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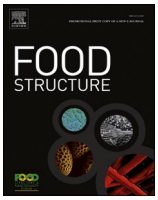

Effects of broiler weight and strain on skin collagen characteristics and their applicability for co-extruded sausage casings

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ABSTRACT

For centuries people around the world enjoyed traditional sausages made from meat stuffed into natural casings. An alternative new technology is to extrude collagen gel, originating from bovine hides, directly onto the product and later cross-link it. Collagen producers are searching for other sources and consequently they are interested in factors influencing extracted collagen quality. One of the alternative sources is chicken skin, where extracted collagen properties have been shown to be influenced by the age of the chickens. In this study, the biochemical and physical properties of chicken skin collagen preparations from two different broiler strains (slow and fast-growing) and two different weights (1.6 and 2.2 kg) were investigated. Rheological measurements showed for all dispersions a decrease in elasticity at 40 °C. Differential Scanning Calorimetry (DSC) measurements of the dispersions showed T_{onset} ranging from 38.7° to 39.1°C. After salt precipitation, the T_{onset} increased to 50.1 – 55.9 °C. Mechanical strength of the films from fast and slow growing chickens ranged from 63 to 67 KPa and 53–57 KPa, respectively. Considering the biochemical and physical properties, all four chicken collagen dispersions have the potential of being a suitable collagen source for the co-extrusion process of sausages.

1. Introduction

Processed intestines from pigs, sheep, and cattle, which can be considered as by-products of the livestock industry, are used as edible natural casings to produce sausages (Wijnker, 2009). Sausage casings play important functional roles from the moment of stuffing right up until the consumer eats the product. The selection of a casing is critical, as it not only influences the integrity, size, and shape of the sausage, but also assists in the conversion of soft meat batter into the desired sausage product during cooking (Savic & Savic, 2016). Casings are designed to accommodate sausage manufacture's quality and processing needs. The ideal collagen sausage casings should provide sufficient strength to undergo processing, while remaining tender enough during consumption (Miller et al., 1983). Natural casings are perceived to have the highest quality and are considered as the golden standard for sausage casings; i.e., this is due to certain characteristics known as: bite, knack, snap and elasticity (Escoubas et al., 2010; Savic & Savic, 2016). However, producers are searching for alternatives, because of the rising costs and availability of sheep and hog intestines as well as an increasing need for kosher/ halal products (Oechsle, 2016). An alternative casing material is collagen gel which, in combination with co-extrusion technology, can be applied directly onto a stream of meat exiting the stuffer's horn, and later splitting the meat rope into individual sausage links (Suurs et al., 2022). Co-extruded casings are produced with a collagen gel, consisting of fibrous and soluble collagenous material (Barbut et al., 2020). Currently, this collagen gel is particularly originating from bovine hides. However, alternative sources, like chicken skin collagen, have also been investigated for their application in co-extrusion technology (Oechsle et al., 2016). Before application is possible, factors that influence collagen quality need to be investigated. Suurs et al. (2022) showed that chicken age is a factor that influences the collagen gel quality for co-extrusion application. Particularly the skins of young broiler chickens appeared to be suitable as a source for collagen gel for co-extrusion. However, within young broiler chickens two other important factors, such as strain and body weight, may influence the collagen properties. The aim of this study was to investigate effects of two broiler strains

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(slow and fast growing) at two different body weights (1.6 and 2.2 kg) on skin collagen quality, by focusing on its application for co-extruded sausage casings.

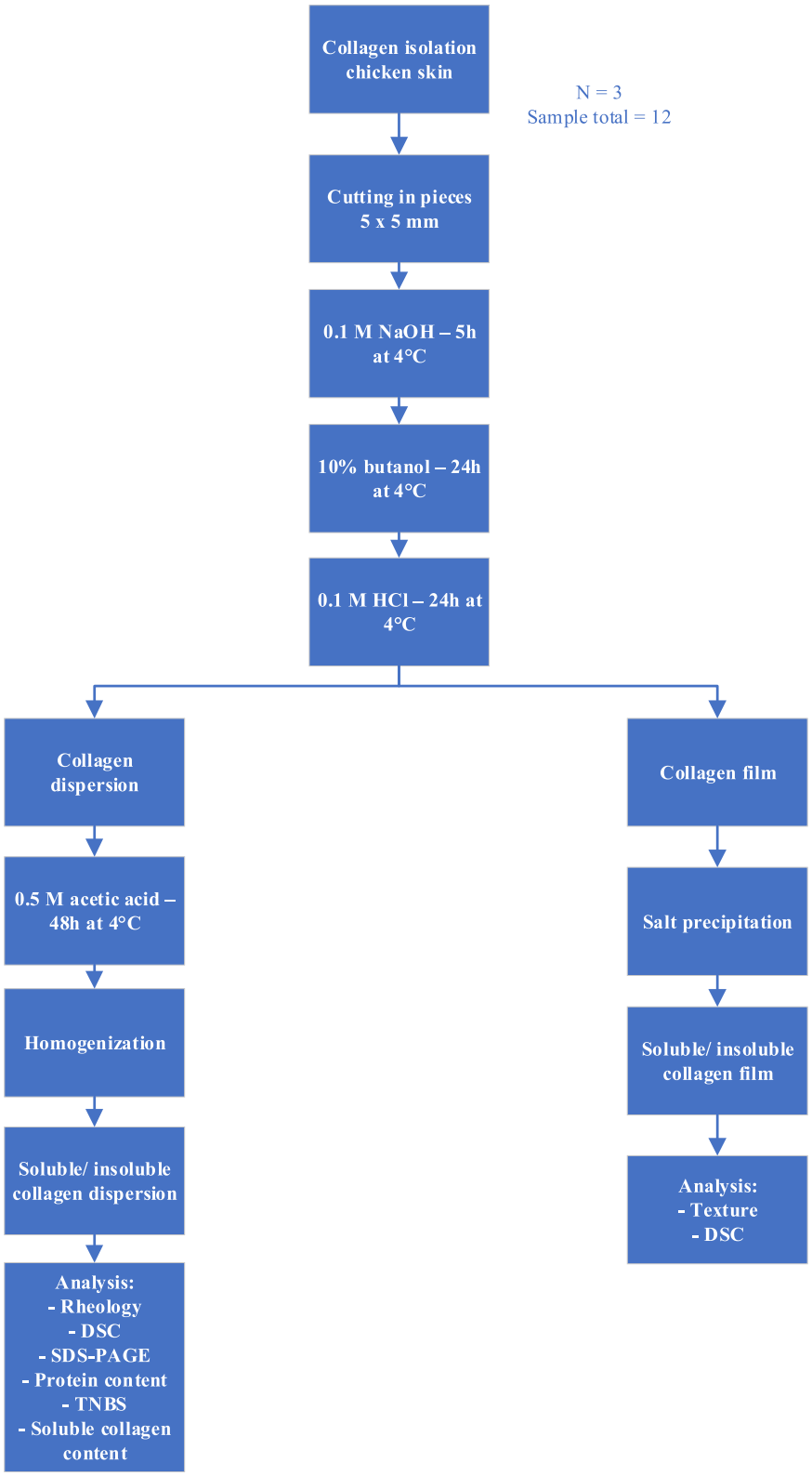

Fig. 1. Flow chart of the extraction methods used to obtain collagen dispersions and films, including an overview of the methods to analyze the dispersions and films. The analysis methods include Differential Scanning Calorimetry (**DSC**), Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (**SDS-PAGE**), and 2,4,6-trinitrobenzenesulfonic acid (**TNBS**).

2. Materials and methods

2.1. Experimental design

The experiment was setup as a 2 × 2 factorial design with two broiler chicken strains (fast growing (**FBC**) and slow growing (**SBC**)) and two different body weights (1.6 and 2.2 kg), resulting in 4 treatments: FBC_1.6 kg, FBC_2.2 kg, SBC_1.6 kg and SBC_2.2 kg. The skins of fast growing (Ross 308) and slow growing (Hubbard JA 757) broiler chickens were obtained from a study described by (Van der Eijk et al., 2022). Skin samples of 5 fast-growing chickens were randomly taken at day 28 (body weight 1.6 kg) and 10 skin samples at day 37 (body weight 2.2 kg). Skin samples of 5 slow growing chickens were randomly taken at day 37 (body weight 1.6 kg) and 10 taken at day 50 (body weight 2.2 kg). The whole skin (breast and neck) of the chickens was collected, followed by feather removal (manual plucking), after which the skins were stored as a pooled sample at − 18 °C, until further processing. No measures were taken to remove the subcutaneous fat. For the preparation of the collagen dispersions, both the dermis and epidermis were used. The pooled skin sample of each chicken source was divided into three portions so that three dispersions (n = 3) could be prepared from scratch.

2.2. Pretreatment of the skins

Dispersions were prepared according to a multistep process from Munasinghe et al. (2014) with slight modifications. Next, they were analyzed for their physical and chemical properties (Fig. 1). Briefly, chicken skins were thawed, rinsed with tap water to remove debris, and cut into small pieces (5 ×5 mm). Skins were incubated in 0.1 M NaOH (Boomlab, Meppel, The Netherlands) at a sample/ NaOH ratio of 1:6 (w/v - based on the wet weight of the original sample). The mixtures were stirred continually for 5 h on a shaker at 4 °C. NaOH was replaced every 2 h, and at the end it was removed by washing with distilled water and filtering (2.5 mm mesh) until neutral pH was reached. Thereafter, fat was removed by adding 10% butanol (Chemlab, Zedelgem, Belgium) at a sample/ butanol ratio of 1:6 (w/v – based on the weight of the swollen chicken pieces). The mixtures were stirred continually for 24 h at 4 °C and the solvent solution was renewed after 12 h. At the end, butanol was removed by washing with distilled water and filtering. The defatted skin samples were soaked in 0.1 M HCl (Boomlab, Meppel, The Netherlands) with a sample/ HCl ratio of 1:6 (w/v). The mixture was stirred continually for 24 h at 4 °C on a shaker, and filtered, followed by rinsing with distilled water until the pH of the wash water reached neutral pH.

2.3. Collagen extraction – preparation of soluble/ insoluble collagen dispersions

The pretreated samples were swollen in 0.5 M acetic acid (Supelco, Zwijndrecht, The Netherlands) for 48 h at a sample/ acetic acid ratio of 2:1 (w/v - based on the weight of the sample after pretreatment) at 4 °C. Samples were then homogenized (JAP blender, Fusionesco, Q-Blend, 1500 W, The Netherlands) until a homogenous collagen dispersion was obtained.

2.4. Collagen extraction – preparation of soluble collagen

Additional samples, from the fast and slow growing broilers with the heaviest weight (2.2 kg), were prepared to obtain an indication of the amount of soluble collagen in these dispersions. Samples were obtained in the same way as described earlier, but after swelling in 0.5 M acetic acid, the skin material was filtered (2.5 mm mesh), while the filtrate was collected and precipitated to obtain soluble collagen. Precipitation was done by adding NaCl crystals (Boomlab, Meppel, The Netherlands) until a final concentration of 2.6 M was reached. The solution was stirred and left for 60 h at 4 °C. The precipitates were collected by centrifuging (Thermo Scientific, IEC, CL10, Fixed Angle Rotor F-G1) at 3700 g for 30 min and supernatant was discarded. The resulting pellet was dialyzed (Ø 17.5 mm, molecular weight cutoff at 14 kDa; Medicell, London, United Kingdom) against 0.1 M acetic acid for 48 h.

2.5. Protein and moisture content of chicken collagen dispersions

Lyophilization (LP-03, IlShinBioBase Europe, Ede, The Netherlands) of the dispersions was done to determine the dry matter content of the collagen dispersions. Approximately 25 g wet material was lyophilized, followed by weighing of the dry material, and calculating the dry matter content.

Protein content of the lyophilized chicken dispersions was assessed (Lowry et al., 1951). Incubation of 10 mg of lyophilized chicken dispersions in 5 U papain (Sigma-Aldrich, Zwijndrecht, The Netherlands) and 1 ml digestion buffer (50 mM $NaPO_4$, 2 mM cysteine, 2 mM ethylenediaminetetraacetic acid (**EDTA**, pH 6.5; Merck, Amsterdam, The Netherlands) was done for 16 h at 65 °C. Followed by centrifugation (5 min; 13,000 g) of the digested samples. The supernatants were used for protein measurements. Bovine serum albumin (**BSA**) (0–250 ng/ml) (Sigma-Aldrich, Zwijndrecht, The Netherlands) and a blank with only papain digestion buffer (50 mM $NaPO_4$, 2 mM cysteine, 2 mM EDTA, pH 6.5; Merck) were used to make a calibration curve. The blue color that is formed in the reaction was measured at A_{max} 750 nm.

2.6. SDS-PAGE analysis

To analyze the molecular weight of the protein chains in the collagen dispersions and to assess the presence of different collagen types or collagen breakdown products sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was conducted. The method was adopted from Faraj et al. (2011). Briefly, 2.5 mg of lyophilized collagen was suspended in 150 µl 2x concentrated sample buffer (4% SDS, 20% (v/v) glycerol, 0.125 M TRIS/ HCl pH 6.8, 20% (v/v) 2-β mercaptho-ethanol, bromo phenol blue) and heated for 10 min at 70 °C. Both samples, and controls were loaded on an 8% (w/v) polyacrylamide gel (0.37 M TRIS, final concentration is 0.1% SDS for both running and stacking gel; running buffer: TRIS/ glycine 2.5 mM/ 0.2 M). Running the gel took 1.5 h at 100 V until the bromo phenol blue front was at the end of the gel. Staining was done with 0.1% (w/v) Coomassie Brilliant Blue solution (50% (v/v) methanol and 10% (v/v) acetic acid; R-250, VWR International BV, Amsterdam, The Netherlands) overnight. Destaining was performed using a solution containing 50% (v/v) methanol (Macron Fine Chemicals, VWR International BV, Amsterdam, The Netherlands) and 10% (v/v) acetic acid (Boomlab, Meppel, The Netherlands) until all bands were clearly visible. Each dispersion (n = 3) was analysed on the gel.

2.7. Rheology

Flow behavior can give an indication of the applicability of the dispersions for use in a co-extrusion system. The flow behavior of the different dispersions was measured with an oscillatory rheometer type AR2000 (TA Instruments, New Castle, USA) equipped with a Peltier plate and a water bath. The rheology measurements were performed based on Oechsle et al. (2016), with slight modifications. Oscillatory measurements, using a 40 mm diameter (hard-anodised aluminium) plate-plate geometry were used to analyse the collagen dispersions. To determine the linear viscoelastic range, stress sweeps were performed at 1 Hz by applying oscillatory stress from 0.01 to 1000 Pa at 5 °C, followed by frequency sweeps performed in the linear viscoelastic range applying 0.1% strain from 1 to 100 rad/s. The complex viscosity η^* was determined as a function of angular frequency ω by applying Eq. (1) (Macosko, 1994):

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

To calculate the dynamic consistency index k^* and the dynamic power law factor n^* by applying Eq. (2) (Keogh & O'Kennedy, 1998), the complex viscosity η^* as a function of the angular frequency ω was plotted.

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

By applying 0.1% strain from 5° to 60°C with ramp rate of 2 °C/ min at 1 Hz temperature sweeps of the dispersions were performed. The temperature at which G' (Pa) started to decrease as a measure of the helix to random coil (i.e., gelatin) transition was determined. The three replicates of each dispersion were measured in duplicate, whereby the average of the duplicates was used in the statistical analysis.

2.8. Determination of primary amine group content

Using 2,4,6-trinitrobenzenesulfonic acid (**TNBS**) the number of primary amine groups present in the lyophilized chicken dispersions was determined. The method was adopted from Buttafoco et al. (2006). An aqueous solution of 4% w/v $NaHCO_3$ (1 ml) was used to incubate the lyophilized samples of 1 mg for 30 min, followed by the addition of a solution of 0.5% w/v TNBS (1 ml) in MilliQ water and incubation at 40 °C for 2 h. HCl (3 ml, 6 M) was added followed by hydrolyzing the samples at 60 °C for 90 min. The mixture was diluted in a 96 well plate 1:1 with MilliQ water. The absorbance was measured at 420 nm, using a spectrophotometer (Bio-Tek, Bad Friedrichshall, Germany). A glycine calibration curve was used to determine the concentration of amine groups. The three replicates of the dispersions were measured in triplicate, whereby the average value was used in the statistical analysis.

2.9. Film preparation

To evaluate the dispersions for film forming ability and strength, films were prepared by placing 5 g of each dispersion on a stainless-steel board, between two layers of plastic sheets, and flattening them with a stainless-steel roller. The roller had a recess of 0.5 mm to achieve uniform film thickness. Sodium chloride solution (24%) was added by a Pasteur pipette to the side of the collagen film between the two plastic sheets, and by carefully lifting the plastic sheet, to the top of the collagen film, the collagen precipitated. Ten films were made (dimension 150 mm × 40 mm) from each of the three replicates (per skin type) and evaluated for their mechanical and thermal properties.

2.10. Thermal transition measurement

Differential Scanning Calorimetry (**DSC**) measurements were performed to study the thermal behavior of the chicken skin dispersions and the precipitated films. The endothermal transition of the dispersions was measured by a DSC (Q1000, TA Instruments, New Castle, DE, USA) equipped with a cooling unit (RCS40). About eight mg of aqueous collagen dispersion or precipitated film were hermetically sealed in a T_{zero} aluminum pan. The sample was equilibrated at 1 °C for 5 min. For the collagen dispersion, a temperature ramp was performed from 1° to 60°C, at 5 °C/ min, while the precipitated collagen films were scanned at a temperature ramp of 1–80 °C, at 5 °C/ min. For all measurements an empty aluminum pan was used as the reference probe. Values determined were: temperature at which denaturation of the collagen helix started (T_{onset}), temperature at which 50% of the collagen helix has unfolded (T_{peak}), and the enthalpy value of denaturation (ΔH), using the DSC software (Universal Analysis 2000 (Version 4.5 A), TA instruments). The three replicates of each skin type were measured in duplicate, whereby the average of the duplicates was used in the statistical analysis.

2.11. Mechanical properties

Strength and elasticity parameters of the films, cut into 150 mm × 40 mm strips and precipitated with 24% NaCl solution, were determined by using a texture analyzer (TA.XT2, Stable Micro Systems, Godalming, UK) equipped with a 5 kg load cell. The tensile measurements were performed by fixing the film with tensile grips (A/TG), employing a crosshead speed of 40 mm/s, gripper initial distance at 30 mm, target distance at 100 mm, and a break sensitivity at 1.00 N. The thickness (0.5 mm) and width (40 mm) of the films were used for the tensile stress calculations. Tensile strength (maximum stress the film can handle prior to breaking) and the stiffness of the casing (maximum elongation the film reached just prior to breaking) were determined from the generated stress-strain curves. Ten films per batch were evaluated, whereby the average value was used in the statistical analysis.

2.12. Statistical analysis

Minitab Version 19 (Minitab Ltd., Coventry, UK) was used for the statistical analysis on all data. Data were checked for normality in both means and residuals. Non-normal distributed data of protein content was transformed (square-root) before analysis. A general linear model was used for analysis of variance (ANOVA) for the dry matter and protein content, thermal, rheological, and mechanical properties, and determination of primary amine group content. The heat flow data of the collagen films required a log transformation (LN) to get normal distributed data. The model used for dry matter and protein content, thermal and rheological properties and primary amine group content was:

$$Y = \mu + Type + Weight + Type*Weight + e$$

We also attempted to look at the age effect. However, it did not work with the original model, due to the small number of birds. Therefore, the following model was also evaluated:

$$Y = \mu + Type + Age + e$$

The model for mechanical properties was:

$Y = \mu + Type + Weight + Type*Weight + Protein + e$ and also the following model was applied:

$$Y = \mu + Type + Age + Protein + e$$

Where Y = dependent variable; μ = overall mean; Type = fast growing or slow growing broiler (FBC or SBC); Weight = 1.6 kg or 2.2 kg; Age = 28, 37 or 50 days; Interaction = 2-way interaction between Type and Weight; Protein = protein content of the dispersion added as a covariate; e = residual error. Data are presented as means ± S.D. Means were compared, after correction for multiple comparisons, using the Bonferroni post-hoc test ($p < 0.05$).

3. Results and discussion

3.1. Evaluation of chicken collagen dispersions

3.1.1. Dry matter and protein content of chicken collagen dispersions

Dispersion prepared from FBC_2.2 kg contained the highest dry matter content of 14.6%, followed by SBC_2.2 kg, SBC_1.6 kg and SBC_1.6 kg, containing 12.3%; 12.2% and 12.2%, respectively. No significant interaction was found between broiler strain and weight and no significant differences in dry matter content was found for broiler strain or weight ($P > 0.05$). A significant difference in dry matter was found between the broiler strains when the factor age was considered in the model ($P < 0.05$), resulting in a significant higher dry matter content for FBC than for SBC. Dispersions prepared from SBC_1.6 kg contained the highest protein concentration of 74 ± 3 μg/ mg followed by the dispersion prepared from FBC_1.6 kg, SBC_2.2 kg, and FBC_2.2 kg with

66 ± 8; 66 ± 6 and 52 ± 6 μg/ mg, respectively. Protein concentration in the dispersions did not show an interaction between chicken type and weight (P = 0.507) but was higher for the SBC than for the FBC chickens (69.39 vs 58.89; P = 0.011) and was also higher for the 1.6 kg chickens than for the 2.2 kg (70.04 vs 58.23; P = 0.003) and was higher for the 28 days old chickens than for the 37- or 50-days old chickens (77.58 vs 62.50 vs 53.96; P = 0.009). Higher protein content for the SBC dispersions was expected as these chickens had more time to reach full growth compared to FBC chickens. This was also expected for the 2.2 kg chickens and for the 50 days old chickens; however, this is not reflected in the results. The values are inconsistent with the previous study by Suurs et al. (2022), where FBC skins at 42 days showed higher protein content (76.4 μg/ mg) compared to SBC skins at 56 days (73.5 μg/ mg). Differences between the two studies may be explained by dietary differences, age at processing, and housing. The chicken skins in the current study were obtained from a study described by (Van der Eijk et al., 2022), where the chickens were raised in The Netherlands, under controlled conditions, while monitoring feed type and intake. The skins from the study by Suurs et al. (2022) were obtained from a local processing plant in Germany. These chickens were raised under commercial conditions with other feed type. Moreover, the chicken skins in the current study were processed at 37 days and 50 days for FBC and SBC, respectively, whereas in the study of Suurs et al. (2022), the chickens were processed at 42 and 56 days for FBC and SBC, respectively.

3.1.2. Soluble collagen content

Collagen dispersions used for the co-extrusion process are a mixture of fibrous and acid soluble collagenous material (Barbut et al., 2020). Soluble collagen can be obtained during the chemical liming treatment (hydrolysis). In the current study, the soluble collagen content was determined to obtain an indication of the dispersion composition regarding soluble and insoluble (fibrous) collagen content. Unfortunately, due to the limited amount of skin material available, it was only possible to analyze this for the SBC_2.2 kg and FBC_2.2 kg. For the SBC_2.2 kg, soluble collagen extracted from the skin was 45.8%. For the FBC_2.2 kg, it was 51.4%.

In future research, it would be good to investigate the ratio of soluble versus insoluble collagen in the dispersion. Especially because in the co-extrusion process, a counter rotating nozzle is used to weave the fibrous collagen material in a crisscross direction to give the casing strength. Particularly, the insoluble collagen fibers in the film are oriented circumferential (Hoogenkamp et al., 2015). So, a greater proportion of soluble collagenous material would result in casings with lower strength as this material cannot be aligned by the nozzle. However, a certain amount of soluble collagen is necessary as it provides a glue to the dispersion to hold the fibers and fibrils together.

3.1.3. SDS-PAGE

The presence of two distinct bands at approximately: 130 kDa and 150 kDa were shown at the SDS-PAGE gel (Fig. 2). These bands indicate α1(I)- and α2(I)-chains of type I collagen (Gojkovic et al., 2014), and a β-chain. The β-chains consists of two α-chains. These findings correspond with data from Munasinghe et al. (2021), who extracted type I collagen from chicken skin and obtained α1 and α2 chains with 148 kDa and 130 kDa molecular weights, respectively. The SDS-PAGE showed no bands visible at the lower molecular weight, indicating the absence of degraded collagen.

For co-extrusion, it is important to have fibrous collagen material, i.e., insoluble fibrils and fibers, present in the dispersion as they can (i.e., with the counter rotating nozzle and the salt treatment) form a stable network that enables the film to shrink and stretch during cooking, as the meat batter contracts and expands during heat processing (Osburn, 2002). Bovine skin, the currently used raw material for casing production, contains type I collagen fibrils and fibers (Oechsle et al., 2016). This study indicates that chicken skin collagen obtained by the current procedure contains collagen polypeptide chains, which in combination with the counter rotating nozzle action (alignment of fibrils and fibers), should be able to provide a stable collagen fibrils/ fibers network formation.

3.1.4. Rheology

3.1.4.1. Temperature profiles. The temperature profiles showed that at approximately 40 °C all dispersions started to display a rapid decrease in elasticity (Fig. 3). This confirms the range of onset temperatures measured by DSC (Table 1). Broiler strain, weight and age had no influence on the temperature stability of the collagen dispersions (P > 0.05). This temperature range is comparable to Suurs et al. (2022), where skin collagen of fast and slow growing broilers was investigated. For bovine collagen dispersions a rapid decrease in elasticity was observed between 30 and 45 °C (Barbut et al., 2020). According to Paul and Bailey (2003) and Covington (2011), the variation in denaturation temperatures is correlated with the proportion of hydroxyproline in the different species. The higher the proportion of hydroxyproline, the

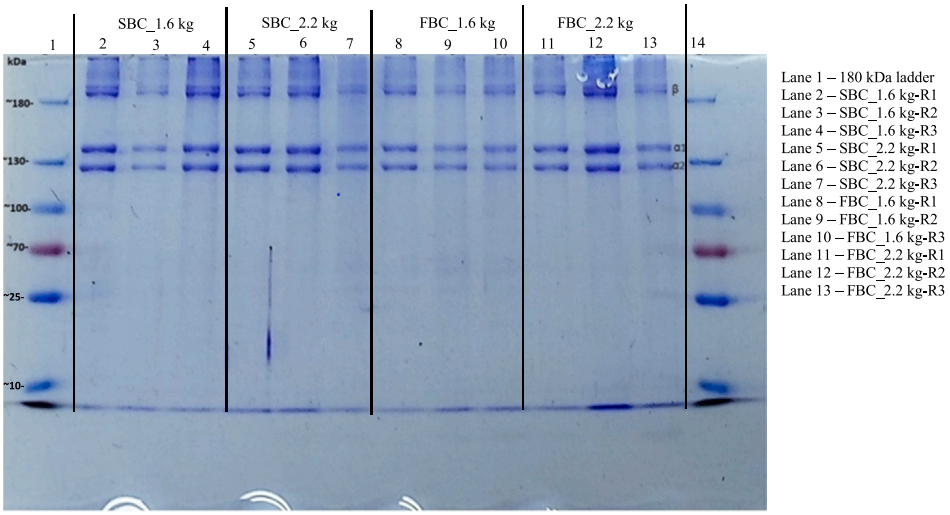

Fig. 2. SDS-PAGE gel of the marker (lanes 1 and 14) and of the dispersions of slow growing broiler chicken of 1.6 kg (SBC_1.6 kg) (lanes 2–4); slow growing broiler chicken of 2.2 kg (SBC_2.2 kg) (lanes 5–7); fast growing broiler chicken of 1.6 kg (FBC_1.6 kg) (lanes 8–10); and fast- growing broiler chicken of 2.2 kg (FBC_2.2 kg) (lanes 11–13). Indicating the presence of collagen β, α1 and α2 collagen bands. R1, R2 and R3 represent the three replicates of each chicken type and weight.

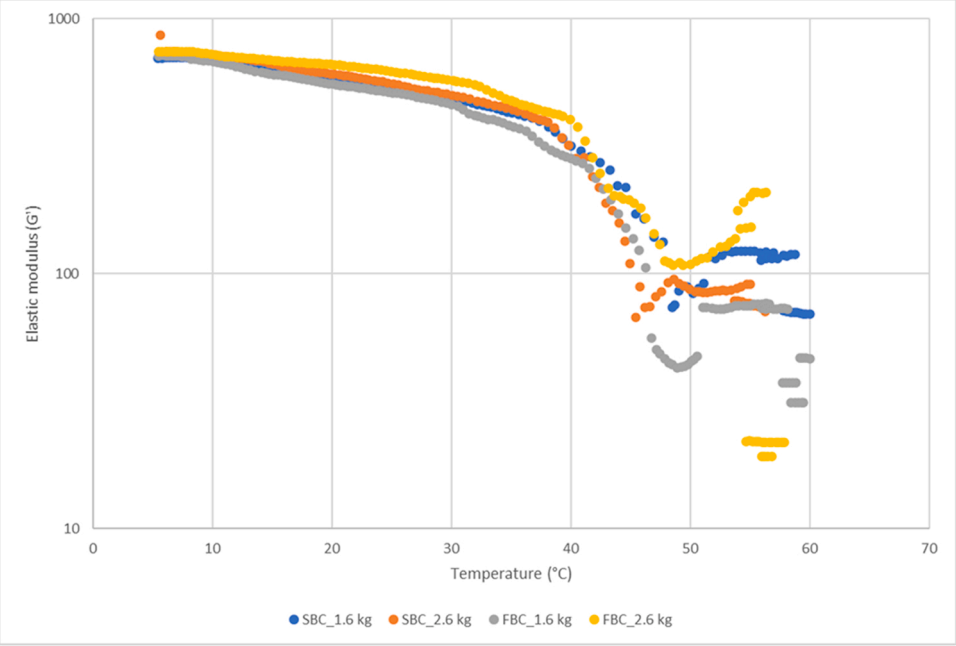

Fig. 3. Elastic modulus (scans 5–60 °C at 2 °C/ min) of four chicken collagen dispersions: slow growing broiler chicken of 1.6 kg (SBC_1.6 kg); slow growing broiler chicken of 2.2 kg (SBC_2.2 kg); fast growing broiler chicken of 1.6 kg (FBC_1.6 kg); and fast growing broiler chicken of 2.2 kg (FBC_2.2 kg), all dispersions show a decrease in elasticity at 40 °C (n = 3 per treatment).

Table 1
Endothermic peaks analysis from the differential scanning calorimeter (DSC) thermograms. Each broiler type and weight [fast growing broiler chicken of 1.6 kg (FBC_1.6 kg); slow growing broiler chicken of 1.6 kg (SBC_1.6 kg); fast growing broiler chicken of 2.2 kg (FBC_2.2 kg); slow growing broiler chicken of 2.2 kg (SBC_2.2 kg)] were determined in duplicate as collagen dispersion and dehydrated films (n = 3 per treatment). Means ± standard deviations.

Type	Dispersion/ Film	T_{onset} (°C)	T_{peak} (°C)	Enthalpy ΔH (J/g)
FBC_1.6 kg	Dispersion	39.06 ± 1.07	41.54 ± 1.46	0.28 ± 0.09
SBC_1.6 kg	Dispersion	39.11 ± 1.13	41.77 ± 1.31	0.22 ± 0.06
FBC_2.2 kg	Dispersion	38.78 ± 0.34	41.33 ± 0.56	0.40 ± 0.33
SBC_2.2 kg	Dispersion	38.65 ± 0.40	41.69 ± 0.63	0.38 ± 0.11
P-value_strain		0.72	0.55	0.77
P-value_weight		0.62	0.70	0.38
P-value_strain*weight		0.49	0.90	0.74
P-value_age		0.69	0.92	0.63
FBC_1.6 kg	Film	55.87 ± 4.86	60.25 ± 5.54	0.23 ± 0.07
SBC_1.6 kg	Film	54.67 ± 5.88	59.22 ± 7.04	0.31 ± 0.13
FBC_2.2 kg	Film	50.07 ± 4.03	54.15 ± 5.08	0.28 ± 0.06
SBC_2.2 kg	Film	54.32 ± 4.74	58.88 ± 4.35	0.41 ± 0.16
P-value_strain		0.43	0.38	0.52
P-value_weight		0.13	0.14	0.54
P-value_strain*weight		0.25	0.25	0.98
P-value_age		0.16	0.16	0.83

higher the denaturation temperature. Hydroxyproline levels of the different broiler strains and weights were not measured. The interest is in comparing the chicken skin collagen with bovine skin collagen as the latter is currently used as the collagen source for dispersions for co-extrusion. Based on the study of Nik et al. (2014), chicken skin collagen would have higher denaturation temperature with hydroxyproline content of 121 residues/1000 compared to porcine and bovine skin containing 91 and 83 residues/ 1000, respectively. However, this is not reflected in the results when comparing the temperatures found by Barbut et al. (2020).

In the sausage production process, a heating step is used to cook the sausages (Suurs & Barbut, 2020). Hereby it must be prevented that the collagen helix unfolds into random coil structures (i.e., transformation into gelatin), since then the casing can feel sticky resulting in poor quality sausages as the casing will stick to the baskets (Suurs et al., 2022). This means that collagen dispersions need a high denaturation temperature to prevent formation of random coil structures. Based on the results of the current study, all collagen dispersions appear to be suitable to be used in the co-extrusion application as their thermal stability is in the temperature range reported for bovine collagen dispersions (Barbut et al., 2020).

3.1.4.2. Complex viscosity. Flow properties of the dispersion provides valuable information for sausage producers using a co-extrusion system. Fig. 4, where the complex viscosity η^* is plotted as a function of angular frequency (ω), shows that all dispersions exhibited a shear-thinning behavior, following a power-law model. Overall, the complex viscosity of the dispersions was comparable to the values reported by Suurs et al. (2022).

Fig. 5a shows the dynamic consistency index (k^*) for the chicken collagen dispersions. No significant interaction was found between broiler strain and weight (both $P > 0.05$) and also no significant differences were found for broiler strain, weight or age ($P > 0.05$). The values measured in the current study are lower than the ones reported by

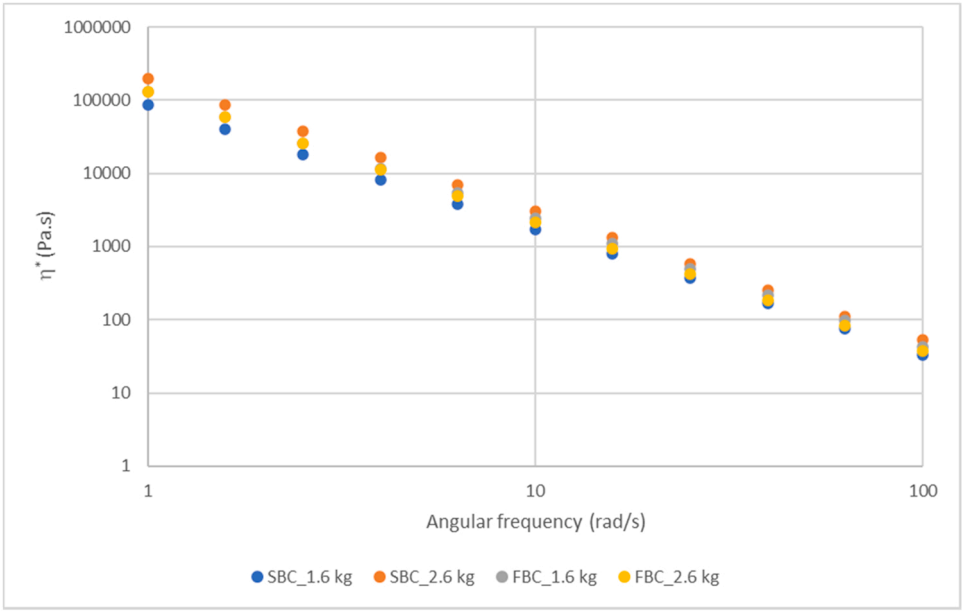

Fig. 4. Complex viscosity η^* calculated from $\left[\left(\frac{G''}{\omega}\right)^2+\left(\frac{G'}{\omega}\right)^2\right]^{1/2}$ of chicken collagen dispersions of slow growing broiler chicken of 1.6 kg (SBC_1.6 kg), slow growing broiler chicken of 2.2 kg (SBC_2.2 kg), fast growing broiler chicken of 1.6 kg (FBC_1.6 kg), fast growing broiler chicken of 2.2 kg (FBC_2.2 kg) as a function of the angular frequency ω (n = 3 per treatment), showing shear thinning behavior following a power-law model for all dispersions.

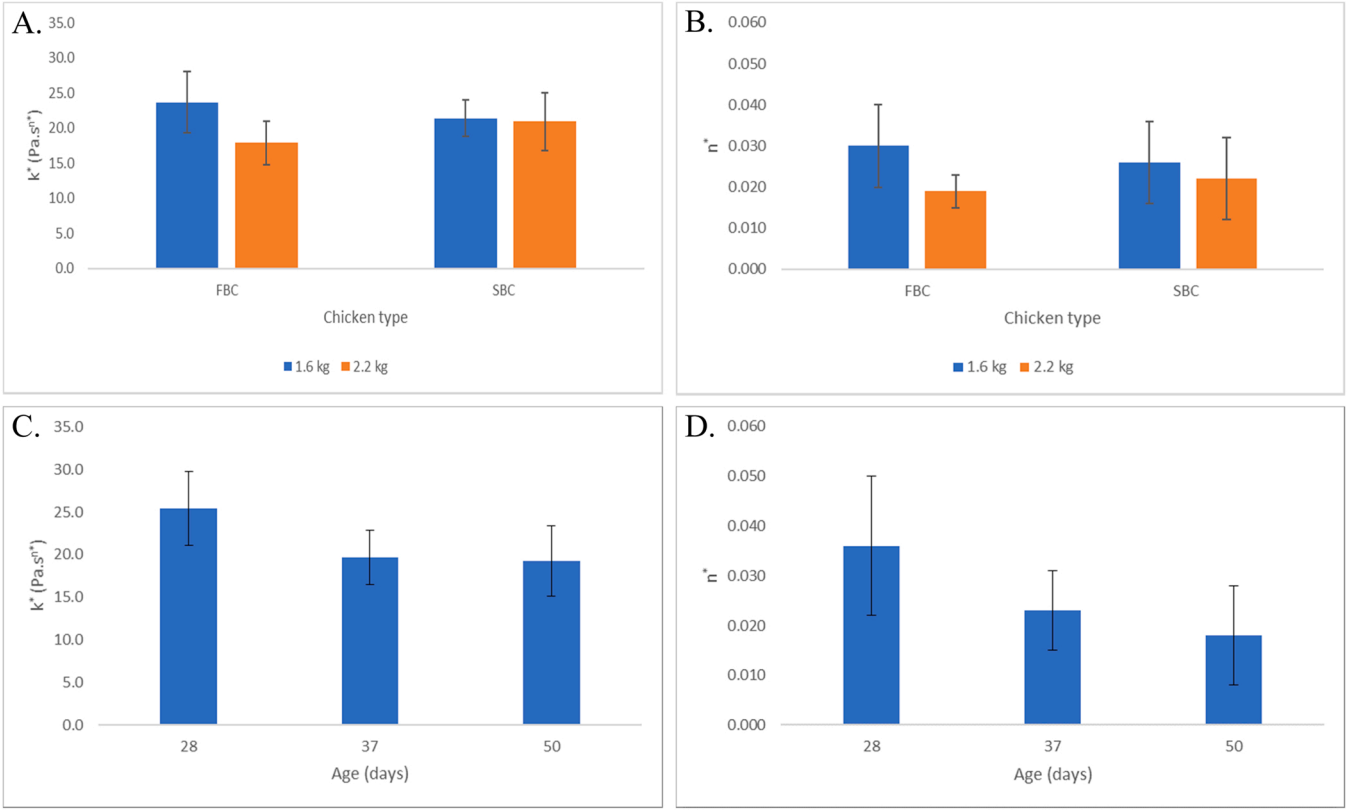

Fig. 5. A and C. Dynamic consistency index (k^*), B and D. Dynamic power law factor (n^*) of collagen dispersions made from FBC (fast growing broiler chicken), and SBC (slow growing broiler chicken) weighing 1.6 and 2.2 kg. Bars represent mean values ± S.D. A. Showing highest consistency value for FBC_1.6 kg, but no significant differences between the dispersions. B. Showing almost only elastic behavior for all dispersions. C. Showing highest consistency value for 28 days old chickens, D. Showing highest n^* value for 28 days old chickens. P-value_strain = 0.88; P-value_weight = 0.23; P-value_strain*weight = 0.30. A. P-value_strain = 0.88; P-value_weight = 0.23; P-value_strain*weight = 0.30. B. P-value_strain = 0.71; P-value_weight = 0.11; P-value_strain*weight = 0.41. C. P-value_age = 0.21. D. P-value_age = 0.27.

Suurs et al. (2022), where values of 41 Pa s^{n^*} for FBC and 44 Pa s^{n^*} for SBC were recorded. Moreover, the values were much lower compared to the study of Oechsle et al. (2016), who reported a value of 606 Pa s^{n^*} for chicken skin collagen. The collagen extraction processes and dispersions' protein content probably resulted in large differences compared to current findings. Compared to Suurs et al. (2022), the acid swelling step in the current study was extended from 24 h to 48 h. This resulted in more swelling of the collagen and therefore probably a lower consistency value. Moreover, in the previous study, there were clearly unswollen parts of collagen visible, while this was not the case in the current study. The skin collagen dispersion made by Oechsle et al. (2016) was prepared according to Bueker et al. (2009), where the dispersion was made by coarsly mincing the acid swollen hide pieces and then forcing it through a plurality of perforated disks arranged in series where the diameter of the individual holes becomes smaller from disk to disk. Finally, the collagen paste is tranferred to large kneaders. So, here they used a different way of producing the chicken collagen dispersion compared to the current study.

Shear-thinning ($n^* < 1$), shear-thickening ($n^* > 1$) or Newtonian ($n^* = 1$) behaviour can be indicated by the dynamic power law factor n^*. The dynamic power law factor n^* indicates shear-thinning ($n^* < 1$), shear-thickening ($n^* > 1$) or Newtonian ($n^* = 1$) behaviour (Keogh & O'Kennedy, 1998). Fig. 5b shows that n^* values for all four dispersions were comparable and almost 0, which means they have almost only elastic behavior. The difference with Suurs et al. (2022) is that the current dispersions also contained small number of viscous components ($n^* = 0.03$), and especially the collagen from the lighter chickens. This is probably due to the younger age of the chickens, where less intra and intermolecular crosslinks are formed and therefore more viscous components could be expected in the dispersion. The viscous components in the dispersion most probably originates from the soluble collagen. Unfortunately, no correlations between the n-value and soluble collagen content could be made, because there was not enough material available to measure the soluble collagen content. Comparable to the k^* and n^* values of two most used commercial bovine collagen dispersions (both dispersions measured: $k^* = 55$ Pa s^{n^*} and $n^* = 0.01$), the values for k^* from the current study are much lower. This could mean that the extrusion of the dispersions would not proceed satisfactorily, i.e., too low viscosity resulting in poor film forming capacity.

3.1.5. Evaluation of the amount of amine groups in chicken collagen dispersions

The number of crosslinkable groups was determined by assaying the free amine groups in the dispersions using the TNBS assay. The more free amine groups, the stronger the casing will be when adding a crosslinker that needs an amine group to react with; i.e. for sausage production liquid smoke is used. Dispersion prepared from SBC_1.6 kg contained the highest number of amine groups 199 ± 20 nmol/ mg, followed by SBC_2.6 kg, FBC_1.6 kg and FBC_2.6 kg, containing 193 ± 28 nmol/ mg, 185 ± 20 nmol/ mg and 175 ± 25 nmol/ mg, respectively (Fig. 6). No significant interaction was found between broiler strain and weight and no significant differences in free amine groups were found for broiler strain or weight ($P > 0.05$). A lack of significant difference was expected for FBC_2.2 kg and SBC_1.6 kg as they were the same age at processing. The age difference between FBC_1.6 kg and FBC_2.2 and SBC_1.6 kg was probably too small to show changes in crosslinking. If a difference was to be expected, it would be for FBC_1.6 kg versus SBC_2.2 kg, as the SBC_2.2 kg chickens were actually older [28 days for FBC_1.6 kg (197 nmol/ mg) versus 50 days for SBC_2.2 kg (181 nmol/ mg)]. However, no significant difference was found for broiler strain or age ($P > 0.05$). According to a study by Pines et al. (1996) no mature stable crosslinks could be detected in chicken breast skin up to 49 days of age.

The amine group content of three commercial bovine collagen dispersions was determined on 284 ± 12 nmol/ mg, 442 ± 152 nmol/ mg, and 378 ± 56 nmol/ mg (unpublished data). The results indicate that there can be a considerable spread in amine group content between different commercial bovine collagen dispersions. Moreover, compared to the chicken collagen dispersions the number of amine groups of the bovine collagen dispersions is higher. The differences among the bovine collagen dispersions and with the chicken collagen dispersions may have to do with the difference in production method, i.e., time in the alkaline solution, whereby deamidation by hydrolysis of the sidechains of glutamine and asparagine residues, the number of amine groups can be reduced as NH_2 groups will be split off and NH_3 is formed (Suurs & Barbut, 2020).

Considering that broiler strain, weight and age had no significant effect on the number of cross-linkable groups, all chicken skin sources in this study appears to be acceptable for casing production and the corresponding co-extrusion technology. However, considering the lower amount of amine groups in the chicken collagen dispersions, the casings would be less strong after crosslinking with liquid smoke compared to bovine collagen dispersions.

3.1.6. Differential Scanning Calorimetry (DSC) of collagen dispersions

DSC measurements were carried out to investigate the collagen transformation temperatures (Suurs et al., 2022). The essence of collagen denaturation is the removal of hydrogen bonds. Denaturation of collagen consists of the disruption of the secondary and tertiary collagen structures. The collagen denaturation temperature increases with the age of the animal due to a higher degree of lysine-hydroxylysine

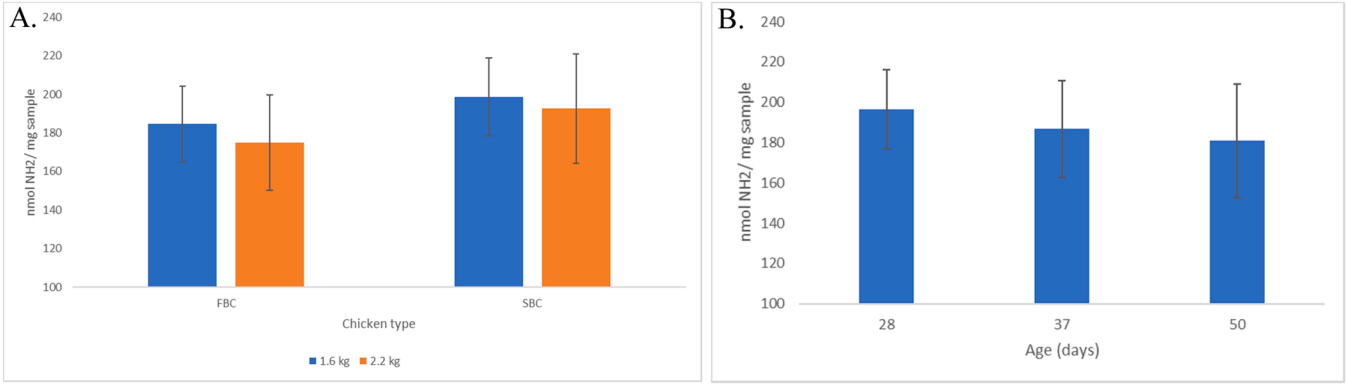

Fig. 6. A. The number of NH_2 groups present in chicken collagen dispersions prepared from skins of slower growing broiler chicken of 1.6 kg (SBC_1.6 kg), slower growing broiler chicken of 2.2 kg (SBC_2.2 kg), fast growing broiler chicken of 1.6 kg (FBC_1.6 kg), and fast-growing broiler chicken of 2.2 kg (FBC_2.2 kg), showing no significant differences in free amine groups between the dispersions; B. The number of NH_2 groups present in chicken collagen dispersions prepared from skins of 28, 37 and 50 days old chickens, showing no significant difference in free amine groups between the ages. n = 3 per treatment. Bars represent means ± S.D. A. P-value_strain = 0.28; P-value_weight = 0.58; P-value_strain*weight = 0.89. B. P-value_age = 0.84.

covalent crosslinks formation between the collagen molecules (Jolanta & Piotr, 2010; Noorzai & Verbeek, 2020). As a result of denaturation, the collagen helix is transformed into a coil (Jolanta & Piotr, 2010). In the current study, no significant interaction was found between broiler strain and weight ($P > 0.05$) on T_{onset}, T_{peak} and enthalpy. Furthermore, no main effects of broiler strain, weight and age were found (Table 1), suggesting that broiler strain, weight and age have no influence on the thermal properties of the prepared chicken skin dispersions. This was expected as the age difference between the chickens investigated was rather small.

The enthalpy corresponds with the number of hydrogen bonds in the triple helix that provide stability to the helix (Schroepfer & Meyer, 2017). Paul and Bailey (2003) mentioned that by measuring the enthalpy, an indication of the integrity of the collagen molecule can be provided. Degradation of the collagen results in lower enthalpy, whilst more crosslinking increases the denaturation temperature, resulting in higher enthalpy values. The current study indicates that the collagen dispersions prepared from the skins of heavier birds had higher integrity compared to the lighter birds, as the enthalpy increases with increasing weight of the birds (Table 1), which in turn may be related to age of the chickens. The data showed an increasing trend for enthalpy value with increasing age of the chickens (28 days 0.49 J/g vs 37 days 0.53 J/ g vs 50 days 0.61 J/g). However, no significant difference was found between the age of the chickens and the enthalpy ($P > 0.05$).

3.2. Evaluation of chicken collagen films

3.2.1. Differential Scanning Calorimetry (DSC) of collagen films

Comparable to Suurs et al. (2022), the chicken collagen dispersions were made into a film by partial dehydration (using 24% NaCl as done in the industry during the co-extrusion process), and this resulted in a higher thermal stability for all dispersions (Table 1). No significant differences in T_{onset}, T_{peak} and enthalpy were found between the different collagen films (Table 1), indicating that broiler strain, weight and age have no influence on the thermal properties of the films. Barbut et al. (2020) and Suurs et al. (2022) also showed a significant increase in thermal stability of chicken and bovine collagen dispersions that were transformed into collagen films by salt (24%) precipitation. A compact structure of the fibers is formed due to the decreased moisture content as the salt induces dehydration. McPherson et al. (1986) also mentioned an increased denaturation temperature resulted in a stronger association of bovine collagen fiber structure. So, from the perspective of the collagen films there is no preference which chicken dispersion should be used for casing production and corresponding co-extrusion technology.

3.2.2. Mechanical properties of collagen films

The ability to form a strong film (from the dispersions) is a very important aspect to make sausage casings in the co-extrusion process. First, the films must be strong enough to withstand the pressure of the meat dough during the partial drying and cooking. Second, the sensory perception of "bite" is determined by the strength of the casing together with the ground meat. No significant interaction was found between broiler strain and weight. Fig. 7a and b demonstrate that there were also

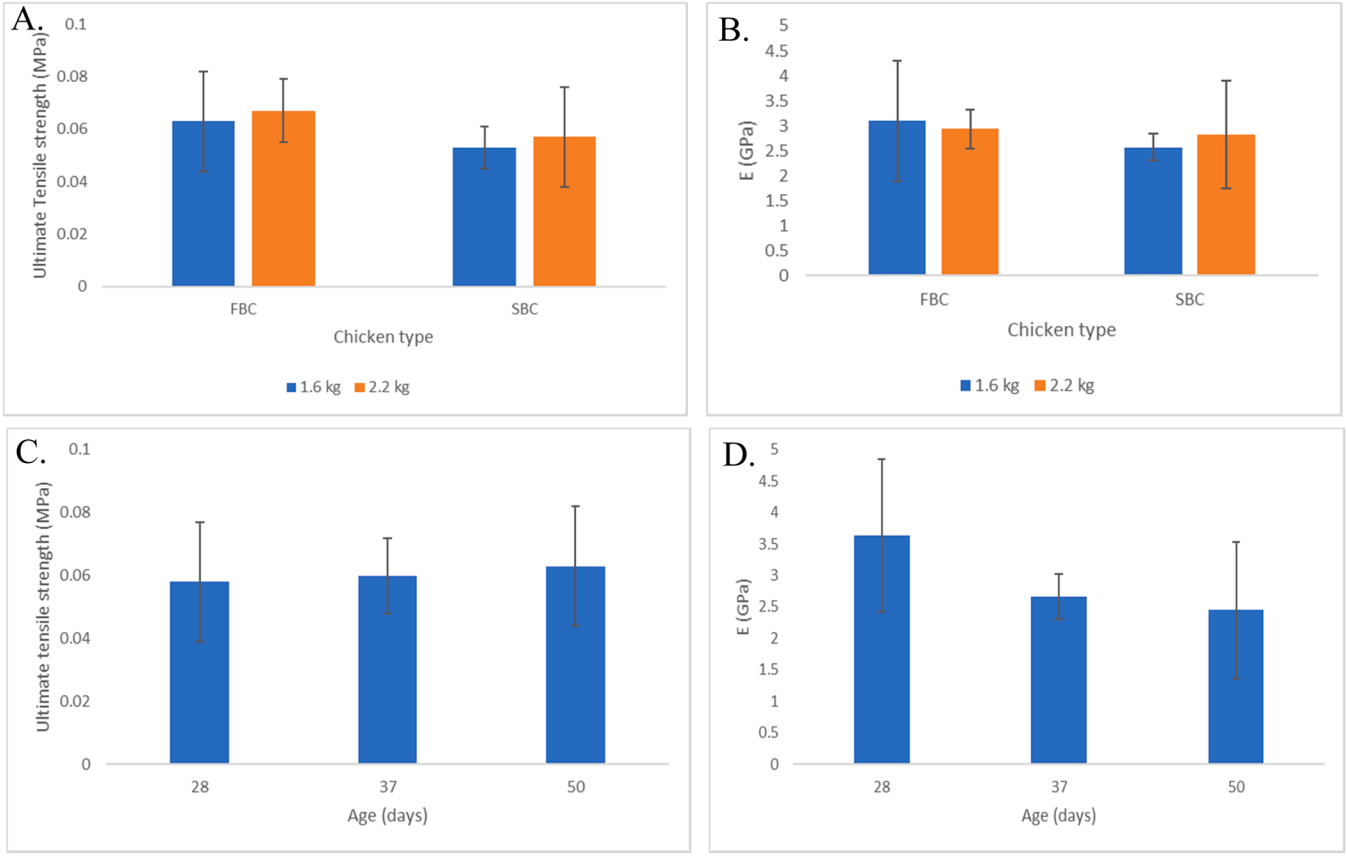

Fig. 7. Mechanical properties of collagen films (produced by exposing to 24% NaCl); a) Ultimate tensile strength (UTS) and b) Stiffness (E) for slow growing broiler chicken of 1.6 kg (SBC_1.6 kg), slow growing broiler chicken of 2.2 kg (SBC_2.2 kg), fast growing broiler chicken of 1.6 kg (FBC_1.6 kg), fast growing broiler chicken of 2.2 kg (FBC_2.2 kg); showing no significant differences in UTS and E values for the different films. c) Ultimate tensile strength (UTS) and d) Stiffness (E) for 28-, 37- and 50-days old chickens, showing no significant differences in UTS and E values. n = 3 per treatment. Bars represent means ± S.D. A. P-value_strain = 0.29; P-value_weight= 0.62; P-value= 0.99. B. P-value_strain = 0.54; P-value_weight = 0.92; P-value_strain*weight = 0.68. C. P-value_age = 0.98. D. P-value_age = 0.65.

no significant main effects of broiler strain and weight (both $P > 0.05$) in the tensile strength and Young's modulus when films prepared from the different dispersions were exposed to 24% NaCl solution. Moreover, the age of the chickens had no effect on the tensile strength or Young's modulus ($P > 0.05$). However, the results indicate that in general the slow growing chickens showed lower tensile strength and stiffness values compared to the fast growing chickens. Moreover, the results also indicate that the tensile strength of films prepared from the skins of lighter chickens were less compared to the heavier chickens. The differences in texture are not originating from differences in protein content between the dispersions as no significant difference was observed ($p > 0.05$) when protein content was added as a co-variate to the statistical models. At this point, no verification can be done to check whether the differences in tensile strength and stiffness values can be attributed to the difference in the proportion of soluble collagen, as there was not enough material to evaluate the amount of soluble collagen of the lighter chickens (FBC_1.6 kg and SBC_1.6 kg).

The co-extruded films containing 4% chicken skin collagen (normalized to protein content) from Oechsle et al. (2017) showed tensile strength values of 0.07 MPa, which are comparable to the values found in our study. Compared to the values measured for bovine collagen dispersions by Barbut et al. (2020), the values for tensile strength found in this study are about 5 times lower. This has probably to do with the orgin (bovine versus chicken collagen) and age of the raw materials when processed. Steers are processed at 18 – 24 months, whereas the chickens in the current study were 28–50 days old. As the collagen fibril diameter increases with age (Miller et al., 1983), stronger films should be produced from dispersions prepared from older animals' skin. Probably, there is an optimum in the animal's age, because at a certain point (depending on the origin) the collagen fibers in the skin are crosslinked and the films prepared from dispersions with these fibers will exhibit lower mechanical strength (Suurs et al., 2022). Moreover, crosslinked fibers show more resistance to swelling and have a lower water-holding capacity (Miller et al., 1983), which strongly influences the film forming ability and tensile properties.

Considering that broiler strain and weight had no influence on mechanical properties of the films, all chicken skin sources evaluated in this study appears to be acceptable for casing production and the corresponding co-extrusion technology. However, sausage manufacturers must consider getting a less tough casing when using chicken skin collagen than when working with bovine skin collagen.

4. Conclusions

Based on the properties measured in the current study the chicken collagen dispersions prepared of skins from slow and fast-growing broilers have the potential of being a suitable source of collagen for the co-extrusion process. However, attention should be given to the rheological properties as the k^* values of the dispersions are rather low. Furthermore, the fact that the strength of the films prepared from the chicken collagen dispersions is lower compared to the films prepared from bovine collagen dispersions, might have the consequence that the bite characteristics of these films will be different, and therefore must be considered when using this material.

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CRediT authorship contribution statement

Patricia Suurs: Conceptualization, Investigation, Formal analysis, Writing – original draft. **Kudakwashe Farawu:** Investigation. **Henry van den Brand:** Validation, Writing – review & editing. **Willeke F. Daamen:** Partly experimental supervision, Writing – review & editing. **Shai Barbut:** Writing – review & editing, supervision.

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.

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