introduced about 50 years ago, the technology has not been used on a large commercial scale for the following 30-40 years. Only recently large dedicated plants, for in-line co-extrusion, have been constructed. Today there is still very little published information about the topic (e.g., collagen, composition, viscosity, shear thinning) and even basic data about the effect of raw material source (e.g., animal age, breed), processing parameters during extraction (e.g., type of acid used), and conditions during application (e.g., drying, cross-linking). However, there has been some effort made to understand the properties of certain co-extruded collagen films and the effects of different additives (TOMIHATA *et al.,* 1994;

OLDE DAMINK *et al.*, 1995; O'SULLIVAN *et al.*, 2006; WOLF *et al.*, 2006; HARPER *et al.*, 2012; OESCHSLE *et al.*, 2014; OESCHSLE *et al.*, 2016; BARBUT and IOI, 2019). Overall, the properties of prepared collagen dispersions and processing conditions of co-extrusion casing production needs more attention. Today the co-extrusion process is highly automated and as such also helps to improve food safety and reduce waste (OSBURN, 2000; BARBUT, 2015). The main goal of this research was to evaluate different processing conditions that can be used to partially dehydrate collagen films. The study is believed to be the first to look at the differences between commercially prepared collagen dispersions that are used in the production of co-extruded meat products. Differences were evaluated by studying the collagen dispersions and films' mechanical, microstructure and thermo-mechanical properties. The results should help researchers and manufacturers to better understand co-extrusion through material selection and process controls.

2. MATERIALS AND METHODS

2.1. Collagen dispersions

Collagen dispersions were evaluated and identified as Collagen 1 through 5 (C1, C2, C3, C4, C5), as they are proprietary blends (see explanation in the Introduction). Information that was provided to the researchers, by the two major manufacturers in the world, and compositional analysis can be found in Table 1 (note: manufacturers did not want to provide any specific information on product number/identity as some of the dispersions are still being modified). Protein content was determined in triplicate by the Dumas method (FP528, Leco, St Joseph, MI) using a nitrogen factor 6.25. Moisture content is reported as the moisture lost when drying (Oven 650G, Fisher Scientific, Toronto, ON) from 5 g samples heated at 105˚C for 24 h.

Table 1. Commercial collagen dispersion specifications and the work needed for extrusion (i.e. measurement of resistance to flow) through a 7mm die: C1 (Collagen 1), C2 (Collagen 2), C3 (Collagen 3), C4 (Collagen 4) and C5 (Collagen 5).

Collagen	pH	Moisture Content		Protein (%)	Swelling Agent[a]	Work of Extrusion[b] (Nm)	Work of Extrusion[b] (Nm/%protein)
		Dispersion (%)	Film (%)				
C1	2.06	93.6	74.4	5.14	-	5.16±0.19	1.00±0.04
C2	2.21	95.7	77.8	3.57	-	3.19±0.21	0.89±0.06
C3	2.01	93.6	74.5	3.68	HCl	4.04±0.05	1.10±0.01
C4	2.67	94.1	74.7	4.37	HCl/Acetic Acid	3.63±0.01	0.83±0.01
C5	2.04	93.7	76.9	4.82	-	4.04±0.13	0.84±0.03

[a]Swelling agent as reported by the manufacturer (see text). [b]n = 9.

2.2. Partially dehydrated film formation

The method of film formation was adapted from HARPER *et al.* (2013) who worked with alginate solutions. Briefly, collagen dispersions were first cooled to 4˚C to reduce the

adhesiveness during film formation. The collagen dispersions were also degassed using a vacuum packaging machine (Multivac Canada Inc., Woodbridge, ON) at 7.3 kPa for 25 s, 50 s and 75 s, consecutively (settings 4, 6 and 8, respectively). This was performed to remove gas bubbles that were incorporated during processing, as they can create weak spots in the films. Following the degassing stage, dispersions were mixed to assure homogeneity of the samples (dispersions were mixed by rolling the material, placed in plastic bags, 10 times in adjacent directions).
Later, approximately 3 g portions of the collagen were rolled on a stainless steel board between two layers of plastic sheets with a stainless steel roller. The roller had a recess of 0.50 mm in order to achieve uniform film thickness. The top plastic sheet was removed and the film was then placed in a salt bath on the remaining plastic sheet. The first study was performed with one collagen sample (C2), to evaluate the effects of brine concentration and contact time on the textural properties of the films (note: C2 was selected as it is one of the most popular dispersions used by the industry). Brine solutions consisted of 15, 20, 25 and 30% NaCl in deionized water. Films were immersed in the brine for 1.0, 2.5, 5.0 and 10.0 min intervals. After formation, films were covered again with a plastic sheet to prevent further dehydration before testing.
After establishing the salt concentration and time (see data and conclusions in Discussion), the second study evaluated all five dispersions and their films, which were dehydrated in 30% NaCl brine for 5 min. These conditions ensured that the films were strong enough to be removed from the plastic sheet and tested.

2.3. Extrusion force of collagen dispersions

The collagen dispersions were evaluated by using a texture analyzer (TA-XT2i, Texture Technologies Corporation, Scarsdale, NY) with a forward extrusion fixture (TA 93, Texture Technologies). Approximately 50 g samples were loaded into the cell (100 mL capacity) fitted with a 7 mm opening die, and brought to 4˚C. The plunger compressed the dispersion at a rate of 1 mm/s. From the generated force-distance curve (n = 9 per dispersion) the work of extrusion was calculated once the readings had stabilized (i.e., after pushing down about 10-35 mm).

2.4. Mechanical properties and film thickness

A standard method for testing film's tensile properties (ASTM, 2010) was performed on the partially dehydrated films. Films were evaluated by using the texture analyzer (TA-XT2i, Texture Technologies) equipped with grippers that were set at a distance of 50 mm, trigger force of 5 g, test speed of 2 mm/s, and an overall test distance of 25 mm. The film's thickness was determined by measuring each film at the top, center and bottom using a digital micrometer (Testing Machines Inc., Islandia, NY). The films were cut into 75 mm × 25 mm strips (JDC Precision Sample Cutter, Thwang-Albert Instrument Comp., Philadelphia, PA). The average thickness and width of the films were used for the tensile stress calculations. From the generated stress–strain curve, the tensile strength (maximum stress the film endured prior to breaking) and the percent elongation (maximum elongation the film reached prior to breaking) were determined. A total of eighteen films were tested for each of the treatments (six films per each of the three trials).
A puncture test was also performed with the texture analyzer. In this test, a 5 mm diameter ball probe was used to puncture films mounted in a film extensibility fixture, which has circular opening of 10 mm diameter (TA-108S5, Texture Technologies). The test

speed was 1 mm/s and the trigger force was set to 5 g. The distance to puncture and work of puncture were determined from the generated force - distance curve. A total of eighteen films were tested for each of the treatments (six films per trial).

2.5. Light microscopy

Collagen dispersions were prefixed in 10% neutral buffered formalin for 10 h at room temperature and then dehydrated in 70% isopropanol for 2 h, 95% for 1 h, and 100% for 4 h. The dehydrated samples were dipped in xylene, prior to embedding in paraffin. Samples were cut into 4-6 μm cross sections. Masson stain was used to differentiate collagen from other meat proteins. In another set of sections, Periodic-Acid Schiff (PAS) stain was used to differentiate carbohydrates, specifically cellulose fibers.
A light microscope (Olympus BX 60, Olympus Corporation, Centre Valley, PA) was used to examine the samples. Representative images (a total of six images per treatment) were taken using Image Pro Plus (Version 6.0, Media Cybernetics Inc., Bethesda, MD) software.

2.6. Rheology of film forming dispersion

Rheological analysis was performed on the collagen dispersions using a Bohlin CS50 (Malvern Instruments Ltd, Worcestershire, UK) with a 25 mm DIN coaxial cylinder bob and cup fixture. The bob was lowered into 13 g of collagen that was preloaded into the bottom of the cup. Excess collagen was removed and mineral oil was then applied on the top to keep the exposed surface from drying. The temperature of the collagen was increased from 20° to 55°C at 1.25°C/min, held for 2 min and returned to 20°C, at the same rate. Continuous oscillating shear (1 Hz and 0.0012 strain) was applied during testing. Test parameters were determined in a pre-trial to evaluate the linear range. The elastic modulus (G') was recorded (Bohlin Zetasizer Series software, version 6.32, Malvern Instruments Ltd.) and used to determine the changes in stiffness of the dispersions (HELARY *et al.*, 2009).

2.7. Differential Scanning Calorimetry (DSC)

The melting profiles of the collagen dispersions and partially dehydrated films were evaluated using a differential scanning calorimeter (DSC Q2000, TA Instruments, New Castle, DE). Samples (~10 mg) were placed in anodized-aluminum hermetically sealed pans. Temperature was ramped from 20° to 80°C at a rate of 5°C/min. Samples were then held at 80°C for 2 mins and then cooled back to 20° at 5°C/min. The same thermal profile was used to rescan samples for identifying reversible peaks. The melting behavior was studied between 30° and 80°C by integrating the endothermic peak (TA Universal Analysis 2000 Software, TA Instruments) to determine the onset temperature, melting temperature and enthalpy. A total of three dispersions or films were tested for each of the treatments.

2.8. Amino acid analysis

Collagen dispersion's amino acid profile was analyzed (Advanced Protein Technology Center, Toronto, ON). Approximately 0.01 g of each collagen dispersion was weighed. HCl (6N) and norleucine (internal standard) were added to each sample and then

hydrolyzed for 24 h at 110˚C. After hydrolysis, an aliquot was transferred to another tube for derivatization.

2.9. Experimental design and statistical analysis

The experiment used to compare the different dispersions was designed as a complete randomized block with three independent trials. Each trial consisted of six measurements, per dispersion, for the mechanical properties of the films (tensile and puncture tests). The statistical analysis was performed using SAS Version 9.2 (SAS Inst., Cary, NC). A General Linear Model was used for the analysis of variance (ANOVA). The film type means and interactions were compared by using Tukey's multiple comparison analysis ($P \leq 0.05$). For setting up dehydration conditions (salt concentration and time) the results from dispersion C2 are reported as averages and standard deviations.

3. RESULTS AND DISCUSSION

3.1. Mechanical properties

During formation and stabilization of co-extruded casings, the newly produced sausage is exposed to a variety of different stresses (e.g., moving on a conveyer belt, vibration). It is therefore crucial to impart sufficient strength shortly after extrusion so that the sausages can also undergo subsequent treatment, such as smoking, drying and cooking (KOBUSSEN *et al.,* 2012). In an industrial setting, the newly formed casings are stabilized by first removing some of the water through brining. Dehydration is driven by osmosis, which helps to increase the density of the collagen fibers and thus shortening the distance among collagen molecules to help improve the mechanical stability of the casing (KOBUSSEN *et al.*, 2000; VISSER, 2012). In the first study we established the best conditions for the brining procedure by evaluating the effects of brine concentration (15 to 30% NaCl) and brining time (1 to 10 min) using a dispersion that is widely used by the industry (Collagen 2). The overall settings used were based on guidelines described in several industry protocols (KOBUSSEN *et al.,* 2000). In order to evaluate the mechanical properties of the wet films, (i.e., as applied to the sausage), tensile and puncture tests were performed (Fig. 1).

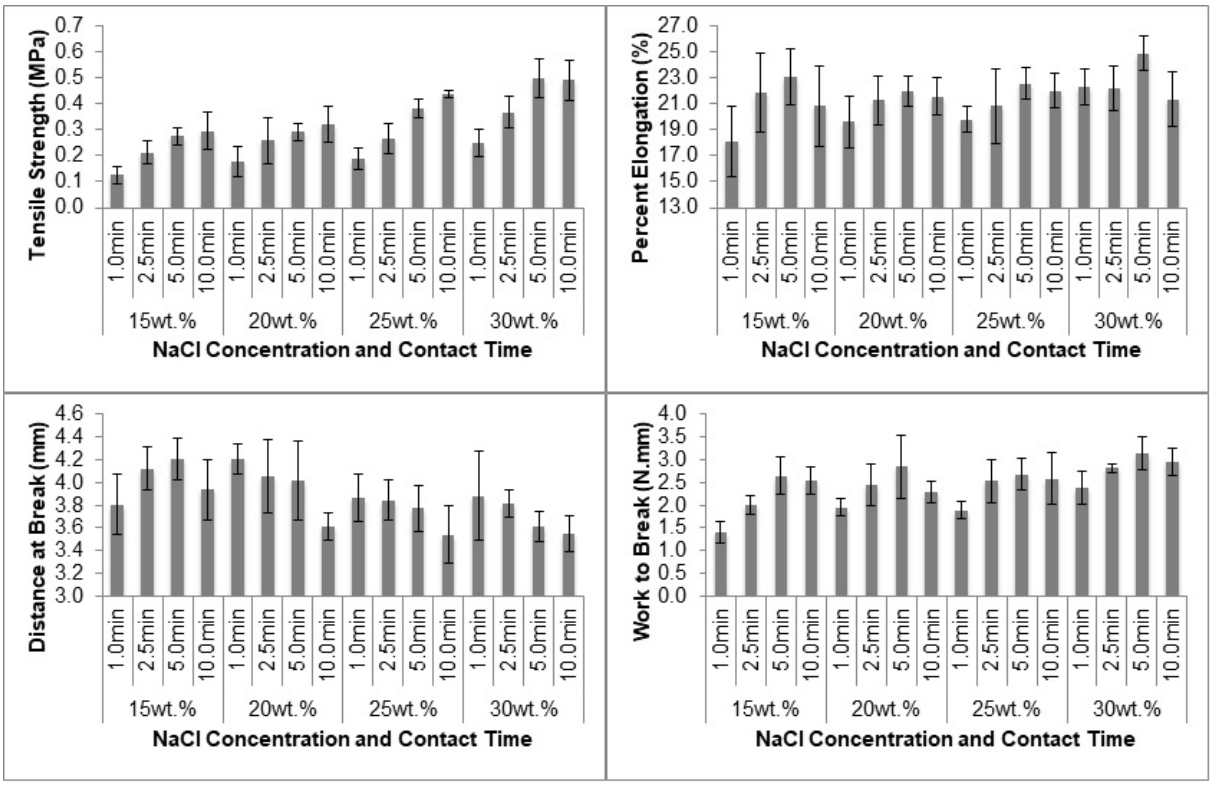

Figure 1. Mechanical properties of collagen films (Collagen 2) produced with increasing concentration of NaCl and contact time (n=18).

Mechanical testing demonstrated that there were no significant interactions ($P>0.05$) between the brine concentration and contact time. Overall, there were some significant differences ($P<0.05$) in the film's mechanical properties when modifying the salt concentration or contact time (Tables 2 and 3). Tensile strength, percent elongation and work to break increased with raising salt concentration and contact time. This indicates that there is a need for a certain level of dehydration to stabilize the collagen network in the films. These results also demonstrate that processors would be able to modify the mechanical properties of their casings by altering the concentration or exposure time.

Table 2. Mechanical properties of collagen films (Collagen 2) produced with increasing concentration of NaCl. Means were averaged across contact times; 1.0, 2.5, 5.0, 10.0 min.

Concentration (%)	Tensile Strength[1] (MPa)	Percent Elongation[1] (%)	Distance to Break[2] (mm)	Work of Break[2] (Nmm)
15	0.23^{c}	21.0^{a}	4.0^{a}	2.2^{b}
20	0.26^{bc}	21.1^{a}	4.0^{ab}	2.4^{b}
25	0.32^{b}	21.3^{a}	3.8^{b}	2.4^{b}
30	0.40^{a}	22.7^{a}	3.7^{b}	2.8^{a}

[1]Tensile test.

[2]Puncture test.

Means, within a column, followed by a similar letter are not significantly different (P>0.05).

Table 3. Mechanical properties of collagen films (Collagen 2) produced with increasing contact times to NaCl. Means were averaged across concentrations; 15, 20, 25, 30% NaCl.

Time (min)	Tensile Strength[1] (Mpa)	Percent Elongation[1] (%)	Distance to Break[2] (mm)	Work of Break[2] (Nmm)
1.0	0.19^{c}	20.0^{b}	3.9^{a}	1.9^{b}
2.5	0.28^{b}	21.5^{ab}	4.0^{a}	2.4^{a}
5.0	0.36^{a}	23.1^{a}	3.9^{ab}	2.8^{a}
10.0	0.39^{a}	21.4^{ab}	3.7^{b}	2.6^{a}

[1]Tensile test.

[2]Puncture test.

Means, within a column, followed by a similar letter are not significantly different (P>0.05).

Studying the effects of dehydration conditions helped us to select the best conditions for processing the different commercially prepared dispersions. The higher salt concentration was selected for the evaluations because it provides the most strength and thus helps avoid damaging the casings during further processing. Overall, the film produced with 30% salt had the highest tensile strength and work of puncture (P<0.05), across all contact times (Table 2). This is also the salt concentration currently used in a number of the industrial settings. Although this salt concentration is prepared at the beginning of the day, processors must ensure that it is maintained, because over time it will be diluted (i.e., moisture coming out from the casings; (KOBUSSEN *et al.*, 2012)). The dehydration effect can also be seen in the data concerning moisture content in the films after exposure to the brine solution (Table 1). Table 3 shows that there was no significant difference in mechanical properties between 5 and 10 min. Therefore, a 5 min exposure time was selected for use in the follow up evaluation (second study). It should be mentioned that under commercial conditions, exposure times have been reported to be even shorter (BONTJER *et al.*, 2011). This is usually due to production pressures. In any case, the products are not rinsed after the industrial brining process so high salt concentration remains on the surface.

In the second study, the properties of the actual films produced from the other commercial collagen dispersions were investigated. Overall, there were some significant differences in the tensile strength and percent elongation among the five preparations (Table 4). Films produced with C4 had the lowest tensile strength and percent elongation of the protein films (0.15 MPa and 16.33%, respectively). As will be discussed below, these observations may be correlated to the sample's protein content, collagen structure, acid used for swelling, and the overall dispersions' composition.

The mechanical properties of the partially dehydrated films were also compared after adjusting for protein concentration (Table 5), to examine the effect of protein content (i.e., important for processors dealing with cost per unit). These data reveal differences in the tensile strength, distance at break and work to break; therefore, it would appear that differences among the dispersions are influenced by more than just protein content (assuming protein content affects mechanical properties linearly). It should be noted that meat processors use the dispersions as they arrive to the plant (i.e., no dilution or ingredients added) and therefore cost per unit is critical for them.

Table 4. Mechanical properties of partially dehydrated films: C1 (Collagen 1), C2 (Collagen 2), C3 (Collagen 3), C4 (Collagen 4) and C5 (Collagen 5).

Collagen	Tensile Strength[1] (MPa)	Percent Elongation[1] (%)	Distance at Break[2] (mm)	Work to Break[2] (Nm)	Thickness (mm)
C1	0.33^{a}±0.03	24.38^{a}±3.82	6.12^{a}±0.51	3.72^{a}±0.88	0.43^{a}±0.01
C2	0.29^{ab}±0.05	18.57^{ab}±2.42	5.57^{a}±0.41	2.75^{ab}±0.48	0.37^{b}±0.00
C3	0.19^{cd}±0.02	19.22^{ab}±2.35	5.26^{a}±0.47	1.99^{b}±0.41	0.41^{a}±0.01
C4	0.15^{d}±0.03	16.33^{b}±1.37	5.33^{a}±0.42	1.89^{b}±0.34	0.41^{a}±0.02
C5	0.24^{bc}±0.02	23.42^{a}±1.81	5.91^{a}±0.24	1.81^{b}±0.26	0.42^{a}±0.01

[1]Tensile test.

[2]Puncture test.

Means, within a column, followed by a similar letter are not significantly different (P>0.05).

Table 5. Comparing mechanical properties of partially dehydrated films: C1 (Collagen 1), C2 (Collagen 2), C3 (Collagen 3), C4 (Collagen 4) and C5 (Collagen 5) based on adjusting percent protein.

Collagen	Tensile Strength[1] (MPa/%protein)	Percent Elongation[1] (%/%protein)	Distance at Break[2] (mm/%protein)	Work to Break[2] (Nm/%protein)
C1	0.046^{b}±0.00	3.30^{a}±0.52	0.83^{c}±0.07	0.50^{ab}±0.12
C2	0.061^{a}±0.01	3.94^{a}±0.51	1.18^{a}±0.09	0.58^{a}±0.10
C3	0.035^{bc}±0.00	3.51^{a}±0.43	0.96^{bc}±0.09	0.36^{bc}±0.07
C4	0.029^{c}±0.01	3.12^{a}±0.26	1.02^{ab}±0.08	0.36^{bc}±0.07
C5	0.037^{bc}±0.00	3.57^{a}±0.28	0.90^{bc}±0.04	0.28^{c}±0.04

[1]Tensile test.

[2]Puncture test.

Means, within a column, followed by a similar letter are not significantly different (P>0.05).

Meat processors must also consider the extrusion properties of the dispersion so that they can adjust the co-extrusion equipment. It is interesting to note that today most collagen suppliers do not provide that information. In the current study, the forward extrusion test was performed to provide insight into whether the five dispersions show differences in flow behaviour. Overall, the values varied by as much as 60% (Table 1). It appeared that samples with lower pH (C1, C3 and C5) required a higher work of extrusion. When the values were adjusted to %protein, C1 and C3 were still the highest. It may suggest that a greater degree of conformational changes increases the stiffness of the dispersion. The conformational changes discussed are a result of lowering the pH from 5 to 2 (away from isoelectric point of 8.26 and 4.56 for collagen and collagen hydrolysate, respectively), which has been reported to increase fiber hydration and swelling (WOLF *et al.*, 2006; OESCHSLE *et al.*, 2014). OESCHSLE *et al.* (2014) demonstrated that collagen entanglement depends strongly on the pH as well as acid used, and indicated that entanglement increases with lowering pH values below the isoelectric point.

Lower tensile strength and elasticity could also be the result of excessive alkaline modification during corium separation. If alkaline modification, or liming, is not controlled then the extracted collagen may be of low molecular weight, which will not

contribute as much to the regeneration of collagen structures (SAVIC and SAVIC, 2016). These authors also indicated that intact fibrillar structures produce higher strength and elasticity in collagen casings.
Commercially prepared collagen dispersions are sometimes modified by the addition of functional ingredients, such as fillers (e.g., cellulose), plasticizers (glycerol), cross-linking agents (smoke condensate), as well as colourants (BARBUT and IOI, 2019). The combination of collagen (commonly used at 3 to 8%) and other modifiers (ranges from 0 to 10%) typically results in dispersions with 3 to 25% dry matter (KOBUSSEN *et al.*, 2000). With such a wide range, one can expect quite a lot of variation among dispersions. In the dispersions evaluated here (representing some of the most commonly used by the industry) dry matter ranged from 4.3 to 6.4% (Table 1).

3.2. Light microscope imaging

Light microscopy was used to identify and characterize the homogeneity and condition of the fibers within the collagen dispersion. As previously discussed, collagen dispersions are mixtures of soluble and insoluble collagen. The proportion is affected by the origin of the material, method of extraction (e.g., extent of liming) and processing (e.g., degree of chopping). Masson trichrome stain was used to differentiate collagen from other proteins, and also help study the condition of the collagen fibers in relation to their mechanical and thermo-mechanical properties. Since cellulose was added to some of the samples, PAS stain was also used to identify and characterize carbohydrate fillers (CARSON, 1997) found in the dispersions. Overall, cellulose fibers are one of the most commonly used additives, as the fibers can contribute to the film's strength and elasticity. MATHEW *et al.* (2012) observed that physical entanglements of cellulose nanofibers could increase the tensile strength of dried collagen films. It appears that some of the dispersions studied here contained a certain amount of cellulose (see micrographs below).
The micrographs (Fig. 2) show some differences in the proportions of long and short fibers, as well as the homogeneity of the collagen network (i.e., shown as areas of varying stain intensity throughout the network) among the dispersions. Collagen 3 (C3) appeared to have the greatest homogeneity and as previously mentioned required the highest work of extrusion (1.10 J/% protein) (Table 1). Therefore, the work of extrusion may be attributed to the homogeneity. HELARY *et al.* (2009) also reported that areas of non-homogeneity affect the mechanical properties of collagen hydrogels. As can be seen, collagens C2 and C5 appeared to have more small circular pockets, which may be small air bubbles that stayed within the dispersions.
All of the dispersions show insoluble fibers (some show typical birefringence, as can be seen on the right side of Fig. 2), of pretty much similar size range and morphology. The fibers are suspended in the soluble collagen network (i.e., background stained blue by the Masson stain). Some of the insoluble fibers were identified as cellulose because they had a ribbon-like morphology with twists down their length (REDDY and YANG, 2004; CRANSTON and GRAY, 2008). The ribbon-like fibers also picked up the PAS carbohydrate stain, providing further evidence that they are cellulose (Fig. 3). It is interesting to note that the stained material on the interface between the collagen matrix and cellulose appeared to be darker (Fig. 3). This may indicate that collagen might have developed interfacial interactions with some of the cellulose fibers. Overall, commercial collagen dispersions often have 0.5% cellulose fiber. Since there does not appear to be major differences in the cellulose concentration, the differences in mechanical properties may not be attributed to the addition of cellulose.

3.3. Thermo-mechanical Properties

Understanding collagen melting temperatures is very important to the meat processors because they often cook the sausages in the co-extruded casings. The rheological tests demonstrated that between 30˚C and 40˚C all of the dispersions start to display a rapid decrease in elasticity (Fig. 4). It was observed that the collagen samples with higher pH (Table 1; C4 and C2) began to lose their elasticity at a higher temperature. This may be attributed to the fact that collagen undergoes significant conformational change as one lowers the pH from 5 to 2. As previously mentioned, conformational changes result in increased hydration and swelling (WOLF *et al.*, 2006), therefore this may have reduced the thermal stability of these dispersions.

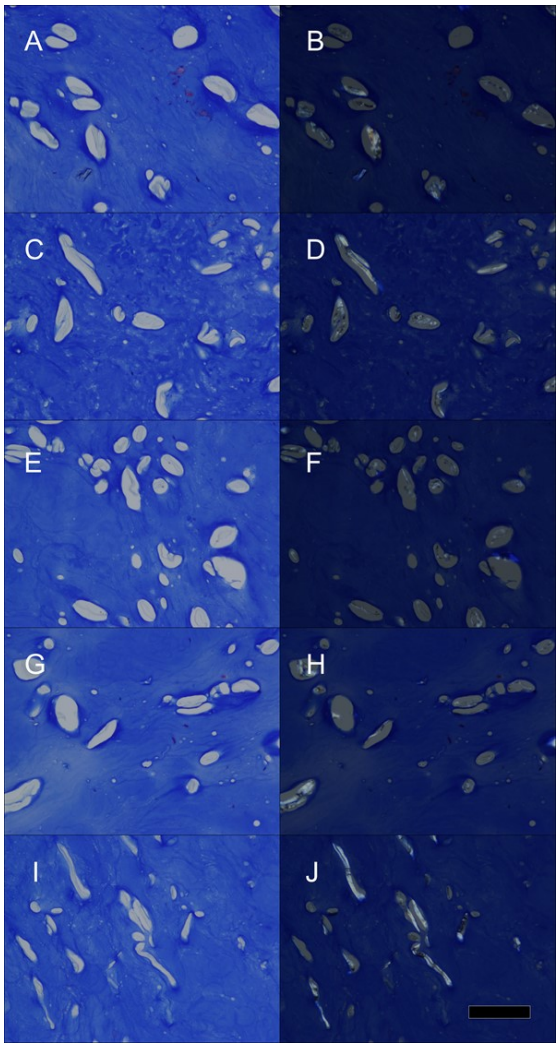

Figure 2. Light micrograph images of commercial collagen dispersions: Collagen 1 under regular illumination (A); Collagen 1 under polarized light (B); Collagen 2 under regular illumination (C); Collagen 2 under polarization (D); Collagen 3 under regular illumination (E); Collagen 3 under polarized light (F); Collagen 4 under regular illumination (G); Collagen 4 under polarized light (H); Collagen 5 under regular illumination (I); Collagen 5 under polarized light (J). Black bar represents 100 μm.

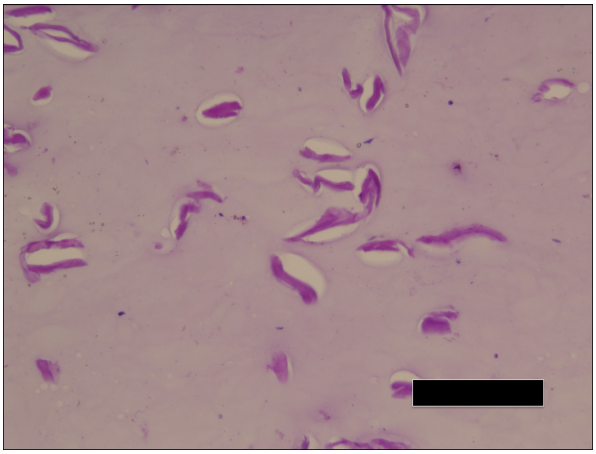

Figure 3. Light microscope image of a commercial collagen dispersion stained with periodic acid schiff. See text for explanation. Black bar represents 100 μm.

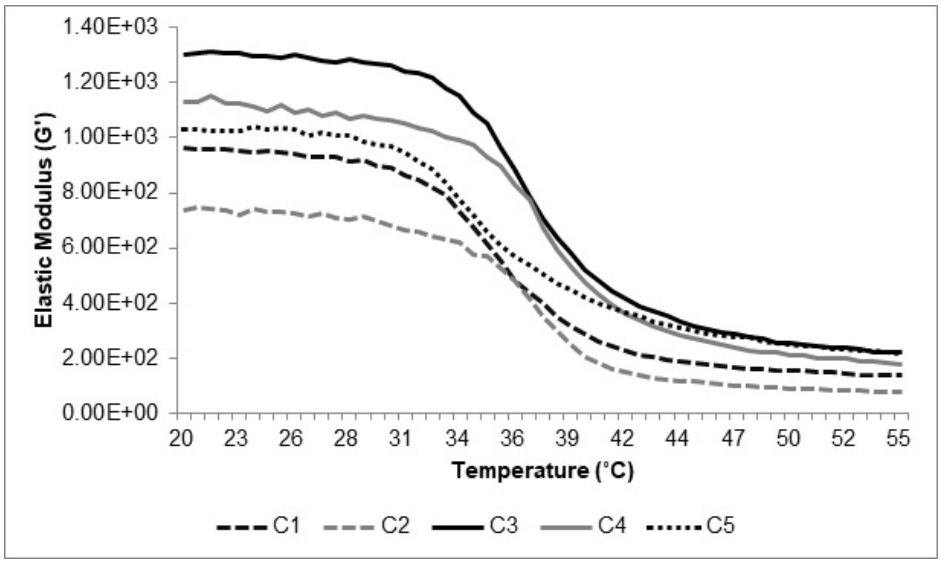

Figure 4. Rheological thermographs (20 to 55˚c at 1.25˚c/min) of five commercial collagen dispersions: C1 (Collagen 1), C2 (Collagen 2), C3 (Collagen 3), C4 (Collagen 4) and C5 (Collagen 5).

DSC scans were performed on the collagen dispersions and partially dehydrated films. The collagen dispersions exhibited an endothermic peak that started between 33.5 to 35.4˚C, with a maximum at 36.7 to 38.9˚C, and had a denaturation enthalpy of approximately 3.1 to 5.3 J/g (Table 6). Once the initial DSC run was completed the samples were cooled down to 4˚C, before a secondary run was performed to look for irreversible changes. The second run of all dispersions resulted in no endothermic peaks, indicating that irreversible denaturation occurred. This is similar to FRIESS and LEE's (1996) observation of insoluble collagen fibers.

The DSC denaturation temperatures are in the same range as the rheological measurements discussed above. Similar to the rheological observations, the collagen with

lower pH displayed lower thermal stability in the DSC thermograms. The dispersions with a pH closer to 2 (Collagens C1, C3 and C5) appeared to have slightly lower denaturation temperatures. As mentioned before, conformational changes at a lower pH may result in a greater hydration (WOLF *et al.,* 2006). GIOFFRE *et al.* (2011) observed similar thermal denaturation behavior when the pH of wet gelatin films was decreased.
The range of onset temperature values corresponds to the rheological transitions observed (Fig. 4) and therefore confirms that the collagen in these dispersions has been significantly modified during the preparation procedure (extraction of collagen from hides, the liming process, and the follow up chopping and acidification procedures). It should be mentioned that in native collagen samples denaturation transitions are seen in the 60˚C range (BERNAL and STANLEY, 1986).
DSC was also performed on partially dehydrated films (brined with 30% salt) to see if there was any effect on the thermal stability of the brined collagen. Overall, dehydrating the films greatly increased the stability. The thermograms of the films showed an endothermic peak that started between 58.2 to 60.3˚C, with a maximum at 63.9 to 65.3˚C and a denaturation enthalpy of approximately 1.7 and 4.1 J/g (Table 6). These denaturation temperatures are fairly similar to those reported by BERNAL and STANLEY (1986) who examined native bovine tendons, and reported denaturation at 61.55˚C. GIOFFRE *et al.* (2011) also observed an increase in the denaturation temperature when wet gelatin films were dried. Thus the difference between the denaturation temperature of the raw collagen dispersions and the partially dehydrated films is the result of some protein denaturation, higher salt concentration and a decrease in moisture content (i.e. 95 to 75%; Table 1). Fiber assembly may also help explain the increased thermal stability of the partially dehydrated and salted films. MCPHERSON *et al.* (1986) suggested that stronger association of collagen fiber structure is correlated with increased denaturation temperature. It has also been demonstrated that high ionic strength conditions result in a greater degree of packed collagen fibers and assembly (WILLIAMS *et al.*, 1978). Overall, since the ionic strength is raised during film dehydration (salt migration into the film), there may be collagen fiber assembly, resulting in a higher thermal stability of the films.

Table 6. Analysis of endothermic peaks from differential scanning calorimetry (DSC) thermograms. Five commercial collagen samples were tested as collagen dispersions and partially dehydrated/brined films: C1 (Collagen 1), C2 (Collagen 2), C3 (Collagen 3), C4 (Collagen 4) and C5 (Collagen 5).

Collagen	Treatment	Onset Temperature (˚C)	Temperature of Denaturation (˚C)	Enthalpy ΔH (J/g)
C1	Dispersion	33.54±0.21	36.71±0.51	5.33±0.61
C2	Dispersion	34.59±0.15	38.44±0.06	3.05±0.31
C3	Dispersion	34.26±0.01	38.09±0.08	4.12±0.10
C4	Dispersion	35.41±0.11	38.94±0.02	3.93±0.26
C5	Dispersion	33.45±0.10	37.30±0.21	4.45±0.03
C1	Film	59.90±0.23	64.87±0.12	3.07±0.55
C2	Film	58.40±0.21	63.88±0.57	1.76±0.38
C3	Film	60.32±1.61	65.00±0.68	3.05±0.21
C4	Film	58.22±0.24	63.94±0.61	3.06±0.82
C5	Film	58.30±0.40	65.34±0.37	4.19±0.37

3.4. Amino acid analysis

In addition to the protein content, the protein quality may also influence the mechanical and thermal behaviour of the collagen fibers. Analyzing the amino acid profile (Table 7) was done to examine potential correlations between the protein quality and performance of the collagen dispersions. Collagen fiber's properties vary as a result of the formation of cross-links between overlapped collagen molecules. Cross-links between molecules and fibers can be formed via a number of different mechanisms: Schiff base cross-links from enzymatic oxidation (lysyl oxidase), or through non-enzymatic processes like glycation (glucose, lysine and arginine). In general, lysine, glutamic acid and hydroxyl groups have been classified as reactive groups because they project radially from collagen molecules, thus providing sites for intermolecular and interfibrillar cross-links to occur (AVERY and BAILEY, 2008). Overall, there appeared to be some differences in the amino acid makeup of the dispersions (e.g., Lys, Glu, OH-Pro, Arg), but there were no observable trends.

Table 7. Amino acid profile (relative molecular mass) of commercial collagen dispersions.

Amino Acid	Relative Molecular Mass (%)				
	Collagen 1	Collagen 2	Collagen 3	Collagen 4	Collagen 5
Asx (Asp+Asn)	4.6	5.0	5.1	5.3	4.7
Glx (Glu+Gln)	7.5	6.9	7.8	8.2	6.9
OH-Pro	14.1	13.1	14.3	14.1	12.8
Ser	3.9	3.9	3.9	3.9	3.9
Gly	23.1	22.9	22.4	22.2	23.4
His	1.1	0.9	1.1	1.1	0.9
Arg	10.6	9.5	10.5	10.6	9.4
Thr	1.7	1.8	1.8	1.8	1.8
Ala	7.0	8.0	6.9	6.9	7.8
Pro	15.8	15.1	15.3	15.1	16.1
Tyr	0.6	0.6	0.7	0.7	0.5
Val	1.7	2.1	1.7	1.8	2.1
Met	2.1	1.7	2.1	1.9	1.8
Ile	1.0	1.3	1.1	1.1	1.1
Leu	1.5	2.0	1.4	1.4	1.9
Phe	1.5	2.2	1.9	1.9	2.1
Lys	2.2	2.8	2.0	2.0	2.9

Note: values based on a single determination.

4. CONCLUSION

Manipulating the dehydration conditions (brine concentration; 15 to 30% NaCl, and contact time; 1 to 10 min) resulted in differences in mechanical properties when the brine concentrations were averaged across contact times and contact times were averaged across salt concentrations. This indicates that meat processors can adjust the performance of their casings through the modification of brine concentration and contact time.

The results also show differences among commercial collagen dispersions and provide actual values (currently not available in the literature), as well as some potential explanations for the differences. The mechanical evaluation of dispersions and films demonstrated that there are significant differences in flow behavior of the raw dispersions as well as tensile strength and percent elongation of the partially dehydrated films (i.e., after the common brining process). It was suggested that a higher degree of intact fibers can result in a collagen film with higher tensile strength and elasticity. Collagen dispersions with pH values closer to 2 seem to exhibit lower thermal stability, as conformational changes in the fiber structure occur at lower pH. Furthermore, the process of partially dehydrating the collagen fibers greatly increases the temperature of denaturation.
Overall, this paper provides research and industry personnel with a better understanding of the parameters important for co-extrusion of collagen dispersions into casings, through material selection and manipulation of brining operations.

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