PREPARATION OF PROTEIN PRODUCTS FROM COLLAGEN-RICH POULTRY TISSUES

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ABSTRACT

Chicken stomachs are by-products obtained from the poultry processing in slaughterhouses. Their amount has been gradually increasing as a consequence of a continually rising poultry consumption. Since these animal tissues are still rich in proteins, mainly collagen, fat, and minerals, it is essential and beneficial to investigate the appropriate management and further processing. Collagen could be extracted from chicken stomachs and used as a raw material in the food, cosmetic, medical, and also pharmaceutical industry. This paper is to investigate possibilities of such extraction of collagen products, gelatines, or alternatively hydrolysates, from chicken stomachs after prior biotechnological treatment with the proteolytic enzyme Protamex. In this experiment, non-collagenous proteins were removed from stomachs using 0.03 M NaOH and 0.2 M NaCl. Subsequently, the tissue was defatted applying acetone and the enzyme Lipolase. Purified and dried collagen was then treated with the proteolytic enzyme Protamex. In the last step, gelatine was extracted from the tissue in hot water. The influence of selected processing parameters on the extraction efficiency and final product quality was monitored. The extraction conditions included the amount of the added enzyme (0.1 – 0.4%) and the extraction temperature of between 60 and 65 °C. The total gelatine yield ranged from 43.80 to 96.45% and the gel strength varied from 2 ±0 to 429 ±8 Bloom. The enzymatic treatment of the raw material is an economical and ecological alternative to traditional acid or alkaline treatments. Extracted gelatine with the gel strength of 100 – 300 Bloom would be suitable for the applications in the food industry in the production of confectionery, marshmallow, aspic or dairy products.

Keywords: biotechnology; chicken stomach; food industry; by-products; gelatine

INTRODUCTION

The consumption of poultry meat has been consistently increasing. The current status in the Czech Republic is approximately 27 kg per person per year. Such a situation emphasizes the importance of management and processing of slaughter by-products (**Český statistický úřad, 2019**). The poultry slaughter process produces two forms of edible and inedible waste, solid and liquid. Solid waste includes skin, feathers, intestines, offal, glands, limbs, and bones and liquid involves blood and various adipose tissues (**Seong et al., 2015; Ockerman and Hansen, 2000**). Poultry waste comprises up to 30% (in several cases even 40%) of the live weight of the animal. Considering their proteinaceous nature and the fact they are produced in large quantities, they must be managed to avoid environmental pollution. However, inedible parts of poultry are mostly incinerated or landfilled. That is undesirable waste management producing up to 100 million tonnes of waste worldwide (**Borowski and Kubacki, 2015; Xiong et al., 2016; Ferraro, Anton and Santé-Lhoutellier, 2016**). Blood is used as an additive in

certain food products and in the production of feed meal. Bones and skins are applied in the production of hydrolysates, gelatine, fertilizers, and feed for livestock; furthermore, in the leather industry in the leather production and in the production of meat-and-bone meal. Adipose tissues are employed in the production of biofuels, industrial lubricants, oils, soaps, and as a functional additive for cosmetic products (**Lee, Lee and Song, 2015; Cruz-Fernández et al., 2017; Sarbon, Badii and Howell, 2013**). Keratin hydrolysates obtained from feathers are used as growth promoters, feed additives, and functional additives in cosmetic products (**Ockerman and Hansen, 2000; Barbut, 2015; Dikeman and Devine, 2014; Wan Omar and Sarbon, 2016**). Other wastes aim for the production of biofuels, composting, anaerobic digestion, or the isolation of valuable substances contained in animal by-products (**Borowski and Kubacki, 2015; Alibardi and Cossu, 2016; Xiong et al., 2016**). The best solution would be to eliminate waste. Even though this is unfortunately very difficult to achieve, the optimal waste management must be pursued. To use slaughter by-

products efficiently, several criteria are vital to be accomplished. Primarily, a process recycling such a material to produce new products must be developed. Equally important is to provide a sufficient amount of slaughter by-products in the locality of new products manufacture together with the appropriate technological and economical background. A potential market where to offer these products is also essential. One of the ideal solutions appears to be the processing of slaughter waste, such as chicken stomachs, into further protein products containing significant amounts of collagen, vitamins, and minerals (**Rafieian, Keramat and Shahedi, 2015; Lee, Lee and Song, 2015; Khalid et al., 2011**). It is important to explain that in the countries of Central Europe (the Czech Republic, Slovakia, Poland, and Hungary) poultry stomachs are considered to be edible offal. However, in Western Europe and America, these animal tissues are not included in a diet and are generally regarded as a slaughter waste. A suitable alternative to the utilization of chicken stomachs is in collagen products of gelatines and hydrolysates possible to apply in the food, pharmaceutical, cosmetic and medical industry. This would facilitate the management of an undesired and unused slaughter waste (**Alao et al., 2017; Toldra, 2006; Schreiber and Gareis, 2007; Rousselot gelatin, 2019**).

Poultry slaughtering produces by-products having extraordinary physico-chemical properties (**Ockerman and Hansen, 2000; Ferraro, Anton and Santé-Lhoutellier, 2016**). The chicken stomach is a part of the digestive system functioning as a smooth muscle bag divided into a muscular and glandular part. Only the muscular part is edible. The chicken stomach represents about 3% of the total weight of poultry. Since stomachs contain a significantly large amount of collagen, suitable methods of extracting gelatine from them have been investigating. Regrettably, chicken stomachs are composted or incinerated rather than used for the consumption in these regions so far (**Marvan, 2017; Huda et al., 2013; Kosseva, 2013**). The viscera, including chicken stomachs, provides extraordinary nutritional value and is highly appreciated in many parts of the world, such as in China, Japan, and India (**Bakar and Harvinder, 2002**).

In practice, type A and type B gelatines are encountered. Type A gelatine is obtained by acid treatment of the raw material, while type B gelatine is extracted using a base. Currently, the extraction is performed using beef and pork skins and bones. This study examines the gelatine extraction after the prior enzyme treatment which seems to be the most convenient method of obtaining gelatine in terms of time and energy savings. Type B gelatine is treated for up to 6 months, type A gelatine for up to 40 hours, but enzyme extracted gelatine is treated for only up to 24 hours.

What is more, this form of gelatine is considerably well digested and absorbed in the gastrointestinal tract (**GMIA Standard Testing Methods for Edible Gelatin, 2019; Schreiber and Gareis, 2007; Mokrejš et al., 2019**).

The aims of this study

As chicken stomachs are solid poultry by-products containing large amounts of collagen (**Marvan, 2017; Ockerman and Hansen, 2000**), this study is to contribute to the investigation of suitable methods for the collagen extraction from such a slaughter waste. To our best knowledge, extraction and application of gelatine obtained from chicken stomachs by enzymatic treatment of the raw material have not been reported yet. Therefore, the aim of this paper is to assess the possibilities of extracting gelatine from chicken stomachs after the preceding biotechnological treatment of tissues with the proteolytic enzyme Protamex. It continues in the previous research "Preparation of protein products from collagen-rich poultry tissues" and "Utilization of protein by-products from poultry slaughterhouses for the preparation of collagen" (**Polaštíková et al., 2019a; Polaštíková et al., 2019b**). This study focuses on monitoring the influence of selected technological conditions on the process efficiency and the final quality of extracted gelatine. The examined factors include the amount of the added Protamex proteolytic enzyme (Factor A; 0.1, 0.25, and 0.4%) and the extraction temperature (Factor B; 60, 62.5, and 65 ° C). Furthermore, it characterizes prepared gelatine by its gel strength and ash content.

Scientific hypothesis

Gelatine with a high gel strength of approximately 200 – 300 Bloom can be extracted from chicken stomachs.

MATERIAL AND METHODOLOGY

Material

Chilled chicken stomachs were provided by Raciola Uherský Brod, the Czech Republic. Stomachs were minced and homogenized to the particle size of 3 mm. The dry matter content was 19.10 ±0.05% and the composition in dry matter was as follows: protein content of 75.6 ±0.8%; fat content of 21.70 ±0.01% and mineral content of 3.900 ±0.005%.

Appliances, tools and chemicals

P-22/82 meat mincer Braher (Brather Internacional, Spain), LT2 shaker Kavalier (Kavalier, Czech Republic), Kern 440 – 47 electronic analytical scale, Kern 770 electronic analytical scale (Kern, Germany), pH meter Multical pH 526 (WTW, Weilhein, Germany), heating block LTHS 250 and 500 (Merci, Czech Republic), WTB Binder E28-TB1 driver (Binder, Germany), Memmert ULP 400 drying device (Memmert GmbH + Co. KG, Germany), SLR heating board with a magnetic stirrer (Schott Gerate GmbH, Germany), Stevens LFRA Texture Analyser for measuring gelatine gel strength (Leonard Farnell and Co Ltd., England), magnetic stirrer IKA Labortechnik PCT Basic with a heating and magnetic stirrer (IKA-Werke, Germany), differential scanning calorimeter DSC 1 (Mettler-Toledo, Germany), Mora hot air oven (Mora, Czech Republic), Nabertherm L9/11 muffle furnace (Nabertherm, Germany), desiccator (Kavalier, Czech Republic), EBA 20 centrifuge including a rotor (Hettich, Germany), vertical mixer ETA 0010 New Line (ETA, Czech Republic), KRUPS grinder and Samsung refrigerator (KRUPS, Czech Republic).

Erlenmeyer flasks of the volume of 2 L and 0.5 L; 2 L PET bottles with a screw cap; 25 mL, 200 mL, 250 mL and
1000 mL graduated cylinder; Petri dishes; pipettes;

weighing bottles; low-density filter papers; metal sieves; sprays with distilled water; scissors; gel strength flasks; non-stick drying pads; PA fabric; silicon crucible; 1 mm and 2 mm metal sieves; laboratory spoons and sticks; beakers; laboratory tongs; self-closing PE bags; funnels; metal sheet and adhesive tape.

Enzym Protamex (*Bacillus* protease complex developed for the hydrolysis of food proteins; declared activity of 1.5 $AU.g^{-1}$), distilled water, 0.03 M and 0.06 M NaOH, 0.2 M HCl, acetone, chloroform, ethanol, the enzyme Lipolase. The enzyme was provided by the Danish company Novozymes and the all chemicals used were provided by the Czech company Verkon.

Factor analysis

Factor analysis refers to a trial method describing the effect of individual factors on the total yield. It is a more time-consuming optimization method sensitive to measurement errors. It provides an extensive range of information; it monitors the impact of several factors on the sample. Factor analysis enables to evaluate not only one factor but also a complex of factors affecting the studied sample. Factor schemes of 2^2 or 2^3 are the most common. The analysis is a matrix creating a combination of input values. And the number of experiments depends on the number of variables (**Antony, 2014; Erge and Zorba, 2018**). In this study, a factor scheme of 2^2 was applied for the experiments, for two levels and two examined quantities. The factors were as follows: the amount of Protamex enzyme added (Factor A; 0.1, 0.25 and 0.4%) and the extraction temperature (Factor B; 60, 62.5, and 65 °C). The enzymatic treatment of the raw material and the extraction time were constant for all laboratory experiments, 30 h, and 2 h, respectively.

Testing of functional properties gelatines

The extraction efficiency was calculated according to the following equation:

$$HY = \frac{m_1}{m_0} . 100$$

$$GY = \frac{m_2}{m_0} . 100$$

$$\eta = HY + GY$$

Where:

HY is the hydrolysate yield (%), m_0 is the weight of the defatted raw material (g), m_1 is the weight of the hydrolysate, GY is the gelatine yield (%), m_2 is the weight of gelatine (g) and η is the total yield (%).

Gelatine analysis providing ash content and gel strength was performed according to the Standard testing methods for edible gelatine (**GMIA Standard Testing Methods for Edible Gelatin, 2019**). The melting temperature of gelatine gel was determined using a differential scanning calorimeter (DSC). After weighing 15 – 30 mg of the sample onto the DSC aluminum dish, it was sealed with a lid. Subsequently, the sample was placed into the measuring cell together with the reference sample. First, the DSC dish was cooled to 5 °C and maintained at this temperature for 5 min. Then, the dish was heated at a heating rate of 5 °C/1 min to the final temperature of 50 °C. Afterward, it was cooled to the initial temperature of 5 °C following the cooling rate of 5 °C/1 min. The melting temperature reflected an endothermic peak during the sample heating (**Höhne, Hemminger and Flammersheim, 2003**).

Preparation of chicken stomach gelatines
Preparation of pure collagen

The purpose was to remove non-collagenous proteins and fat from the raw material to obtain isolated collagen which was then processed in gelatine extraction. First, the raw material was washed in water which removed albumins from the raw material. The treatment in 0.2 M NaCl at the ratio of 1:6 for 1.5 h followed to remove globulins. Then, the treatment with 0.03 M NaOH at the ratio of 1:6 for 20 h removed glutelins. And finally, the treatment with the enzyme Lipolase (the amount of 5% enzyme) with water 1:10 for 3 days defatted the material. Afterward, the defatted tissue was dried at 35 ±1 °C in the oven for 24 h. Thereafter, solvent defatting of the material was performed using acetone at the ratio of 1:9 for 20 h. This was followed by grinding pure collagen on a vertical mixer to the particle size of 1 mm.

Extraction of gelatine from pure collagen

The purified raw material was mixed with distilled water at the ratio of 1:10 and gently shaken at room temperature for 45 min. Then, the pH was adjusted to 6.5 – 7.0. Subsequently, the Protamex enzyme was added in the amount following *Factor A*, which is 0.1% or 0.25% or 0.4% of the enzyme (Table 1). The enzymatic treatment time of 30 h was constant for all experiments. In the next step, the raw material was filtered through a metal sieve, which was provided with 3 layers of PA fabric, and washed thoroughly with water to inactivate the enzyme partially. The material was then subjected to gelatine extraction. First, the washed material was placed into a beaker and mixed with distilled water at the ratio of 1:8. Subsequently, it was heated to the temperature of 60 °C, 62.5 °C, or 65 °C following *Factor B*. After reaching a defined temperature, the gelatine was extracted for 2 h. Finally, 200 mL of gelatine solution was poured onto a 330 cm^2 sheet provided with a non-stick film and dried in an air circulation drier at the temperature of 45 ±1 °C for 2 days.

Table 1 provides the list of experiments including the technological conditions, process characterization, and the list of prepared gelatines following the factor scheme of 2^2.

Statistical analysis

The results of all experiments were processed in MiniTab® 17.3.1 software (Fujitsu Ltd., Tokyo, Japan) for Windows. The statistical significance of the investigated process factors within the observed limits was evaluated on the significance level of $p = 95\%$. Factors with a value lower than $\alpha = 0.05$ influenced the evaluated variables with a 95% significance. The lower the p value, the greater the influence of process factors on the sample. Subsequently, the coefficient of determination characterizing the quality of the regression model was established and the data was graphically expressed.

Table 1 Characteristics of the experiments defining technological conditions, process characterization and attributes of prepared gelatines.

Exp. No.	Factor A Enzyme addition (%)	Factor B Extraction temperature (°C)	Yield of hydrolysate, η_H (%)	Yield of gelatine (main fraction), η_G (%)	Yield of gelatine (minor fraction), η_G (%)	Total extraction efficiency, $\sum\eta$ (%)	Gel strength, F ±SD (Bloom)	Ash content, AC ±SD (%)
1	0.10	60.0	7.76	24.39	32.70	64.85	192 ±10	1.87 ±0.04
2	0.10	65.0	6.65	23.84	13.31	43.80	429 ±8	1.60 ±0.30
3	0.40	60.0	8.87	86.47	1.11	96.45	8 ±0	1.10 ±0.90
4	0.40	65.0	6.65	88.69	0.55	95.89	2 ±0	1.00 ±0.30
5	0.25	62.5	7.76	63.19	9.67	80.62	96 ±4	1.40 ±0.30

RESULTS AND DISCUSSION

The evaluated variables included the degree of conversion, i.e. the percentage of conversion of the raw material into collagen products, the degree of purity of the final products in terms of ash content, and the quality of the extracted gelatine expressed in gel strength in Blooms.

The equation (1) of total extraction efficiency was:
$$\sum\eta = 177 + 139.5\,A - 2.16\,B \qquad (1)$$

The amount of added enzyme performed a statistically significant ($p = 0.035$) influence on the total extraction efficiency, whereas the extraction temperature showed no statistical significance ($p = 0.309$); $R^2 = 93.58\%$.

Figure 1 depicts the effects of factors A and B on the total extraction efficiency. It reveals that the overall yield is the least (less than 50%) with the enzyme addition of 0.1% and the extraction temperature of 65 °C. Conversely, the highest total efficiency of more than 90% was recorded with the enzyme addition of 0.4% and the extraction temperature of 60 and 65 °C. At the temperature of 62.5 °C, the yield declined below 90% again. In general, the total efficiency increases with a rising amount of added enzyme and growing extraction temperature. Thus, the highest efficiency of 96.45% was monitored when 0.4% enzyme was added and the extraction temperature was 60 °C; the lowest efficiency of 43.80% was determined with 0.1% added enzyme and the extraction temperature of 65 °C.

The yield of the gelatine extraction from chicken stomachs varied between 23.84 and 88.69%. **Du et al. (2013)** treated chicken and turkey heads in acetic acid and achieved gelatine yields ranging from 21.1 to 38.0%. Lower gelatine yield of 21.1% was obtained for chicken gelatine extracted at 60 °C and higher gelatine yield of approximately 38.0% was established for turkey gelatine extracted at 50 °C. In both studies, a lower gelatine yield was established if compared to the present experiment. **Almeida, Calarge and Santana (2013)** treated chicken feet at 120 °C for 20 min and extracted gelatine with a yield of about 36% which is in accordance with the yields determined in this study. **Cheng et al. (2009)** treated chicken feet in hydrochloric, acetic, and lactic acid and established the gelatine yield of 5.6 (HCl), 7.3 (acetic acid), and 8.3 (lactic acid) which is less than in this experiment. **Sarbon, Badii and Howell (2013)** extracted gelatine from chicken skin using both the acid and alkaline method with the total yield of only 16%. Therefore, it is evident that the acid and alkaline method may not be optimal to apply for skin processing. A higher yield of gelatine was achieved using the enzymatic treatment of the raw material **(Mrázek et al., 2019)**. Duck gelatine yield examined by **Huda et al. (2013)** was 28.4% which is a lower yield compared to the chicken gelatine yield of 31% achieved by **Liu, Lin and Chen (2001)**. **Abedinia et al. (2017)** treated duck feet using the acid, alkaline and enzymatic methods with the yields of 12.76, 11.39, and 17.94%, respectively. Even though their study confirmed the highest yield of gelatine by enzymatic treatment, it is still less than it was established in this experiment.

The equation (2) of gelatine gel strength was as follows:

$$F = -1044 - 1018\,A + 23.1\,B \qquad (2)$$

The amount of added enzyme and the extraction temperature did not show a statistically significant ($p = 0.084$; $p = 0.346$) influence on gel strength; $R^2 = 85.69\%$.

Figure 2 depicts the impact of Factor A and B on gelatine gel strength. It is evident that to obtain high values of gelatine strength it is essential to apply higher extraction temperatures together with a lower amount of the enzyme. With 0.1% of the added Protamex enzyme and extraction temperature of 65 °C (Experiment 2), gelatine with the gel strength of more than 400 Bloom was extracted which is significantly high. Generally, the gel strength grows with a decreasing amount of enzyme and rising extraction temperature. In this study, it ranged from 2 ±0 to 429 ±8 Bloom. The lowest gel strength value was recorded with 0.4% of the added enzyme and at the extraction temperature of 65 °C. The highest values of gel strength
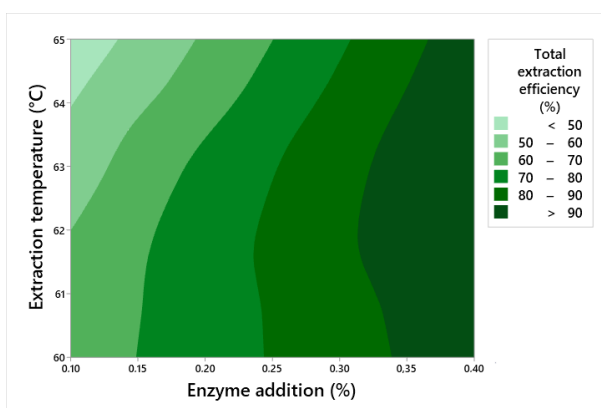

Figure 1 The impact of the amount of added enzyme and extraction temperature on the total extraction efficiency.
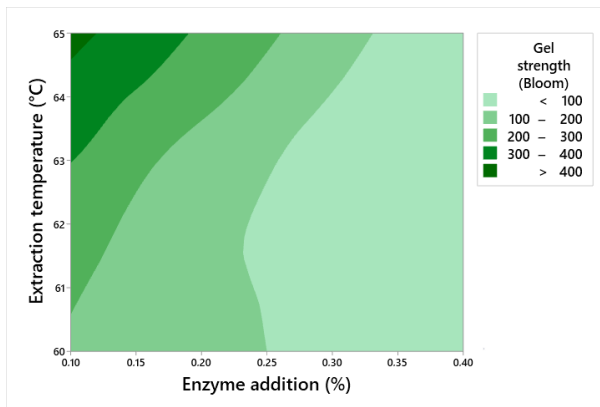
Figure 2 The impact of the amount of added enzyme and extraction temperature on the gel strength.
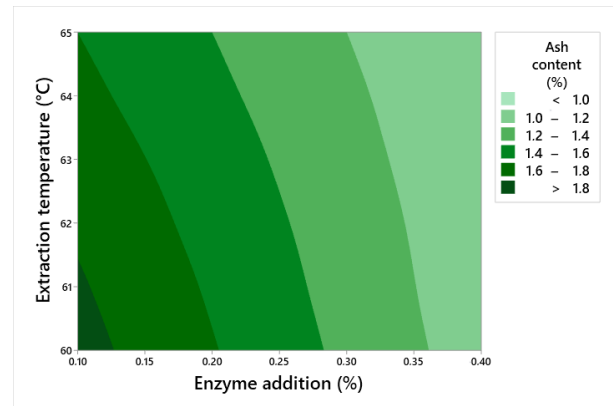

Figure 3 The impact of the amount of added enzyme and extraction temperature on the ash content.

were achieved in the extraction conditions of Experiment 2.

Du et al. (2013) extracted gelatine from turkey and chicken heads with a prior treatment in acetic acid and established the gel strength of 367 Bloom of turkey gelatine extracted at the temperature of 50 °C and the gel strength of 248 Bloom of chicken head gelatine extracted at the temperature of 60 °C which corresponds with this study. **Sarbon, Badii and Howell (2013)** stated bovine gelatine gel strength of 229 Bloom and chicken gelatine gel strength of 355 Bloom. High gel strength values ranging between 320 and 550 Bloom were established in the study by **Rafieian, Keramat and Kadivar (2013)**. **Rafieian, Keramat and Shahedi (2015)** examined chicken bone waste of mechanically deboned meat and determined the gel strength of 520 Bloom which exceeded the results of this experiment. **Sarbon, Badii and Howell (2013)** extracted chicken skin gelatine using both acidic and alkaline extraction methods and recorded the gel strength of 355 Bloom. Such a gel strength value confirms they have obtained the gelatine of considerably good quality. Compared to other alternative gelatine sources, such as fish, chicken gelatine achieves higher gel strength values; mackerel gelatine showed the gel strength of 280 Bloom and tilopia gelatine of about 220 Bloom (**Bakar and Harvinder, 2002**). In the last decade, an interest in both poultry and fish gelatines has increased. Gel strength of fish gelatines may reach up to 420 Bloom. Such a significant gel strength was measured in gelatine extracted from tuna skin according to the study by **Zhou, Mulvaney and Regenstein (2006)**.

The equation (3) of the ash content in gelatine was as follows:

$$AC = -4.277 - 2.283\,A - 0.0370\,B \qquad (3)$$

For the ash content, the amount of added enzyme was statistically significant ($p = 0.008$). In contrast, the extraction temperature was statistically insignificant ($p = 0.092$); R2 = 98.58%.

Figure 3 shows the effects of Factors A and B on ash content. It is evident that to obtain a low amount of ash content in % it is vital to apply a lower/higher extraction temperature and a higher amount of the added enzyme. With 0.4% of the added enzyme Protamex and the extraction temperature of 60 and 65 °C, the ash content is

approximately 1.1%. The ash content generally grows with a decreasing amount of the added enzyme and rising extraction temperature. The highest value corresponds with 0.1% of the added enzyme and the extraction temperature of 60 °C which reflects the extraction conditions in Experiment 1.

In the present study, the ash content ranged from 1.0 ±0.3 to 1.87 ±0.04%. **Du et al. (2013)** published a smaller ash content of only 0.03 to 0.06% in turkey and chicken gelatine extracted at 50 °C and 60 °C. **Almeida and Lannes (2013)** established the ash content in chicken feet gelatine of 1.9%. According to **The United States Pharmacopeial Convention (2018)** the maximal content of ash in gelatine must not exceed 2.0%; therefore, this factor has been accomplished in this study. **Bueno et al. (2011)** determined approximately 0.3% of ash in pork gelatine and **Sarbon, Badii and Howell (2013)** established 1.1% of ash in beef gelatine. In contrast to this study, **Rafieian, Keramat and Shahedi (2015)** recorded the ash content in chicken bone waste of 2.6%. **Sarbon, Badii and Howell (2013)** affirmed a lower ash content of 0.4% in chicken skin gelatine extracted using both acid and alkaline methods. **Huda et al. (2013)** extracted gelatine from duck feet using 5% lactic acid in the rate of 1:8 and established the ash content of 28.6% which is fourteen times higher than the required limit for gelatine application in the food industry.

Melting temperatures of gelatine gels

Figure 4 depicts DSC curve of gelatine gels melting temperatures. Experiment 4 (the gel strength of 2 ±0 Bloom) failed to identify the melting temperature of the gel since a hydrolyzate was formed. The gelatine of Experiment 1 (0.1% of the added enzyme and the extraction temperature
of 60 °C) performed a gelatine gel melting temperature of approximately 35 °C (the gel strength of 192 ±10 Bloom). Very similar melting temperature was achieved in Experiment 5 (0.25% of the added enzyme and the extraction temperature of 62.5 °C; the gel strength of 96 ±4 Bloom). The melting temperature of 36 °C was recorded in Experiment 3 with the gel strength of 8 ±0 Bloom (0.4% of the added enzyme and the extraction temperature of 60 °C). The highest melting temperature of gelatine gel with a strength of 429 ±8 Bloom (40.5 °C) was identified in Experiment 2 (0.1% of the added enzyme and
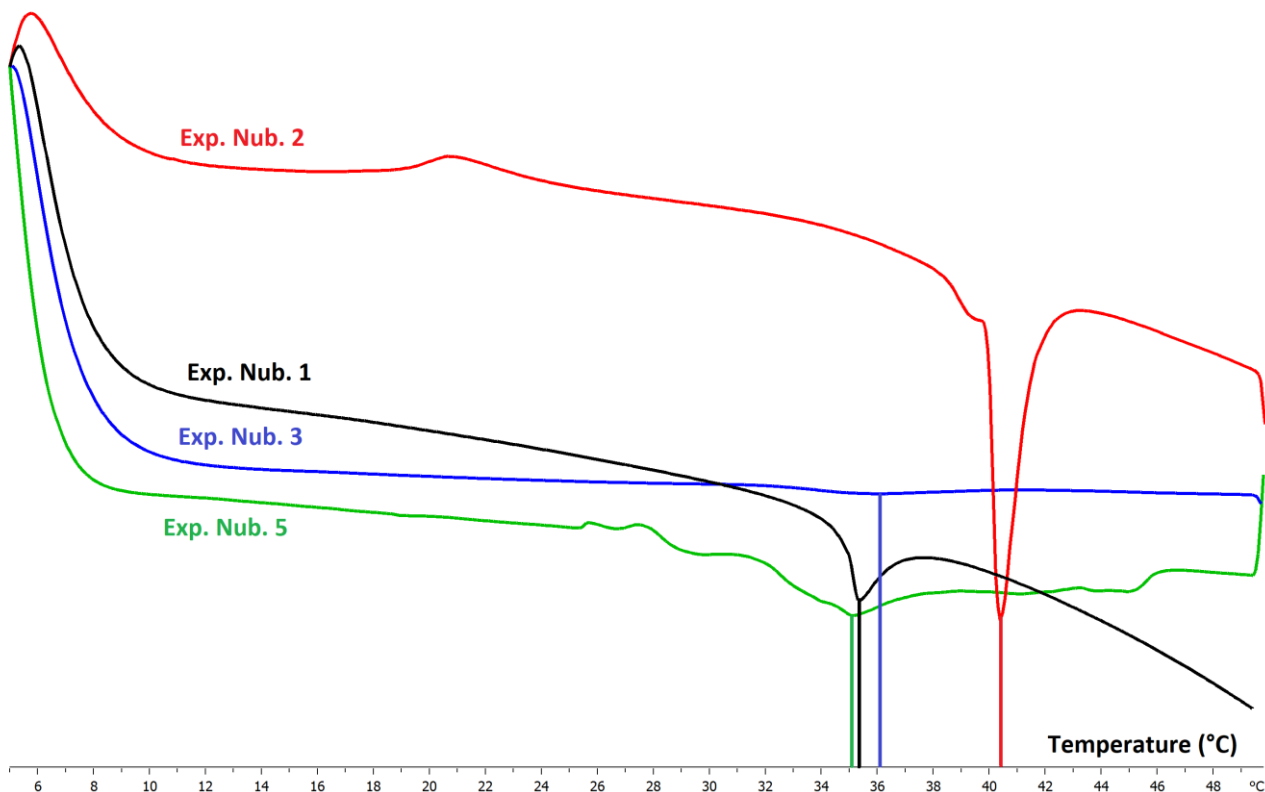
Figure 4 DSC curve of gelatine gel melting points.

the extraction temperature of 65 °C). Melting temperatures of commercial gelatine gels vary in the range from 30 to 40 °C. Their values are important not only from a technical point of view, but also considering the particular application of gelatines influencing various factors, such as the management of gelatine products, maintanance of the final shape of gelatine products and the stability of the products during the storage. Concerning gelatines extracted from chicken stomachs, their melting temperatures ranged from 35 to 40 °C which is comparable with commercial gelatines **(Schreiber and Gareis, 2007)**. **Du et al. (2013)** determined the melting temperature between 33.7 and 34.2 °C. That is slightly lower than the melting temperature of 35 – 40 °C established using DSC in this study reflecting the trend that melting temperature increases with a rising gelatine gel strength.

CONCLUSION

The study examines the possibility of extracting gelatine from chicken stomachs after the prior treatment by the proteolytic enzyme Protamex. The main objective was to propose technological conditions for processing stomachs into collagen products, either gelatines or hydrolysates, with a maximum yield. The influence of Factor A and B on the final efficiency and quality of extracted gelatine was monitored. Factor A represents the amount of added enzyme of 0.1, 0.25 and 0.4% and factor B represents the extraction temperature of 60, 62.5 and 65 °C. The extraction time of 2 h was constant. The final extraction efficiency ranged from 43.83% with 0.1% of added enzyme and the extraction temperature of 65 °C to 96.45% with 0.4% of added enzyme and the extraction temperature of 60 °C. The highest gel strength of about 430 Bloom was

measured within the conditions of the enzyme addition of 0.1% and extraction temperature of 65 °C. On the other hand, the lowest gel strength of 2 Bloom was established with the enzyme addition of 0.4% and extraction temperature of 65 °C. The ash content in prepared gelatines was less than 2%; it ranged between 1.0 (0.4% of added enzyme and the extraction temperature of 65 °C) and 1.9% (0.1% of added enzyme and the extraction temperature of 60 °C). Edible gelatine with the gel strength of 96 Bloom (with the yield of 63%) is suitable for the applications in the production of confectionery, such as meringues, toffee, licorice and also deposited marshmallow. To produce jelly, gummy bears, aspic and dairy products it is preferable to employ gelatine with a higher gel strength (192 Bloom) despite its lower yield (approximately 24%). Both types of gelatine performed the ash content lower than 2.0% and the melting temperature of about 35 °C which means that such gelatines would be soluble in the mouth and simultaneously it would maintain the product shape during the storage, particularly during the summer months. Gelatine with a high gel strength of more than 220 Bloom is applicable in the production of desserts, extruded marshmallow, fish aspic and reduced fat spreads and in the pharmaceutical industry in the production of soft gelatine capsules.

This study has proved that it is possible to obtain high quality gelatine from chicken stomachs with the gel strength of up to 430 Bloom if appropriate technological conditions are set. The method applied in this study is quite prompt and efficient. Therefore, it has also confirmed that effective processing of valuable poultry slaughter by-products is accesible.

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