

Assessment of possibilities of food grade gelatines preparation from chicken skin

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Abstract: Chicken skin is a product obtained from the poultry meat processing. This tissue contains mainly fat and proteins, especially collagen. Collagen could be gained from a purification process in which undesirable components, such as fats, pigments and globular proteins, are extracted from the skin. Purified collagen might be used as a starting material for the preparation of high gel strength gelatines applied in food, pharmaceutical and cosmetic industry. Chicken skins are also suitable for processing into collagen hydrolysates of various molecular weights with different purposes as food and cosmetic additives. The aim of this study was to prepare gelatines from chicken skins. Prior to the extraction, non-collagen parts from chicken skin were removed with 1M NaCl, 0.5% NaOH; fats were removed with mixture of Petroleum Ether and Ethanol (1:1). 5 samples of gelatines were prepared by extraction with hot water after pre-treatment with proteolytic enzyme. Effect of extraction conditions, different extraction temperatures (40–80 °C) and fixed extraction time (60 min), on gelatine gel strength and yield of the process, were examined. Yields of extracted gelatines ranged between 42–72%. Gel strengths of prepared gelatine samples were between 252–354 Bloom. What is more, prepared chicken skin gelatines were compared with commercial pork and beef gelatines. Results displayed that prepared chicken skin gelatines have comparable gel strength with commercial gelatines.

Key Words: chicken skin, collagen, collagen hydrolysate, extraction, food grade gelatine, protease

INTRODUCTION

Chicken meat is currently one of the most often consumed types of meat and its consumption has been increasing steadily respecting the global population growth. The global chicken meat consumption was 66 million tonnes in 2000, 91 million tonnes in 2009 and 94 million tonnes in 2013 (Seong et al. 2015). Naturally, enormous amounts of by-products, such as viscera, feet, heads, bones, blood, feathers and skins, are produced as well. In general, these by-products are composted or used in the livestock feed production. Poultry by-products contain significant amounts of proteins, enzymes and lipids possible to be processed further into valuable products with a wide range of applications in medicine, pharmacy, food and cosmetic industry (Bueno-Solano et al. 2009).

Gelatine is an exceptional food hydrocolloid with diverse possibilities of usage. This polypeptide is obtained by partial hydrolysis of collagen, a protein found in animal tissues. In gelatine production, prior to the extraction of collagen in hot water, tissue is treated by acid (type A gelatine) or alkali (type B gelatine). Treatment with acid or alkali causes collagen breakage to produce collagen soluble in warm water (gelatine). There are three key factors during the gelatine production influencing its properties: time, temperature and pH. The process can be accelerated if higher temperatures and longer time period are applied. Traditional sources of collagen are skins, connective tissues and bones of beef or pork origin. However, alternative sources, such as fish bones, skins and scales have become more important as well. Also, poultry by-products including chicken, turkey or duck skin are considered as another alternative (Schrieber and Gareis 2007).

Several studies have been devoted to preparation and properties of gelatines obtained from chicken skin. Sarbon et al. (2013) characterized chicken skin gelatines as a possible alternative to mammalian gelatine. Rasli and Sarbon (2015) studied effects of different drying methods on rheological, functional and structural properties of these gelatines compared to beef gelatine.

Sarboň et al. (2015) examined the effect of interactions between gelatine and whey protein on rheological and thermal properties. Wan Omar and Sarboň (2016) analysed the effect of drying methods on functional properties and antioxidant activity of chicken skin gelatine hydrolysate. Nor et al. (2017) investigated the influence of plasticizers concentration on chicken skin gelatine films.

Gelatine shows excellent emulsifying, foaming, film-forming and stabilizing properties which determine it to many different applications, particularly in food industry. Gelatine also performs a unique ability to bind large amounts of water and form a thermo-reversible gel with a melting point close to the temperature of the human body (Schrieber and Gareis 2007). Nevertheless, the origin of gelatine may be problematic for many consumers because of the risk of BSE (Bovine Spongiform Encephalopathy) or religious aspects as both Judaism and Islam do not accept the consumption of pork skin (Badii and Howell 2003). Thus, gelatine prepared from alternative sources has been gaining more significance and attention.

The aim of the work is to assess possibilities of processing chicken skin into gelatines and investigate effects of proposed technological conditions on the yield of gelatine and gelatine gel strength.

MATERIALS AND METHODS

Chicken skins, by-products of chicken breast cuttings processing, were purchased from Raciola Ltd., Uherský Brod, Czech Republic. Prior to experiments, they were stored at $-20\text{ }^{\circ}\text{C}$. They were analysed and results can be seen in Table 1. Dry matter content was determined by drying the sample at $105 \pm 2\text{ }^{\circ}\text{C}$ and is defined as the percentage of sample weight after and before drying. Kjeldahl method was applied to analyse protein content (ISO 937:1978) and lipid content was determined using Soxhlet method (Cruz-Fernández et al. 2017). Hydroxyproline content was established to calculate the amount of collagen (ISO 3496:1978). Samples were annealed to determine their mineral content (Gelatin Manufacturers Institute of America 2013). Each test was repeated three times. Results were reported as arithmetic mean with standard deviation.

Regarding commercial gelatine, beef (type B) and pork (type A) gelatines of the grain size of 2 mm were supplied by IPL (Uherský Brod, Czech Republic).

Table 1 Composition of the chicken skin

	Dry matter (% $\pm$ SD)	Proteins (% $\pm$ SD) ^a	Collagen ^b (% $\pm$ SD) ^a	Fat (% $\pm$ SD) ^a	Minerals (% $\pm$ SD) ^a
Chicken skin	53.6 ± 1.5	16.5 ± 1.3	92.6 ± 0.1	85.0 ± 2.4	0.9 ± 0.3

Legend: ^a based on dry weight of the raw material; ^b from total protein content

Appliances, tools and chemicals

These appliances were employed in the experiment: Stevens LFRA Texture Analyser for measuring the strength of gel (Leonard Farnell and Co Ltd., England), SPAR Mixer SP-100AD-B meat cutter (TH Industry RD, Taiwan), Memmert ULP 400 drying device (Memmert GmbH + Co. KG, Germany), LT 43 shaker (Nedform, Czech Republic), Kern 440 – 47 electronic scale and Kern 770 electronic analytical balance (both Kern, Germany), A 10 labortechnik analytical mill (IKA-Werke, Germany), Nabertherm muffle furnace (Nabertherm GmbH, Germany), Samsung refrigerator and Parnas-Wagner distillation apparatus.

Chemicals used in analyses were: NaCl, NaOH, petroleum ether, ethanol and chloroform (Verkon, Czech Republic); all of analytical grade. Polarzyme 6.0 T – serine endoprotease manufactured by fermentation of microorganisms that are not present in the final product (Novozymes, Denmark) with declared enzyme activity of 6 KPPU/g (kilo protease unit/g).

Processing of chicken skins into gelatine

Processing of chicken skins (flow chart in Figure 1) into gelatine involved 6 steps as follows:

1. First, chicken skins were obtained from chicken processing company. Then they were immediately washed with tap water, cleaned and cooled to $0\text{--}5\text{ }^{\circ}\text{C}$ to avoid bacterial contamination.

They were ground and homogenized. Temperature of the material should not exceed 12 °C. Afterwards, the material was frozen to the temperature of about -3 °C.

2. Next step of grinding involved two phases, pre-grinding and pulping. It was performed in an industrial meat cutter using a 20–30-mm kidney-shaped cutter head and 3-mm cutter head. Afterwards, raw material was packed into PE bags and frozen to -36 °C. Chicken skins were stored at -20 °C and thawed at 10 °C in a refrigerator for 12 h (overnight) before further processing.

3. Then, separation of undesirable non-collagen components followed. Firstly, raw material was treated by shaking in 1M NaCl at a ratio of 1:10 (w/v) for 3 h at room temperature, then filtered through PA fabric and rinsed with water. The process was repeated two times. In the next step, raw material was shaken in 0.5% NaOH at the ratio of 1:10 (w/v) for 18 h at room temperature and then filtered and rinsed with water again.

4. Separation of fat was next, very important step as chicken skins contain a high amount of fat (Table 1). Raw material was dried at 35 °C in a forced circulation oven and afterwards defatted with a solvent mixture of Petroleum Ether + Ethanol (1:1) at the ratio of 1:10 (w/v). The mixture was shaken for 96 h at room temperature, filtered and a new solvent mixture was used. This was repeated four times. Defatted chicken skin was air dried overnight. Then it was ground using A 10 labortechnik analytical grinder with a grain size set to 1–2 mm.

5. Pre-treatment with proteolytic enzyme (Polarzyme 6.0 T) followed. Material was mixed with distilled water at the ratio of 1:20 (w/v) in a 2-litre Erlenmayer flask, pH was adjusted to 7.5 ± 0.3 and 0.5% (based on dry matter of starting material) of enzyme was added into the mixture. It was shaken for 20 h, filtered and rinsed with water properly.

6. Five experiments with different extraction temperatures of 40, 50, 60, 70 and 80 °C were performed. Conditions were set based on preliminary experiments. Pre-treated material (25.0 ± 0.5 g) was mixed with distilled water at the ratio of 1:20 (w/v) in a 2-litre glass vessel, heated to the extraction temperature and stirred continuously by magnetic stirrer at a speed of 350 rpm for 60 min. Then, the mixture was heated to 100 °C for 5 min to inactivate residual content of enzyme (added in step 5). It was filtered through G3 filtering crucible and gelatine solution was dried on an anti-adhesive plate, 500 mL on a square of 22 mm x 32 mm at 45 °C for approximately 48 hours. Resulting gelatine film was ground using A 10 Labortechnik analytical grinder with a grain size of 1–2 mm and weighed.

Yield of gelatine and gelatine gel strength

The yield of extraction (η) was calculated using the formula:

$$\eta = \frac{m_1}{m_0} \cdot 100 (\%),$$

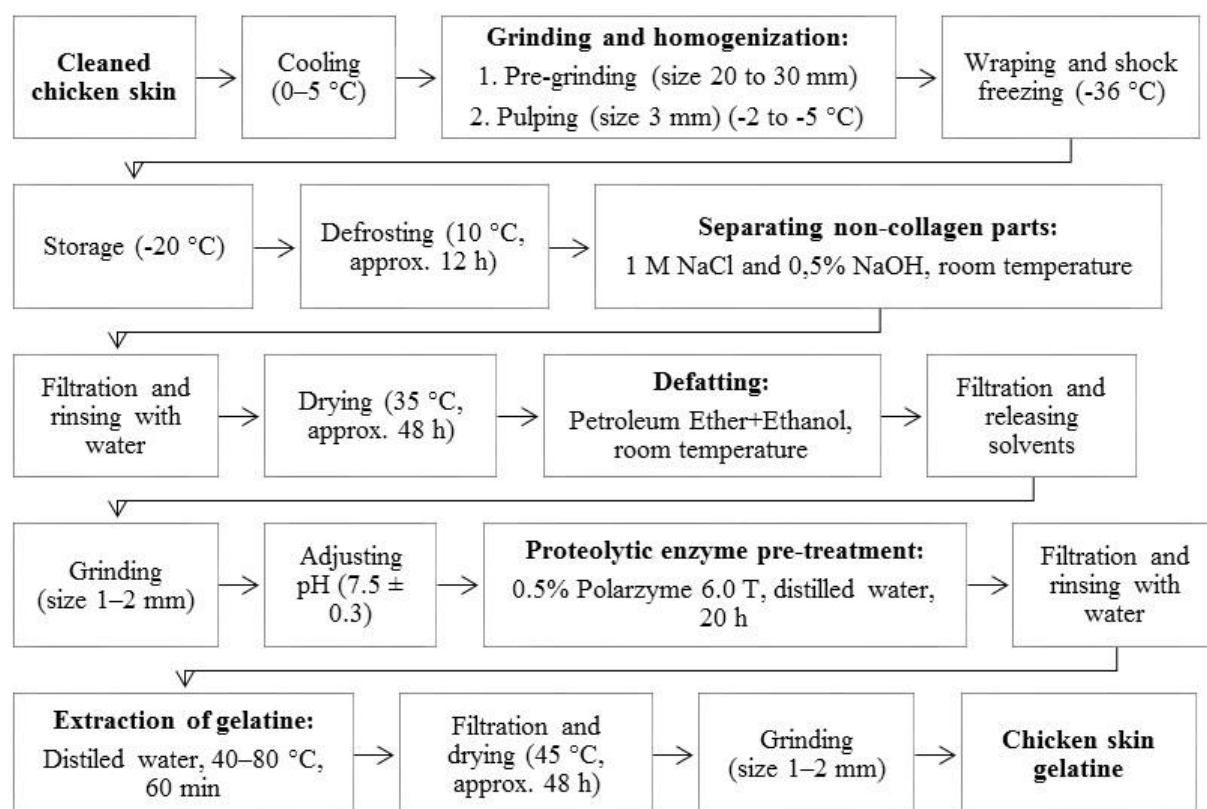
where m_1 is weight of dried gelatine (g) and m_0 is weight of air dried of chicken skin (g) after step 4.

Gelatine gel strength was determined according to the Gelatin Manufacturers Institute of America (2013). Gelatine gel strength (Bloom value) was measured by rigidity of gel of 6.67% gelatine solution prepared as follows. 7.5 g of gelatine was weighed and placed into a bloom jar with a volume of 150 mL and dimensions as follows: overall height of 85 mm, inside diameter of 59 mm, outside diameter of 66 mm, neck inside diameter of 41 mm and shoulder height of 65 mm. Then 105 mL distilled water were added. Resulting 6.67% gelatine solution was allowed to swell at room temperature for 3 h. The bloom jar was heated in water bath at 65 °C and magnetic stirrer was used in order to dissolve all pieces of gelatine. After that, the bloom jar was cooled at room temperature and kept in a refrigerator for 16–18 h (overnight) to form gelatine gel. Then the bloom jar was placed into Stevens LFRA Texture Analyser. Gel strength was expressed as a force (weight in g or Bloom) required to depress a plunger of 0.5" diameter (sharp edge) by penetration speed of 1 mm/s to a prescribed area of the surface of the gelatine sample to a distance of 4 mm. The analysis was repeated three times.

Statistical analysis

One-way ANOVA was applied to all results using Minitab 18 statistical software for Windows (Minitab 213 Inc., USA).

Figure 1 Flow chart of processing of chicken skin into gelatine



RESULTS AND DISCUSSION

Results of all experiments are shown in Table 2.

Table 2 Results of experiments

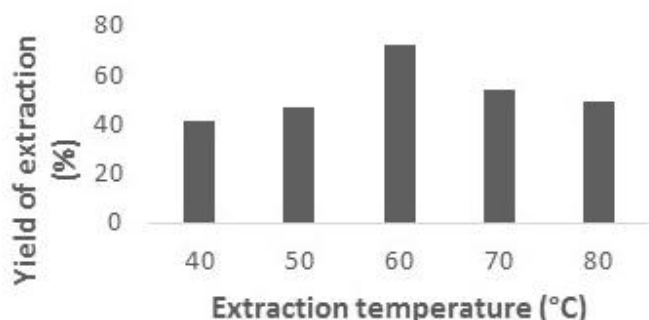
Exp. No.	Extraction temperature (°C)	Yield of extraction (%)	Gel strength (Bloom ± SD)
1	40	41.9	354 ± 2.49
2	50	47.1	352 ± 0.94
3	60	72.4	252 ± 3.85
4	70	53.8	312 ± 1.25
5	80	49.8	306 ± 1.69

Yield of extraction

The yield of gelatine obtained from extraction is generally dependent on particular species, age of animals, collagen type (amino-acid composition), degree of intermolecular collagen cross-linking and method of extraction (Widyasari and Rawdkuen 2014). The yield of gelatine extracted from chicken skin initially grew with an increasing extraction temperature. This might be due to the fact that higher temperature causes higher degree of collagen hydrolysis. Dramatic increase in the yield was observed between the temperatures of 50 °C and 60 °C. However, at 70 °C the yield of extraction significantly decreased to almost the same level as at 50 °C. This decline could be caused by partial collagen denaturation. A further increase of temperature to 80 °C caused a small decline in a yield. Nevertheless, the yield of extraction was considerably high (over 40%) at all extraction temperatures which was probably due to the fact that chicken skin collagen was very young and thus cross-linked weakly. The highest yield of extraction of 72% was achieved at 60 °C. This may be considered as the optimal extraction temperature for this type of collagen as can be seen in Figure 2. Sarbon et al. (2013) reported the yield of extraction of chicken skin gelatine of only 16% in contrast to these results. Sompie and Triasih (2018) determined the yield of extraction of chicken legskin gelatine as very low

with the values ranging from 12 to 14%. Similar results were reached by Widyasari and Rawdkuen (2014) in their study of chicken feet gelatine (13%).

Figure 2 Yield of extraction of chicken skin gelatines at different extraction temperatures

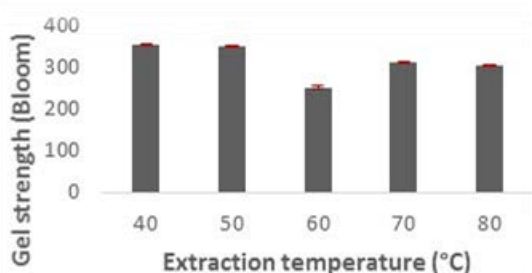


Gelatine gel strength

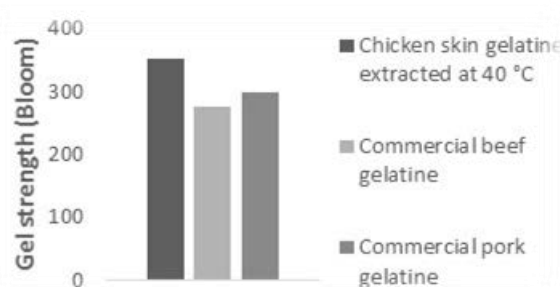
Gelatine gel strength (Bloom value) is one of the most crucial properties of gelatine and is a decisive indicator of its quality. Commercial gelatine from mammalian sources performs its gel strength typically within a range of 50–300 Bloom (Schrieber and Gareis 2007). Results of gel strength of chicken skin gelatines are shown in Figure 3A. Significantly high values of gel strength were recorded during all experiments. Gel strength declined with an increasing extraction temperature with the only exception of the temperature of 60 °C when a sudden drop of gel strength was registered. This may be caused by higher extraction temperature shortening the length of collagen chains of gelatine (a lower molecular weight of gelatine). The drop at 60 °C can be related to a very high extraction yield. Nevertheless, connection between gel strength and yield of extraction has been observed: the higher yield of extraction, the worse gel strength. Higher extraction yield is caused by higher level of hydrolysis, thus resulting in a decline of gelatine gel strength. The highest gel strength of 352 Bloom was recorded at the temperature of 40 °C. The values of gel strength of commercial beef and pork gelatines were 275 Bloom and 300 Bloom, respectively. Figure 3B shows a comparison of these gelatines with prepared gelatine extracted at 40 °C. Even though prepared chicken skin gelatines and commercial gelatines have fairly similar gel strengths, gelatine extracted at 40 °C showed gel strength by 17% higher. Sarbon et al. (2013) reported gel strength of chicken skin gelatine of 355 Bloom, which is almost the same value as in this study. Sompie and Triasih (2018) examined gel strength of chicken leg skin gelatine with the values of only around 78 Bloom. And Widyasari and Rawdkuen (2014) determined gel strength of chicken feet gelatine ranging between 79 and 185 Bloom.

Figure 3 Gel strength of chicken skin gelatines (A) and comparison with beef and pork gelatines (B)

3A)



3B)



CONCLUSION

Composition of chicken skins, by-products of chicken breasts processing, were analysed. What is more, gelatine was prepared. Undesirable components, such as non-collagen proteins and fat, were eliminated. Gelatine extractions were performed in water at 5 different temperatures at constant time. Also, the influence of extraction temperature on gelatine yield and gelatine gel strength (the most

important parameter of gelatine quality) was examined. The highest yield of 72% was achieved at 60 °C and the highest gel strength of 352 Bloom was obtained at 40 °C. Generally, gel strength of all prepared gelatines reached considerably high values of over 250 Bloom. Further quality characteristics important for food industry including water holding and fat binding capacity, emulsifying and foaming properties will be the subject of further analyses.

Results of these experiments have proved that high quality chicken skin gelatine with comparable gel strength to commercially available gelatines from pork and beef should be considered as an important, accessible alternative for mammalian gelatine.

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