



Enzymatically Treated Spent Cellulose Sausage Casings as an Ingredient in Beef Emulsion Systems

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Abstract: The objective of this research was to incorporate an ingredient obtained from spent cellulose casings in beef emulsion modeling systems. The test ingredient (residual sausage casing [RSC]) was procured from cellulose sausage casings following thermal processing of the sausages. The casings were cleaned of contaminants before a combination of enzymatic hydrolysis and high-speed homogenization was conducted in an effort to improve the functional attributes of the cellulose casing residue (i.e., recycling/upcycling of the spent casings). The beef emulsion modeling systems used in this study consisted of 57.30% beef, 20% water, 15% olive oil, 6% of the combination of RSC and an all-purpose binder, 1.45% NaCl, 0.40% sodium tri-polyphosphate, 0.15% sodium nitrite cure, and 0.0035% sodium erythorbate. The overlying goal was to test the ability of the RSC ingredient as a partial or full replacement of binder ingredients in a beef emulsion system. Therefore, the beef emulsion model systems were prepared with 5 different levels of the RSC ingredient (0% RSC, 25% RSC, 50% RSC, 75% RSC, and 100% RSC). This study was independently replicated in its entirety 3 times ($n = 3$) in a completely randomized design, and data were analyzed using a generalized linear mixed statistical model. Emulsion samples were tested for proximate composition, cooking loss, emulsion stability, texture profile analysis, and instrumental color. Overall, technological properties and emulsion stability were lost as the level of the RSC ingredient increased, but low levels of the RSC ingredient (25% RSC) may help maintain acceptable levels of yield and emulsion stability while improving the sustainability of the sausage production system.

Key words: beef emulsion, cellulose casings, cellulosic fibers, emulsion modeling systems, recycled sausage casings, spent casings

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Introduction

Cellulose sausage casings are one of the most popular types of artificial casings used in the meat industry (Marchello and Garden-Robinson, 2017). When sausages are manufactured with artificial cellulose casings, the casings are usually removed and landfilled following thermal processing. At the time of their disposal, spent cellulose casings contain mostly cellulose

and a small amount of liquids generated during thermal processing; however, other components may include proteins, lipids, and meat curing salts (e.g., sodium chloride, sodium phosphate, sodium nitrite/nitrate) (Gentry et al., 1996). If these other non-casing components are removed from the casings, there may be potential to use the cellulose from the spent cellulose casings as an ingredient in meat processing. This process would be termed as upcycling, or when discarded

objects or materials are reused to create a product or an ingredient of greater quality or value.

Processed meat products have been criticized in recent years due to their high levels of calories and low levels of dietary fiber (Schmiele et al., 2015). The meat industry has a significant opportunity to introduce new strategies to reformulate and improve the nutritional properties of processed meat products by reducing the use of unhealthy ingredients and replacing those ingredients with healthier alternatives that may function similarly from a processing standpoint (Toldrá and Reig, 2011). Dietary fiber consists of carbohydrate polymers with the main physiological characteristic of resistance to digestibility and absorption in the small intestine of humans. Consumption of dietary fiber has been established as an efficient way to reduce the risk of cardiovascular disease, diabetes, and cancer of the digestive tract (Anderson et al., 2009; Chuang et al., 2012; Threapleton et al., 2013).

There are several sources of dietary fiber capable of inclusion in processed meat product formulations, many of which have been tested for their use in processed meat products (Schmiele et al., 2015; Talukder, 2015). Therefore, the basis for many of the research studies that have investigated dietary fiber ingredients in meat processing was to provide technological function similar to existing binder ingredients that are used in commercial formulations, such as improved water-binding capacity and maintenance of characteristic textural and visual properties (Zhao et al., 2018). Taking this a step further, several of these sources of dietary fiber may have the potential to be recycled or upcycled from other agro-food applications (López-Marcos et al., 2015; Luo et al., 2018). Thus, the incorporation of dietary fiber into processed meat products is a noteworthy concept, and the additional notion of obtaining these ingredients from recycled or upcycled agro-food applications only adds to the appeal.

With the rationale of reducing environmental impact and costs associated with landfilling or composting spent cellulose sausage casings (Sanders et al., 2000), it is necessary to investigate the use of the residual (spent) sausage casings (RSC) as a value-added food ingredient in an applied setting. Research has been previously completed by Gentry et al. (1996), who investigated the application of spent cellulose casings as a potential feed ingredient in ruminant livestock; however, limited research has been conducted in food science applications. Therefore, the goal of this research was to test the application of an ingredient obtained from RSC in a beef emulsion modeling system.

It was hypothesized that partial or full replacement of a commercial binder ingredient with RSC could be accomplished without sacrificing technological properties of meat emulsions. To test this hypothesis, beef emulsion modeling systems were formulated to test the ability of the residual (spent) cellulose casings to partially or fully replace an all-purpose binder ingredient (which consisted of wheat and dairy ingredients) at inclusion levels of 0%, 25%, 50%, 75%, and 100%.

Materials and Methods

Collection of raw materials

The RSC samples used in this study were provided by a meat processing company located in the southern state of Rio Grande do Sul in Brazil. The sausage casings were 20- to 24-mm caliber size cellulose casings that were used during the manufacture of emulsified chicken sausage. The RSC samples were collected after sausages were peeled immediately following the thermal processing step of sausage manufacture. Following collection, RSC samples were packaged in polyethylene bags, vacuum sealed, and frozen at -18°C . The RSC samples were subsequently thawed prior to the milling process, which consisted of grinding with a knife mill and washing with cold distilled water (2°C) multiple times to remove meat residues from the sausage manufacturing process. After washing, the RSC samples were oven dried at 50°C for 8 h, sifted with a 4.5-mm sieve, vacuum packaged in polyethylene bags, and stored at -18°C until further use in this study. The compositional changes during the washing steps were previously reported by Gabiatti et al. (2020a).

Cellulase enzyme cocktail Celluclast from *Trichoderma reesei* was provided by LNF Latino Americana (Bento Gonçalves, Brazil). The assay kit used for quantification of total dietary fiber was supplied by Megazyme International Ltd. (Wicklow, Ireland). All the reagents used in this study were of analytical standard grade.

Beef inside round subprimals (*semimembranosus*, *adductor*, and associated muscles) were procured commercially, diced, and ground using an industrial meat grinder (Master 90 Y12, Sirman, Marsango, Italy) with an 8-mm plate. One master batch of ground beef was used for this study, and the composition of the mix was 66.36% moisture, 18.30% protein, 14.73% lipid, and 1.05% ash, and the pH of the mix

was 5.80. The same master batch of beef was used in all cases to control variation of the raw material among replication and treatments. Following mixing, samples were packaged and stored at -20°C until further use. The salts (NaCl, sodium tri-polyphosphate, sodium nitrite cure, and sodium erythorbate) used in this study were procured from a local ingredient supplier (Hela [Herman Laue] Spice Company Inc., Uxbridge, Ontario, Canada). The all-purpose binder ingredient (wheat flour, modified milk ingredients, and salt; 11.12% moisture, 7.89% protein, 2.06% lipid, and 12.15% ash, Hela Spice internal ID = 320020) was procured from Hela Spice Canada Inc., and the olive oil used in this study was purchased immediately before the study began from a commercial vendor (Kirkland Signature, Costco Wholesale, Issaquah, WA).

Preparation of test ingredient

The test ingredient (RSC) used in this study was previously characterized for composition, binding ability, rheology, and microscopy (Gabiatti et al., 2020b). The RSC samples were submitted to an enzymatic process using a cellulase cocktail (Celluclast 1.5L®) with the concentration of 50 mg of RSC/mL of buffer⁻¹. The composition of the RSC ingredient used in this study was $4.90\% \pm 0.40\%$ moisture, $30.56\% \pm 1.67\%$ reducing sugars, $45.27\% \pm 3.88\%$ insoluble dietary fiber, and $4.76\% \pm 0.90\%$ soluble fiber (Gabiatti et al., 2020b).

Samples were weighed in glass beakers and then were mixed with 50 mL of sodium citrate buffer 50 mM (pH 4.8). The beakers were placed on heated magnetic stirrers (Thermo Fisher Scientific, Ottawa, Ontario, Canada), and 0.375 U·mL of buffer⁻¹ of the enzyme cocktail was added when the temperature reached 50°C . The samples were held for 6 h with constant stirring (200 revolutions per minute [rpm]) and then blended in a blade homogenizer (PowerGen 1000, Thermo Fisher Scientific) for 5 min (1 min at speed 7,500 rpm, 3 min at speed 12,000 rpm, and 1 min at 7,500 rpm). The solutions with the RSC samples were placed in petri dishes and stored in a freezer at -80°C for 24 h. After 24 h, the RSC was freeze dried with a condenser temperature of -40°C and a pressure of 200 millitorr for 48 h (Genesis freeze drier; Virtis, Los Angeles, CA). Finally, the dried RSC ingredient was placed in plastic containers, sealed with plastic wrap (Fisherbrand clear 100% ultraclean polyethylene wrap; Thermo Fisher Scientific), and stored in desiccators for further use.

Emulsion preparation

For the preparation of the emulsions, ground beef was thawed at $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for at least 16 h. The emulsions were prepared following methodology from Vasquez Mejía et al. (2019) with modifications in the emulsion formulations (Table 1). Five treatments of 300 g of emulsion each were formulated and manufactured for each of the 3 replications. The emulsions

Table 1. Formulations of meat emulsions prepared with RSC as a partial or full replacement of a commercial binder ingredient

	Treatment ¹				
	0% RSC	25% RSC	50% RSC	75% RSC	100% RSC
Beef ² , %	57.60	57.45	57.30	57.15	57.00
Water, %	20.00	20.00	20.00	20.00	20.00
Olive oil, %	15.00	15.00	15.00	15.00	15.00
RSC ³ , %	0.00	1.50	3.00	4.50	6.00
All-purpose binder ⁴ , %	6.00	4.50	3.00	1.50	0.00
NaCl, %	0.85	1.00	1.15	1.30	1.45
Sodium tri-polyphosphate, %	0.40	0.40	0.40	0.40	0.40
Sodium nitrite cure ⁵ , %	0.15	0.15	0.15	0.15	0.15
Sodium erythorbate, %	0.0035	0.0035	0.0035	0.0035	0.0035
Total	100.00	100.00	100.00	100.00	100.00

¹Treatment was defined as the percentage replacement of all-purpose binder with the residual sausage casing (RSC) ingredient.

²One master batch of ground beef was used for this study, and the composition of the ground beef was 66.36% moisture, 14.73% lipid, 18.30% protein, and 1.05% ash.

³Residual sausage casing was procured from RSC followed by enzymatic hydrolysis and high-speed homogenization.

⁴All-purpose binder ingredients included wheat flour, modified milk ingredients, and salt (Hela Spice Canada Inc., Uxbridge, Ontario, Canada).

⁵Sodium nitrite cure contained 6.25% sodium nitrite and 93.75% NaCl, therefore the final product contained 0.01% sodium nitrite.

contained the following: beef (57.00%–57.60%, based on NaCl inclusion), water (20%), olive oil (15%), the combination of RSC and the all-purpose binder (6%), NaCl (0.85%–1.45%, balanced in the formulation depending on calculated salt in the all-purpose binder), sodium tri-polyphosphate (0.40%), sodium nitrite cure (0.15%; targeted to 0.010% or 100 parts per million of sodium nitrite), and sodium erythorbate (0.0035%). The study was replicated a total of 3 times, so there were a total of 15 experimental units represented. The RSC samples were added as a replacement of the all-purpose binder at the percentage replacement levels of 0%, 25%, 50%, 75%, and 100% or at the replacement ratios of 0.0%:6.0%, 1.5%:4.5%, 3.0%:3.0%, 4.5%:1.5%, or 6.0%:0.0%, respectively.

Emulsions were prepared and mixed in a food homogenizer (Ninja BL780C, Mississauga, Ontario, Canada). First, the meat and salts were mixed with 50% of the water (in the form of ice) for 30 s. The olive oil was then added and mixed for 20 s. After this, the fiber/binder (defined by treatment) and 50% of the remaining water were added and mixed for the final 30 s until a homogeneous meat mass was obtained. Temperature of the meat batter was controlled by replacing water with ice and ensuring that the temperature of the final emulsion did not exceed set thresholds of 10°C.

Following emulsion mixing, emulsions were separated into 5 samples of approximately 50 g per treatment, 4 of which were designated for cooked evaluation and 1 of which was designated for uncooked evaluation. The 4 samples used for cooking were designated for the following evaluations: (1) cooking loss, (2) emulsion stability, (3) proximate composition, pH, and instrumental cooked color analysis, and (4) texture profile analysis. The one sample not used for cooking was designated for uncooked instrumental color analysis.

Cooking loss

Evaluation of cooking loss was determined following the methodology described by Álvarez and Barbut (2013) with slight modifications primarily related to batch size. Batches of 30 ± 0.5 g of the uncooked samples were weighed into a tared 50-mL centrifuge tube and centrifuged at 1000g/40 s to eliminate air bubbles/pockets in the emulsions. The tubes were cooked in a water bath (model 80932, VWR, Radnor, PA) initially set to 50°C until samples reached an internal temperature of 45°C, and then the water bath was set to 80°C until samples reached an internal temperature of 72°C.

Internal temperature of the meat samples was measured with a thermocouple (Traceable thermocouple, Thermo Fisher Scientific) that was inserted inside the sample, and the entire cooking process lasted approximately 40 min. Following cooking, the tubes were chilled in an ice bath for 5 min before being inverted and stored at refrigeration temperatures (4°C) for 14 h to release the free exudate. Finally, the tubes were weighed again, and the difference between final weight and raw weight was reported.

Emulsion stability

Emulsion stability parameters were evaluated as described by Tahmasebi et al. (2016). Approximately 15 ± 0.5 g of the uncooked samples was weighed into a tared 50-mL centrifuge tube and centrifuged at 2500g/40 s to eliminate air bubbles/pockets in the emulsions. Cooking procedures followed the aforementioned cooking protocol that was used for cooking loss, and final internal temperature of the samples for this test was again 72°C. Following cooking, the tubes were immediately cooled in an ice bath and placed upside-down for 45 min to release the exudate into pre-weighed aluminum dishes. The total amount of fluid released was expressed as a percentage of the sample weight. The content of water versus lipid released was determined by the difference in the total liquid released before and after drying in a forced-air convection drying oven (Fisherbrand Isotemp 180 L drying oven, Thermo Fisher Scientific) at 100°C for 16 h.

Proximate composition and pH

Proximate composition and pH was determined for the cooked emulsion samples. Following cooking, the tubes were immediately cooled in an ice bath and then placed in a refrigerator at <4°C. The entire contents of each tube (stable emulsion and exudative liquids) were used for the analysis of proximate composition and pH. Moisture content was measured following the methods described by Vasquez Mejía et al. (2019). Briefly, samples were air dried at 75°C until samples maintained a constant weight using a forced-air convection drying oven (Fisherbrand Isotemp 180 L drying oven, Thermo Fisher Scientific). Lipid content was measured by AOAC method 991.36 (AOAC, 2006c) using petroleum ether as the solvent. Protein content was measured following the Dumas method (LECO FP-528 protein/nitrogen analyzer) using a multiplication factor of 6.25 according to the methodology described in AOAC method 990.03 (AOAC, 2006b). Ash content

was quantified by AOAC method 920.153 (AOAC, 2006a) using a muffle furnace at 525°C for 16 h. Total dietary fiber (including insoluble dietary fiber and soluble fiber) was quantified using AOAC method 991.43 (AOAC, 2006d). pH values of the emulsion samples were measured in a homogenate prepared with 2 g of the emulsion sample in 20 mL of distilled water. A pH meter (Accumet AR15, Thermo Fisher Scientific) equipped with a liquid-filled combination electrode (accuTupH Plus rugged bulb variable temperature pH combination electrode, 13-620-185, Thermo Fisher Scientific) was used for quantification of numerical pH values. The pH meter was calibrated before taking measurements using standardized solutions for a pH of 4 and a pH of 7.

Texture profile analysis

Texture profile analysis of cooked emulsion samples was determined with a texture analyzer (TA.XT2; Texture Technologies Corp., Scarsdale, NY). Meat emulsion samples were cooked using the same methodology as previously described for cooking loss section. Following cooking, the tubes were immediately cooled in an ice bath and then placed in a refrigerator at <4°C. The texture profile analysis tests were completed approximately 18 to 24 h after the emulsions were initially prepared and cooked. The protocol for the texture profile analysis tests used in this study has been previously described by Álvarez and Barbut (2013) and Huang and Bohrer (2020). The emulsion samples were cut into cylindrical cores (10-mm length × 20-mm diameter), and 5 equivalent cylindrical samples were obtained and tested for each treatment within each replication. The samples were compressed twice to 75% of their original height using a cylindrical acrylic probe of diameter 101.6 mm × height 10 mm (TA-40A; Texture Technologies Corp.). The test speed was 1.5 mm/s, and the post-test speed was 1.5 mm/s. The parameters used for this study were hardness (N), chewiness (no units/unitless), springiness (%), and cohesiveness (%).

Color measurements

Instrumental color of the meat emulsions was performed on the uncooked and cooked samples. Color of uncooked samples was evaluated immediately following preparation of the emulsions. Color of the cooked samples was evaluated following cooking as previously described in the cooking loss section. Following cooking, the tubes were immediately cooled in an ice bath. A Chroma Meter CR-400 colorimeter (Konica Minolta, Osaka Japan) with a 10° viewing

angle, an aperture size of 8 mm, and with illuminance set to D65 was used to obtain 5 readings for each sample. The CIELAB color space (also known as CIE L*a*b* or sometimes abbreviated simply “Lab” color space) was used in this study, whereas L* reported lightness of the color (L* = 0 indicates black, and L* = 100 indicates white), a* reported position between red and green (negative values indicate green, while positive values indicate red), and b* reported position between yellow and blue (negative values indicate blue, and positive values indicate yellow). Based on these readings, chroma and hue angle were calculated using the following equations:

$$\text{chroma} = \sqrt{a^{*2} + b^{*2}}.$$

$$\text{hue angle} = \tan^{-1}(b^*/a^*).$$

Statistical analysis

Fifteen experimental units were used for this study, which consisted of 3 independent replications ($n = 3$) of the 5 treatments. Each replication was completed on an independent day, which was defined as emulsion manufacture/testing. All 5 treatments were prepared on each independent day; thus, this experiment was conducted in 3 true replicates. A generalized linear mixed model (“PROC GLIMMIX” SAS procedure; SAS version 9.4, SAS Institute Inc., Cary, NC) with a fixed effect of treatment and a random effect of replication was used for statistical determination of all parameters in this study. Contrast statements using orthogonal polynomial coefficients were used to test linear effects for the percentage of RSC in the formulations. Least-square means were separated using the “PDIFF” option with a Tukey-Kramer adjustment, and the “LINES” option was used for means separation. Differences were considered statistically different at $P < 0.05$.

Results and Discussion

Cooking loss and stability

Water-holding capacity of a meat product is used to measure the ability of the meat system to retain moisture during processing or cooking (Hayes et al., 2011). Inherent to their functional properties, formulation of meat products with ingredients high in dietary fiber usually has a profound effect on water-holding capacity. When added to meat products, dietary fiber can elicit improved water-holding capacity with several

different mechanisms, including physical entrapment of water, an alteration in pH, and improved binding of other components (proteins, lipids, and other carbohydrates). Cooking loss measures the ability of a meat system to bind water and lipids after the product experiences protein denaturation and aggregation during cooking. While cooking loss explains the ability of a meat system to hold water, it is also connected to water-holding capacity along with the amount of lipids lost during cooking, or lipid-holding capacity. An additional test that can be used to further interpret water-holding capacity and lipid-holding capacity is emulsion stability testing. Greater emulsion stability is very important in meat products, both from a technological and an economical point of view (Mehta et al., 2015). The incorporation of fiber into meat products may allow for increased water-holding capacity and increased lipid-holding capacity and thus decrease the migration of water and lipids from a cooked meat matrix (Petracci et al., 2013).

It is with this rationale that the hypothesis that partial or full replacement of a commercial binder ingredient with RSC could be accomplished without sacrificing technological properties of meat emulsions. This was unfortunately not the case in this study, and the root cause of this observation was undoubtedly the inability of the RSC ingredient to effectively hold water and lipid within the meat system.

The values obtained for cooking loss and emulsion stability (water released, lipids released, and total liquids released) are presented in Figure 1 and Figure 2, respectively. The results differed ($P < 0.01$) between the treatments, and there was a significant linear effect for all parameters. Cooking loss increased as the inclusion level of RSC increased from 0% to 100%. Cooking loss was not different between 0% RSC and

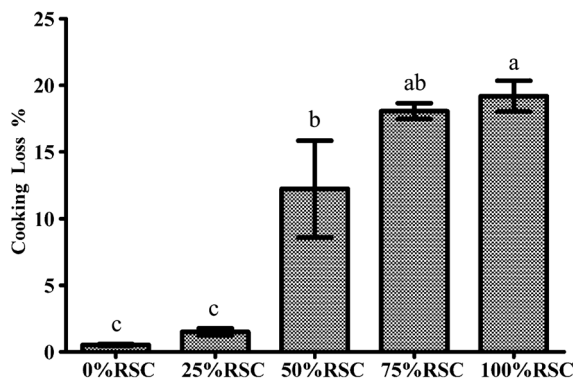


Figure 1. Cooking loss of meat emulsions prepared with residual sausage casing (RSC) as a partial or full replacement of a commercial binder ingredient. Means within a row for experimental treatments without a common superscript differ ($P \leq 0.05$).

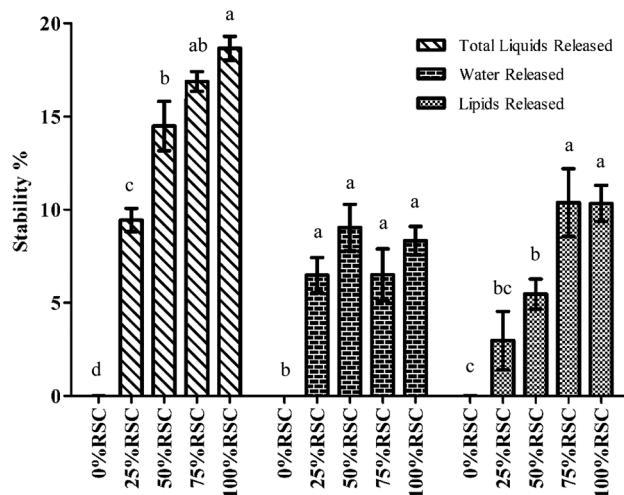


Figure 2. Emulsion stability of meat emulsions prepared with residual sausage casing (RSC) as a partial or full replacement of a commercial binder ingredient. Means within a row for experimental treatments without a common superscript differ ($P \leq 0.05$).

25% RSC, but as the RSC ingredient was increased to 50% replacement of the commercial all-purpose binder, cooking loss increased from 1.50% at 25% RSC inclusion to an unacceptable level of 12.22% at 50% RSC inclusion. From there, cooking loss was at even greater levels at 75% RSC inclusion (18.07%) and 100% RSC inclusion (19.18%). The unacceptable level of cooking loss observed with greater than 25% RSC inclusion could have been caused by several different factors, including pH nearing the isoelectric point of meat (Table 2) and the low ability of the unorganized cellulose fragments to bind and hold water in the meat system (Mehta et al., 2015). Furthermore, Schuh et al. (2013) implied that the charge of hydrocolloid ingredients (e.g., carboxymethyl cellulose and microcrystalline cellulose) can have an integral role in water activity. While these researchers (Shuh et al., 2013) described cellulose as a nonionic polymer (neutrally charged), they also reported that modification of cellulose can affect the charged state. Thus, it is hypothesized that the modification performed with the RSC ingredient (enzymatic hydrolysis and high-speed homogenization treatment) created anionically charged monomers as indicated in Gabiatti et al. (2020a) in their characterization of the formation of various reducing sugars, yet the state of these charged groups did not create a conducive environment for binding with the positively charged meat proteins.

Similar to the results of cooking loss, water released, lipids released, and total liquids released differed ($P < 0.01$) among treatments, and there was a significant linear effect. Water released, lipids released, and total liquids released increased as inclusion level

Table 2. Proximate composition and pH of cooked meat emulsions prepared with RSC as a partial or full replacement of a commercial binder ingredient

	Treatment ¹					SEM ²	<i>P</i> values	
	0% RSC	25% RSC	50% RSC	75% RSC	100% RSC		Treatment	Linear effects
Replications, <i>n</i>	3	3	3	3	3			
Moisture, %	58.91 ^{a,b}	59.55 ^a	58.89 ^{a,b}	58.93 ^{a,b}	57.81 ^b	9.99	0.01	0.01
Lipid, %	14.91 ^a	14.41 ^b	14.14 ^b	13.55 ^c	13.55 ^c	9.01	<0.0001	<0.0001
Protein, %	13.63 ^a	13.28 ^b	13.12 ^b	12.39 ^c	11.68 ^d	0.06	<0.0001	<0.0001
Ash, %	2.39 ^d	2.54 ^c	2.66 ^b	2.71 ^b	2.83 ^a	0.02	<0.0001	<0.0001
TDF ³	2.72 ^d	3.41 ^c	3.87 ^c	4.57 ^b	5.85 ^a	0.10	<0.0001	<0.0001
TIF ³	2.72 ^d	3.41 ^c	3.84 ^c	4.54 ^b	5.76 ^a	0.10	<0.0001	<0.0001
TSF ³	0.00 ^c	0.00 ^c	0.05 ^b	0.06 ^b	0.13 ^a	0.01	<0.0001	<0.0001
pH	6.04 ^a	5.70 ^b	5.58 ^{b,c}	5.54 ^c	5.37 ^d	0.03	<0.0001	<0.0001

^{a,b,c,d}Means within a row for experimental treatments without a common superscript differ ($P \leq 0.05$).

¹Treatment was defined as the percentage replacement of all-purpose binder with the residual sausage casing (RSC) ingredient.

²SEM = standard error of the mean.

³TDF = total dietary fiber; TIF = total insoluble fiber; TSF = total soluble fiber.

of RSC was increased. For each parameter, there was a significant effect between 0% RSC and 25% RSC, indicating the loss of water-holding capacity and lipid-holding capacity when RSC was included in the formulation of meat emulsion models at all levels. Water released was not different ($P > 0.05$) between 25% RSC, 50% RSC, 75% RSC, and 100% RSC. Lipid released was not different ($P > 0.05$) between 25% RSC and 50% RSC, yet there was a difference between 25% RSC and 50% RSC compared with 75% RSC and 100% RSC. To summarize, there were unacceptable levels of total liquid released (as measured with the emulsion stability tests) with all treatments in which the RSC ingredient was included in the formulation, and this level was increased linearly as more of the RSC ingredient was included in the formulation.

Proximate composition, dietary fiber content, and pH

The proximate composition of macronutrients (moisture, lipid, protein, and ash), dietary fiber content, and pH differed among treatments ($P < 0.01$) (Table 2). For each parameter, there was a linear effect ($P < 0.01$) as RSC inclusion increased.

Moisture, lipid, and protein content decreased as inclusion level of RSC increased, while ash, total dietary fiber, total insoluble fiber, and total soluble fiber increased as inclusion level of RSC increased. The compositional differences between the all-purpose binder and the RSC ingredient can likely be attributed to the observed differences in the macronutrient

composition of the emulsions. The liquids released (with lipids and soluble proteins) during the cooking process were actually not fully accounted for in the proximate composition analyses as samples and exudative liquids were evaluated together. Even so, all the formulations were in agreement with the protein requirement for sausage products ($>9.5\%$), according to the Canadian Food Inspection Agency (CFIA, 2018).

As expected, dietary fiber content increased with the increased inclusion of RSC, with the greatest inclusion level (100% RSC) having the greatest dietary fiber content. Most of this dietary fiber content was from insoluble fiber sources. However, soluble fiber content was also greatest for the 100% RSC treatment (0.13%), yet this was likely below levels that would be meaningful for nutritional benefits. Dietary fiber can improve technological attributes in meat products, such as the water-holding capacity and the lipid-holding capacity, thereby reducing the shrinkage, improving cooking loss, and improving purge loss during storage periods. However, the dietary fiber component in the RSC ingredient used in this study did not provide these benefits.

pH was the emulsion property perhaps most altered by inclusion of the RSC ingredient, as pH was greater as RSC inclusion level increased, with a difference of 0.67 units between 0% RSC and 100% RSC. The pH values obtained for the emulsions varied from 5.37 to 6.04. The high substitution level of the RSC ingredient (100% RSC) presented the lowest value for pH compared with other treatments. This was due to the methodology used to obtain the RSC samples.

The RSC ingredient was obtained with enzymatic hydrolysis in which pH was controlled at 4.8 using a 50 mM sodium citrate buffer. The final pH of the RSC ingredient alone was 4.76. In meat systems, variation in pH can have major influence on the water-binding capacity and emulsion stability (Young et al., 2005; Hemung et al., 2013). The general consensus is that, as the pH of a meat mixture approaches the isoelectric point of the major meat proteins (myosin = 5.4; actin = 4.8), water-holding capacity is significantly compromised.

Texture profile analysis

The values obtained for the texture profile analysis are shown in Table 3. All parameters measured (hardness, chewiness, springiness, and cohesiveness) differed ($P < 0.05$) among the treatments, and there was a significant linear effect of the RSC inclusion level. Hardness and chewiness decreased as RSC was increased, whereas springiness and cohesiveness increased as RSC was increased.

It is worth noting that the texture profile was unique in the 0% RSC samples in this study compared with previous research performed by our lab on meat emulsions. Values were 2- to 3-fold greater in this study compared with those presented in other studies (Youssef and Barbut, 2009; Huang et al., 2019; Vasquez Mejía et al., 2019). A likely cause for this could be the formulation of the emulsion models in this study, particularly the high levels of the all-purpose binder ingredient (6%) that resulted in very high emulsion stability in the 0% RSC treatment.

According to Vasquez Mejía et al. (2019), hardness was generally greater when formulations included high levels of dietary fiber. This is because of the entrapment of water and fat within the fiber network, and these interrelations between fiber/hydrocolloid, water, and



Figure 3. Prepared and cooked meat emulsions using residual sausage casing (RSC) as a partial or full replacement of a commercial binder ingredient. T1 = 100% RSC; T2 = 75% RSC; T3 = 50% RSC; T4 = 25% RSC; and T5 = 0% RSC.

meat proteins have a direct impact on the elasticity and hardness properties of an emulsion system. However, in the current study, the event of high levels of dietary fiber incorporation and improved emulsion stability was not apparent. The poor emulsion stability probably resulted in the low hardness values in the emulsions with 50% to 100% substitution of the all-purpose binder with the RSC ingredient. With greater inclusion of the RSC ingredient, all attributes for texture profile analysis were directly affected, and the overall visual texture of the meat emulsion was negatively affected (Figure 3).

Chewiness was reduced significantly between 0% RSC and 25% RSC treatments, indicating the remarkable impact that the RSC ingredient had on texture even at low inclusion levels. Springiness was lowered with greater inclusion of the RSC ingredient. Huang et al. (2019) previously reported that springiness was greater with inclusion of different types of flours in meat

Table 3. Texture profile analysis of meat emulsions prepared with RSC as a partial or full replacement of a commercial binder ingredient

	Treatment ¹					SEM ²	<i>P</i> values	
	0% RSC	25% RSC	50% RSC	75% RSC	100% RSC		Treatment	Linear effects
Replications, <i>n</i>	3	3	3	3	3			
Hardness, N	184.18 ^a	80.22 ^b	44.07 ^c	31.91 ^c	31.61 ^c	5.92	<0.0001	<0.0001
Chewiness	102.74 ^a	25.85 ^b	11.55 ^c	6.24 ^c	4.63 ^c	2.25	<0.0001	<0.0001
Springiness, %	85.70 ^a	86.73 ^a	73.47 ^{a,b}	60.43 ^{b,c}	50.70 ^c	4.76	<0.01	<0.0001
Cohesiveness, %	65.50 ^a	37.40 ^b	33.07 ^b	30.73 ^b	28.30 ^b	2.35	<0.0001	<0.0001

^{a,b,c}Means within a row for experimental treatments without a common superscript differ ($P \leq 0.05$).

¹Treatment was defined as the percentage replacement of all-purpose binder with the residual sausage casing (RSC) ingredient.

²SEM = standard error of the mean.

Table 4. Instrumental color of meat emulsions prepared with RSC as a partial or full replacement of a commercial binder ingredient

	Treatment ¹					SEM ²	<i>P</i> values	
	0% RSC	25% RSC	50% RSC	75% RSC	100% RSC		Treatment	Linear effects
Replications, <i>n</i>	3	3	3	3	3			
Color of uncooked samples								
L*	69.81 ^a	66.85 ^b	66.47 ^b	65.41 ^{b,c}	63.88 ^c	0.399	<0.0001	<0.0001
a*	5.26 ^d	6.66 ^c	6.91 ^{b,c}	7.78 ^b	9.15 ^a	0.215	<0.0001	<0.0001
b*	13.24	13.52	13.17	12.81	12.65	0.217	0.10	0.02
Chroma	1.19 ^a	1.11 ^b	1.09 ^b	1.03 ^c	0.95 ^d	0.013	<0.0001	<0.0001
Hue angle	14.25 ^b	15.07 ^{a,b}	14.88 ^{a,b}	14.99 ^{a,b}	15.62 ^a	0.235	0.03	0.01
Color of cooked samples								
L*	66.03 ^a	64.18 ^{a,b}	62.17 ^{b,c}	60.19 ^c	58.01 ^d	0.47	<0.0001	<0.0001
a*	8.68 ^d	11.24 ^c	12.54 ^{b,c}	13.94 ^{a,b}	14.84 ^a	0.32	<0.0001	<0.0001
b*	12.82 ^a	11.01 ^b	10.62 ^{b,c}	10.31 ^c	10.07 ^c	0.15	<0.0001	<0.0001
Chroma	0.94 ^a	0.78 ^b	0.70 ^{b,c}	0.64 ^{c,d}	0.60 ^d	0.02	<0.0001	<0.0001
Hue angle	14.67 ^d	15.73 ^{c,d}	16.44 ^{b,c}	17.35 ^{a,b}	17.94 ^a	0.24	<0.0001	<0.0001

^{a,b,c,d}Means within a row for experimental treatments without a common superscript differ ($P \leq 0.05$).

¹Treatment was defined as the percentage replacement of all-purpose binder with the residual sausage casing (RSC) ingredient.

²SEM = standard error of the mean.

emulsion modeling systems, with the idea that compositional changes are involved in these differences. This was likely the cause of the differences observed in the current study. Cohesiveness was greatest in the 0% RSC treatment and was significantly lower in the other 4 treatments. This indicated that any level of inclusion of the RSC ingredient lowered cohesiveness of the emulsion system; however, greater inclusion of the RSC ingredient did not appear to be detrimental to cohesiveness. Overall, these data indicate that more research is warranted to obtain acceptable levels of texture with the incorporation of the RSC ingredient. Future research endeavors should focus on improving the technological function of the RSC ingredient in optimal meat system environments (i.e., appropriate pH and compositional levels).

Color measurements

Instrumental color parameters (L*, a*, b*, chroma, and hue angle) for uncooked and cooked emulsions are presented in Table 4. All the values obtained for both uncooked and cooked samples were different ($P < 0.05$) among the treatments, with the exception of the values for b* in the uncooked samples. There was a significant linear effect for all parameters. In uncooked samples, L* decreased, a* increased, b* decreased, chroma decreased, and hue angle increased as inclusion level of the RSC ingredient was increased. In cooked samples, L* decreased, a* increased, b* decreased, chroma decreased, and hue angle increased

as inclusion level of the RSC ingredient was increased. The differences in instrumental color were likely attributed to the differences in the inclusion level of the all-purpose binder as much as they were attributed to the differences in the inclusion level of the RSC ingredient. The RSC ingredient was colorless in its own right, while the all-purpose binder contained wheat flour and modified milk ingredients, both of which have previously been reported to elicit color differences in meat emulsions. Huang et al. (2019) reported that wheat flour caused greater L* and greater b* in meat emulsions. Barbut (2008) reported that modified milk ingredients caused variation of L*, a*, and b* depending on their components and overall composition. An additional factor that could have influenced cooked color was the composition of the meat emulsions. Greater water and lipid loss during cooking would cause greater lean muscle and less fat resulting in darker (lower L*) and redder (greater a*) color.

Conclusions

This research studied the application of a cellulose fiber ingredient obtained from RSC from sausage manufacture. The meat emulsion models used in this study were formulated by substituting a commercial binder ingredient with an RSC ingredient at varying inclusion levels. The hypothesis that partial or full replacement of a commercial binder ingredient with RSC could be accomplished without sacrificing

technological properties of meat emulsion systems was not supported by the data generated in this study. Incorporation of the RSC in meat emulsion systems had technological flaws stemming from poor emulsion stability. Nevertheless, this research opened a new perspective for the application of a low-value raw material ingredient in meat processing. Future research should continue these efforts in order to create a more functional and better suited ingredient from RSC that can be effectively used as an upcycled ingredient in meat products. This could include different types of modification to spent cellulose casings or additional steps beyond that of enzymatic treatment and high-speed homogenization. Such steps should consider pH of the ingredient and meat system reactivity before incorporation.

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