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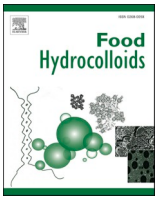

Evaluation of cattle skin collagen for producing co-extrusion sausage casing

Patricia Suurs [a,b,*], Henry van den Brand [a], Robin ten Have [b], Willeke F. Daamen [c], Shai Barbut [a,d]

[a] Adaptation Physiology Group, Wageningen University and Research, Wageningen, the Netherlands
[b] Marel Further Processing B.V, Boxmeer, the Netherlands
[c] Department of Biochemistry, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, the Netherlands
[d] Food Science Department, University of Guelph, Canada

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ABSTRACT

Co-extrusion is a fully automated sausage production process that employs a continuous stream of collagen dispersion to encase the extruded meat mass to form an endless sausage rope, that is later crimped into links of selected sizes. Fibrous and soluble type I collagen dispersions obtained from bovine skins of animals aged 18–36 months is used as the raw material for these dispersions.

In this study, the chemical and physical properties of cattle skin collagen preparations from four sources [American Calf (AC), Dutch Heavy Veal (DHV), Danish Ox and/or Heifer (DOH), and Heavy German Cow (HGC)] were investigated for their potential application as collagen source for co-extrusion.

All dispersions exhibited shear-thinning behavior, following a power-law model with k^* values for HGC, DHV, AC, and DOH dispersions of 59, 68, 95 and 114 Pa s^{n*}, respectively. Rheological measurements showed for all dispersions a decrease in elasticity and loss modulus at 35–40 °C. SDS-PAGE indicated the presence of α1(I)- and α2(I)-chains of type I collagen for all dispersions. The mechanical strength of the films was 1.6, 1.6, 1.3 and 1.2 MPa for films prepared from AC, DOH, DHV and HGC dispersions, respectively. After crosslinking a 27% reduction of free amine groups was found for HGC and DOH, followed by 26 and 19% for AC and DHV, respectively.

Based on the properties of the dispersions and the films in relation to the co-extrusion process AC, DHV and DOH are potentially suitable as an alternative collagen source.

1. Introduction

For many centuries, consumers around the world have enjoyed traditional sausages made by stuffing meat into natural sheep/hog casings (Wijnker, 2009, pp. 1–114). Today many sausages are still made this way and then hung on sticks, which are loaded onto carts, and placed in a smokehouse for further processing by drying/smoking and heating. From an industry's perspective there are basically two alternatives to the smoking and cooking processes: 1) a semi-continuous operation, using sticks that are automatically loaded into the smokehouse; 2) co-extrusion technology in which a semi liquid material (i.e., collagen paste) is introduced onto an endless stream of meat dough (coming out of a meat stuffer), and the collagen is initially gelled in place by the application of a saturated salt solution (typically NaCl, that quickly withdraws water from the gel) and later the continuously filled casing (filled with the meat) is split into individual links/sausages. The follow up steps of thermal drying and the use of liquid smoke (contains aldehydes that crosslink protein molecules) are used to 'strengthen/cement' the casings structure. Unlike the traditional way of sausage making, co-extrusion eliminates the intermediate stages of preparing and storing pre-made casings as in natural or manufactured casings (Suurs & Barbut, 2020). The collagen dispersion used for co-extrusion is initially prepared the same way as the dispersion used for traditional fabricated casings (i.e., arrive to the plant as dried fully prepared to be stuffed casings). The difference lies in the fact that the co-extrusion dispersion (arrive to the plant as gel) is prepared from the same raw materials and goes through the initial processing stages (deliming the skins, washing, acidification and homogenization of the collagen fibers which yields a solid content of 4–5%). At this point the dispersion is packed and sent to the meat plant, while the traditional fabricated casing is going through extrusion to create the casing shape (using a special tunnel), which is then dried and crosslinked (Sobanwa, 2021). To

* Corresponding author. Adaptation Physiology Group, Wageningen University and Research, Wageningen, the Netherlands.
E-mail address: patricia.suurs-hoekstra@wur.nl (P. Suurs).

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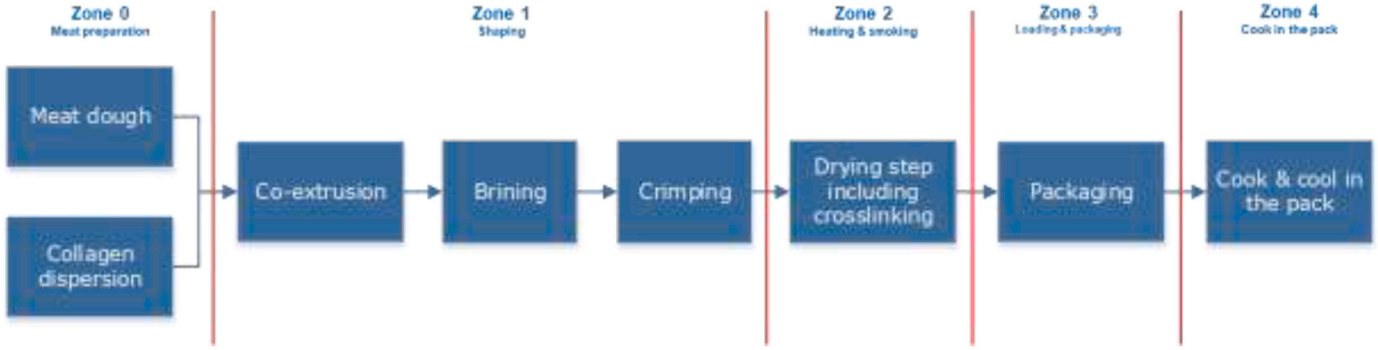


Fig. 1. Schematic overview of the co-extrusion process for preparing cooked smoked sausages, indicating the different zones. Zone 0: meat preparation; Zone 1: shaping of the sausage; Zone 2: heating and smoking; Zone 3: automatic loading and packaging of the sausages; Zone 4: cook the sausages in the pack until T_{core} is 74 °C and chill afterwards.

produce traditional manufactured collagen casing a coagulation bath of sodium or ammonium salt at a pH of 12 (to neutralize the acid and to promote collagen fiber shrinkage), a plasticizer such as glycerol and sorbitol and crosslinking agents such as glutaraldehyde or liquid smoke are used to reduce brittleness and improve casing strength. In the co-extrusion process, a sodium salt bath at neutral pH is used to precipitate the collagen dispersion into a collagen casing and no plasticizers are used. Furthermore, crosslinking with glutaraldehyde is not allowed in the co-extrusion process as it is not approved by the Food and Drug Administration (FDA) as an ingredient for food products. After crosslinking, the casings produced according to the traditional process are inflated with air and dried to a final moisture content of about 13–18%. The casings are ready to be stuffed with meat batter, whereas in the co-extrusion process the casing is being formed as the sausage is produced (Savic & Savic, 2016; Sobanwa, 2021).

The co-extrusion process is characterized by several steps, which are

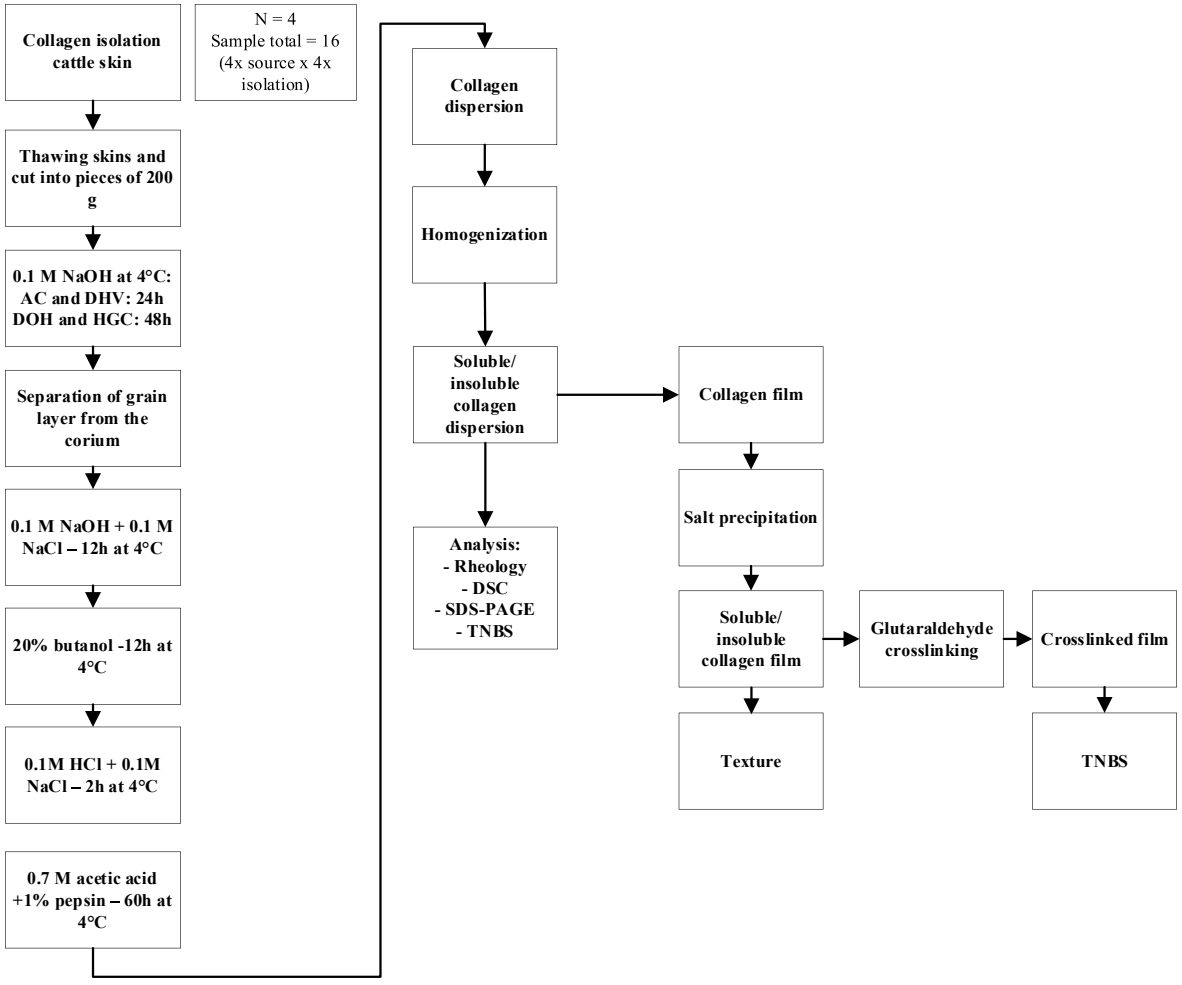


Fig. 2. Flow chart of the extraction methods used to obtain collagen dispersions and films from the different cattle skins, including an overview of the analysis methods for both the dispersions and films. The abbreviations of the methods used are as follows: Differential scanning calorimetry (**DSC**), Sodium dodecyl sulfate polyacrylamide gel electrophoresis (**SDS-PAGE**), and 2,4,6-trinitrobenzenesulfonic acid (**TNBS:** amine group content).

generally divided into five zones (Fig. 1), to obtain a cooked smoked sausage. Zone 0 is related to the meat preparation step and receiving the raw collagen dispersion (as it is being delivered by the supplier). Zone 1 is related to shaping of the product, creating a casing as the sausage is being produced. Here a saturated salt solution is used to quickly remove water from the gel and help precipitate the collagen dispersion into a film. In Zone 2, the sausages enter the drying cabinet to further stabilize the casing by both air-drying and crosslinking with smoke condensates (Kobussen et al., 2000; Suurs & Barbut, 2020). The aldehydes present in the smoke condensate are the most important ones for this process, as they are capable to induce covalent crosslinkages between collagen fibers and fibrils (Toledo, 2007). The covalent bonding strengthen the network structure thereby enhancing the mechanical properties of the collagen film (Bontjer et al., 2011; Morgan et al., 1988; Zhang et al., 2022). In Zone 3, sausages are automatically loaded into packages. In Zone 4, packed sausages are cooked to an internal temperature of 74 °C to obtain a safe product (Savic & Savic, 2016).

In every step of the process, the collagen paste must have certain properties to achieve a well-accepted sausage product. An important feature of the co-extrusion process is that a collagen paste is used, consisting of fibrous and soluble type I collagen with approximately 3–5% protein (Barbut et al., 2020). The main source of collagen for co-extrusion is bovine skin/hide, and more specifically the corium layer of animals aged 18–36 months, a by-product of the leather industry (Sobanwa, 2021). According to Sobanwa (2021) the hide's corium of animals at this age is more fibrous, stronger and contains less soluble collagen compared to corium of younger animals. However, different skin types are available at the leather tanneries, e.g., of veal calves, heifers and oxes. Their hide off-cuttings, which cannot be used by the leather industry (Noorzai et al., 2019), usually end up in landfill. Extracting collagen from these off-cuttings can greatly increase their value (Matinong et al., 2022; Noorzai et al., 2019). However, as mentioned by Noorzai et al. (2019) and Matinong et al. (2022), the structure of a hide is influenced by age, sex, diet, and environment. In this study, we examined whether cattle skins, obtained from alternative sources, may be used to produce high-quality co-extrusion casings. The study compared the chemical composition of skins' collagen from two different veal calf breeds (used for meat production), a mixture from Danish ox and heifers (used for working [ox] and meat production [heifer]), and heavy German cows (used for meat/milk production), employing both biochemical and physical measurements (note: a mixture of hides from oxen and heifers was used, because this was offered as such by tannery). The overall aim of the study was to evaluate potential sources of cattle collagen for the co-extrusion process.

2. Material and methods

2.1. Experimental design

Cattle skins from different types [(American Calf (**AC**), Dutch Heavy Veal (**DHV**), Danish Ox or Heifers (**DOH**) and Heavy German Cow (**HGC**)] were collected from a local tannery (ECCO, Dongen, The Netherlands). The main difference between DOH versus HGC is that cows are used for meat production and breeding purposes, whereas the oxen are especially used as working animals (Noorzai et al., 2019). Furthermore, there is a difference in slaughter age between the different cattle species: calves 6–8 months, ox/heifers at a maximum age of 30 months, and cows 36–60 months. One skin was available per animal species, the origin on the animal's body was unknown i.e., of the spine region or abdomen. The whole skin (including the hair/coat) of each group was collected and divided into four portions to prepare four dispersions (n = 4) per source. The four portions were stored at −18 °C, until processing.

2.2. Preparation of skin collagen dispersions

A multistep process was used to obtain the dispersions prior to determining their physical and chemical properties (Fig. 2). The procedure was performed according to Noorzai et al. (2019) with slight modifications. Briefly, skins were thawed and cut into pieces of approximately 200 g. Skins were incubated in 0.1 M NaOH (Boomlab, Meppel, The Netherlands) at a sample: solution ratio of 1:6 (w/v - based on the wet weight of the original sample, 1 g of sample with 6 ml of solution). The mixtures of AC and DHV were soaked for 24 h, and DOH and HGC were soaked for 48 h in NaOH at 4 °C. Given the age difference between AC and DHV versus DOH and HGC, more crosslinks are expected in the latter two and therefore the time in the NaOH was extended. Due to swelling of the skin, it was possible to separate the grain layer (including the coat) from the corium with a knife. NaOH was removed by washing with distilled water and filtering (2.5 mm mesh) until neutral pH was reached. Thereafter, fat was removed by adding first 0.1 M NaOH +0.1 M NaCl (Boomlab) at a sample: solution ratio of 1:6 (w/v – based on the weight of the swollen skin pieces), whereby the solution was renewed every 2 h for at least 12 h, and then 20% butanol (Chemlab, Zedelgem, Belgium) at a sample: solution ratio of 1:6 (w/v – based on the weight of the skin pieces) was added. Mixtures were stored overnight at 4 °C. Samples were later washed and filtered (2.5 mm mesh) with distilled water until the pH of the wash water reached neutral pH. After defatting, the skin pieces were cut into smaller pieces (1 × 1 cm) and soaked in 0.1 M HCl (Boomlab) + 0.1 M NaCl demineralization solution with a sample: solution ratio of 1:6 (w/v) for 2 h at 4 °C. The mixture was then filtered through a double layer cheese cloth, followed by rinsing with distilled water until the pH of the wash water reached neutral pH. Samples were swollen in 0.7 M acetic acid (Supelco, Zwijndrecht, The Netherlands) and 1% (w/w) pepsin (Merck, Darmstadt, Germany) for 60 h at 4 °C at a sample: solution ratio of 1: 2 (w/v). The pepsin will only attack the non-triple helical domains of native collagen and leaves the helical portions intact (Matinong et al., 2022; Zhang et al., 2006). Acid not taken up by the collagen was filtered out (2.5 mm mesh), collected, and kept for mixing. Samples were pre-mixed in a food processor (Braun, type 3202, Kronberg im Taunus, Germany) with a sample: solution ratio of 2:1 (w/v, based on the weight of swollen skin pieces), followed by homogenization with a water-cooled high shear mixer (Marel, Boxmeer, The Netherlands) until a homogenous collagen dispersion was obtained.

2.3. Soluble collagen content of cattle skin collagen dispersions

Additional samples from the AC, DHV, DOH and HGC skins were prepared to obtain an indication of the amount of soluble collagen in each dispersion. Samples were obtained similarly, but after swelling in 0.7 M acetic acid plus 1% pepsin (w/w), the skin material was filtered (2.5 mm mesh), and the filtrate was collected and precipitated to obtain soluble collagen. NaCl crystals (Boomlab) were used for the precipitation until a final concentration of 2.6 M was reached. The solution was stirred and left for 24 h at 4 °C. The precipitates were collected by centrifuging (Thermo Scientific, IEC, CL10, Fixed Angle Rotor F-G1, Waltham, Massachusetts, USA) at 3700 g for 30 min. The pellet was dialyzed (Ø 17.5 mm, molecular weight cutoff at 14 kDa; Medicell, London, United Kingdom) in approximately 20 vol of 0.7 M acetic acid for 24 h at 4 °C.

2.4. SDS-PAGE analysis of collagen dispersions

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was conducted to determine the molecular weight of the protein chains in the collagen dispersions (soluble part), and to assess the presence of collagen breakdown products. The method was obtained from Faraj et al. (2011). Briefly, 2.5 mg of lyophilized dispersions was suspended in 150 μl sample buffer followed by heating for 10 min at

70 °C. An 8% (w/v) polyacrylamide gel was prepared to load both samples and controls. The gel was run at 100 V until the Bromophenol blue front was at the end of the gel. Gels were stained overnight with 0.1% (w/v) Coomassie Brilliant Blue solution (R-250, VWR International BV, Amsterdam, The Netherlands). A solution containing 50% (v/v) methanol (Macron Fine Chemicals, VWR International BV) and 10% (v/v) acetic acid (Boomlab) was used for de-staining until bands were clearly visible. Each dispersion prepared from the different skin type (n = 4) was analyzed on gel.

2.5. Moisture and protein content of collagen dispersions

Dry matter content was determined by lyophilization (LP-03, IlShin BioBase Europe, Ede, The Netherlands), where approximately 25 g of the collagen dispersion was lyophilized, after which the dry material was weighed and calculated. Four replicates of each skin type were measured once, whereby the average per skin type was used in the statistical analysis.

Protein was assessed according to Lowry et al. (1951). In short, 10 mg of lyophilized dispersions were incubated in 1 ml papain digestion buffer of 50 mM phosphate buffer at pH 6.5 containing 2 mM cysteine, 2 mM ethylenediaminetetraacetic acid (EDTA) and 5 U papain (Sigma-Aldrich, Zwijndrecht, The Netherlands) for 16 h at 65 °C. After digestion, the samples were centrifuged (5 min; 13,000 g) and supernatants were used for protein measurements. A calibration curve was made, using bovine serum albumin (BSA) at concentrations of 0–250 ng/ml in papain digestion buffer. The color reaction that occurs has a characteristic blue color with A_{max} at 750 nm. Four replicates of each skin type were measured in triplicate, whereby the average of the triplicates was used in the statistical analysis.

2.6. Thermal transition measurement of cattle skin collagen dispersions

Differential Scanning Calorimetry (DSC) measurements were performed to assess endothermal transitions of collagen (DSC Q1000, TA Instruments, New Castle, DE, USA). Eight to thirteen mg of collagen dispersion was hermetically sealed in a T_{zero} pan (aluminum) and equilibrated at 1 °C for 2 min. A temperature ramp was performed from 1 to 80 °C, at 5 °C/min. An empty aluminum pan was used as the reference. Values determined included temperature at which helix to random coil transition of the collagen started (T_{onset}), temperature where 50% of the collagen has unfolded (T_{peak}), and the denaturation enthalpy (ΔH) using the DSC software (Universal Analysis 2000; Version 4.5A). Four replicates of each skin type were run in duplicate, whereby the average of the duplicates was used in the statistical analysis.

2.7. Rheology of collagen dispersions

The flow behavior of the different dispersions was investigated, as this could indicate the applicability of the dispersions for use in a co-extrusion system. The rheology measurements were performed based on Oechsle et al. (2016), with slight modifications. Measurements were conducted with an oscillatory rheometer (TA Instruments AR2000, New Castle, USA), which was equipped with a Peltier plate and a water bath. The collagen dispersions were analyzed by oscillating measurements, using a 40 mm diameter plate-plate (hard-anodised aluminium) geometry. Stress sweeps were performed at 1 Hz applying an oscillatory stress from 0.01 to 1000 Pa at 5 °C (linear viscoelastic range determination). Subsequently frequency sweeps were performed by applying 0.1% strain, determined from the linear viscoelastic range, from 1 to 100 rad/s. Next, the complex viscosity η^* could be determined as a function of angular frequency ω by applying Eq. (1) (Macosko, 1994, pp. 109–133):

Fig. 3. Procedure for collagen film preparation. A) Collagen dispersion on a stainless-steel board between two layers of plastic sheets. B) Flattening of the collagen dispersion with a stainless-steel roller. C) Addition of saturated sodium chloride solution to the side of the collagen film, thereby gently lifting the plastic sheet. D) Collagen film precipitated with saturated sodium chloride solution.

$$\eta^* = \left[\left(\frac{G''}{\omega}\right)^2 + \left(\frac{G'}{\omega}\right)^2\right]^{1/2} \quad (1)$$

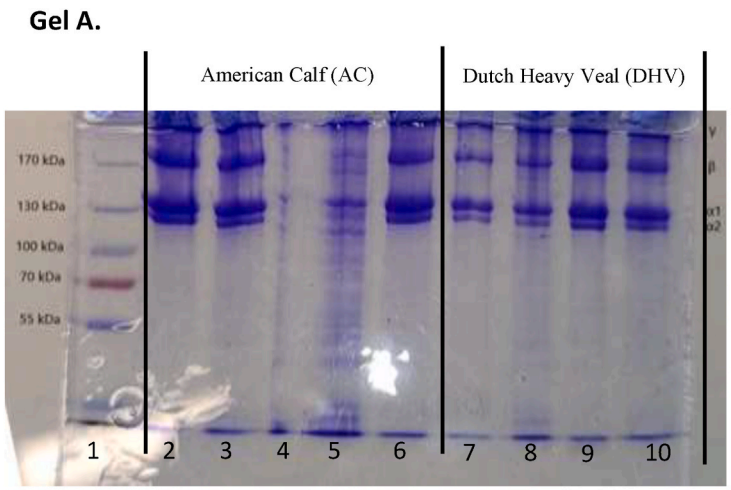
By plotting the complex viscosity η^* as a function of the angular frequency ω the dynamic consistency index k^* (Pa s^{n*}) and the dynamic power law factor n^* (−) were calculated by applying Eq. (2) (Keogh & O'Kennedy, 1998).

$$\eta^* = k^* \omega^{n^*-1} \quad (2)$$

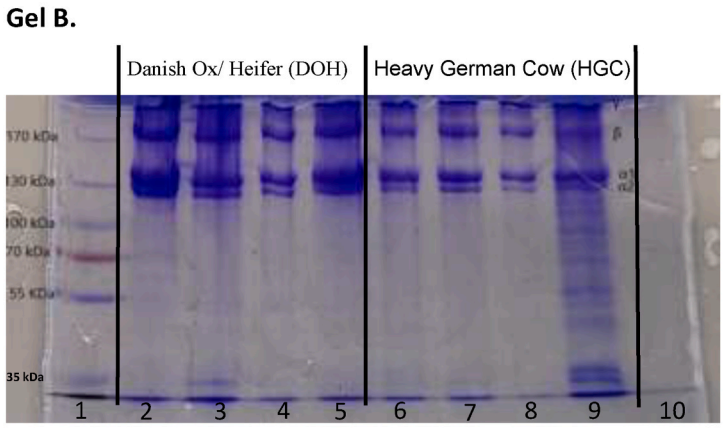
Temperature sweeps of the dispersion were performed in the linear viscoelastic range by applying 0.1% strain from 5 to 60 °C at 2 °C/min at 1 Hz. The temperature at which G' (Pa) started to decrease (at least one decade) was determined as a measure of the helix to random coil transition. Four replicates of each skin type were run in duplicate, whereby the average of the duplicates was used in the statistical analysis.

2.8. Primary amine group content of collagen dispersions

The number of primary amine groups present in the lyophilized dispersions were determined using 2,4,6-trinitrobenzenesulfonic acid (TNBS). The methodology originated from Buttafoco et al. (2006). Briefly, 1 mg lyophilized samples were incubated for 30 min in 1 ml aqueous solution of 4% w/v $NaHCO_3$. A solution of 0.5% w/v TNBS (1 ml in MilliQ water) was added to the mixture and incubated at 40 °C for 2 h. After adding HCl (3 ml, 6 M), hydrolyzation started at 60 °C for 90



Lane	Sample and run (R) number
1	Prestained protein ladder
2	AC-R1
3	AC-R2
4	Empty
5	AC-R3
6	AC-R4
7	DHV-R1
8	DHV-R2
9	DHV-R3
10	DHV-R4



Lane	Sample and run (R) number
1	Prestained protein ladder
2	DOH-R1
3	DOH-R2
4	DOH-R3
5	DOH-R4
6	HGC-R1
7	HGC-R2
8	HGC-R3
9	HGC-R4
10	Empty

Fig. 4. SDS-PAGE gel of the marker (A and B lane 1) and of the dispersions on gel A: American Calf (AC-R1 to AC-R4) (lanes 2 to 6); Dutch Heavy Veal (DHV-R1 to DHV-R4) (lanes 7 to 10). Gel B: Danish Ox/Heifer (DOH-R1 to DOH-R4) (lanes 2 to 5); Heavy German Cow (HGC-R1 to HGC-R4) (lanes 6 to 9), indicating the presence of β, α1 and α2 collagen bands, whereby AC-R3 and HGC-R4 showing bands at the lower molecular weight, most likely due to degraded collagen as during mixing a too high temperature was generated in the high shear mixer. DHV-R2 and DOH-R2 show bands at 35 kDa, indicating the presence of pepsin. The four lanes per animal type represent four replications.

min. A 96 well plate was used to dilute the mixture 1:1 with MilliQ water, followed by mixing and measuring the absorbance at 420 nm (Bio-Tek Spectrophotometer, Bad Friedrichshall, Germany). A glycine calibration curve was used to calculate the concentration of free amine groups. Four replicates of each skin type were run in triplicate, whereby the average value was used in the statistical analysis.

2.9. Film preparation from cattle skin collagen dispersions

Film forming ability, strength and Young's modulus of the films were evaluated by putting 5 g of each dispersion on a stainless-steel board, between two layers of plastic sheets, and flattening them with a stainless-steel roller (Fig. 3). To achieve uniform film thickness, a roller with a recess of 0.5 mm was used. Sodium chloride solution (24%) was added by a Pasteur pipette to the side of the collagen film, which was rolled flat between two plastic sheets. Then the collagen precipitated, by gently lifting the plastic sheet to the top of the collagen film. Ten films were made (dimension 150 mm × 40 mm) from each of the four replicates (per skin type) and evaluated for their mechanical properties.

2.10. Mechanical properties of collagen films

Films were determined by texture analyzer (TA.XT2, Stable Micro Systems, Godalming, UK) equipped with a 5 kg load cell to evaluate the Young's modulus and strength of the films after exposure to 24% NaCl (mimicking industrial settings). Therefore, the films were cut into 150 mm × 40 mm strips and precipitated in 24% NaCl solution. The tensile measurements were performed by fixing the film with tensile grippers, employing a crosshead speed of 40 mm/s, gripper initial distance was set at 20 mm, target distance at 120 mm, and a break sensitivity at 1.0 N. The initial thickness (0.5 mm) and width (40 mm) of each film was used for the tensile stress calculations. Tensile strength (maximum stress the film can handle prior to breaking) and the Young's modulus (maximum elongation the film reached just prior to breaking) were determined from the created stress-strain curves. Ten films per batch were evaluated, and the average value was used in the statistical analysis.

2.11. Primary amine group content of crosslinked collagen films

Films were crosslinked overnight with 5% glutaraldehyde (Merck, Darmstadt, Germany), followed by lyophilization. The number of primary amine groups present in the lyophilized crosslinked collagen films was determined using TNBS as described. Four replicates of each skin type were measured in triplicate, whereby the average value was used in the statistical analysis.

2.12. Statistical analysis

In the experiment, different cattle types were used, each being slaughtered at a different age. This means that the cattle type was confounded with age. Therefore, only type was included as a factor in the statistical model.

Statistical analysis was performed (Minitab Version 19, Minitab Ltd., Coventry, UK). Data were checked for normality in both means and residuals. A general linear model was used for analysis of variance (ANOVA) for protein and soluble collagen content, thermal, rheological, and mechanical properties, and the primary amine group content. The model used was:

$$Y = \mu + Type + Run + e,$$

where Y = dependent variable; μ = overall mean; Type = American Calf (AC), Dutch Heavy Veal (DHV), Danish Ox/Heifer (DOH), or Heavy German Cow (HGC); Run = 1, 2, 3 or 4; e = residual error. Data are presented as means ± SD. Means were compared after correction for multiple comparisons using the Bonferroni post-hoc test ($p \leq 0.05$).

Spearman correlation analysis was used to determine the relationship between soluble collagen content and viscoelastic and mechanical properties of both dispersions and films ($p \leq 0.05$).

3. Results and discussion

3.1. Co-extrusion technology: Zone 0

3.1.1. Collagen dispersion

Zone 0 of the co-extrusion process relates to receiving the raw collagen dispersion and in our study to determine its composition and quality. Soluble collagen content is of importance for understanding the flow properties (i.e., providing the glue to hold the fibers and fibrils together). The amount of soluble collagen (tropocollagen) in the dispersion depends on the age of the animal and chemical processing. For example, collagen derived from young animal's skin has a high level of soluble collagen, and the liming process will also solubilize some of the collagen. The amount of soluble collagen in commercial dispersions is not always fixed and depends on processing conditions.

Soluble collagen extracted from the skins of HGC (1.6%) was lower ($P < 0.05$) than for DOH (9.8%), AC (9.5%) and DHV (6.6%). It was expected that the soluble collagen content of HGC would be lower compared to AC and DHV since these animals are older. Miller et al. (1983) reported that with increasing the animal age, a lower soluble collagen content was found in corium samples. This is related to increased development of stable intramolecular and intermolecular crosslinks, which are more resistant to destruction by heat or acids (Miller et al., 1983).

To produce collagen dispersions, type I collagen which is present in the bovine skin is used (Oechsle et al., 2016). Type I collagen triple helix contains two α1(I) molecules and one α2(I) polypeptide chains (Chambers, 2015). The dispersions prepared from AC, DHV, DOH and HGC showed a double band at approximately 130 kDa (Fig. 4), probably indicating α1(I)- and α2(I)-chains of type I collagen (molecular mass of $\alpha_2 < \alpha_1$ with approximate mass ratio 1:2). In addition, a band above 170 kDa was present, which may indicate a β-chain. The dispersions prepared from DHV-R2 and DOH-R2 showed bands at 35 kDa, most likely pepsin used during preparation of the collagen dispersions. All other dispersions showed no low molecular weight bands, indicating no major presence of degraded collagen, except for samples AC_R3 and HGC_R4. Here bands were visible at lower molecular weight, indicating collagen breakdown products and/or small protein contaminants. As these two isolations suffered from temperature built-up in the high shear mixer, it is most likely degraded collagen, resulting in heat denaturated collagen (gelatin). For this reason, it was decided to exclude dispersions AC_R3 and HGC_R4 from further analyses.

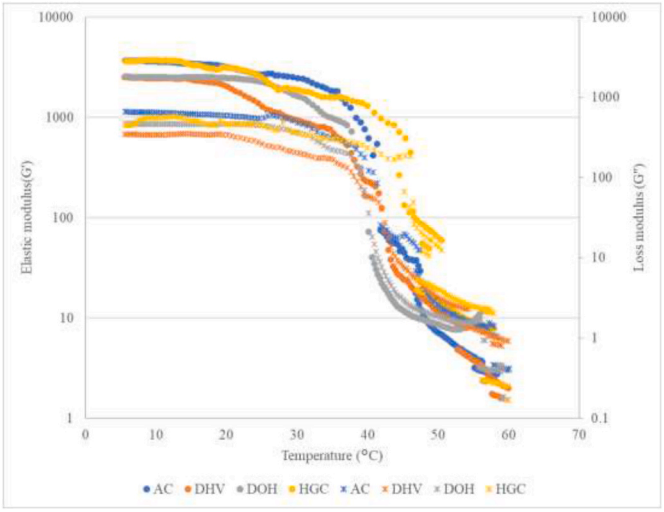


Fig. 5. Representative curve of elastic (•) and loss modulus (x) (scans 5–60 °C at 2 °C/min) of the cattle collagen dispersions: American Calf (AC); Dutch Heavy Veal (DHV); Danish Ox/Heifer (DOH); and Heavy German Cow (HGC); showing for all dispersions a decrease in elasticity between 35 and 40 °C (n = 4 per treatment for DHV and DOH; n = 3 per treatment for AC and HGC).

Dry matter content was analyzed as it indicates about all the other ingredients present in the dispersion. In general, a commercial collagen dispersion consists of 4–10% collagen (Bueker et al., 2016) and 0–10% of modifiers (Barbut et al., 2020), resulting in dispersion with 3–25% dry matter (Kobussen et al., 2000). Dispersions in the current study did not differ in dry matter content and averaged 8.2 ± 2.7, 6.7 ± 1.6, 6.6 ± 1.5 and 6.1 ± 1.4 g/100 g, for AC, DOH, HGC and DHV, respectively. The values of AC, DOH and HGC were somewhat higher than those of commercial bovine collagen dispersions reported by Barbut et al. (2020), where the dry matter ranged from 4.3 to 6.4%.

Protein content was analyzed since it provides information useful to control the texture characteristics of the sausage, e.g., first bite in sausage. In general, a higher protein content in the casing results in a stronger casing. Protein concentrations in the dispersions did not show significant differences between cattle types ($P = 0.560$) and averaged 24.2 ± 1.1, 23.4 ± 1.3; 23.0 ± 0.8 and 22.7 ± 3 g/100 g for HGC, DHV, DOH and AC, respectively. Expressed on a dry matter basis the protein concentrations averaged 1.9 ± 0.7, 1.5 ± 0.2, 1.6 ± 0.3 and 1.4 ± 0.3 g/100 g for AC, DOH, HGC and DHV respectively. They are different compared to reported commercial bovine dispersions ranging from 3.5 to 5.1% (Barbut et al., 2020). This difference is probably due to the procedure to calculate the protein content. Barbut et al. (2020) used the Dumas procedure, whereas this study used a Lowry assay.

3.2. Co-extrusion technology: Zone 1

3.2.1. Counter rotating cone extrusion

Zone 1 of the co-extrusion process (Fig. 1) is dealing with shaping of the product, i.e., creating a casing as the sausage is being produced while a collagen dispersion is added to the sausage surface, i.e., to be later gelled in place (Suurs & Barbut, 2020). As some heat can also be generated within the nozzle head (due to friction of the counter rotating action of the nozzles), water-cooled nozzles are used. However, it is still important to know the transition temperature of the collagen dispersion from the helical to random coil form (i.e., transformation to gelatin due to breakage of hydrogen bonds between adjacent polypeptide chains of collagen molecules (Zhang et al., 2006), since the mechanical strength of a film is critical for the process (i.e., expansion of the meat during heating) and the final product (sensory perception). Fig. 5 shows that in all the temperature profiles between 35 °C and 40 °C all dispersions started to display a rapid decrease in elasticity and the loss modulus,

Table 1
Endothermic peaks analysis from the differential scanning calorimeter (DSC) thermograms cattle collagen dispersions made of American Calf, Dutch Heavy Veal, Danish Ox/Heifer, and Heavy German Cow determined in duplicate as collagen dispersion (n = 4 runs per treatment for DHV and DOH; n = 3 runs per treatment for AC and HGC). Mean ± standard deviation.

Skin type	T_{onset} (°C)	T_{peak} (°C)	Enthalpy ΔH (J/g)
American calf	33.58 ± 0.31	36.34 ± 0.31	3.30 ± 1.10
Dutch heavy veal	33.38 ± 0.41	36.59 ± 0.79	2.82 ± 0.97
Danish ox or heifer	33.41 ± 0.45	36.21 ± 0.42	4.00 ± 1.27
Heavy German cow	33.52 ± 0.55	36.82 ± 0.66	2.32 ± 0.23
P-value_type	0.78	0.76	0.27
P-value_run	0.39	0.64	0.74

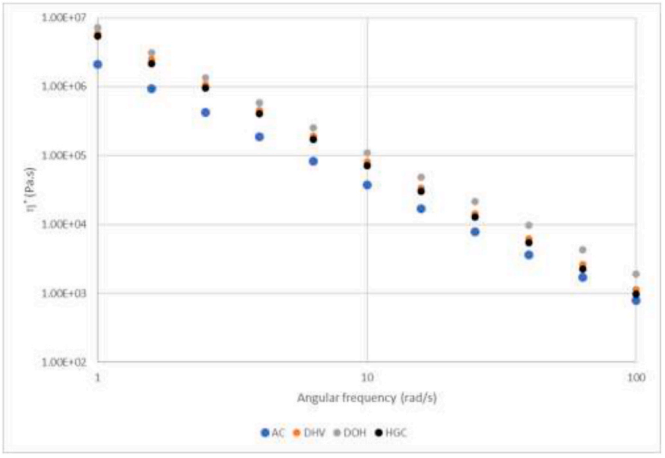


Fig. 6. Complex viscosity η^* calculated from $\left[\left(\frac{G''}{\omega}\right)^2+\left(\frac{G'}{\omega}\right)^2\right]^{1/2}$ of cattle collagen dispersions made of American Calf (AC), Dutch Heavy Veal (DHV), Danish Ox/Heifer (DOH), and Heavy German Cow (HGC) as a function of the angular frequency ω (n = 4 runs per treatment for DHV and DOH; n = 3 runs per treatment for AC and HGC). Overall, showing shear thinning behavior following a power-law model for all dispersions.

reflecting intact trimers (γ) of collagen turning into individual chains (α) or dimers (β) in the transition from helical to random coil (Zhang et al., 2006). Barbut et al. (2020) reported a rapid decrease in elasticity between 30 and 45 °C for commercial bovine collagen dispersions. Our findings confirm the range of onset temperatures also measured by DSC (Table 1). Overall, DSC measurements allow to determine the helix-to-coil transition temperature of the dispersions (Horgan et al., 1991). No significant differences in T_{onset} and T_{peak} were found between the different collagen dispersions, indicating that cattle type had no influence on the thermal properties of the dispersions. However, the current study indicates differences in the enthalpy values among the cattle types, with lower values for HGC than for AC, DHV and DOH (P = 0.27). According to Schroepfer and Meyer (2017), the enthalpy corresponds to the number of hydrogen bonds in the triple helix that actually provides stability to the helix. Lower enthalpy values correspond to degraded collagen, while crosslinking increases the denaturation temperature and results in higher enthalpy values (Paul & Bailey, 2003). A higher enthalpy value for the DOH dispersion may be related to the animal's type/purpose (e.g., meat/milk production versus working animals), having more collagen development due to excercise and the environment conditions where they were kept (e.g., outside). A low enthalpy value from HGC dispersions was not expected as German heavy cows were used for milk production and breeding purposes and the higher slaughter age and older animals have more intramolecular and intermolecular crosslinks (Miller et al., 1983).

Based on the thermal stability in the current study, all collagen dispersions appear suitable to be used in a co-extrusion application, as this is in the temperature range reported for commercial bovine collagen dispersions.

3.2.2. *Extrusion of the dispersion*

The collagen dispersion should have the desired flow properties, i.e., it should be viscous enough that it flows out of the nozzle and forms a thin layer around the meat dough, and it must also be elastic enough so that the thin layer does not run off the sausages' surface. Determining the complex viscosity (η^*), dynamic consistency index (k^*), and dynamic power law factor (n^*) gives insight into these rheological properties of dispersions. All dispersions exhibited shear-thinning behavior, following a power-law model, as can be seen when the complex viscosity η^* is plotted as a function of the angular frequency (ω) (Fig. 6). Overall, the complex viscosity of all dispersions was comparable. The k^* value showed no significant difference for cattle type (P > 0.05, Fig. 7A), but large variations in k^* were found within the different cattle types, which may be due to variability of skin tissue within the cattle type used to make the dispersion. Skin parts located closer to the spine of the animal are tougher (stronger fiber bundles) than parts located closer to the abdomen (loose fiber bundles) (Covington, 2009, pp. 29–71). As tougher bundles will swell less compared to loose fiber bundles, this could result in differences in viscosities. The k^* values of HGC, DHV, AC, and DOH were 59, 68, 95 and 114 Pa s^{n*}, respectively. In the production of commercial bovine collagen, large kneaders are used, including several homogenization steps, which results in a smooth homogenous dispersion (Bueker et al., 2016), unlike our protocol. Different acids and

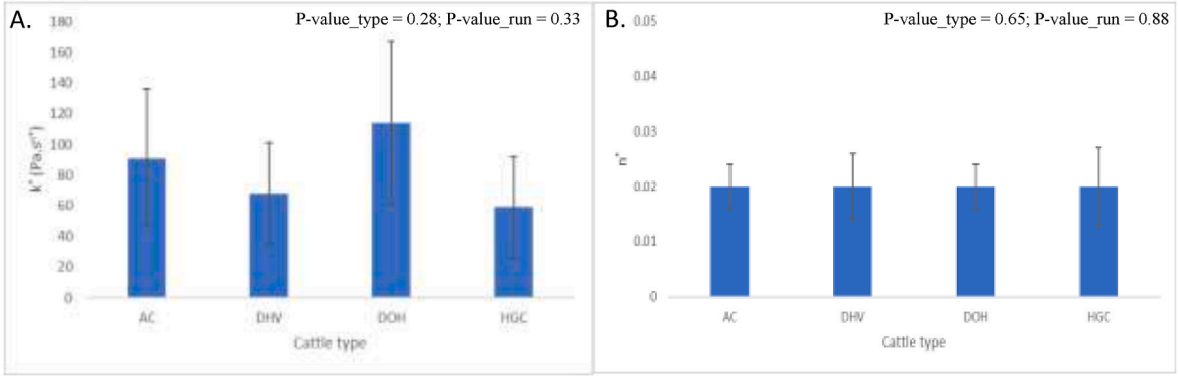


Fig. 7. A) Dynamic consistency index (k^*) and B) the dynamic power law factor (n^*) of collagen dispersions made from AC (American Calf), DHV (Dutch Heavy Veal), DOH (Danish Ox/Heifer), and HGC (Heavy German Cow). Bars represent mean ± standard deviation. A) Highest consistency value (k^*) was found for DOH, but no significant differences between the dispersions. B) all dispersions shows almost only elastic behavior (n). n = 4 runs per treatment for DHV and DOH; n = 3 runs per treatment for AC and HGC.

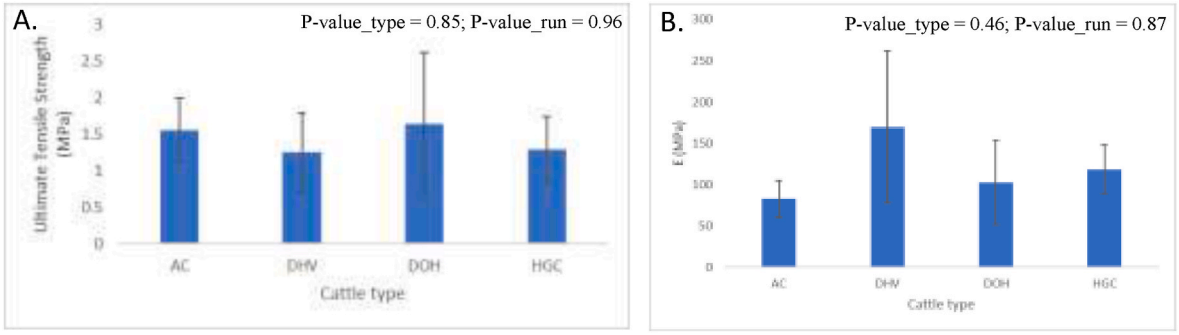


Fig. 8. Mechanical properties of collagen films prepared from cattle dispersions produced by exposing to 24% NaCl; A) Ultimate tensile strength (UTS), and B) Young's modulus (E) for American Calf (AC), Dutch Heavy Veal (DHV), Danish Ox/Heifer (DOH), and Heavy German Cow (HGC). Overall, showing no significant didfferences in UTS and E values for the films. n = 4 runs per treatment for DHV and DOH; n = 3 runs per treatment for AC and HGC. Bars represent mean ± standard deviation.

concentrations have an effect on the rheological behaviour of the dispersions. Hydrochloric acid for example is the preferred acid, used by the industry, as it yields a more viscous paste and improves the swelling rate of the collagen fibers (Ioi, 2013; Ratanavaraporn et al., 2008; Sobanwa, 2021). Oechsle et al. (2014) reported that collagen entanglement and network formation depends strongly on the pH and acid type. They found enhanced collagen entanglement with increased ionic strength of the solvent (in case pH < pI). This can influence the production of the collagen dispersion, depending on what is needed for the co-extrusion process. According to Oechsle et al. (2014) it is likey that the highly entangled collagen matrices will probably result in co-extruded collagen casings with high elasticity and tensile strength.

The power law factor (n^*) (Fig. 7B) showed no significant difference for cattle type ($P > 0.05$). The n^* values for AC, DHV, DOH and HGC were comparable (0.02), which means they demonstrate an elastic behavior. Viscoelastic systems have a n^* value between 0 and 1, where 0 means completely elastic and 1 means Newtonian behaviour (Keogh & O'Kennedy, 1998). The values found in our study 0.02 ± 0.004, 0.02 ± 0.006, 0.02 ± 0.004 and 0.02 ± 0.007 (−) for AC, DHV, DOH and HGC, respectively are comparable to the commercial bovine collagen dispersions ($n^* = 0.01$).

Spearman correlation between soluble collagen content and viscoelastic properties of all dispersions showed a positive relationship ($p = 0.009$) with the dynamic consistency index k^* ($r = 0.670$). Changes in soluble collagen content are associated with changes in the dynamic consistency index k^*. No relationship of soluble collagen content was found with the power law factor n^* ($r = 0.213$, $p = 0.464$).

Considering that not only the k^* value is responsible for flow properties of the dispersion, but the amount of soluble collagen as well (Barbut et al., 2020), it appears that the HGC skin is less suitable as collagen source for co-extrusion casings. Moreover, it is expected that the flow properties of the dispersions prepared from AC, DHV and DOH skins are not optimal for co-extrusion, because of the high k^* values, and therefore require viscosity adjustment by dilution with aqueous acetic acid.

3.2.3. *Brining of the dispersion - creating mechanical strength*

The next step in the co-extrusion process (Fig. 1) is creating mechanical strength by immersion of the sausage rope in a sodium chloride solution (e.g., 20–30% NaCl), which dehydrates the casing by water removal. This results in a denser collagen fiber network that provides the initial strength to the casing (Kobussen et al., 2000; Visser, 2012). This is important as the sausage is later exposed to different stresses, as it undergoes subsequent treatments, like smoking, drying and cooking with additional moving and shaking on a conveyor, and pushing into baskets (Kobussen et al., 2000; Morgan et al., 1988). The sodium chloride solution doesn't affect the sensory properties of sausages produced under commercial conditions. As a matter of fact, the salt concentration at the sausage surface will be low as water is being pulled out of the product/"expelled by the casing". Overall, in industrial co-extrusion applications salt must be added on a continuous basis to the circulation solution to keep it always saturated. The general observation was that films could easily be made from the AC, DHV and DOH dispersions, but films from HGC dispersions showed poor film forming ability, probably due to the fibrous structure of the dispersion resulting in films with small fractures. However, no significant differences ($P > 0.05$) were found among the different skin types Ultimate Tensile Strength (UTS) values were 1.6 ± 0.4, 1.6 ± 0.9, 1.3 ± 0.5, and 1.2 ± 0.5 MPa, for AC, DOH, DHV and HGC, respectively; Fig. 8A. It was expected that the dispersion of HGC would yield weaker films (i.e., tensile strength), possibly due to the low soluble collagen content, which is necessary to hold the fibers and fibrils together. The amount of soluble collagen was low as the raw material originated from animals that have formed quite some crosslinks due to age (Miller et al., 1983).

The Young's modulus of the films was calculated to gain insight into the behavior of the films when subjected to a force. A higher Young's modulus is associated with brittle films, whereas a low modulus reflects flexible films (Chakravartula et al., 2019). No significant differences ($P > 0.05$) were found among the different skin types in Young's modulus and averaged 170 ± 92, 119 ± 30, 103 ± 51, and 83 ± 23 MPa, for DHV, HGC, DOH and AC, respectively; Fig. 8B. Large variations in strength and Young's modulus were found within the different cattle types, probably due to the large variation in parts of the skin being used to prepare the dispersions. Barbut et al. (2020) analyzed five commercial bovine collagen dispersions and reported tensile strength values that were approximately 6 times lower. This difference is most probably due to differences in exposure time in the NaCl. Barbut et al. (2020) used an exposure time of 5 min in 30% NaCl, whereas in this study maximal exposure time in 24% NaCl was applied (overnight storage of the films between two plastic sheets without rinsing, so high salt concentration remains on the surface) before measuring the mechanical properties.

Spearman correlation between the soluble collagen content and the texture properties of all the films prepared showed a negative relationship with the Young's modulus of the films.

($r = -0.512$; $p = 0.043$). This means that a higher proportion of soluble collagen content results in a lower Young's modulus and thus a more flexible film. No relationship with soluble collagen content was found for the tensile strength values ($r = 0.418$, $p = 0.107$).

Overall, from the film strength perspective it is preferred to use dispersions prepared from AC, DHV and DOH. Although the films from HGC dispersion could be evaluated for their mechanical properties, the film forming capacity of the HGC dispersion was not sufficient.

Table 2
Number of NH_2 groups present in collagen dispersions and crosslinked films (5% glutaraldehyde) determined with TNBS assay.

Type	Amine groups in dispersion (nmol/mg sample)	Amine groups in x-linked films (5% glutaraldehyde) (nmol/mg sample)	Reduction of free amine groups
American calf	436 ± 53	324 ± 51	26%
Dutch heavy veal	439 ± 56	355 ± 39	19%
Danish ox/heifer	440 ± 34	324 ± 34	27%
Heavy German cow	482 ± 32	354 ± 40	27%

Dispersions and films were prepared from skins of American calf (AC), Dutch heavy veal (DHV), Danish ox/heifers (DOH), and heavy German cow (HGC). Overall, showing no significant differences in amine groups between the different dispersions and films. n = 4 runs per treatment for DHV and DOH; n = 3 runs per treatment for AC and HGC. Mean ± standard deviation.

Table 3
Properties of dispersions and films per skin type [American Calf (AC), Dutch Heavy Veal (DHV), Danish Ox/Heifer (DOH) and Heavy German Cow (HGC)] and their performance with respect to co-extrusion process and/or final product.

Measurement	Properties in relation to co-extrusion process/final product	American calf	Dutch heavy veal	Danish ox/heifer	Heavy German cow
SDS-PAGE	Type I collagen	+	+	+	+
DSC	Helix-to-coil temperature	+	+	+	+
Rheology	Extrudability	±	±	±	–
Texture	Film forming capacity	+	+	+	–
Texture	Casing strength	+	+	+	–
Amine groups	Crosslinking potential	+	+	+	+

± = property of the dispersions/films meets the criteria.

3.3. Co-extrusion technology: Zone 2

3.3.1. Crosslinking the films

For sausage manufacturers it is important to have information regarding the presence of crosslinkable groups in the dispersions and the final strength of the film as it is related to the sensory properties of the sausage. The TNBS assay was used to determine the free amine groups after crosslinking with glutaraldehyde. In this study, the choice was to use glutaraldehyde as crosslinker as its aldehyde content is known, so that it can serve as a reference for the efficiency of liquid smoke available on the market (Barbut & Ioi, 2019). Dispersions did not differ in the number of amine groups ($P > 0.05$) and averaged 482 ± 32, 436 ± 53, 440 ± 34, and 439 ± 56 nmol/mg, for HGC, AC, DOH and DHV, respectively (Table 2). Significant differences were expected between the younger AC and DHV versus older DOH and HGC (Miller et al., 1983), since the AC and DHV skins were soaked in NaOH for 24 h, while for DOH and HGC skins this was 48 h. Longer time in high alkaline solution can result in higher reduction of NH_2 groups (Suurs & Barbut, 2020).

After crosslinking the films, with 5% glutaraldehyde, no statistical differences were observed between AC, DHV, DOH and HGC crosslinked films. AC films showed 324 ± 51 nmol/mg free amine groups, whereas for DHV, DOH and HGC it was 355 ± 39, 324 ± 34, and 354 ± 40 nmol/mg, respectively, corresponding to a reduction of 26, 19, 27 and 27% primary amine groups (Table 2). Considering the ability of the dispersions to create crosslinks with glutaraldehyde, it is obviously expected that liquid smoke will also be able to form crosslinks, and therefore all four dispersions are considered suitable to produce cooked smoked sausages by the co-extrusion technology.

4. Conclusions

The properties of the dispersions and films are summarized in Table 3. Skins of the AC, DHV and DOH were found to have the best potential to serve as a suitable collagen source for the co-extrusion process. HGC skin appears less suitable since its film forming ability was poor, which is an important property for co-extruded films. The study also showed that optimization of the preprocessing treatments appears to play an important role and that different skin sources may need to be treated differently to achieve the best suitability. In addition, soluble collagen in the dispersion is a factor to be considered as it influences the viscoelastic and mechanical properties of the dispersions and films.

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Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Barbut, S., & Ioi, M. (2019). An investigation of the mechanical, microstructural and thermomechanical properties of collagen films cross-linked with smoke condensate and glutaraldehyde. *Italian Journal of Food Science, 31*(3), 644–660. https://doi.org/10.14674/IJFS-1290

Barbut, S., Ioi, M., & Marcone, M. (2020). Co-extrusion of collagen casings. Effects of preparation, brining and heating on strength, rheology and microstructure. *Italian Journal of Food Science, 32*, 91–106. https://doi.org/10.14674/IJFS-1557

Bontjer, M. B. H., Kuijpers, M. W. J. T., & Berg, van den K. W. (2011). Method for preparing food products by co-extrusion. In *In particular sausage and food products obtained with this method WO 2006/135238*.

Bueker, M., Bueker, G., & Grolig, G. (2016). In *Collagen concentrate, use thereof and also process for production thereof, Kalle GmbH*. US9,504,262.

Buttafoco, L., Engbers-Buijtenhuijs, P., Poot, A. A., Dijkstra, P. J., Daamen, W. F., Kuppevelt van, T. H., Vermes, I., & Feijen, J. (2006). First steps towards tissue engineering of small-diameter blood vessels: Preparation of flat scaffolds of collagen and elastin by means of freeze drying. *Journal of Biomedical Materials Research Part B: Appl. Biomater., 77*(2), 357–368. https://doi.org/10.1002/jbm.b.30444

Chakravartula, S. S. N., Soccio, M., Lotti, N., Balestra, F., Dalla Rosa, M., & Siracusa, V. (2019). Characterization of composite edible films based on pectin/alginate/whey protein concentrate. *Materials, 12*, 2454. https://doi.org/10.3390/ma12152454

Chambers, R. (2015). *Basic collagen info*. Paper from Stanford University. http://www.rdchambers.net/wp-content/uploads/2015/02/collagen.pdf. (Accessed 6 September 2022) accessed.

Covington, A. D. (2009). *Tanning chemistry – the science of leather, skin and its components*. Cambridge, United Kingdom: The Royal Society of Chemistry, RSC publishing.

Faraj, K. A., Brouwer, K. M., Geutjes, P. J., Versteeg, E. M., Wismans, R. G., Deprest, J. A., Chajra, H., Tiemessen, D. M., Feitz, W. F. J., Oosterwijk, E., Daamen, W. F., & Kuppevelt, van T. H. (2011). The effect of ethylene oxide sterilization, beta irradiation and gamma irradiation on collagen fibril-based scaffolds. *Tissue Engineering and Regenerative Medicine, 8*(5), 460–470.

Horgan, D. J., Kurth, L. B., & Kuypers, R. (1991). pH effect of thermal transition temperature of collagen. *Journal of Food Science, 56*(5), 1203–1204. https://doi.org/10.1111/j.1365- 2621.1991.tb04734.x

Ioi, M. (2013). *An investigation of commercial collagen dispersions and their use in co-extrusion sausage manufacturing*. Guelph, Ontario, Canada: MSc-thesis, University of Guelph, 1 – 97.

Keogh, M. K., & O'Kennedy, B. T. (1998). Rheology of stirred yoghurt as affected by added milk fat, protein and hydrocolloids. *Journal of Food Science, 63*(1), 108–112.

Kobussen, J., Kobussen, M., Kobussen, J., & Alexander, D. (2000). Brine formulation for curing extruded sausage strand. *U.S. patent no., 6*, 54–155.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Macosko, C. W. (1994). *Rheology: Principles, measurements, and applications*. Hoboken: WILEY VCH.

Matinong, A. M. E., Chisti, Y., Pickering, K. L., & Haverkamp, R. G. (2022). Collagen extraction from animal skin. *Biology, 11*(6), 905–920. https://doi.org/10.3390/biology11060905

Miller, A. T., Karmas, E., & Lu, M. F. (1983). Age-related changes in the collagen of bovine corium: Studies on extractability, solubility and molecular size distribution. *Journal of Food Science, 48*(3), 681–685. https://doi.org/10.1111/j.1365-2621.1983.tb14875.x

Morgan, T. F., Frame, G., & Kobussen, P. J. (1988). Process for producing a linked co-extruded edible product. *U.S. patent no. 5, 795*, 605.

Noorzai, S., Verbeek, C. J. R., Lay, M. C., & Swan, J. (2019). Collagen extraction from various waste bovine hide sources. *Waste Biomass Valori, 11*, 5687–5698. https://doi.org/10.1007/s12649-019-00843-2

Oechsle, A. M., Akgün, D., Krause, F., Maier, C., Gibis, M., Kohlus, R., & Weiss, J. (2016). Microstructure and physical–chemical properties of chicken collagen. *Food Structure, 7*, 29–37. https://doi.org/10.1016/j.foostr.2016.02.001

Oechsle, A. M., Wittmann, X., Gibis, M., Kohlus, R., & Weiss, J. (2014). Collagen entanglement influenced by the addition of acids. *European Polymer Journal, 58*, 144–156. https://doi.org/10.1016/j.eurpolymj.2014.06.015

Paul, R. G., & Bailey, A. J. (2003). Chemical stabilization of collagen as a biomimetic. *The Scientific World Journal, 3*, 138–155. https://doi.org/10.1100/tsw.2003.13

Ratanavaraporn, J., Kanokpanont, S., Tabata, Y., & Damrongsakkul, S. (2008). Effects of acid type on physical and biological properties of collagen scaffolds. *Journal of Biomaterials Science, Polymer Edition, 19*(7), 945–952. https://doi.org/10.1163/156856208784613505

Savic, Z., & Savic, I. (2016). *Sausage casings* (2nd ed.). Vienna, Austria: Victus, Inc.

Schroepfer, M., & Meyer, M. (2017). DSC investigation of bovine hide collagen at varying degrees of crosslinking and humidities. *International Journal of Biological Macromolecules, 103*, 120–128. https://doi.org/10.1016/j.ijbiomac.2017.04.124

Sobanwa, M. O. (2021). Redesign of collagen casings for high quality performance using food grade polysaccharides. In *PhD-thesis*. Loughborough, UK: University of Nottingham, 1 – 316.

Suurs, P., & Barbut, S. (2020). Collagen use for co-extruded sausage casings – a review. *Trends in Food Science & Technology, 102*, 91–101. https://doi.org/10.1016/j.tifs.2020.06.011

Toledo, R. T. (2007). Wood smoke components and functional properties. In D. E. Kramer, & L. Brown (Eds.), *International smoke seafood conference proceedings, Alaska sea grant college program* (p. 55). USA: Anchorage.

Visser, P. R. (2012). *Casings for foodstuffs*. U.S. patent no. 20120114807A1.

Wijnker, J. J. (2009). *Aspects of quality assurance in processing natural sausage casings*. Utrecht, The Netherlands: PhD-thesis Utrecht University.

Zhang, Z., Li, G., & Shi, B. (2006). Physicochemical properties of collagen, gelatin and collagen hydrolysate derived from bovine limed split wastes. *Journal of the Society of Leather Technologies and Chemists, 90*(1), 23–28.

Zhang, T., Yu, Z., Ma, Y., Chiou, B., Liu, F., & Zhong, F. (2022). Modulating physicochemical properties of collagen films by cross-linking with glutaraldehyde at varied pH values. *Food Hydrocolloids, 124*. https://doi.org/10.1016/j.foodhyd.2021.107270, 107270 1 – 12.