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Biotechnological preparation of chicken skin gelatine using factorial design of experiments

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ABSTRACT

Poultry meat production steadily increases and its by-products including legs or skins may be further utilized to prepare gelatine, generally by using alkali or acid substances. Since they are toxic, different methods are desirable, e.g. applying proteolytic enzymes. Using enzymes is beneficial as they shorten processing time and reduce the required water amount. Processing conditions (the enzyme amount during the pre-treatment, first and second extraction temperature and time) of the process may influence gelatine quality and its yield. In this study, gelatines were prepared from chicken skin at different conditions in the series of combined factorial design of experiments. The significance of the influence of the conditions on the quality (gel strength, viscosity, melting and gelling point) and yield of chicken skin gelatine (CSG) was observed. The highest yield (31.5%) was obtained at the conditions of the enzyme amount of 0.8%, first extraction temperature 80 °C and time 30 min, second extraction temperature 90 °C and time 60 min. Furthermore, the highest CSG quality of gel strength (190 Bloom), viscosity (4.06 mPas), melting point (38.3 °C) and gelling point (20.5 °C) was obtained at the conditions of 0.2%/ 50 °C/30 min in the first extraction.

Keywords: Collagen, chicken skin, enzyme pre-treatment, gelatine, gelling properties

1. Introduction

Gelatine is a water-soluble fibre-based substance known as collagen (**Schrieber & Gareis, 2007**). It is unique due to its chemical and physical properties, including stabilisation, gelation, texturisation, emulsification, sedimentation, clarification, thickening, viscosity enhancement, film-forming ability, biodegradability, adhesiveness, biomaterial-based packaging and encapsulation or foaming ability; and is thus widely used in many applications in the food and non-food industries (**Martucci & Ruseckaite, 2010; Cebi et al., 2016**). Gelatine gels that contain a low concentration of gelatine are often used to prepare films or coatings due to their functional and rheological properties, whereas gelatine gels with

a high concentration of gelatine are applied in tissue and material engineering because of their high mechanical properties (**Dash, Foston, & Ragauskas, 2013**). Gelatine is highly digestible with a low content of calories, therefore, it is perfectly suitable for supplementation of proteins in body-building foods or the reduction of carbohydrates in curing diabetes or as drug and gene delivery systems. Furthermore, it contains all essential amino acids except for tryptophan (**Ranganathan et al., 2019; Saber, 2019**).

Generally, gelatine is industrially produced using alkali or acid pretreatment of the raw material, followed by the extraction in water at the temperatures from 50 to 100 °C. However, pre-treatment of collagenous material can be accelerated by proteolytic enzymes. Since enzymes contain no toxic substances, they pose no risk for the environment (**Nalinanon et al., 2008**). Another advantage of using enzymes in the pre-treatment of the raw material is the significant shortening of the pre-treatment time. Commonly used industrial alkaline pre-treatment lasts for weeks or even months, whereas the enzyme pre-treatment takes approximately only a day. In addition, enzyme pre-treatment does not require any adjustment of the aqueous solution which is often necessary for alkaline pre-treatment. Therefore, water consumption is significantly lower as well as the consumption of the electric energy. Enzymes, such as pepsin, trypsin, chymotrypsin, alkalase, properase E, pronase, bromelain, papain, proctase or neutrase, have already been used in the experiments isolating collagen from various tissues (**Yang et al., 2008; Aleman et al., 2011; Lassoued et al., 2014**).

Concerning the raw material, gelatine is commonly extracted from two kinds of raw materials containing a significant amount of collagen: pigskins or cowhides (more than 98%). However, consuming pork gelatine is banned in Judaism and Islam and beef gelatine is allowed providing it has been prepared according to the specific religious requirements. Hindus are not allowed to consume cow-derived products at all.

In addition, some other countries where Buddhism is widespread, such as India or East Asia, must also respect certain restrictions on pork meat or gelatine due to the specific religious believes (**Karim & Bhat, 2008; Gómez-Guillen et al., 2011**). The population of Muslims, Jews and Hindus has been rising and is expected to be around 53% of the global population in the next 20 years counting almost 4 billion people (**PRC, 2020**). Moreover, beef gelatine may be associated with bovine spongiform encephalopathy (BSE) or foot-and-mouth disease (FMD) (**Jeevi-than et al., 2014**). Therefore, alternative sources of gelatine, such as poultry or fish by-products, are highly desirable for the production of halal gelatine. However, especially concerning fish gelatine, there are some drawbacks including its characteristic fishy odour, worse thermal stability and poorer rheological properties if compared to gelatines derived from land mammals. Hence fish gelatine meets desired application parameters only insufficiently (**Kim & Wijesekara, 2012**). On the other hand, gelatine derived from poultry appears to be a very promising alternative.

The physico-chemical properties of gelatine may be affected by many factors including the type of the tissue, age and species of animal, applied pre-treatment method and extraction conditions (particularly temperature and time) (**Dunconseille et al., 2015**). Increasing extraction temperature and lengthening extraction time during the gelatine production process generally result in a yield enhancement of the process, accompanied by the deterioration of gelling properties (**Kittiphattana-bawon et al., 2012**). Therefore, the extraction conditions, such as temperature and time, should be optimized in order to produce both high process yield and quality of gelatine.

Basic physical properties of gelatine are translucency, colourlessness, brittleness (in a dried state), tastelessness and the fact it is odourless and edible (**Ahmad et al., 2017**). Nonetheless, one of the most notable physical properties of gelatine in commercial applications is its ability to form a gel. A few-

percent solution of gelatine in water forms a transparent elastic gel at temperatures below 35 °C causing an arrangement of native collagen forming a network of collagen fibres capable of incorporating large amounts of water into its structure. This characteristic makes gelatine unique in terms of its sensory aspects, particularly the release of flavour utilized in the food industry. Transformation between the solution and gel is thermo-reversible and the gelatine gel possesses a unique ability to "melt in the mouth" (**Choi & Regenstein, 2000**).

The lack of traditional raw materials stemming from a constantly growing consumption of gelatine encouraging the rise of their price, as well as cattle diseases or religious reasons have led to the investigation of non-mammalian sources of gelatine. Therefore, preparation of gelatine from innovative sources, such as chicken skin, has been explored as a promising substitution for traditional sources (**Cliché et al., 2003; Sarbon et al., 2015; Bichukale et al., 2018; Mrázek et al., 2019**). Chicken skin contains approximately 75% of collagen type I and 15% of type III (**Abedin & Riemschneider, 1984**). Predominantly, it is processed into animal feed while a minor proportion is added to meat emulsions or used as a fat source in the preparation of soups (**Cliché et al., 2003**). Chicken skin gelatine exhibits similar properties to conventional commercial gelatine and contains a high proportion of amino acids (especially proline and hydroxyproline), which is of great importance for the gelling effect as well as for the gel strength (**Wantgueai & Noomhorn, 2009; Gomez-Guillón et al., 2011**).

In this study, combined factorial design of experiments was employed to establish the significance of the influence of individual processing factors (the extraction time and temperature, amount of enzyme) on the yield and quality (the gel strength, viscosity, melting and gelling point) of prepared gelatines in order to optimize the gelatine extraction process. Using this method enables to examine more than one factor at two or more levels. Experimental design generally involves combinations of different factor levels, allowing to evaluate interactions between different factors, to assess the significance of each factor to identify a more effective solution to the issue by using only a small number of experiments. Factorial experiments use orthogonality that enables a description of the phenomenon without having to examine all variants of the solution. Therefore, the factorial design of experiments can be employed to determine the optimal values of individual factors influencing the process (**Kennedy & Kraus, 1999; Antony, 2014**). At the moment, scientific literature offers only few reports concerning the use of factorial experiments in the optimization of gelatine manufacture. However, possible applications of such a design of experiments have already been described in technical practice, for example at work by Štraus and Dolejs using factorial experiments in water treatment issues (**Straus & Dolejs, 2010**). Further studies have been recently published conducted on pharmaceutical and analytical quality design (**Fukuda et al., 2018**) and seed-mediated growth of gold nanorods (**Borrows et al., 2017**).

The aims and hypotheses of this work: this paper aims to continue in the research of the processing of chicken skins into gelatines (**Mrázek et al., 2019**) and to perform two-stage extraction of chicken skin gelatines (CSG) under different processing conditions. Furthermore, using a combined factorial design of experiments, it evaluates the effect of processing conditions (the amount of enzyme during the pre-treatment, extraction temperature and time) of CSG preparation on the extraction yield and gelling properties of prepared gelatines mainly represented by the gel strength, viscosity, gelling and melting point. Moreover, it proposes the optimal processing conditions of gelatine preparation to ensure high efficiency of gelatine extraction as well as its quality. Finally, it examines commonly available commercial pork and bovine gelatines to provide a wider comparison of the results. The main hypothesis of this study assumes that the CSG yield increases with a higher intensity of processing conditions during the CSG preparation (a higher level of enzyme during the pre-treatment of the raw material, higher extraction temperature and longer extraction time). However, at the same time,

deterioration of CSG gelling properties has occurred. The secondary hypothesis presumes that the experiment will establish processing conditions for the preparation of CSG that provide gelling properties of CSG comparable to conventional commercial beef and pork gelatines, as well as a comparable yield of CSG with the findings of similarly focused studies.

1.1. Materials

Chicken skins (Raciola, Czech Republic); enzyme: Polarzyme 6.0 T -proteolytic serine endoprotease manufactured by fermentation of microorganisms not presented in the final product (Novozymes, Denmark) with declared enzyme activity of 6 KPU/g (kilo protease unit/g); commercial gelatines: Pork D526 and D012119, Beef D529 and Halal (Via Naturae, Czech Republic). Chemicals: sodium chloride, sodium hydroxide, hydrochloric acid, suluric acid, petroleum ether, ethanol, chloroform, Ehrlich's reagent (Verkon, Czech Republic). All chemicals were at analytical type of grade.

1.1.1. Preparation and analysis of raw material

Chicken skins were stored at − 20 °C prior to the experiments. The composition of chicken skins was determined: dry matter: 53.6 ± 1.5%; in dry matter: proteins: 16.5 ± 1.3%, collagen: 92.6 ± 0.1%, fats: 85.0 ± 2.4%, inorganic solids: 0.9 ± 0.3%.

Determinations were proceeded as follows: the amount of dry matter was established by sample drying at 103 °C to the constant weight (**AOAC, 2000**). Protein was determined according to the Kjeldahl method: the sample was heated in the presence of concentrated sulfuric acid and reduced nitrogen was released in the form of ammonium sulfate. Sodium hydroxide was added resulting in conversion to ammonia, which was distilled and collected in hydrochloric acid solution. The amount of nitrogen present in the sample was determined by titration and protein content was determined by calculation using a conversion factor (**ISO 937-1978**). Collagen was determined by spec-trophotometric determination of hydroxyproline content after the reaction with Ehrlich's reagent: the sample was subjected to hydrolysis with sulfuric acid at 105 °C, hydrolysate was filtered, diluted and oxidized, product was decarboxylated and the absorbance of the resulting compound measured at the wavelength of 558 nm. The collagen content was calculated on the basis of the percentage content of hydroxyproline using the equation:

$$\text{Collagen content (\%)} = 8 \times W \qquad (1)$$

where W is the hydroxyproline content, calculated as percentage by mass, 8 is the transformation factor (**Ignat'eva et al., 2007; ISO 3496-1994**). The amount of fat was determined by Soxhlet extraction in two cycles using two solvents (chloroform and ethanol). Mineral content was determined by sample burning and annealing at 650 °C for 60 min in a muffle furnace (**Nollet & Toldra, 2015**).

Grinding of raw material (chicken skin), separation of non-collagen parts and defatting were performed according to the previous study (**Mrázek et al., 2019**). Briefly, the raw material was first ground using a meat grinder. Non-collagenous proteins and pigments were removed by treating of the raw material in sodium chloride and sodium hydroxide solution in an Erlenmayer flask. This was followed by rinsing and drying of the raw material. Fats were removed by treating the raw material in the mixture of

petroleum ether and ethanol (1:1). The following stages of the gelatine preparation process include the pre-treatment of the raw material and the extraction of chicken skin gelatines using factorial experimental design described in detail in the following chapters.

1.2. Methods

1.2.1. Concept of combined factorial design of experiments

A combined design of experiments was applied to investigate the influence of processing conditions on chicken skin gelatine preparation and to evaluate the process optimization.

Based on the previous research, following four processing factors were selected: the amount of enzyme during the pre-treatment of raw material (factor A), temperature (factor B) and time (factor C) of first stage of gelatine extraction and temperature of second stage of gelatine extraction (factor D) (**Schrieber & Gareis, 2007; Abedinia & Nafchi, 2017; Mokrejš et al., 2019**). Concerning the evaluation of gelatine gel-forming properties, four basic parameters were selected: gel strength, viscosity, melting and gelling point. For factor A, two levels were chosen: 0.2% (min. level) and 0.8% (max. level). According to the previous experiments, higher values led to excessively high levels of hydrolysis and denaturation of collagen (**Gál et al., 2020**). Based on the recent study, factor B is the most important factor affecting the properties of gelatine. Therefore, seven levels of this factor were considered: 50, 55, 60, 65, 70, 75 and 80 $\pm$ 0.5 °C. Low temperature is expected to ensure a high quality; on the contrary, high temperature should provide a high yield of gelatine of sufficient quality. For factor C, two levels were selected: 30 min (min. level) and 120 min (max. level). According to the previous experiments, 30-min extraction is assumed to result in a high gelatine quality, whereas four times longer time should guarantee a high yield of gelatine. For factor D, the levels were selected 10 °C higher when compared to the first stage of gelatine extraction: 60, 65, 70, 75, 80, 85 and 90 $\pm$ 0.5 °C. Two-stage extraction was chosen to examine whether the yield from one dosage enhances as it is common in the industrial production. The time of the second stage was set on 60 min which is expected to balance the CSG yield and quality based on the previous research. Prolonging the extraction time would negatively affect the CSG quality and result in a low gelatine yield (**Mokrejš et al., 2021**).

1.2.2. Processing of chicken skins into chicken skin gelatines (CSG)

Ground, stripped of non-collagenous parts and partially dried chicken skin sample (25 g) was pre-treated by enzyme Polarzym 6.0 T, then mixed with distilled water at the ratio of 1:20 (w/v) in Erlenmeyer flask and the amount of enzyme (factor A) based on dry matter of the raw material prior to the pre-treatment was added to the mixture (the exact is displayed in **Table 1**). pH was set to 7.5 $\pm$ 0.3 to optimize the enzyme working conditions. The mixture was shaken for 20 h using LT 43 shaker (Nedform, Czech Republic) followed by the filtration with a polyamide fabric and proper rinsing with distilled water.

The series of 28 experiments of two-stage gelatine extraction were performed in distilled water at the ratio of 1:20 (w/v) at defined extraction temperature (factor B) for the specific extraction time (factor C - **Table 1**) using SLR heating board (Schott Gerate, Germany). After the first extraction, the second extraction was performed. During the second extraction, the temperature was increased by 10 °C (compared to the first extraction - factor D - **Table 1**) and the extraction time was set to 60 min for optimal quality of gelatine.

Similar conditions were maintained in the chicken feet gelatine preparation (**Mokrejš et al., 2019**) and 60 min-extraction was used in some further studies as well (**Kolodziejska et al., 2008; Kim et al., 2012; Hidayati et al., 2021**). After every stage of the extraction, the filtration of resultant gelatine solution using polyester filtration fabric was proceeded. Afterwards, drying of the obtained gelatine solution in a thin layer at 50 $\pm$ 0.3 °C was performed using ULP 400 drying device (Memmert, Germany). Finally, gelatine powder was prepared by grinding of the resulting gelatine film to the size of particles of 1 -2 mm using A 10 Labortechnik analytical mill (IKA-Werke, Germany). 56 samples of gelatines from the first and second extractions were then subjected to testing of the properties connected with gelation of gelatines (gel strength, viscosity, melting and gelling point). The efficiency of every extraction was calculated using the equation:

$$\text{Efficiency (\%)} = (m_1/m_0) \times 100 \qquad 2$$

where $m1$ is the initial weight of dried chicken skin gelatine (g) and m0 is weight of chicken skin (g) prior to the enzyme pre-treatment.

1.2.3. Analysis of gelatines

The study analyses the samples of chicken skin gelatine and commercial pork and beef gelatine (Via Naturae, Czech Republic) providing data about their gel strength, viscosity, melting and gelling point.

1.2.4. Gel strength and viscosity

The value of gelatine gel strength was determined according to the Gelatine Manufacturer's Institute of America (**Gelatine Manufaturer's Institute of America GMIA-Standard Testing Methods for Edible Gelatine, 2019**) using Stevens LFRA texture analyser (Leonard Farnell, England). Gelatine gel strength (or Bloom value) is a force (weight in g) required to depress a measuring probe by specific penetration to a definite area of the gelatine surface to a particular distance. The viscosity determination was also proceeded following the GMIA using Ubbe-lohde' s viscometer (Fisher Scientific, Czech Republic).

Time required for 100 ml of 6.67% gelatine solution to pass through the capillary tube of the pipette was measured and viscosity of the gelatine solution was calculated. The analyses were repeated three times.

1.2.5. Melting and gelling point

The melting point of the gelatine gel was determined according to the procedure described by Schrieber and Gareis with a slight modification (**Schrieber & Gareis, 2007**). A 6.67% gelatine solution was prepared and transferred to a capillary with an inner diameter of 2 mm; the height of the gelatine column was 1 cm. The capillary was cooled at 10 $\pm$ 0.5 °C for 3 h to form a gelatine gel. The capillary was inserted into a test tube as depicted in **Fig. 1**; a thermometer was added and the test tube was placed into a beaker heated to 55 $\pm$ 0.3 °C (rate of 3.5 °C/min). The melting point of gelatine was determined when the water pressure forced the formed gelatine solution out of the capillary. **Fig. 1** shows the equipment used for determination.

The gelling (setting) point of the gelatine solution was determined using the same equipment and procedure described by Schrieber and Gareis with a slight modification (**Schrieber & Gareis, 2007**). The same equipment as for the melting point determination was used.

Gelatine solution was prepared according to the method described above (viscosity determination). In this case it was transferred directly to a test tube (up to half of its height) and the temperature of the solution was measured. Once the temperature decreased to 30 $\pm$ 1.0 °C, cooled water was added to the beaker so that the gelatine sample was completely surrounded by water. Then, steel balls weighing 0.10 g were thrown into the test tube each time the temperature decreased by 0.5 °C. The gelling point was determined when the ball became fixed inside or on the surface of the formed gelatine gel.

The analyses were repeated three times and the arithmetic means with standard deviations were used to express the results.

Table 1 Arrangement and results of factorial design experiments

Exp. No.	A (%)	B (°C)	C (min)	D (°C)	1st Y	1st GS	1st V	1st M_p	1st G_p
1	0.2	50	30	60	16.8 ± 0.1	190 ± 1	4.06 ± 0.03	38.3 ± 0.1	20.5 ± 0.5
2	0.2	55	30	65	17.2 ± 0.2	189 ± 2	3.76 ± 0.03	38.1 ± 0.2	20.0 ± 0.5
3	0.2	60	30	70	17.5 ± 0.1	187 ± 2	3.18 ± 0.01	37.8 ± 0.1	19.5 ± 0.5
4	0.2	65	30	75	17.8 ± 0.1	172 ± 1	3.92 ± 0.02	37.3 ± 0.2	19.0 ± 0.5
5	0.2	70	30	80	17.1 ± 0.3	163 ± 2	3.38 ± 0.03	37.2 ± 0.2	19.0 ± 0.5
6	0.2	75	30	85	18.2 ± 0.1	163 ± 3	3.12 ± 0.03	37.1 ± 0.1	18.5 ± 0.5
7	0.2	80	30	90	18.5 ± 0.3	162 ± 2	3.54 ± 0.02	36.5 ± 0.3	18.0 ± 0.5
8	0.2	50	120	60	17.1 ± 0.2	182 ± 2	3.53 ± 0.02	37.3 ± 0.2	19.5 ± 0.5
9	0.2	55	120	65	17.8 ± 0.2	178 ± 3	3.82 ± 0.03	37.0 ± 0.1	19.0 ± 0.5
10	0.2	60	120	70	18.1 ± 0.3	145 ± 1	4.02 ± 0.03	37.1 ± 0.1	18.5 ± 0.5
11	0.2	65	120	75	18.5 ± 0.1	134 ± 3	3.51 ± 0.01	37.0 ± 0.2	18.0 ± 0.5
12	0.2	70	120	80	19.3 ± 0.3	134 ± 2	3.91 ± 0.01	37.0 ± 0.2	18.0 ± 0.5
13	0.2	75	120	85	19.5 ± 0.3	133 ± 1	4.02 ± 0.02	36.2 ± 0.1	17.0 ± 0.5
14	0.2	80	120	90	19.8 ± 0.1	126 ± 2	3.40 ± 0.03	35.1 ± 0.3	16.5 ± 0.5
15	0.8	50	30	60	17.9 ± 0.1	145 ± 2	4.03 ± 0.03	37.8 ± 0.3	18.5 ± 0.5
16	0.8	55	30	65	18.0 ± 0.3	139 ± 1	4.01 ± 0.02	37.5 ± 0.3	18.5 ± 0.5
17	0.8	60	30	70	18.5 ± 0.2	135 ± 2	3.21 ± 0.02	36.8 ± 0.2	17.5 ± 0.5
18	0.8	65	30	75	19.0 ± 0.1	130 ± 3	3.53 ± 0.01	35.2 ± 0.1	17.0 ± 0.5
19	0.8	70	30	80	19.1 ± 0.1	127 ± 1	3.51 ± 0.03	35.1 ± 0.2	17.0 ± 0.5
20	0.8	75	30	85	20.8 ± 0.2	125 ± 2	3.22 ± 0.03	34.7 ± 0.3	16.0 ± 0.5
21	0.8	80	30	90	22.7 ± 0.3	117 ± 2	3.03 ± 0.02	34.5 ± 0.1	15.0 ± 0.5
22	0.8	50	120	60	18.6 ± 0.1	110 ± 1	3.94 ± 0.02	36.3 ± 0.1	17.5 ± 0.5
23	0.8	55	120	65	19.9 ± 0.2	107 ± 3	3.02 ± 0.02	35.7 ± 0.2	17.0 ± 0.5
24	0.8	60	120	70	20.1 ± 0.3	91 ± 2	3.91 ± 0.03	35.3 ± 0.3	17.0 ± 0.5
25	0.8	65	120	75	20.7 ± 0.1	87 ± 2	3.18 ± 0.01	35.0 ± 0.1	16.5 ± 0.5
26	0.8	70	120	80	22.4 ± 0.2	85 ± 1	3.15 ± 0.03	34.7 ± 0.2	15.0 ± 0.5
27	0.8	75	120	85	22.2 ± 0.1	80 ± 1	3.13 ± 0.02	34.5 ± 0.2	14.0 ± 0.5
28	0.8	80	120	90	23.6 ± 0.3	75 ± 2	2.31 ± 0.03	34.2 ± 0.1	13.5 ± 0.5

Exp. No.	A (%)	B (°C)	C (min)	D (°C)	2nd Y	2nd GS	2nd V	2nd M_p	2nd G_p
1	0.2	50	30	60	5.21 ± 0.1	175 ± 3	2.71 ± 0.03	34.9 ± 0.3	15.0 ± 0.5
2	0.2	55	30	65	5.35 ± 0.2	172 ± 2	2.65 ± 0.02	34.8 ± 0.1	14.5 ± 0.5
3	0.2	60	30	70	5.62 ± 0.2	171 ± 2	2.43 ± 0.01	34.4 ± 0.2	14.5 ± 0.5
4	0.2	65	30	75	5.71 ± 0.1	170 ± 1	2.15 ± 0.01	35.1 ± 0.1	14.5 ± 0.5
5	0.2	70	30	80	6.01 ± 0.2	159 ± 3	2.16 ± 0.02	33.9 ± 0.2	14.0 ± 0.5
6	0.2	75	30	85	8.42 ± 0.3	120 ± 2	2.13 ± 0.03	33.1 ± 0.1	14.0 ± 0.5
7	0.2	80	30	90	7.63 ± 0.2	111 ± 2	2.45 ± 0.02	32.1 ± 0.3	13.0 ± 0.5
8	0.2	50	120	60	3.71 ± 0.1	153 ± 1	2.51 ± 0.01	33.5 ± 0.1	14.5 ± 0.5
9	0.2	55	120	65	3.72 ± 0.2	133 ± 2	2.71 ± 0.01	32.9 ± 0.2	14.5 ± 0.5
10	0.2	60	120	70	3.91 ± 0.1	137 ± 3	2.39 ± 0.03	31.7 ± 0.2	14.0 ± 0.5
11	0.2	65	120	75	4.13 ± 0.2	111 ± 2	2.51 ± 0.02	31.5 ± 0.1	14.0 ± 0.5
12	0.2	70	120	80	4.37 ± 0.2	95 ± 2	2.73 ± 0.02	31.3 ± 0.3	13.5 ± 0.5
13	0.2	75	120	85	6.91 ± 0.3	83 ± 2	2.49 ± 0.03	31.3 ± 0.3	13.0 ± 0.5
14	0.2	80	120	90	6.79 ± 0.2	81 ± 1	2.39 ± 0.02	30.1 ± 0.1	12.0 ± 0.5
15	0.8	50	30	60	3.18 ± 0.1	125 ± 2	2.93 ± 0.01	31.2 ± 0.1	14.0 ± 0.5
16	0.8	55	30	65	3.72 ± 0.2	122 ± 1	2.88 ± 0.03	30.1 ± 0.2	14.0 ± 0.5
17	0.8	60	30	70	5.23 ± 0.3	105 ± 2	2.77 ± 0.02	29.9 ± 0.2	13.5 ± 0.5
18	0.8	65	30	75	6.02 ± 0.2	95 ± 1	2.39 ± 0.01	29.2 ± 0.1	13.5 ± 0.5
19	0.8	70	30	80	7.06 ± 0.2	91 ± 3	2.12 ± 0.02	26.8 ± 0.1	13.0 ± 0.5
20	0.8	75	30	85	7.91 ± 0.1	75 ± 2	2.33 ± 0.03	26.1 ± 0.1	13.0 ± 0.5
21	0.8	80	30	90	8.81 ± 0.3	77 ± 3	2.27 ± 0.02	25.7 ± 0.2	12.0 ± 0.5
22	0.8	50	120	60	3.52 ± 0.2	67 ± 2	1.75 ± 0.03	30.9 ± 0.1	13.0 ± 0.5
23	0.8	55	120	65	3.11 ± 0.1	63 ± 1	1.73 ± 0.01	29.5 ± 0.1	13.0 ± 0.5
24	0.8	60	120	70	3.53 ± 0.3	67 ± 2	1.81 ± 0.02	29.1 ± 0.3	13.0 ± 0.5
25	0.8	65	120	75	4.43 ± 0.2	57 ± 2	1.65 ± 0.03	28.7 ± 0.3	12.5 ± 0.5
26	0.8	70	120	80	7.15 ± 0.3	51 ± 1	1.61 ± 0.01	26.3 ± 0.1	12.0 ± 0.5
27	0.8	75	120	85	6.47 ± 0.2	50 ± 2	1.51 ± 0.03	25.1 ± 0.1	12.0 ± 0.5
28	0.8	80	120	90	7.69 ± 0.1	52 ± 2	1.41 ± 0.02	25.1 ± 0.2	11.5 ± 0.5

Where A-factor A (amount of enzyme), B-factor B (extraction temperature of 1st stage of extraction), C-factor C (extraction time of 1st stage of extraction), D-factor D (extraction temperature of 2nd stage of extraction), 1st Y-yield of gelatine of 1st stage of extraction (%), 1st GS-gel strength of gelatine extracted in 1st stage of extraction (Bloom), 1st V-viscosity of gelatine extracted in 1st stage of extraction (mPa.s), 1st Mp-melting point of gelatine extracted in 1st stage of extraction (°C), 1st Gp-gelling point of gelatine extracted in 1st stage of extraction (°C), 2nd Y-yield of gelatine of 2nd stage of extraction, 2nd GS-gel strength of gelatine extracted in 2nd stage of extraction, 2nd V-viscosity of gelatine extracted in 2nd stage of extraction, 2nd Mp-melting point of gelatine extracted in 2nd stage of extraction, 2nd Gp-gelling point of gelatine extracted in 2nd stage of extraction.

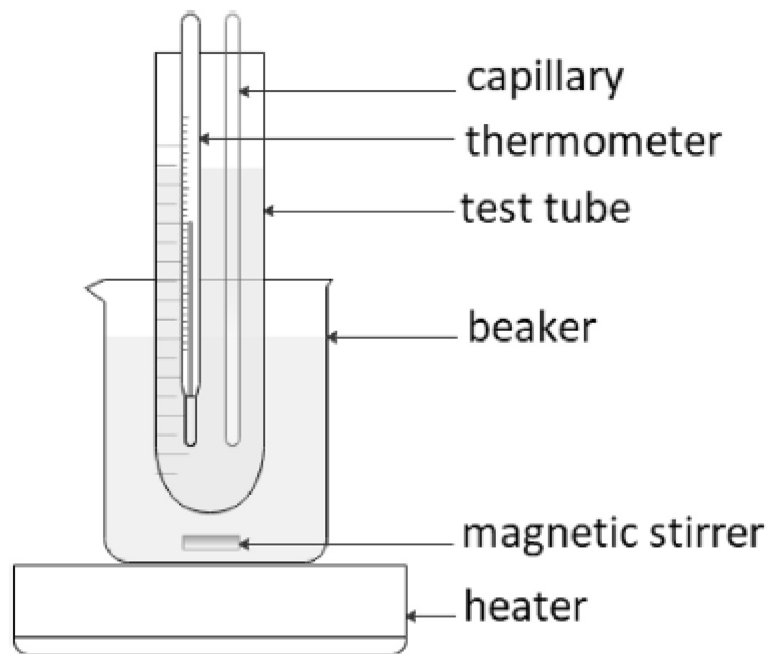

Fig. 1. Equipment for melting and gelling point determination.

1.2.6. Statistical analysis

Minitab 18 statistical software for Windows (Minitab Fujitsu Ltd., Japan) was applied to evaluate of all results using ANOVA. The level of significance was set at $p < 0.05$. Values are expressed as mean ± standard deviation.

2. Results and discussion

The results and arrangements of factorial design experiments of chicken skin gelatines (CSG) preparation are depicted in **Tables 1**a and **1**b.

Table 2 represents gelling properties of two types of beef and two types of pork commercial gelatines. As can be seen, beef gelatines have better gelling properties when compared with pork gelatines. The significant difference is noticeable between Beef Halal and Pork D526.

The statistical significance of processing factors was evaluated using the analysis of variance and p-values at the significance level of 95%. For the applied factorial design, the critical F-value is F = 5.32. That is why, the higher F-value is above the critical one, the more process factors influence the monitored variables. Similarly, factors with a p-value lower than 0.05 have a greater effect on the variables with 95% probability.

Table 2 Gelling properties of commercial beef and pork gelatines.

Type of gelatine	Gel Strength (Bloom)	Viscosity (mPa. s)	M_p (°C)	G_p (°C)
Beef D529	280 ± 3	4.72 ± 0.01	33.5 ± 0.3	19.5 ± 0.5
Beef Halal	300 ± 2	4.85 ± 0.03	34.1 ± 0.2	19.5 ± 0.5
Pork D526	200 ± 3	2.53 ± 0.02	32.1 ± 0.1	19.0 ± 0.5
Pork D012119	275 ± 1	3.10 ± 0.02	32.2 ± 0.2	19.0 ± 0.5

Where M_p-melting point, Gp-gelling point.

Table 3 shows the results of the analysis of variance for the gelatine yield, gel strength, viscosity, melting and gelling point. The influence of all processing factors was statistically significant with the only exception of factors A (the amount of enzyme) and C (the extraction time) in the case of viscosity.

2.1. Yield of chicken skin gelatine (CSG)

The yield of gelatine during the first extraction ranged from 16.8 to 23.6%. The lowest yield was recorded at min. levels of factors (the amount of enzyme of 0.2; extraction temperature of 50 °C and extraction time of 30 min); on the other hand, the highest yield was established at max. levels of factors (0.8%/80 °C/120 min). Therefore, the results were in the alignment with the assumptions as the level of collagen hydrolysis was lower in the mild conditions (thus less disruption and loosening occurred which means collagen chains were available for the extraction) and vice versa. The difference in the yield using min. and max. conditions was 6.8%. The increase of the extraction temperature from 50 to 80 °C was reflected by the rise in the gelatine yield of 1.7-5%. The growth of the enzyme amount from 0.2 to 0.8% during the pre-treatment enhanced the yield from 1.1 to 3.8% and the extension of the extraction time increased the yield ranging between 0.3 and 3.3%.

The effects of individual processing factors on the CSG yield in the first extraction is shown in **Fig. 2**. Factor B (the extraction temperature) has the most considerable effect on the yield. The dependence of the yield on temperature is nearly linear; however, the difference between the values was relatively low. The gelatine yield during the second extraction was in the range of 3.11-8.81% with the difference between the values of 5.7% which was slightly lower than within the first extraction. The sum of the yields of the first and second extractions varied from 20.8 (exp. no. 8) to 31.5% (exp. no. 21). This shows that significant CSG amounts may be obtained using the two-stage extraction.

Sompie and Triasih tested the effect of the temperature on the yield and properties of chicken leg skin gelatine with the results between 12.3 and 14.1% which is less than was reached in this study (**Sompie & Tri-asih, 2018**). The four-stage extraction was employed with the extraction temperatures of 50, 55, 60 and 65 °C for 5 h at every extraction stage. Longer extraction time resulted in a higher yield of gelatine only in the further extraction stages. Taufik et al. investigated the effects of the extraction temperature on the yield of chicken leg gelatine with the results of 15.3-16.5% which is comparable with exp. no. 1 in this study (**Taufik et al., 2010**). They applied the extraction temperatures of 45, 50 and 55 °C for 24 h. Du et al. studied the properties of chicken head gelatine with the yield of 24.8% which is in accordance with exp. no. 28 (Du et al., 2013). Gelatine was extracted at 50 °C for 18 h.

Sarbon et al. prepared chicken skin gelatine at the extraction temperature of 45 °C for 8 h with the yield of 16.1% which is equivalent to exp. no. 1 in this study (**Sarbon et al., 2013**).

Table 3 Analysis of variance of the experimental design for gelatine yield, gel strength, viscosity, melting and gelling point (study of the influence of processing factors in 1st gelatine extraction).

	DF	SS	MS	F-value	p-value
Response: Yield = 9.078 + 3.607 A - 0.1105 B - 0.01468 C; R^2 = 0.8802					
Factor A	1	32.79	32.7889	73.01	0.000
Factor B	6	34.21	34.2108	76.18	0.000
Factor C	1	12.22	12.2232	27.22	0.000
Response: Gel strength = 289.3–83.93 A - 1.275 B - 0.3786 C; R^2 = 0.9531					
Factor A	1	17751	17750.9	284.90	0.000
Factor B	6	4552	4551.7	73.02	0.000
Factor C	1	8126	8126.0	130.42	0.000
Response: Viscosity = 5.232–0.474 A - 0.02221 B - 0.00052 C; R^2 = 0.3987					
Factor A	1	0.56573	0.56573	3.82	0.065
Factor B	6	1.38173	1.38173	11.20	0.003
Factor C	1	0.015556	0.01556	0.13	0.726
Response: Melting point = 43.240–2.583 A - 0.07643 B - 0.00913 C; R^2 = 0.8982					
Factor A	1	16.817	16.8175	94.01	0.000
Factor B	6	16.356	16.3557	91.43	0.000
Factor C	1	4.723	4.7232	26.40	0.000
Response: Gelling point = 17.317 + 3.631 A - 0.10625 B - 0.01389 C; R^2 = 0.9511					
Factor A	1	33.223	33.2232	204.47	0.000
Factor B	6	31.609	31.6094	194.54	0.000
Factor C	1	10.938	10.9375	67.32	0.000

Where DF-Degree of freedom, SS-sum of squares, MS-means of squares, factor A-amount of enzyme, factor B-1st extraction temperature, factor C-1st extraction time.

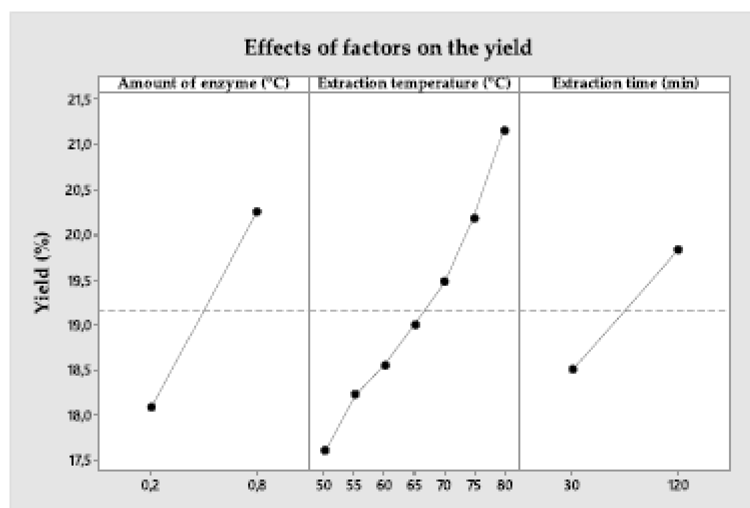

Fig. 2. The main effects of individual process factors (amount of enzyme, extraction temperature and time) on the gelatine yield (1st extraction).

None of these studies employed enzymes during the extractions which proves the importance of the enzymes effect. To obtain comparable results as in this study, much longer extraction times within the similar extraction temperatures had to be applied. Therefore, the energy consumption of the extraction process in this study was significantly lower.

2.2. CSG gel strength

Gelatine gel strength is a critical factor for the evaluation of the gelatine quality. Strength of commercial pork and beef gelatine gels ranges from 100 to 300 Bloom; the most preferred is the value between 200 and 250 Bloom. As high gel strength gelatines are classified gelatines with the values over 200 Bloom; the gel strength of bovine or pork gelatine with typical values between 200 and 240 Bloom serves as the standard. Middle gel strength gelatines include gelatines with Bloom values between 100 and 200 and gelatines with gel strength below 100 are classified as low gel strength gelatines (**Schrieber & Gareis, 2007**).

The CSG gel strength from the first extraction ranged from 190 to 75 Bloom. The highest strength was monitored in exp. no. 1 and the lowest in exp. no. 28. In accordance with the assumptions, gel strength decreases with higher values of processing factors. Therefore, the trend is contrasting to the gelatine yield behaviour. These conditions probably caused a higher level of hydrolysis initiating a decrease of collagen chains molecular weight. Shorter chains of collagen molecules seem to provide fewer junction zones reorganising native collagen structure. This presumably results in deterioration of the gel strength and other gelling properties of CSG (**Finer et al., 1975**). The increase of the enzyme amount from 0.2 to 0.8% generated the decrease of gel strength by 36-72 Bloom. The growth of the extraction temperature from 50 to 80 °C caused the reduction by 28-56 Bloom and the extension of the extraction time from 30 to 120 min stimulated a decline by 8-48 Bloom.

The effect of individual processing factors on the CSG gel strength from the first extraction is represented in **Fig. 3**. Factor A (the amount of enzyme) performed the highest effect on the yield while the impact of other factors was only slightly lower or even comparable. The gel strength is influenced more significantly by a change of processing conditions than the yield since the difference between min. and max. yield was 6.8%, while the decline of the gel strength in dependence on the strenuousness of the conditions was up to 39.5%.

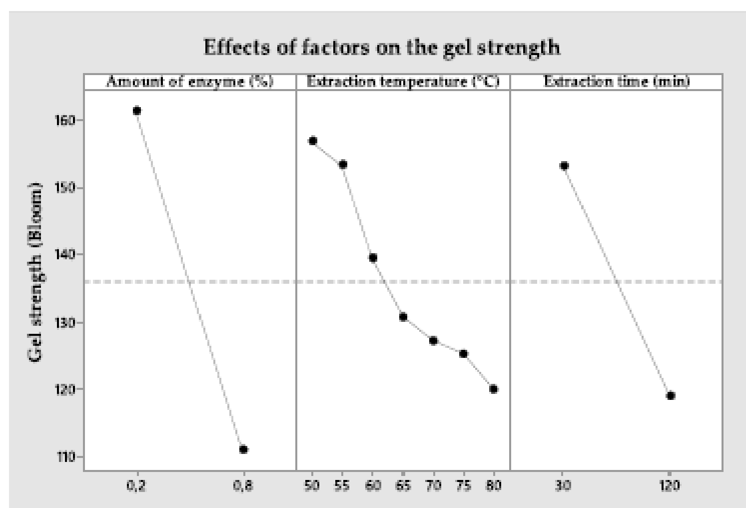

Fig. 3. The main effects of individual process factors (amount of enzyme, extraction temperature and time) on the gel strength (1st extraction).

The CSG gel strength prepared within the second extraction varied between 175 and 52 Bloom. Therefore, the second extraction comprised a reduction of the gel strength by 15-51 Bloom. It is conceivable to prepare middle gel strength CSG of a good quality with a smaller enzyme amount or shorter extraction temperature (exp. no. 1-11 and 15-17).

Two types of beef and pork commercial gelatines were tested with the values varying from 200 to 300 Bloom for Pork D526 and Beef Halal gelatine, respectively. Thus, Pork D526 gelatine showed a comparable gel strength as CSG prepared in exp. no. 1.

Poultry gelatine gel strength has been recently studied and reported ranging from 185 to 355 Bloom. Kim et al. prepared chicken skin gelatine and reported its gel strength from 218 to 270 Bloom with the extraction conditions of 75-100 °C and 60 min (**Kim et al., 2012**). A higher gel strength in comparison with this study was achieved by using a much longer extraction time. Widyasari et al. extracted gelatine from chicken feet with the gel strength of 185 Bloom which is comparable with the results of exp. no. 3 in this study with the extraction conditions of 60 °C and 30 min (**Widyasari & Rawdkuen, 2014**). However, Widyasari et al. employed more energy-intensive conditions: 70 °C and 90 min. Almeida and Lannes extracted gelatine from chicken feet with the gel strength of 295 Bloom which is higher than in this study (**Almeida & Lannes, 2013**). This higher gel strength was obtained by using the extraction temperature of 55 °C for 6 h which is much longer extraction time than applied in this study. Du et al. extracted gelatine from chicken heads with the value of 248 Bloom which is a higher gel strength as well; nevertheless, gelatine was extracted for a significantly longer period (18 h) with the extraction temperature of 55 °C (**Du et al., 2013**). Finally, Ee et al. gained chicken heads gelatine with the very high gel strength of 355 Bloom within the extraction conditions of 70 °C and 3 h. Since a longer extraction time was applied, the process required a higher energy consumption (**Ee et al., 2019**).

2.3. Viscosity of CSG

Viscosity is another significant indicator of gelatine quality important particularly in the processing, such as moulding or soaking in the production of gelatine candies or capsules.

Viscosity of CSG solution was established from 4.06 to 2.31 mPa s in the first stage of extraction. As with the gel strength, the highest viscosity was recorded in exp. no. 1 and lowest in exp. no. 28; however, the dependence of viscosity decrease on the intensity of the extraction conditions was not as distinct as within the gel strength behaviour. A similar hypothesis applies to the viscosity assumes that longer collagen chains are able to form complex entanglements with a greater resistance to flow.

The influence of individual processing factors on viscosity of CSG extracted in the first extraction is depicted in **Fig. 4.** Similar to gel strength, viscosity was reduced with higher values of processing factors which proves the connection with gel strength. The extraction temperature showed a very pronounced influence, the amount of enzyme was less significant and the extraction time performed only a very little effect on gelatine viscosity. The viscosity of CSG solution gained in the second extraction declined from 2.99 to 1.15 mPa s which implies that the use of bigger enzyme amounts in combination with long extraction times (exp. no. 22-28) provided gelatine with a very low viscosity. The reason is probably a large disruption of the collagen molecular structure and reduction in molecular weight resulting in shorter molecule chains which do not form complex entanglements that would cause substance resistance to flow (**Gresham, 2008**).

Commercial gelatines performed viscosity in the range from 4.85 (Beef Halal) to 2.53 mPa s (Pork D526) which is comparable with the values obtained in the first extraction (4.06-2.31 mPa s) and also with the results of exp. no. 1-21 in the second extraction. Beef gelatines showed higher viscosity values.

Sompie and Triasih recorded the viscosity of chicken legskin gelatine in the range of 6.52-7.02 mPa s, which is higher than monitored in this study (**Sompie & Triasih, 2018**). Nevertheless, the longer extraction time together with a different pre-treatment method applying acetic acid for 24 h may have

caused these higher viscosity values. Taufik et al. reported chicken feet gelatine viscosity of 6.5-7.7 mPa s comparable with the previous study by Sompie and Triasih (**Taufik et al., 2010**). Taufik et al. applied a longer extraction time (24 h) and different pre-treatment method (sodium hydroxide for 40 min and citric acid for 40 min) as well. Rafienian et al. extracted gelatine from chicken deboner residues and reported gelatine viscosity of 5.85 mPa s, which exceeded the values determined in this study (**Rafienian et al., 2015**). Hydrochlorid acid was used in the pre-treatment and the extraction was proceeded under these conditions: 86.8 °C for 1.95 h which is equivalent to the conditions in exp. no. 14 and 28 in this study.

2.4. Melting and gelling point of CSG

Melting and gelling points are further important quality indicators. Providing these values are too low, gelatine cannot be used as a gelling agent under room temperature (23 $\pm$ 1 °C). Melting and gelling points are affected by molecular weight of collagen, concentration, distribution of amino acids, type of the raw material and the amount of amino acids proline and hydroxyproline (**Gundem & Tarhan, 2020**).

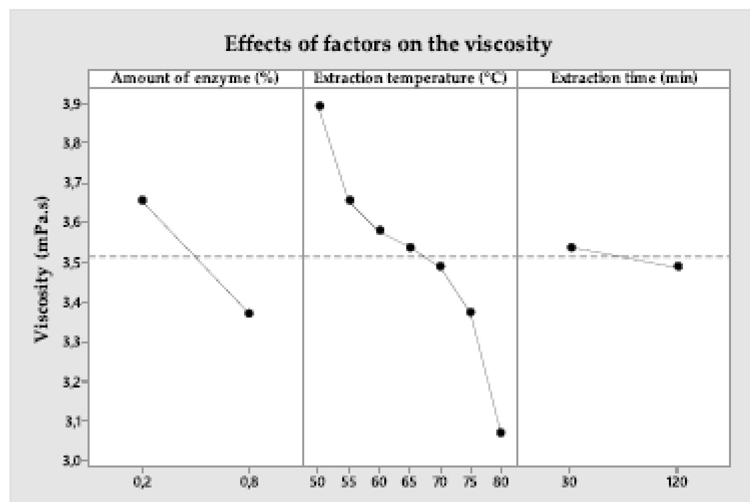

Fig. 4. The main effects of individual process factors (amount of enzyme, extraction temperature and time) on the viscosity (1st extraction).

Melting point was established from 38.3 to 34.2 °C in the first extraction. Even though the decline in a melting is minor, it reflected the intensity of the process parameters. Gelatine with a lower gel strength performed a lower melting point and vice versa which is in the alignment with the assumptions. The more ordered gel structure (a finer network of collagen fibres) formed at milder process conditions is closely related to a higher strength of the structure, thus melting at higher temperatures. A higher amount of enzyme caused a decrease of a melting point by 0.5-2.4 °C. A rise of the extraction temperature from 50 to 80 °C represented a drop from 1.8 to 3.3 °C. Finally, longer extraction times resulted in a decrease by 0.2-1.8 °C.

The influence of individual processing factors on the melting point of CSG gained in the first extraction is shown in **Fig. 5**. Regarding viscosity and gel strength, a decrease of the monitored factor was observed with rising values of processing factors. According to the presumptions, the melting point decreased with the intensity of the extraction conditions since it is another parameter associated with gelling properties of gelatine. The extraction temperature showed a significant effect, similarly as the

influence of the enzyme amount, and the impact of the extraction time on the gelatine melting point was slightly milder. However, the difference between the min. and max. value is relatively small (38.3 vs 34.2 °C). That is why, the melting point is affected less significantly by the extraction conditions if compared with the gel strength or viscosity.

The melting point of CSG obtained in the second extraction varied between 34.9 and 25.1 °C with a decrease of 2.2-9.4 °C in comparison with the first extraction. Additionally, the decrease was pronounced more with higher values of processing factors. This may stem from the thermal history of the sample from the first extraction. However, increasing the temperature in the second extraction by 10 ° C is probably more significant because chicken skin collagen is very young (35 days only) and thus, very sensitive to thermal stress. Furthermore, the degree of crosslinking is very low compared to, for example, cowhide (**Elhar-faoui et al., 2007**). Therefore, the second extraction had a more significant impact on the gelatine melting point than the first one.

The melting point of commercial beef and pork gelatines was determined in the range of 34.1-32.1 °C which is comparable with the values of CSG extracted in the second extraction in exp. no. 5-9. All CSGs extracted in the first extraction performed a higher melting point than commercial gelatines which means that CSG is appropriate for the food applications, such as gummy bears, aspics, jellies and other products stored at room temperature.

Melting point was examined in the paper by Bichukale et al. who prepared chicken skin gelatine with a melting point reaching from 33.0 to 31.9 ° C (**Bichukale et al., 2018**). That is comparable with CSG extracted in the second extraction in exp. no. 6-12 in this study. All CSG extracted in the first extraction showed a higher melting point.

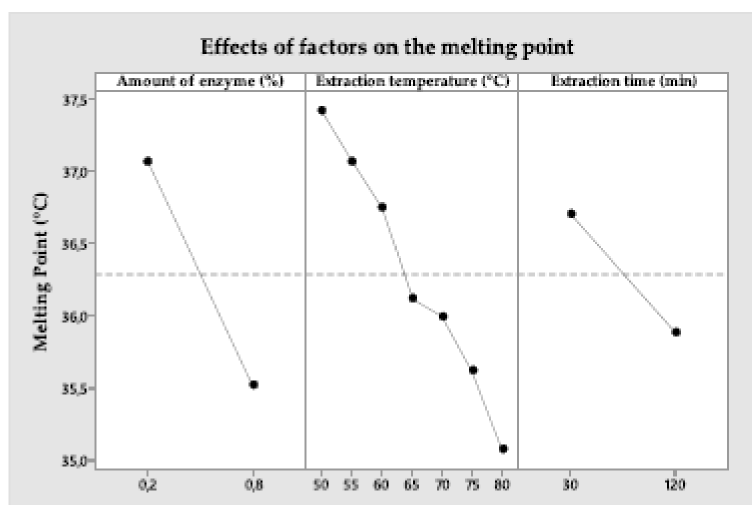

Fig. 5. The main effects of the individual process factors on the melting point of gelatine (1st extraction).

Bichukale et al. used alkali and acid pre-treatment and extracted at 40, 45, 50, 55 and 60 °C overnight. The significantly longer extraction time probably caused a lower CSG melting point. Xin et al. examined gelling properties and gelling points of tilapia skin gelatine (31 °C) and CSG (38 °C) which is greatly comparable to the best results of this study (**Xin et al., 2021**). Gelling point of fish gelatine is comparable with some CSG extracted in the second extraction in this study. Xin used a combined alkali-acid pre-treatment and extracted gelatine at 55 °C for 3 h, much longer time again. Rahman et al. reported a melting point of chicken feet gelatine of 26.7 °C which is a significantly lower value when compared to the results of the gelatines extracted in the second extraction in the more intensive

processing conditions in this study (**Rahman & Jamalu-lail, 2012**). Rahman et al. used alkali pre-treatment and extracted gelatine at 60 ° C for 5 h, thus, such a longer extraction time negatively affected the melting point. Choe and Kim prepared chicken feet gelatine using acid pre-treatment at different extraction temperatures (65, 75, 85 and 95 °C) for 120 min obtaining the melting points of 38.5, 37.8, 36.5 and 36.4 °(**Choe & Kim, 2018**). The extraction times and temperatures were comparable with those in some experiments of this study. Therefore, the acid pre-treatment in the Choe and Kim's study seems to have a similar impact on the melting point as the enzyme pre-treatment in this study.

Gelling point (also known as setting point) in the first extraction was established from 20.5 to 13.5 °C. The decrease in values was pronounced more in comparison with the melting point. Similar hypothesis as for the melting point applies to the gelling point. Within mild extraction conditions, gelatine seemed to be formed with a more organized fibre network and showed a better ability to form a gel in the aqueous solution at a higher ambient temperature than gelatine prepared in more intensive extraction conditions. A higher amount of enzyme resulted in a temperature drop of 1.5-3.0 °C. An increase in the extraction temperature from 50 to 80 ° C represented a reduction in the range from 2.5 to 4.0 °C and a growth in the extraction time caused a decrease of 0.5-2.0 °C.

The influence of individual processing factors on the gelling point of CSG extracted in the first extraction is shown in Fig. 6. The gelling point as well as the melting point declined with higher values of processing factors. The extraction temperature and the amount of enzyme performed a significant effect on the CSG gelling point while the extraction time showed less considerable impacts. Therefore, the influence of these processing factors is very similar to the behaviour of the melting point.

Gelling point in the second extraction varied from 15.0 to 11.5 °C. It is clear that the second extraction performed less significant effect on the CSG gelling point than the first extraction. The decrease between the first and second extraction ranged between 2.0 and 5.5 °C.

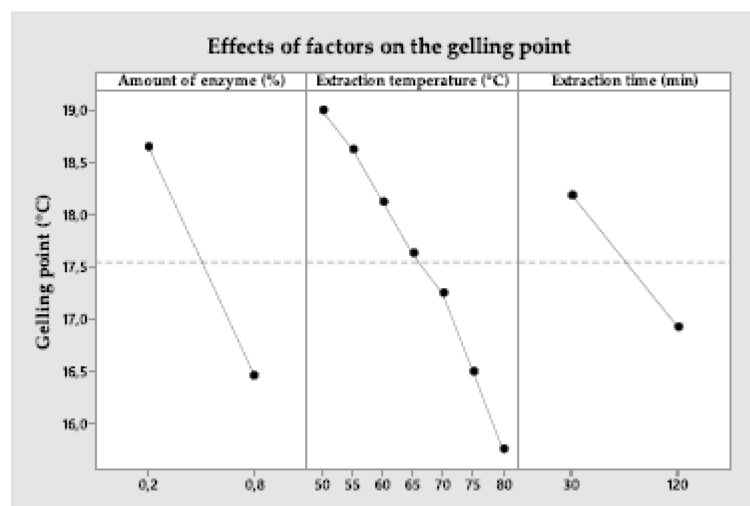

Fig. 6. The main effects of the individual process factors on the gelling point of gelatine (1st extraction).

Gelling point of commercial gelatines was 15.0 °C for pork and 15.5 °C for beef gelatines. These values are comparable to prepared CSGs concerning particularly those prepared using lower enzyme amounts, extraction temperatures and shorter extraction times (exp. no. 1-11, 15-16).

CSG gelling point was examined by Sarbon et al. who obtained the value of 25 °C (**Sarbon et al., 2013**). They applied alkali-acid pre-treatment and extracted at 45 °C overnight. Such a lower extraction temperature appeared to have a positive effect on CSG gelling point. A recent study conducted by Xin et al. investigated CSG and tilapia skin gelatine with gelling points of 22 and 14.5 °C which is in alignment with the results of this study (**Xin et al., 2021**). The gelling point of fish gelatine is considerably lower and comparable to the CSG extracted in the second extraction in this study which confirms generally poorer gelling properties of fish gelatines. Kuan et al. determined the duck feet gelatine gelling point of 20.5 ° C which is exactly the same value as in exp. no. 1 of this study (**Kuan, Nafchi, Huda, Ariffin, & Karim, 2017**). However, a different method of gelatine preparation was employed: acid pre-treatment and 12 h-extraction at 55 °C. Thus, the notably longer extraction time seemed to have no impact on the results of their study.

The lowest CSG total yield (the sum of the first and second extraction yields) of 20.8% was achieved under these processing conditions: the amount of enzyme of 0.2%, extraction temperature of 50 °C and extraction time of 120 min for the first extraction and 0.2%/60 °C/60 min for the second extraction. The highest total yield of 31.5% was obtained within the conditions of 0.8%/80 ° C/30 min and 0.8%/90 °C/ 60 min. Monitored gelling properties were the highest at the min. levels of factors and the lowest at the max. levels. The hypothesis that gelling properties decrease with higher values of factors has been verified. Factor A had the highest effect on the gel strength, whereas factor B showed the most significant influence on the yield and factor C had the highest effect on the gel strength. The hypothesis assuming that the optimal processing conditions provide CSG gelling properties comparable to gelling properties of commercial gelatines has not been proven for the gel strength (190 vs 200-300 Bloom). It has been confirmed for the viscosity (4.06 vs 2.53-4.72 mPa s), melting (38.3 vs 32.1-34.1 °C) and gelling point (20.5 vs 19.0-19.5 °C). The hypothesis that comparable yield of CSG is achieved in similar experimental conditions has been accomplished. The highest yield of CSG in this study was 23.6%. Sompie and Triasih established the yield of chicken legskin gelatine of 14.1% (Sompie & Triasih, 2018), Sarbon reported the CSG yield of 16.1% (**Sarbon et al., 2013**), Taufik published the chicken leg gelatine yield of 16.5% (**Taufik et al., 2010**) and Du recorded the chicken head gelatine yield of 24.8% (Du et al., 2013). Comparable results have been obtained under less energy intensive conditions.

3. Conclusions

Chicken skin gelatines (CSG) were prepared under different processing conditions using two-stage extraction with the food-grade enzyme pre-treatment. Combined factorial design was used for the draft of experiments and assessment of processing factors influence. The effect of four factors on the CSG quality was monitored: the amount of enzyme, first extraction temperature, time and second extraction temperature. The CSG quality was represented by four indicators: gel strength, viscosity, melting and gelling point. In addition to non-toxicity of the process, the enzyme pre-treatment reduces the extraction time and water consumption. Two-stage extraction ensures a more efficient utilisation of the raw material. Therefore, biotechnological preparation of CSG has been proved as a very promising method alternative to the traditional pre-treatment (acid or alkali) and raw materials (beef and pork skins) used in the commercial production of gelatines. Prepared CSG have shown the potential to be utilized in many applications, especially in the food industry, such as gums, liquorice, meringues or toffees, as well as in pharmacy (soft gelatine capsules, tablets) or cosmetics (creams, ointments).

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