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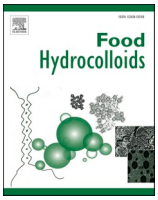

Properties of different poultry skins sources in relation to co-extruded sausage casings

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ABSTRACT

Casings are an essential component in the transformation of comminuted meat into a finished sausage. Their strength and the texture of the ground meat determine the "bite" perception when eating a sausage. Traditionally, meat has been stuffed into natural casings, but alternatives, such as cellulose and co-extruded collagen casings are emerging. Bovine hide split collagen is the primary source for co-extruded casings. However, an increase in meat products consumption puts pressure on the supply of collagen casings, and therefore producers are searching for alternatives. In this study, the properties of chicken skin collagen preparations from four types of birds [fast-growing broilers (42 d), slower-growing broilers (56 d), broiler breeders (52 wk), and laying hens (100 wk)] were investigated by biochemical, and physical analyses to obtain properties important in designing new dispersions for co-extrusion. SDS-PAGE, rheology, DSC and TNBS showed little difference in parameters between the different chicken types. However, after salt precipitation, creating strong films from the broiler breeder and laying hen skins' dispersions was not possible. Creating films was possible with the dispersions of fast and slower-growing broiler skins, particularly after precipitation with saturated NaCl.

In conclusion, chicken skin collagen from slower and fast growing broilers have the potential of being a suitable collagen source for the co-extrusion process. Overall, it was feasible to form stronger films with broiler skins than with skins of broiler breeder and laying hens. This is important as the casings' strength dictates the initial sensory perception when eating a sausage.

1. Introduction

Sausages have an important place in our diet, due to their nutritional density, shelf life, unique texture, and flavor (Savic & Savic, 2016). In the process of sausage making, a casing is an essential component in the transformation of comminuted meat into a finished product (Osburn, 2000; Suurs & Barbut, 2020; Barbut et al., 2020). Traditionally, sausage meat has been stuffed into intestines derived from pigs, sheep, and cattle, known as natural casings (Adzaly et al., 2015; Savic & Savic, 2016). However, the limited availability of natural casings, the challenges of working with them, and their relatively high price enhanced the development of alternatives, such as cellulose casings, manufactured collagen, and the more recently developed co-extrusion casings (Barbut, 2010). Co-extrusion technology eliminates the intermediate stages of preparing and storing pre-made casings (Suurs & Barbut, 2020). Unlike traditional sausage production, where casings are stuffed with meat, co-extrusion introduces a thin layer of semi-liquid material that is extruded onto the meat batter as it is being discharged from the extrusion nozzle. This is later followed by the so called "gelled in place" to harden the sausage casing. Currently, beef collagen is the primary source for this semi-liquid material. It is a by-product of the meat packing industry; obtained from the skins of slaughtered beef cattle (Hoogenkamp et al., 2015; Suurs & Barbut, 2020). However, as meat and meat products consumption have significantly increased over the past two decades, there has been a shortage of natural collagen casings, which has been paralleled by a significant price increase (Oechsle, 2016). Consequently, collagen producers are searching for alternative sources. One of these include poultry by-products, such as chicken skins, feet and bones, as currently mostly turned into low value products (Arunmozhivarman et al., 2017). Oechsle et al. (2016) indicated that chicken skin could be

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an alternative source. Studies on collagen from avian species by Liu et al. (2001) and Lin and Liu (2006) reported that the molecular structure of collagen from avian species is different from mammalian species. This is obviously a species effect, but may also be partially related to the age of the animal. For example, beef cattle are processed at 18–40 months (Miller et al., 1983; Burke, 1980), whereas broilers are processed at 5–6 weeks of age. The animal's age influences the collagen structure, and as the animal grows older, the degree of covalent crosslinks increases, due to lysyl oxidase-initiated crosslinking (Oechsle, 2016); Noorzai, 2020, pp. 1–284). Collagen originating from younger animals is easily solubilized during the first separation stage in alkaline environment (Noorzai, 2020, pp. 1–284) because the crosslinks are not so stable, but acid soluble. Consequently, beef collagen from older animals has more crosslinks than broiler collagen and this may considerably affect the applicability of the collagen for casing production. It can be hypothesized that collagen originating from older poultry, such as broiler breeders (average 52 weeks old) and laying hens (average 100 weeks old), may be more suitable for casing production than collagen from young broiler chickens (Gojkovic et al., 2014). However, there seems to be a limit to the age of animals that can be used for collagen casing production, as the stiffness of collagen fibers continues to increase with more crosslinking, and this eventually results in higher brittleness and a lower tensile strength of the fibers (Varnali, 2002).

Oechsle et al. (2016), evaluated the application of various chicken collagen samples (chicken skin and bone collagen) within the co-extrusion process, and reported that broiler skin collagen was the most suitable source. However, there is no scientific data indicating whether commercially available skins, of different poultry species, are suitable to serve as a collagen source for co-extruded sausage casings. Collagen obtained from various poultry species may feature different gel properties, yielding films with different functionalities (e.g. stretch ability, appearance, bite). Different gel properties could also lead to differences in performance. Therefore, the goal of this study was to explore whether chicken skins, obtained from chickens of varying ages, could be used for the production of high quality co-extrusion casings. The experiment compared the biochemical composition of skin collagen from fast and slower-growing broilers, broiler breeders and laying hens, employing both biochemical and physical measurements. The overall aim of this study was to determine essential properties required for selecting potential poultry collagen for the co-extrusion process.

2. Materials and methods

Chicken breast skin from different age birds: fast-growing broiler chickens (**FBC**, 42 days old), slower-growing broiler chickens (**SBC**, 56 days old), broiler breeders (**BB**, 52 weeks old) and laying hens (**LH**, 100 weeks old) were collected from local processing plants in Germany and The Netherlands. The dermis and epidermis were used for preparing the dispersions. Breast skin (10 kg) was collected of each age group, and then stored at −18 °C until further processing.

2.1. Collagen isolation

A multistep process was used to obtain the dispersions prior to determining their physical and chemical properties. Sample preparation and pre-treatment were performed according to Munasinghe et al. (2015) with slight modifications. Briefly, chicken skins were thawed and cut into small pieces (0.5 × 0.5 cm). Non-collagenous proteins were removed by adding 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). The mixtures were shaken for 24 h at 4 °C. NaOH was then removed as samples were washed with distilled water and filtered (2.5 mm mesh) until neutral pH was reached. Fat was removed by adding 10% butanol (Chemlab, Zedelgem, Belgium) at a sample/solution ratio of 1:6 (w/v). The mixtures were shaken for 24 h, at 4 °C. Butanol was then removed as samples were washed five times with distilled water and filtered (2.5 mm mesh). The defatted samples were swollen in 0.5 M acetic acid (Supelco, Zwijndrecht, The Netherlands) for 24 h at a sample/solution ratio of 1:6 (w/v - based on the wet weight of the original sample), at 4 °C. The skin samples were collected by filtering with a fine sieve (2.5 mm mesh). Subsequently, the collected acetic acid was added to the mixture whereby the addition was calculated based on the protein content of the raw material. This was done to obtain a dispersion with a protein content of 3.5–4% protein. The samples were homogenized in a food processor (UMC5 Stephan Food Processing Machinery, Hameln, Germany) for 2 min at 1500 rpm under vacuum for FBC and SBC. BB and LH samples were homogenized for 3 min at 1500 rpm and 1 min at 3000 rpm under vacuum, as these samples were more viscous. The temperature of the mixture was kept below 20 °C throughout the whole procedure. As a final step, the mixture was sieved (2.5 mm mesh). The dispersions were stored at 4 °C for 2 days until further analysis. For each chicken source, four dispersions (replicates) were produced, whereby on day 1 two dispersions of each chicken source were prepared and on day 2 the next two dispersions.

2.2. Protein and moisture content of raw materials

Protein content in the skin samples was determined by the Kjeldahl method using a conversion factor N of 6.25 (NEN-EN-ISO 8968-1). Dry matter content (NEN-ISO 1442) was determined by using ~2.5 g homogenized skin samples that were dried (103 °C) for 16hr. Both analyses were done by a commercial laboratory. The four different skin types were evaluated in duplicates.

2.3. Protein and moisture content of chicken collagen dispersions

Dry matter content of the collagen dispersions was determined by lyophilization (Scanvac, Coolsafe 55-4, LaboGene, Denmark), where approximately 25 g were lyophilized for 72 h, after which the amount was weighed and the dry matter content calculated.

Protein content of the dispersions was measured by the Lowry method (Lowry et al., 1951). In short, 50 mg of lyophilized chicken dispersions were incubated in 2.5U papain (Sigma-Aldrich, Zwijndrecht, The Netherlands) in 1 mL digestion buffer (50 mM $NaPO_4$, 2 mM cysteine, 2 mM EDTA, pH 6.5) for 16 h at 65 °C. The digested samples were centrifuged (5 min; 13,000 g) and supernatants used for protein measurement. A calibration curve was made, using BSA (0–250 ng/mL) and a blanc with only papain digestion buffer (50 mM $NaPO_4$, 2 mM cysteine, 2 mM EDTA, pH 6.5). The reaction that occurs has a characteristic blue color with A_{max} at 750 nm.

2.4. Film preparation

For evaluation of the dispersions for film forming and textural abilities, films were made by placing 4 g of the dispersions onto plastic sheets, which were then covered with a second sheet. A round mold with a raised edge of 0.3 mm was used to form 0.3 mm thick circles, with a diameter of 90 mm. Later, salt solutions were added to allow collagen to precipitate and to form a firm film. From each of the four replicates per skin type (FBC, SBC, BB, LH), six films were made with 24% NaCl and six were made with 40% K_2HPO_4 solution. In total 24 films per skin and salt type were produced and evaluated. Note that both NaCl and K_2HPO_4 are used in industry to harden co-extruded beef collagen casings.

2.5. SDS-PAGE analysis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was conducted to analyze the molecular weight of the protein chains in the collagen dispersions, and to assess the presence of other collagen types or collagen breakdown products. The method was adopted from Faraj et al. (2011). Briefly, 2.5 mg of collagen was suspended in 150 μl sample buffer and heated for 10 min at 70 °C. Samples

and controls were loaded on an 8% (w/v) polyacrylamide gel. After running at 150 V, the gels were stained with 0.1% (w/v) Coomassie Brilliant Blue solution (R-250 (VWR International BV, Amsterdam, The Netherlands). Each of the four skin type replicates (FBC, SBC, BB, LH) was used to produce a gel.

2.6. Thermal transition measurement

To study the thermal behavior of the chicken skin dispersions and the precipitated films Differential Scanning Calorimetry (DSC) measurements were performed. The endothermal transition of collagen was measured (DSC Q1000, TA Instruments, New Castle, DE, USA) equipped with a cooler (RCS40). Five to ten mg of collagen dispersion or precipitated film was hermetically sealed in a T_{zero} aluminum pan. The system was equilibrated at 1 °C for 5 min. For the collagen dispersion, a temperature ramp was performed from 1 to 80 °C, at 5 °C/min, while the precipitated collagen films were treated with a ramp of 1–100 °C, at 5 °C/min. In both cases, an empty aluminum pan was used as the reference probe. Temperature at which helix to random coil transition of the collagen started (T_{onset}), temperature at which 50% of the collagen has unfolded (T_{peak}), and the value of denaturation enthalpy (ΔH) were determined using the DSC software (Universal Analysis 2000 (Version 4.5 A), TA instruments). The four replicates of each skin type were measured in duplicate.

2.7. Rheology

To investigate differences in flow behavior between the different raw materials, which could indicate the applicability of the dispersions on a co-extrusion system, rheology measurements were performed based on Oechsle et al. (2016), with slight modifications. Experiments were conducted using an oscillatory rheometer type AR2000 (TA Instruments, New Castle, USA) equipped with Peltier plate and water bath. The collagen dispersions were analyzed by oscillating measurements using a plate-plate geometry of 40 mm diameter hard-anodised aluminium. Stress sweeps were performed at 1 Hz in order to determine the linear viscoelastic range applying oscillatory stress from 0.01 to 1000Pa 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):

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

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

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

Temperature sweeps of the dispersion were performed by applying 0.1% strain from 5 to 60 °C with ramp rate of 2 °C/min at 1 Hz. The temperature at which G' (Pa) started to decrease as a measure of the helix to random coil transition was determined. The four replicates of each skin type were measured in duplicate.

2.8. Texture

Puncture force parameters were determined of the four replications per skin type (FBC, SBC, BB, LH), whereby per skin type six films prepared with 24% NaCl solution and six films prepared with 40% K_2HPO_4 solution were evaluated. The puncture force was done by fixing the films to a holder and using a spherical probe (P/5 S, Stable Micro Systems, Surry, United Kingdom), attached to a texture analyzer (TA.XT2, Stable Micro Systems) equipped with a 5 kg load cell while employing a crosshead speed of 10 mm/s. Maximum peak force and area under the peak were determined (Bourne, 1978).

2.9. Determination of primary amine group content

The concentration of primary amine groups present in the lyophilized chicken dispersions were determined using 2,4,6-trinitrobenzenesulfonic acid (TNBS). The method was adopted from Buttafoco et al. (2006). Samples of 1.5 mg were incubated for 30 min in an aqueous solution of 4% w/v $NaHCO_3$ (1 mL). Then a solution of 0.5% w/v 2,4,6-trinitrobenzenesulfonic acid (TNBS) (1 mL) in MilliQ water was added and the mixture was incubated at 40 °C for 2 h. After the addition of HCl (3 mL, 6 M), samples were hydrolyzed at 60 °C for 90 min. The reaction mixture was diluted in a 96 well plate 1:1 with MilliQ water, mixed well and the absorbance was measured at 420 nm using a spectrophotometer (Bio-Tek, Bad Friedrichshall, Germany). The concentration of free amine groups was determined using a glycine calibration curve. The four replicates of each skin type were measured in triplicate.

2.10. Statistical analysis

Statistical analysis on all data was performed in 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 the thermal transition temperature, mechanical characterization and determination of primary amine group content. The model used for the thermal transition temperature and primary amine group content was Y = μ + Skin type + Day + e. Where Y = dependent variable, μ = overall mean, Skin type = type of skin (FBC, SBC, BB, LH), Day = production day of the dispersion (1 or 2), e = residual error. The model used for the mechanical characterization was Y = μ + Skin type + Day + Salt type + Salt type*Skin type + Day + e. Where Y = dependent variable, μ = overall mean, Skin type = type of skin (FBC, SBC, BB, LH), Day = production day of the dispersion, Salt type = type of salt (NaCl, K_2HPO_4), Salt type*Skin type = interaction between skin type and salt type, Day = production day of the dispersion (1 or 2), e = residual error. Data are presented as means ± SE. Means were compared, after correction for multiple comparisons, using the Bonferroni post-hoc test ($p < 0.05$).

3. Results and discussion

3.1. Chemical characterization of raw materials

The aim of the research was to investigate whether skins of different avian sources and ages can be used to produce high quality collagen casings. To allow comparing their performance in the dispersions, skins were first analyzed for protein, and moisture content. The fat content was calculated based on the protein and moisture content, Protein content (w/w) of FBC, SBC, BB and LH skins were 9.1%, 11.5%, 10.2% and 16.4%, respectively. On dry weight basis, these values translate to a protein content of 15%, 21%, 22% and 36%, respectively. Fat content (w/w) of FBC, SBC, BB and LH skins were calculated on 51%, 44%, 35% and 29%, respectively. On dry weight basis, these values translate to a fat content of 83%, 76%, 75% and 62%, respectively. Inconsistent results are found for the protein and fat content of chicken skins in literature. Munasinghe et al. (2014), reported protein and fat contents of 22.6% and 31.6%, respectively, on dry weight basis. Although the protein content is comparable to our FBC sample, the fat content was remarkably lower. Additionally, Bonifer and Froning (1996), reported 9.1% protein and 37.2% fat in the fresh skins of 6–8 weeks old broilers. The protein content is in accordance with the 42-day old chicken skins in our study, however the fat content again much lower. Swatland and Barbut, (2007) reported chicken skin to contain 45.1% fat and 6.6% protein. It is evident that results can vary quite a lot. This is explained by the fact that the chicken skin composition might be affected by several

factors, such as age, dietary differences and location; e.g. back or breast skin (Bonifer & Froning, 1996). Often the body part and the age of the chickens are not mentioned in the literature. Based on the current findings it can be concluded that at least age is an important factor affecting protein concentration of the skin. Protein content of the older animals in our study (LH) was in the same range as found for bovine skin that is typically used for sausage casing production; i.e. 30% on dry weight basis, which also depends on the animal age (Noorzai et al., 2019).

3.2. Chicken collagen dispersions

The dry matter content of the dispersions prepared from FBC and SBC skins differed from the BB and LH skins. The former had a higher dry matter content (12.83 ± 0.22% and 12.93 ± 1.13%, respectively). Compared to the BB and LH with lower dry matter ($p < 0.00001$) (8.62 ± 1.31% and 10.84 ± 1.23%, respectively). Moreover, a significant difference ($p < 0.05$) was found between the BB and LH dispersions. Differences in dry matter content are probably caused by the higher amount of acid addition to the BB and LH skins. Overall, the FBC and SBC collagen was already more swollen after soaking in acetic acid than the collagen of the BB and LH skins.

On the contrary, the protein content of FBC, SBC and LH freeze-dried skin samples was lower than the protein content of the BB samples (7.6 ± 1.4%, 7.4 ± 1.5%, 10.4 ± 2.8% vs 15.8 ± 2.6%, respectively, ($p < 0.004$). It should be noted that the values are probably an underestimation of the actual protein content, because not all the gel was digested by papain. In addition, after centrifugation, some fat was floating on top of the suspension and the presence of fat in the samples could made protein extraction more difficult.

3.3. SDS-PAGE

The SDS-PAGE gels (Fig. 1) clearly show the presence of two distinctive bands: 100kD and 130kD, indicating α1(I)- and α2(I)-chain monomers of type I collagen (molecular mass of $\alpha_2 < \alpha_1$ with approximate mass ratio α_1: α_2 = 1:2) (Gojkovic et al., 2014). According to Abedin and Riemschneider (1984) chicken skin contains types I and III collagen. However, α1(III) bands were not detected on our gels. α1(III) collagen was expected at 138 kDa (Oechsle et al., 2016). The gels also clearly show a difference in contrast of the bands of SBC and FBC, in comparison with BB and LH. The BB and LH dispersions were likely more intra- and intermolecularly crosslinked than the FBC and SBC dispersions, as crosslinks increases with age (Oechsle et al., 2016; Pines et al., 1996; Yamauchi et al., 1988). Furthermore, the SDS-PAGE showed that no major denaturation of the collagen had occurred, due to the pretreatment of the skins, since there were no bands visible at the lower molecular weight.

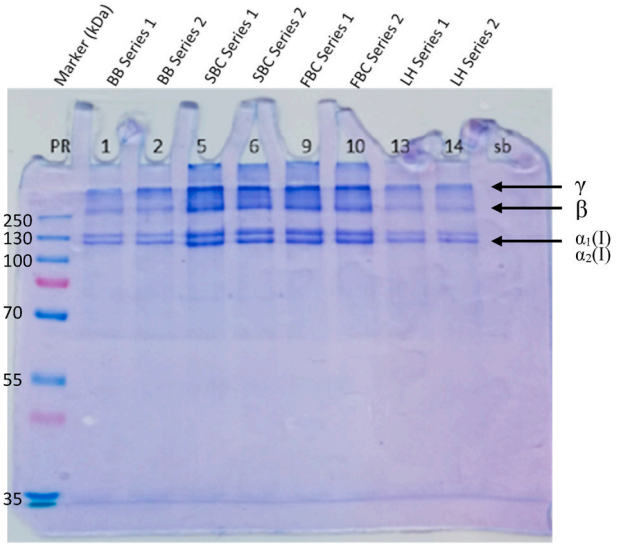

Fig. 1. SDS-PAGE gel of the marker and of the dispersions of broiler breeder (BB), slower growing broiler chicken (SBC), fast growing broiler chicken (FBC) and laying hen (LH) indicating the presence of α1(I) and α2(I) bands which are particular for type I collagen. It should be noted that only a small fraction of the collagen is soluble and placed on the gel. Most of the collagen is insoluble, resulting in a pellet left in the sample buffer.

3.4. Differential Scanning Calorimetry (DSC) of collagen dispersions and films

Measurements were made to study the thermal behavior of chicken skin dispersions from different ages. In the sausage making process, the collagen dispersion is converted into a film by partial dehydration (precipitation) with a saturated salt (e.g., NaCl) solution, followed by crosslinking and finally a heating step to cook the sausage (Suurs & Barbut, 2020). During this transformation process, the collagen is transformed from a helical to a crystalline structure (Bianchi & Conio, 1967). During the heating step, it is important that the collagen does not change from the crystalline structure to the random coil phase, as in that phase the collagen can unfold (i.e., transformed to gelatin), and result in sausages with a sticky/mushy casing. This means that collagen dispersions and films need a high helix-to-coil transition temperature to prevent random coil formation, and this correlates with a higher stability at a high temperature environment (Miles et al., 2005). In general, chicken dispersions investigated here showed higher T_{onset} temperatures (Table 1) compared to the commercial bovine collagen dispersions, studied by Barbut et al. (2020). The current study show that T_{onset} (average 40.95 °C), T_{peak} (average 44.02 °C) and enthalpy (0.241 J/g) of the dispersions prepared from young chicken skins (SBC and FBC) were not significantly different from the dispersions made from old chicken skins (BB and LH; Table 1). T_{onset} values for bovine collagen dispersions ranged from 33.5 to 34.5 °C (Barbut et al., 2020).

The transition from a chicken collagen dispersion to a film by partial dehydration (exposure to 24% NaCl) resulted in a higher thermal stability for all dispersions. The increase is due to decreasing moisture content; i.e., during the salt dehydration step. This results in a compact structure formation, in which the fibers are closer to each other. T_{onset} of films prepared from the LH skins was 61.77 °C. It was significantly higher ($P = 0.004$) than the T_{onset} of the films prepared from SBC, FBC and BB skins (57.33 °C, 56.08 °C and 57.93 °C, respectively). Barbut et al. (2020), also showed a significant increase in thermal stability of commercial bovine collagen dispersions that were transferred into collagen films by salt precipitation: endothermic peaks starting at 58.2–60.3 °C, with maximum values at 63.9–65.3 °C. This trend is in accordance with the current chicken collagen films. McPherson et al. (1986), suggested that stronger association of bovine collagen fiber structure is correlated with increased denaturation temperature.

The films prepared from skins of older chickens (BB and LH) clearly show two helix-to-coil-transitions, whereas the FBC and SBC films only show one transition (Table 1, $T_{onset\ 1}$ Fig. 2). The second transition, at approximately 70 °C, for BB and LH (indicated by $T_{onset\ 2}$ Fig. 2), could be related to the lysine-aldehyde derived crosslinks that are replaced by mature crosslinks in the collagen of the aging the bird (Miles et al., 2005). Given the higher thermal stability of chicken skin dispersions (ranging from 40.9 to 41.5 °C) compared to bovine skin dispersions (ranging from 33.5 to 34.5 °C), all four types of chicken skin sources should be acceptable for casing production. However, when considering the thermal stability of the collagen films, the films made of the LH skin is preferred over the others as it shows the highest thermal stability. Overall, we need to have a high helix-to-coil transition temperature to prevent random coil formation of the collagen film in the sausage production process.

Table 1
Analysis of endothermic peaks from differential scanning calorimetry (DSC) thermograms. The four replicates of each chicken source [fast growing broiler chicken (FBC), slower growing broiler chicken (SBC), broiler breeder (BB) and laying hen (LH)] were determined in duplicate both as collagen dispersion (n = 4 per type) and as dehydrated films (n = 4 per type). Skins of fast growing broiler chicken (FBC), slower growing broiler chicken (SBC), broiler breeder (BB) and laying hen (LH) were measured (n = 1 per type). Means ± standard deviation.

Type	Dispersion/Film/Skin	T_{onset} (°C)	T_{peak} (°C)	Enthalpy ΔH (J/g)	T_{onset} (°C)	T_{peak} (°C)	Enthalpy ΔH (J/g)
FBC	Dispersion	41.02 ± 0.42	44.16 ± 0.67	0.232 ± 0.082	–	–	–
SBC	Dispersion	40.88 ± 0.33	43.87 ± 0.39	0.249 ± 0.127	–	–	–
BB	Dispersion	41.11 ± 0.32	45.28 ± 0.62	0.162 ± 0.103	–	–	–
LH	Dispersion	41.50 ± 0.25	44.52 ± 0.92	0.048 ± 0.025	–	–	–
P-value		0.42	0.37	0.12			
FBC	Film	56.08 ± 0.48 ^{y}	61.78 ± 1.62	0.455 ± 0.103 ^{x}	–	–	–
SBC	Film	57.33 ± 0.55 ^{y}	62.85 ± 0.96	0.379 ± 0.052 ^{x}	–	–	–
BB	Film	57.93 ± 2.13 ^{y}	62.15 ± 3.21	0.037 ± 0.005 ^{y}	69.72 ± 1.54	74.75 ± 1.82	0.282 ± 0.115
LH	Film	61.77 ± 1.78 ^{x}	64.61 ± 1.66	0.017 ± 0.007 ^{y}	70.12 ± 0.94	75.98 ± 1.56	0.110 ± 0.038
P-value		0.00	0.20	0.00			
FBC	Skin	61.64 ± 1.76	66.93 ± 0.83	7.231 ± 0.383	–	–	–
SBC	Skin	62.55 ± 0.49	65.44 ± 1.89	2.567 ± 0.724	–	–	–
BB	Skin	64.43 ± 0.03	66.42 ± 0.76	5.875 ± 2.775	–	–	–
LH	Skin	65.75 ± 1.32	68.39 ± 2.04	3.455 ± 4.235	–	–	–

T_{onset} = onset temperature; T_{peak} = temperature at 50% heat flow; ΔH = total enthalpy change in melting of collagen.
^{a,b,x,y} = Means within a column and dispersion/film not sharing a common superscript differ (P < 0.05).
- = Not available.

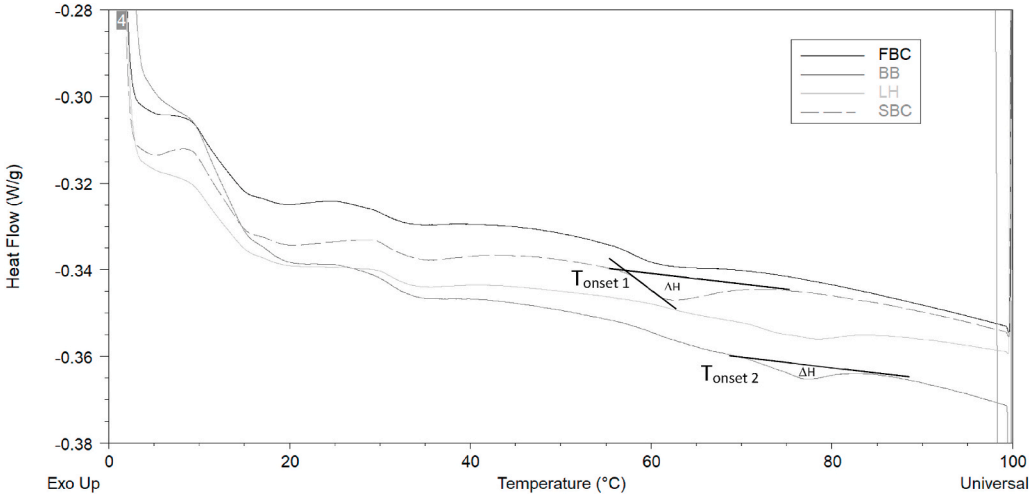

Fig. 2. Differential scanning calorimetry showing representative helix-to-coil transition of chicken collagen dispersions obtained from FBC (fast growing broiler chicken), SBC (slower growing broiler chicken), BB (broiler breeder) and LH (laying hen) skins. . Heating rate 5 °C/min, indicating T_{onset1} for a dispersion prepared from SBC skins, and $T_{onset\ 2}$ for a dispersion prepared from BB skins.

3.5. Rheology

3.5.1. Temperature profiles

In the sausage making process, the dehydration of co-extruded collagen follows a heating step to cook the sausage. Consequently, it is important to measure the collagen melting temperature. The rheological tests showed that between 40 °C and 45 °C all dispersions started to display a rapid decrease in elasticity (Fig. 3). This temperature range was not found for bovine collagen dispersions where a rapid decrease was reported between 30 and 45 °C (Barbut et al., 2020). This may be attributed to a two times higher lysine content in chicken skin collagen compared to bovine skin collagen (Gojkovic et al., 2014). Lysine provides thermal stability as Lysine – Glycine –Y links are involved in side-chain interactions (Brodsky & Persikov, 2005; Gojkovic et al., 2014).

The rheological transitions observed for the dispersions (loss of elasticity, Fig. 3) were in the range of the onset temperatures measured by DSC. This means that all four chicken skin sources appears to be suitable for making casings, as indicated earlier. The elasticity loss of the dispersions at 40–45 °C showed that the collagen in these dispersions were significantly modified by the preparation procedure (NaOH treatment and acidification), as all chicken skins (starting material) showed denaturation temperatures above 60 °C (Table 1).

3.5.2. Complex viscosity

Viscosity measurements can provide valuable information (e.g., flow properties of dispersions) to the sausage producers using a co-extrusion system. Fig. 4 shows that all dispersions show shear-thinning behavior following a power-law model, and that they all reveal the same complex viscosity. The complex viscosity η^* is a measure of the total resistance to flow as a function of angular frequency (ω), so the higher the value for the complex viscosity, the more resistance the material is to flow. The expectation was that the complex viscosity of the collagen dispersions made from the older chicken skins (BB and LH) would be higher than those from young chickens (SBC and FBC). This is because the presence of intra- and intermolecular crosslinks in the BB and LH dispersions, making them more resistant to flow. Oechsle et al. (2014) found contradictory results when comparing a highly crosslinked native collagen

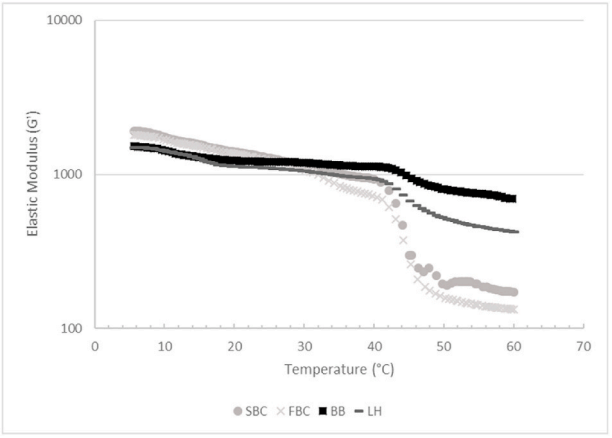

Fig. 3. Elastic modulus (5–60 °C at 2 °C/min) of four chicken collagen dispersions varying in age: SBC (slower growing broiler chicken), FBC (fast growing broiler chicken), BB (broiler breeder), and LH (laying hen), showing a rapid decrease in elasticity for all dispersions between 40 °C and 45 °C.

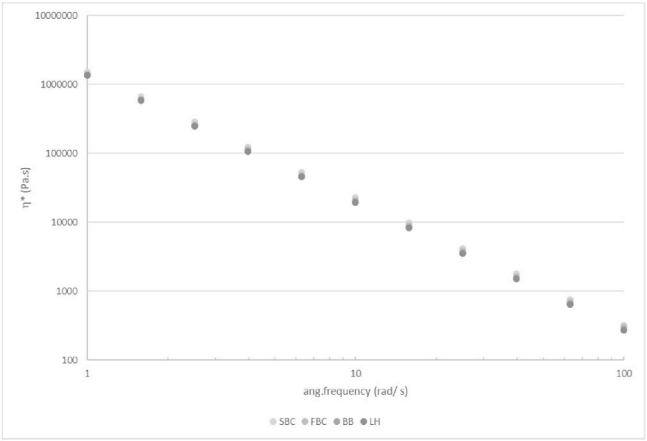

Fig. 4. Complex viscosity η^* calculated from Eq. (1) of chicken collagen dispersions of SBC (slower growing broiler chicken), FBC (fast growing broiler chicken), BB (broiler breeder) and LH (laying hen) as a function of the angular frequency ω (n = 4).

with a telopeptide-poor collagen. The former showing a lower η than telopeptide-poor collagen without crosslinks present. They attributed this to the presence of crosslinks in the material.

Table 2 shows the dynamic consistency index (k^*) and dynamic power law factor (n^*) for the chicken collagen dispersions. The k^* describes the consistency of the dispersion, where a higher value means higher consistency of the material. Table 2 shows that dispersions made from the older chicken skins were lower than the younger ones. Dispersions made from BB and LH skins showed lower k^* values, probably caused by the higher number of crosslinks in the older skin. These

Table 2
Dynamic consistency index (k^*) and dynamic power law factor (n^*) calculated from Eq (2). by making a double logarithmic plot of complex viscosity (η^*) versus angular frequency (ω) of collagen dispersions made from FBC (fast growing broiler chicken), SBC (slower growing broiler chicken), BB (broiler breeder), and LH (laying hen).

Collagen dispersion	k^* (Pa s^n*)	n^*
Fast growing broiler chicken	41	0.01
Slower growing broiler chicken	44	0.01
Broiler breeder	34	0.01
Laying hen	35	0.01

crosslinks are affecting with the rheological measurements, thereby probably underestimating the value (Oechsle et al., 2016). Oechsle et al. (2016), also measured a dispersion made from chicken skin and reported a consistency value of 606 Pa s^n*. Their collagen extraction process and the higher dispersions' protein content probably resulted in the large difference compared to current findings.

The dynamic power law factor n^* indicates shear-thinning ($n^* < 1$), shear-thickening ($n^* > 1$) or Newtonian ($n^* = 1$) behaviour. Shear-thinning behaviour means that n^* decreases with increasing values of ω. Viscoelastic systems are intermediate between 0 and 1 (Keogh and O'Kennedy, 1998). Table 2 shows that n^* values for all four dispersions were similar and almost 0, which means that they have almost only an elastic components. Oechsle et al. (2016), reported a n^* value of 0.13 in their experimental chicken skin dispersion. This means that their dispersion also had some viscous components. Unfortunately, no data is present regarding the dynamic consistency index k^* and n^* for commercial bovine collagen dispersions. This makes it difficult to compare current results with most used (by the industry) bovine collagen dispersions, and to judge whether the chicken collagen dispersions have similar flow properties.

3.6. Texture

An important aspect of the collagen dispersion is the ability to form a strong film. A strong film is desired because the final goal is to co-extrude the dispersion onto meat dough to form a casing. The strength of the casing determines, together with the texture of the ground meat, the sensory perception "bite" when eating a sausage. Puncture tests were used as a tool to evaluate the force and energy required to puncture the films after exposing the dispersion to 24% NaCl or 40% K_2HPO_4 (as is also done under industrial settings). The general observations was that the SBC and FBC dispersions resulted in fairly firm and manageable films that performed well in the puncture test. However, the films made from BB and LH dispersions were difficult to handle (mushy texture) making puncture measurements difficult to execute (Fig. 7).

No significant interaction was found between skin type and salt type (Fig. 5) and no significant difference in puncture force was found between the different skins (Fig. 5). A significant difference ($p < 0.05$) was found between the different salt types (NaCl or K_2HPO_4), used for precipitating the dispersions. No significant difference in total energy required to puncture the films was found between the different salts and skin types (Fig. 6). Munasinghe et al. (2015) also extracted collagen from broiler skins and evaluated the strength of these films. They reported a higher film strength, ranging from 5 to 158 N compared to the current study. However, their films were prepared by casting and drying instead of precipitation (note: drying results in about at least 4× higher

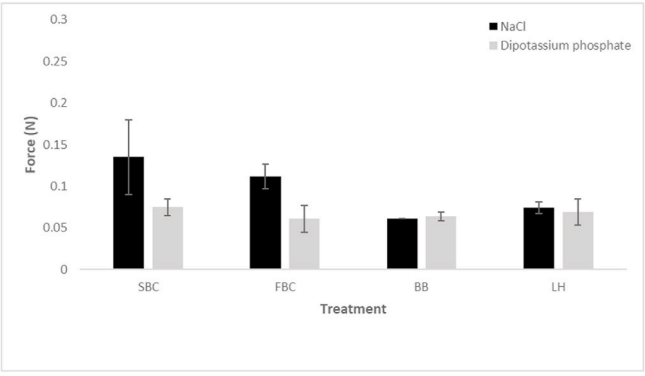

Fig. 5. Ultimate force (N) to puncture films made from chicken collagen dispersions treated with 24% NaCl or 40% K_2HPO_4 solutions for SBC (slower growing broiler chicken), FBC (fast growing broiler chicken), BB (broiler breeder) and LH (laying hen); n = 4. Bars represent means ± standard deviations.

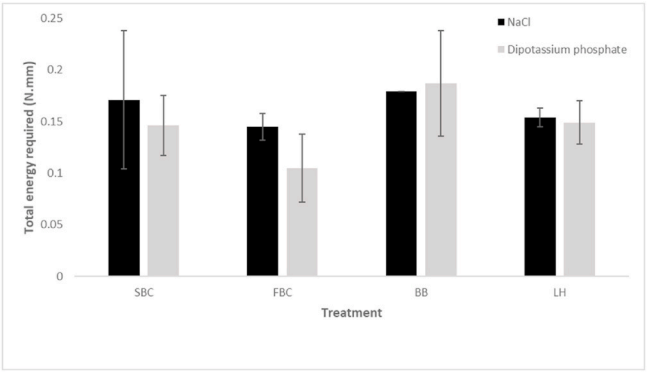

Fig. 6. Total energy required (N.mm) to puncture films made from chicken collagen dispersions treated with 24% NaCl or 40% K_2HPO_4 solutions for SBC (slower growing broiler chicken), FBC (fast growing broiler chicken), BB (broiler breeder) and LH (laying hen) n = 4. Bars represent means ± standard deviations.

Fig. 7. Images of films prepared from dispersions originating from broiler breeder skins (A) and from fast growing broiler chickens (B) precipitated with 24% NaCl. The figure shows the resulting semi transparent film (image A), and opaque (B). Note: an opaque appearance is associated with a salt-precipitated dispersion forming a strong film.

dry content level). Moreover, collagen was crosslinked with glutaraldehyde; i.e., known as a very efficient crosslinker that also raises strength of protein films/gels. Barbut et al. (2020) evaluated five commercial bovine collagen dispersions by preparing films by using a stainless steel roller with a recess of 0.50 mm, followed by partial dehydration in a 30% NaCl solution. They found that the amount of work to break the films (puncture test) was approximately 16 times higher compared to the values found in the current study. The difference might have been caused by several factors. First, the protein concentration (3.5–4.0% vs. 4.0–5.0% in their study). Second, the preparation procedure of the dispersions; e.g., small scale equipment versus large scale industrial preparations, where homogeneity of the dispersions is more difficult to obtain in small scale lab equipment. Third, the origin of the material and therefore differences in collagen structure of the raw materials; bovine versus chicken collagen. Gojkovic et al. (2014) investigated the amino acid composition of chicken skin collagen and beef skin collagen of animals at different ages. They found that bovine collagen had a higher glycine content (13.7 g/kg versus 6.2 g/kg of protein for skin of a 5 year old hen) and proline (10.31 g/kg versus 4.2 g/kg of protein for skin of a 5 year old hen). This contributes to a higher rigidity of the collagen coils and fibril strength, because Glycine –Proline -Y units were most stabilizing in the triple helix. A fourth factor could be the thickness of the bovine films which was ~1.5 times higher compared to our chicken films. Based on our texture findings, young chicken skins (SBC and FBC) are preferred over old chicken skins (BB and LH) as a source for making co-extrusion casings. Optimization of the skin pretreatment procedure is important in obtaining high quality dispersions and producing strong casings. This can include an enzyme solubilisation method for extracting collagen from mature tissue as described by Noorzai et al. (2019).

3.7. Evaluating the amount of crosslinkable groups in raw chicken skins

In the co-extrusion process, casings are crosslinked by applying liquid smoke, which contains aldehydes, reacting with the collagen free amine groups. The more free amine groups, the stronger the casing will eventually be. The number of crosslinkable groups was determined by assaying the free amine groups using a trinitrobenzene sulfonic acid (TNBS) assay. Data are expressed as the average values of nmol free amine groups per mg protein (Fig. 8). No significant differences in free amine groups were found between skin types, although it was expected to find higher numbers for the SBC and FBC chicken skins, as with raising collagen age there will be a higher degree of crosslinking (Gojkovic et al., 2014). The reason that no significant difference was found may be due to: a) the pretreatment procedure with NaOH swelling resulted in cleavages of the collagen structure, b) the dispersions contained fat, which was not completely removed in the pretreatment, c) the age difference between the FBC and SBC versus BB and LH was not enough to show any significant changes in crosslinking. In a chicken, skin integrity is dependent on the collagen content (Granot, Plavnik, et al., 1991,b) and crosslinking (Ramshaw et al., 1986). Pines et al. (1996) showed that up to 49 days of age no mature stable crosslinks such as pyridinoline or deoxypyridinoline could be detected, and only negligible amount of histidinohydroxylysinenorleucine (**HHL**) was detected in male and female chicken breast skin. In any case, there is a possibility that the lifespan of BB and LH chickens is not enough to develop these mature crosslinks. However, optimization of the pretreatment procedure would be necessary to get the maximum value out of these raw materials.

4. Conclusions

Dispersions and associated films suitable for co-extrusion application should have a number of properties (Table 3). In the evaluation of the dispersions and the films, the emphasis was on the performance, assessed by texture measurements, as a casing dictates the initial sensory perception when eating a sausage. Based on the current study, chicken skin collagen from slower and fast growing broilers have the potential of being a suitable source of collagen for the co-extrusion process. As it is better possible to form firmer films with young broiler chicken skins than with old chicken skins of broiler breeder and laying hens.

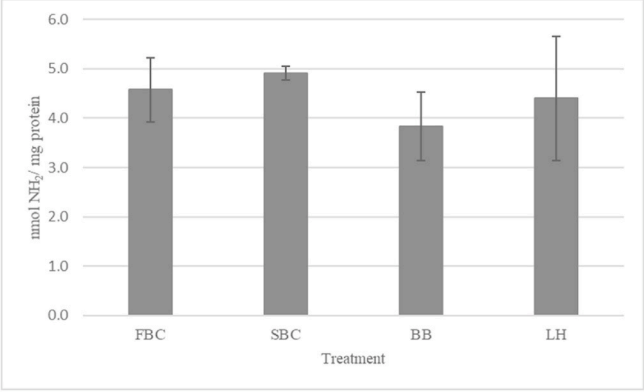

Fig. 8. The number of crosslinkable groups present in chicken collagen dispersions prepared from skins of FBC (fast growing broiler chicken), SBC (slower growing broiler chicken), BB (broiler breeder) and LH (laying hen) n = 4. Bars represent means ± standard deviations.

Table 3
Properties of dispersions and films per skin type [FBC (fast growing broiler chicken), SBC (slower growing broiler chicken), BB (broiler breeder) and LH (laying hen)] and their performance with respect to co-extrusion process and/or final product.

Measurement	Properties in relation to co-extrusion process/final product	Fast growing broiler skins	Slower growing broiler skins	Broiler breeder skins	Laying hen skins
SDS-PAGE	Type I collagen	+	+	+	+
DSC	Helix-to-coil temperature	+	+	+	++
Rheology	Extrudability	+/−	+/−	+/−	+/−
Texture	Casing strength	+	+	−	−

+/- = property of the dispersions/films meets the criteria.

Author statements

Patricia Suurs: Conceptualization, Investigation, Formal analysis, Writing – Original Draft. **Henry van den Brand:** Validation, Writing – review & editing. **Willeke F. Daamen:** Writing – review & editing. **Shai Barbut:** Conceptualization, Writing – review & editing, supervision.

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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.

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