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Proposal of processing chicken by-products tissues into food-grade collagen

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Abstract

The consumption of poultry has been on rise and shows no signs of diminishing; hence greater importance is placed on subsequently treating animal by-products, which are divided into 3 categories. Such materials are produced in large quantities, and with respect to its biological nature, needs to be handled in a way that prevents polluting the environment. Finding an effective use for this by-products, for example, as a raw material which could be processed into other products, would constitute an ideal way to address the issue. Indeed, it contains large amounts of protein, especially collagen, as widely applied in the food sector and other industries. By-products that boast a high proportion of collagen include the skin of poultry, which can be obtained by a procedure involving controlled isolation of any undesirable components, i.e. fats, soluble non-collagenous proteins and pigments. Hence, what was once considered waste is turned into a valuable raw material rich in collagen, and further transformation of the latter translates into soluble collagen and collagen hydrolysate. As an example, chicken skin, which is high in fats (84%), can be effectively defatted by shaking of grinded raw material in a mixture of solvents for a certain period of time, the result being collagen with the residual fats content of approximately 14% using mixture of solvents petroleum ether and ethanol. The use of acetone brought a similar effect (18%). However, the use of $NaHCO_3$ solution did not lead to the acceptable result (81%). The shaking of the raw material in water solution with lipolytic enzymes was also tested. Three types of enzymes in different concentrations were used for this purpose, but the residual fats content was also much higher compared to chemical solvents (48 – 69%). Possibilities for further processing of the raw material into products with potential applications in industry were also proposed.

Keywords: *category III material; by-products; chicken; collagen; hydrolysate; lipolytic enzyme; processing; skin*

Introduction

It is estimated that 20 to 100 million tons of food waste is produced globally every year. In the EU, the production of fish results in 5.2 million tons of waste, while the figure for the meat-processing industry stands at 16.5 million tons[1]. These materials are defined as a by-products of animal origin and are divided into 3 categories according to Regulation (EC) No. 1069/2009 of the European Parliament and of the Council. Category 1 materials are, for example, animal bodies or their parts suspected or confirmed of TSE (Transmissible Spongiform Encephatolaphaties) infection, bodies of non-farmed and wild animals, experimental animals and others suspected of being infected with a disease communicable to

humans or animals containing specified risk material. Category 2 materials includes, for example, manure, unmineralised guano and digestive tract content; animal by-products collected during waste water treatment, animal by-products containing residues of permissible substances or contaminants in excess of permitted levels, products of animal origin which have been declared unfit for human consumption due to the presence of impurities in these products. Category 3 materials are, for example, bodies and parts of slaughtered animals which are suitable for human consumption but are not intended for human consumption for commercial reasons, bodies of animals slaughtered in a slaughterhouse and found suitable for human consumption, poultry heads, skins, horns and limbs and feathers, or poultry by-products slaughtered on a farm which did not show signs of a disease portable to humans[2]. This study deals with category 3 materials.

By-products from the meat-processing sector include blood, bones, meat scraps, skin, fat, horns, hooves, limbs and guts. The cost of disposal can be compensated by raising the price of end products, thus increasing their profitability. Nevertheless, a large quantity of these by-products contain important nutrients such as proteins, fats, minerals and vitamins, which potentially could increase the revenues of meat-processing businesses[3].

More than seven million tons of by-products bones per year are generated through slaughtering mammals. For centuries, these surplus bones have been used to prepare soup and stock. Moreover, efforts currently exist to develop techniques to raise the percentage of meat obtained during processing by slaughterhouses. In many countries, meat that is mechanically separated is added into meat products, but the quantity of this tends to be capped[4]. As mentioned above, blood represents an important by-product of the meat industry. In healthy animals, blood is a sterile material and comprises 2.4 to 8.0% of the live weight of an animal. Blood is used in foodstuffs, for instance, as an emulsifier, stabilizer, coloring agent or nutritional ingredient. The greatest proportion of such blood is destined for producing blood-based foodstuffs in the animal feed industry. Other applications include medical and pharmaceutical products. One of the most sought-after by-products is the skin of mammals, which comprises 4 to 11% of the live weight of the animal. Collagen sourced from bovine and porcine skin is used in the primary packaging of food products, in particular the casings or skins of meat-based items (sausages, etc.), as well as an ingredient in cosmetics. Moreover, the skin of the pig is similar in composition to that of humans; thus it has been utilized medically to cover burns or ulcers or in skin transplantation surgery. Additionally, the guts of animals are high in nutritional value and are deemed fit for human consumption, particularly in Southeast Asian cuisine. In China, Japan and India, guts form an ingredient in traditional medicine. Animal fat is also considered an important by-product, traditionally in the preparation of meals, but it is applied in margarines and hydrogenated vegetable fats as well. Lard is added to goods such as sausages, processed meat-based items and emulsified products[5,6].

The poultry industry is one of the fastest growing agri-food industries in the world, with an annual production of over 100 million tonnes of chicken meat worldwide. (FAO, 2019).[7] The meat of poultry represents 5.5% of total agricultural production, and 12.7% of the output of the meat-processing industry in the EU. In 2014, the EU produced 13 million tons of meat from poultry, 9% more than in 2007. The largest producers are Poland (13.9%) and France (12.9%), closely followed by the U.K. (12.6%) and Germany (11.8%). Of this, chicken (79.8%) and turkey (14.8%) make up the largest proportion of such meat; while duck only stands at 3.6% of total production of poultry meat. Waste from the manufacture of meat, poultry, cattle and porcine products causes excessive pollution if the process is carried out through inadequate methods. Otherwise, serious environmental complications could occur that lead to degradation of the soil, surface water and groundwater. A major problem also arises in animals through the presence of pathogenic micro-organisms. Repurposing such waste is the only option for reducing the environmental impact of inappropriate disposal[8]. By-products of the poultry industry account for approximately 22 to 30% of total production of poultry, and include edible tissues, blood, guts and other materials. Animal by-products from farming are bedding litter and manure, which can be recycled into feed or fertilizers, as well as those from hatcheries, particularly egg shells, unhatched eggs and dead/discarded chickens, which can be added to feedstuffs in the form of meal to the extent of 3 to 5%[5].

The literature mentions the possibilities of using the feet of poultry, skin and heads as raw materials for preparing soluble collagen. For example, Almeida et al. researched the effect of treating chicken feet with 4% acetic acid for 16 h[9]. Kim et al. reported the process of preparation (0.1 mol/L HCl for 48 h at

room temperature) and characterization of duck feet gelatin[10]. Yeo et al. conducted similar experiments; used 0.1 mol/L HCl as well, although the duration of processing equaled 5 days[11]. Rafieian et al. utilized 1% NaCl for treating remnants from mechanically processed chicken for 30 minutes, in addition to 3 – 7% HCl for 24 h at room temperature[12]. In case of chicken skin, Sarbon et al. investigated 0.15% NaOH for 120 minutes, and 0.15% H_2SO_4 or 0.7% citric acid for 120 minutes[13]. Du et al. looked into treating poultry heads with 0.015 mol/L $NaHCO_3$ solution for 3 hours, 0.1 mol/L NaOH for 6 hours and 0.05 mol/L acetic acid for 18 h at 4 °C[14].

The university department of the authors has previously been active in finding applications for poultry paws processed by means of microbial proteases under moderate reaction conditions (i.e. neutral pH, temperature not exceeding 75 °C, and atmospheric pressure); experiments resulting in preparation of collagen hydrolysate[15]. Poultry feathers are a significant source of keratin, a chemically and mechanically highly durable protein due to the presence of disulphide bridges. Hydrolytic cleavage of disulphide (–S–S–) and peptide (–CO–NH–) bonds in keratin facilitates soluble products of varying molecular weight[16,17,18]. Processing poultry feathers into keratin hydrolysates, under alkaline conditions with proteolytic enzymes, is also something the authors have engaged in previously[19,20]. In the food industry, hydrolysed keratins have been employed variously, e.g. in nutritional supplements, as a means to adjust the viscosity of dairy products, in energy drinks, and as a stabilizing agent for emulsions. Furthermore, the cosmetic sector has witnessed major adoption of keratin hydrolysates. Adding them into cosmetics emulsions for the skin improves barrier and moisturizing properties, while in hair cosmetics (shampoos or conditioners) keratin hydrolysates enhance the structure and characteristics of the hair[21,22].

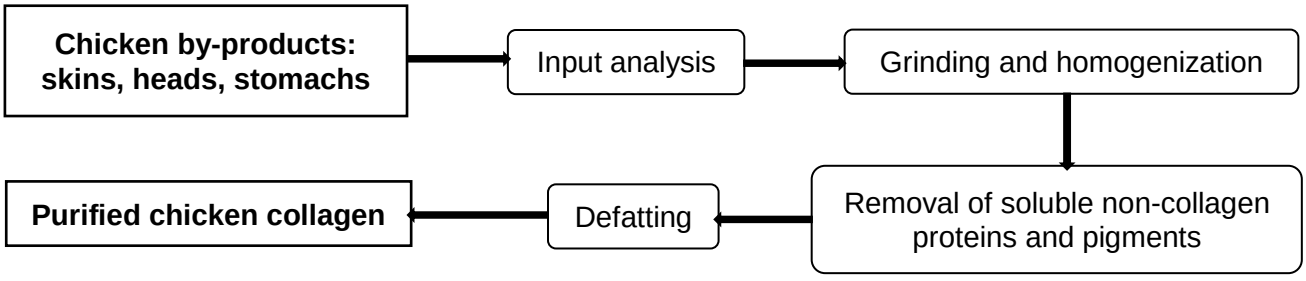
Interestingly, little information is available on turning of some poultry by-products into a raw material for generating collagen; probably due to the complexities involved in cleaning raw materials, e.g. undigested remnants of food, digestive enzymes and the extremely sensitive nature of the tissues. Consequently, the authors decided to dedicate research to this type of by-products.

The aims of the paper

The objectives of this paper are as follows, to: 1*)* select appropriate by-products high in collagen from a slaughter house for chickens; 2*)* analyze the composition of selected tissues (especially with regard to protein content and the proportion of collagen); 3*)* propose a suitable method for the preparation of tissues for further processing; in particular, by focusing on temperature and the grinding technique during homogenization; 4*)* process tissues high in collagen and devise a simple procedure to facilitate controlled removal of undesirable constituents in studied tissues (fats, soluble non-collagenous proteins and pigments) with a view to generating collagen; and to focus on the effective removal of fats, which are an important component of biological tissues; 5*)* assess the possibilities of processing collagen into a soluble collagen or other products.

Materials and methods

A general process layout of the procedure for processing chicken by-products from slaughter into purified collagen is shown in Scheme 1.



Scheme 1: The flow chart of preparation of purified chicken-sourced collagen

Appliances, tools and chemicals

The equipment utilized in the laboratory comprised the following: SPAR Mixer SP-100AD-B industrial meat cutting machine with four-arm knife (TH Industry RD, Taiwan), Memmert ULP 400 dryer (Memmert GmbH + Co. KG, Germany), LT 3 shaker (Nedform, Czech Republic), desiccator, , metal filtering screen (mesh size 1 mm), PA cloth (mesh size 200 μm), muffle furnace (Nabertherm GmbH, Germany), and Parnas-Wagner distillation apparatus (Fisher Scientific, Czech Republic). The list of chemicals was as follows: NaCl, NaOH, $NaHCO_3$, petroleum ether, ethanol, chloroform (Verkon, Czech Republic)**;** all chemicals were of analytical grade.

Additionally, the authors utilized enzymes delivered by Novozymes, Denmark. Lipozyme TL - an enzyme produced through immobilizing microbial lipase from *Thermomyces lanuginous* onto a granulated silica carrier; the lipase is produced through submersion fermentation of *Aspergillus oryzae*, a genetically modified organism with the declared activity of 100 KLU/g, the optimal operating values of which stand at 70 °C and pH 6.0 – 9.0. Lipex 100 L - a lipase produced through submersion fermentation of a genetically modified strain of *Aspergillus* with the declared activity of 100 KLU/g, the optimal operating values of which stand at 30 °C and pH 7.0. Lipolase 100 T - a lipase with *Thermomyces lanuginosus* produced through submersion fermentation of a genetically modified micro-organism *Aspergillus oryzae* with the declared activity of 100 KLU/g, the optimal operating values stand at 30 °C and pH 11.0. Polarzyme 6.0 T - a protease produced through fermentation of a micro-organism not present in the final product, its declared activity equals 100 KPPU/g, and the optimal operating values stand at 10 – 60 °C and pH 7.0 – 11.0.

Raw Materials and Input Analysis

Skins, heads and stomachs were chosen as prospective by-products from the slaughter of chickens since they were deemed suitable for generating collagen; they were supplied by Raciola Uherský Brod, Ltd. (Czech Republic). Analysis was subsequently carried out to determine the composition of the raw materials. Further to this, the content of dry matter, protein, collagen, fats and minerals was determined. Each analysis was repeated three times and mean values were calculated.

Determining the content of dry matter involved applying an indirect method to determination of moisture (AOAC, 2000)[23], wherein the sample was dried at 103.0 ±2.0 °C until constant weight was reached. The amount of protein present was arrived at by the Kjeldahl method, through mineralization in concentrated H_2SO_4 for around 1.5 h, followed by extraction of ammonia released from boric acid solution via distillation apparatus. Subsequently, titration was carried out with a solution of hydrochloric acid, and the proportion of protein content in the total amount of nitrogen was calculated[24].

The principle process to determine the extent of collagen is as follows: a portion of the tissue was mixed with 0.1 mol/L NaOH, at the ratio 1:30 (w/v), and the mixture was heated for 45 minutes at 95.0 ±2.0 °C. Then filtration and drying of any solid matter occurred at 103.0 ±2.0 °C until constant weight was reached. The figure for loss of collagen was identified by a calculation; since collagen is soluble in NaOH, the observed loss of material represents the proportion of collagen in the raw material[25,26]. The fats content was discerned by weighing out 10.0 g of the sample, putting it into a thimble and placing into extraction apparatus, in accordance with Soxhlet. The extraction process took place in two cycles: in the first, fats were extracted with chloroform, whereas ethanol was applied in the other; the combined duration of both lasted 6–8 h. The amount of residual extracted fats were identified by gravimetry. The content of mineral substances was determined as follows: a portion of the dried sample (1.0 g) was placed in a platinum ashing crucible and incinerated above a pilot flame (about 30 minutes); this was followed by annealing the sample at 650.0 ±5.0 °C in a muffle furnace for 2 hours. The quantity of mineral substances present was identified using gravimetry[27]. The results from analyses of studied tissues are shown in Table 1 (see below).

Isolation of Chicken Collagen

Process of isolation of collagen from the slaughter-house chicken by-products comprises three main technological steps:

1. Grinding and homogenization of the raw material. After sampling, the raw material was rinsed with water in the transport pipeline system, where it was also cooled off. Any excess water was left to drain away before the raw material was placed in collection containers. These containers were located in a cooler plant to prevent microbial contamination of the raw material; they were stored at 0 – 5.0 °C for a maximum of 36 hours. Prior to grinding and homogenizing the raw material, it was chilled or frozen to a low temperature, i.e. –6.0 to –4.0 °C, which varied in accordance with the given material. Grinding was carried out swiftly to prevent the temperature exceeding 12.0 °C. An industrial meat cutter was utilized for this purpose, which had been fitted with a four-arm knife unit; various holes of different shape and size on the cutting plate were tested, depending upon the type of raw material, along with assessment of the optimum number of grinding cycles. This technique brought about a rise in temperature to a maximum of 3.0 °C. Subsequently, the raw material was put into a Polyethylene packaging material with a wall thickness of 100 μm, and then frozen to –36.0 ±2.0 °C and stored in the freezer at –20.0 ±2.0 °C. Prior to the steps described below, the raw material was placed in a cooler box and defrosted at 10.0 ±2.0 °C for 12 hours.

2. Removal of soluble non-collagenous proteins and unwanted pigments. Extraction took place in two phases by applying two different solutions. The crude tissue was mixed with 1 mol/L solution of NaCl in an Erlenmeyer flask, at the ratio 1:10 (w/v); then the flask was placed on a shaker in an incubator at 5.0 ±2.0 °C. Shaking lasted 6 hours in total; after the initial period of 3 hours the raw material was filtered on a filtering screen fitted with one layer of Polyamid cloth and rinsed with water. Afterwards, the raw material was again treated for the remaining three hours with 1 mol/L solution of NaCl. After further filtration, the raw material was mixed with 0.5% NaOH solution, at the ratio 1:10 (w/v), the sample undergoing such treatment for a period of 18 hours; the temperature was the same as in the preceding period of treatment, i.e. 5.0 ±2.0 °C. Finally, the raw material was filtered through the screen with a layer of PA cloth.

3. Defatting. This was required due to the high amount of fats (84 %) in the raw material. Four possible methods were tested for the purpose, utilizing: a*)* diluted $NaHCO_3$; *b)* lipolytic enzymes; c*)* solvents; and *d)* combination of enzyme + solvent and $NaHCO_3$ + enzyme.

a) During defatting with $NaHCO_3$, steps were taken according to the slightly modified method devised by Du et al., who had tested this procedure using chicken heads[14]. Immediately after removing unwanted proteins and pigments, the raw material was mixed with 0.1 mol/L $NaHCO_3$ in an Erlenmeyer flask, at the ratio 1:4, placed on a shaker and treated for one hour at 5.0 ±2.0 °C. This was followed by filtration through a layer of PA cloth. This sequence was repeated in triplicate. Finally, the raw material was dried in a drying oven with forced convection at 35.0 ±2.0 °C.

b) As part of the enzyme defatting technique, three types of lipolytic enzymes were investigated. The process proceeded thus: the raw materials were first mixed with distilled water in an Erlenmayer flask, the ratio being 1:10 (w/v), and an enzyme was added, at the amounts (related to the weight of the raw material, w/w) of 2% for Lipex 100 L, 4% (w/w) for Lipozyme TL and 5% (w/w) for Lipolase 100 T. The level of pH was adjusted to 7.0 ±0.3 (Lipex 100 L and Lipozyme TL) or 11.0 ±0.3 (Lipolase 100 T) in order to achieve optimum enzymatic activity. The Erlenmayer flask was fixed on a shaker and shaken at room temperature. Treatment with the enzyme lasted 72 hours; twice per day the mixture was filtered through a PA cloth and the mixture was supplemented with a fresh dose of the enzyme. The level of pH was monitored and adjustments to adhere to the specified level were made when necessary. Finally, the raw material was dried in a drying oven with forced convection at 35.0 ±2.0 °C.

c) For the solvent method, the poultry skins were initially dried at 35.0 ±2.0 °C for approximately one day in a drying oven with air circulation. The Petroleum ether and Ethanol solvent system was selected by the authors since its effectiveness had been proved previously in the processing of chicken paws[15]. The mixture of solvents was prepared so that the volume ratio stood at 1:1. The raw material was combined with the solvent in an Erlenmeyer flask, the ratio of this equaling 1:10 (w/v), and shaken on

a shaker at 23.0 ±2.0 °C in three cycles (to allow for testing in triplicate) for 72 hours in total. After 24 and 48 hours of such shaking, the solvents that had been applied were filtered out and a fresh mixture of solvents were used. The defatted material was left in a fume hood for any remaining solvent to evaporate (about 30 minutes in duration).

d) Regarding the combined method, two systems were tested: As part of combination of enzyme + solvent, the chicken skins were treated in an Erlenmeyer flask with 2% (w/w) Lipex 100 L in distilled water, at the ratio 1:10 (w/v); the mixture was shaken for 72 hours at room temperature; twice per day the mixture was filtered through a PA cloth and a new dose of mixture was prepared with a fresh enzyme. The level of pH was regulated and adjusted to 7.0 ±0.3 when necessary. After filtration, the skins were mixed with acetone in an Erlenmeyer flask, at the ratio 1:10 (w/v), and shaken for 32 hours in three cycles at room temperature. After 8 and 24 hours, the solvent was filtered out and a fresh dose of solvent added. Afterwards, the solvent was filtered out using a filter screen, and the defatted material was left in a fume hood for any remaining solvent to evaporate (about 30 minutes in duration). As part of combination of $NaHCO_3$ + enzyme, the poultry skins were first defatted in an Erlenmeyer flask (in triplicate for 1 hour each) by applying 0.1 mol/L solution of $NaHCO_3$, at the ratio 1:4 (w/v) at room temperature. After filtration, the skins were mixed with distilled water, Lipolase 100 T was added at the amount of 1% (w/v), and the mixture was shaken for 72 hours at room temperature; twice per day, the mixture was filtered through a PA cloth and a new dose of mixture was prepared with the fresh enzyme. The level of pH was regulated and adjusted to 11.0 ±0.3 when necessary. Finally, the raw material was dried in a drying oven with forced convection at 35.0 ±2.0 °C.

For the defatted raw material, the content of remaining fats were determined via the Soxhlet method of two extraction steps. Firstly, the fats were extracted for eight hours from the raw material by chloroform; the same extraction time was then applied for ethanol extraction after the chloroform had been distilled out. The residual content of fats were calculated by gravimetry. The determination was carried out three times; the results are presented as an arithmetic mean.

Results and discussion

Input Analysis

Table 1 shows the composition of the chicken skins, heads and stomachs.

Table 1: Composition of the chicken skins, heads and stomachs

Chicken:	**Dry matter** [%]	**Proteins** [%] *	**Collagen** [%] *#	**Fat** [%] *	**Minerals** [%] *
Skins	53.6 ±1.51	15.5 ±1.32	92.6 ±0.11	84.0 ±2.41	0.91 ±0.31
Heads	23.0 ±0.10	50.3 ±0.51	88.8 ±1.01	32.9 ±0.44	16.8 ±0.13
Stomachs	19.1 ±0.50	74.6 ±0.84	98.5 ±0.14	21.7 ±0.10	3.92 ±0.10

**based on the dry weight of the raw material, #from total protein content, n=3*

Upon analyses of the composition discerned of the sampled tissues, the authors decided to use skin to isolate collagen, as it contains a high proportion of collagen (≈ 93% of total protein) and a low content of mineral substances (< 1%), see Table 1; the limited extent of inter-molecular netting of collagen can also be assumed to simplify any further processing of collagen.

Grinding and Homogenization

A two-step procedure was devised for grinding and homogenizing raw materials. When processing the skins, the raw material is to be frozen to –4.0 to –2.0 °C; for the initial grinding phase, a cutting plate with kidney-shaped elements is recommended, while a circular plate with elements 3 mm in diameter should be used in the second phase. When processing the heads, the raw material is frozen to –6.0 to

–4.0 °C; for the first stage of grinding, the kidney-shaped cutting plate is applicable, whereas the circular plate with elements of 3-mm diameter is most suitable for the second stage. For processing stomachs, it is sufficient to freeze the material to –2.0 to 0°C, since grinding them does not generate warmth as it does for the heads. Again, the initial grind should be carried out with the kidney-shaped cutting plate, while a circular plate with elements of 5-mm diameter is optimum in the second stage. Alternatively, a single grinding cycle is possible for processing the stomachs, which requires the circular cutting plate with elements of 5-mm diameter; the temperature of the stomachs has to range between –6.0 to –4.0 °C.

Deffating

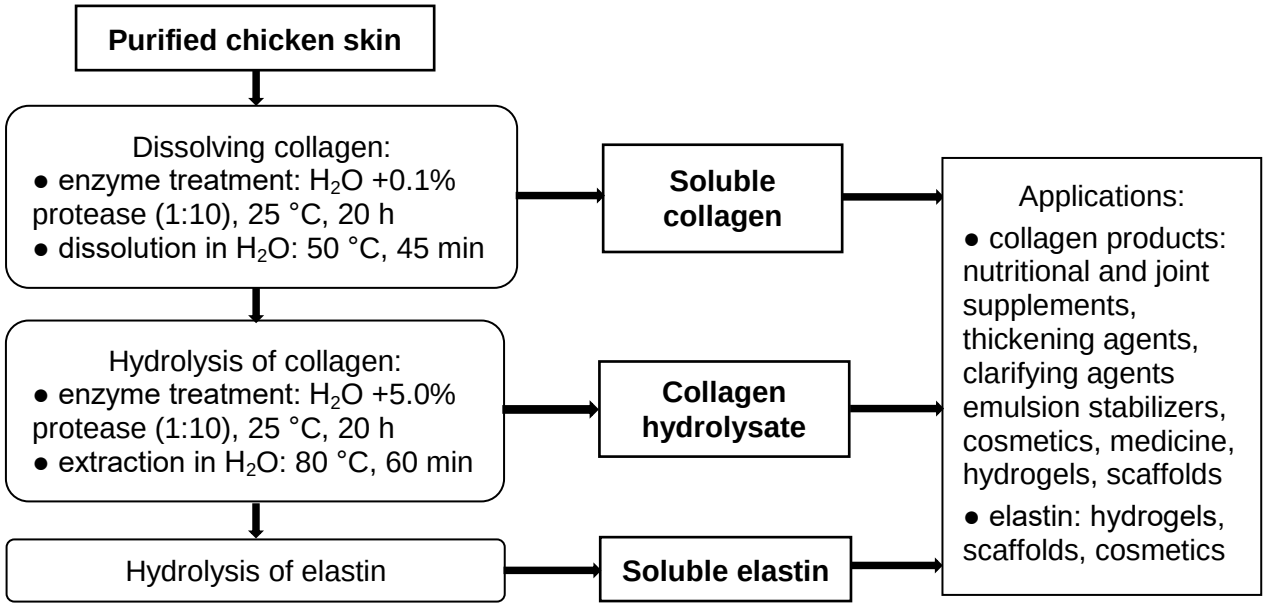
Results from the defatting of skins are detailed in Table 2. The first method investigated herein involved defatting with 0.1 mol/L solution of $NaHCO_3$. Findings showed that this method was not suitable for this form of tissue (i.e. skin) since the residual content of fats exceeded 80.0 %. This method was used by Du et al. (2013) as well for defatting of turkey and chicken heads, but the result of defatting was not reported[14].The second defatting method required three types of lipolytic enzymes. Therein, the effect of defatting was extremely low as this method reduced the fats content from the initial figure of approx. 84.0 % to levels of 68.7 % (Lipozyme TL), 62.0 % (Lipex 100 L) and 48.3 % (Lipolase 100 T). The enzyme Lipolase 100 T showed the highest activity of the tested enzymes, probably due to the highest enzyme concentration used. Lipex 100L was found more effective than Lipozyme TL, despite the enzyme concentration was half. If the residual content of fat in the raw material is low, then this method would be advantageous in reality. Indeed, compared with methods necessitating chemicals, it would represent an option for carrying out the process free of solvents. Defatting by solvents was another method under test. In this case, defatting with a mix of petroleum ether and ethanol was investigated. In fact, this method demonstrated the greatest defatting effect. The content of residual fats equaled 14.5%, which is an acceptable value for the further processing of collagen. The systems of combined methods were researched last. Of these, the initial one pertained to a combination of enzyme (Lipex 100 L) and solvent (acetone), for which residual fats stood at 18.1%, while the combination of $NaHCO_3$ solution and an enzyme (Lipolase 100 T) exhibited the figure of 70.3% for residual fats. Nadalian et al. (2013) performed defatting of chicken skin by shaking the tissue in acetone three times for 1 h, but did not report the result of defatting efficiency[28]. Acetone defatting has already been tested in the previous study of the authors (Mrázek et al. 2018)[29] for defatting chicken paws with a result of 7.74% residual fat; a combination of petroleum ether and ethanol with a result of defatting 4.97% was tested as well. Low values of residual fat in chicken paws compared to chicken skins are probably due to much lower fat content in chicken paws (34.8%)[29].

Table 2: The residual fats content in chicken skins for the various defatting methods investigated

Defatting method	Residual fats (% ±SD)
0.1 mol/L $NaHCO_3$	81.2 ±3.3
Lipozyme TL (4%, w/w)	68.7 ±4.1
Lipex 100 L (2%, w/w)	62.0 ±2.9
Lipolase 100 T (5%, w/w)	48.3 ±4.7
Petroleum ether and Ethanol	14.5 ±3.8
Lipex 100 L (2%, w/w) + acetone	18.1 ±3.5
0.1 mol/L $NaHCO_3$ + Lipolase 100 T (1%, w/w)	70.3 ±4.3

The Proposal of Processing of Purified Chicken Skin

Purified chicken-sourced collagenous materials can be further processed in several steps to obtain soluble collagen, collagen hydrolysate and (soluble) elastin by utilizing technology intended for zero-waste. Authors of this study suggest the following possibilities of procedures as is depicted in Scheme 2.



Scheme 2: General example layout for processing purified chicken skin collagen into collagenous (and elastin) products and examples of their utilization.

Under moderate reaction conditions (temperature not exceeding 50.0 °C, potentially with the slight addition of a proteolytic enzyme, the collagen was first treated in distilled water, at the ratio 1:10 (w/v), and supplemented with 0.1 % (w/w) Polarzyme 6.0 T (a proteolytic enzyme), this treatment occurred at 25.0 ±2.0 °C over a period of 20 hours. Once filtered and rinsed with water, dissolution of the collagen followed for 45 minutes with distilled water at 50.0 ±2.0 °C. Any soluble collagen was filtered out, and the non-degraded part (residual collagen and elastin) was further treated for 20 hours at 25.0 ±2.0 °C by water supplemented with the same proteolytic enzyme (5.0 %, w/w). After filtering and rinsing the same with water, extraction in distilled water took place for 60 minutes at 80.0 ±2.0 °C in order to prepare collagen hydrolysate, which was separated out by filtration. Any elastin that remains at this stage has the capacity to be further hydrolysed to obtain elastin hydrolysate.

Conclusion

Three by-products from the slaughter of chickens were sampled - skins, heads and stomachs. After cleaning and cooling them, identification was made as to the composition of studied by-products. Chicken skin was selected for further processing since it was high in collagen and contained minimal mineral substances; subsequently, collagen was isolated from the skin by gradually removing any undesirable constituents from the material. The process of grinding and homogenizing was optimized with regard to temperature and the size of the holes in the cutting plate. Unwanted soluble non-collagen proteins and pigments were also removed from the skins. Additionally, several methods were tested as regards defatting the raw material, out of which the combination of solvents (petroleum ether and ethanol) was most effective at defatting the skins, which contained approximately 14.5% residual fat. Isolated chicken collagen was subjected to sequential processing in water alone, then water that had been supplemented with a proteolytic enzyme. As part of this first phase, soluble collagen was obtained, the approximate yield being 24%; afterwards, (low-molecular) collagen hydrolysate was prepared, resulting in a yield of around 74%. Such products could be used, for example in the food industry as

nutritional supplements and agents to thicken milk products, stabilize emulsions and clarify beverages (beer/wine); further applications includes cosmetics (humectants) or pharmacy (e.g. joints nutrition supplements)[30,31]. Out of the part left non-degraded, 2% pertains to elastin that can be further processed into soluble elastin, an additive in cosmetics, which is also utilized to create scaffolds in human medicine[32,33]. From the work reported in this manuscript following patent resulted: Patent CZ 307665 - Biotechnology-based production of food gelatine from poultry by-products (2019).

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Návrh zpracování vedlejších kuřecích produktů na potravinářský kolagen

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Souhrn

Celosvětová spotřeba drůbežího masa neustále roste. Z tohoto důvodu je v současnosti kladen velký důraz na následné zpracování vedlejších produktů, které vznikají při zpracování masa a jsou rozděleny do 3 kategorií. Vzhledem k tomu, že jsou produkovány ve velkém množství a s ohledem na svou biologickou povahu je třeba s nimi zacházet způsobem, který je šetrnný k životnímu prostředí. Nalezení účinného využití těchto vedlejších produktů, např. jako suroviny, která by mohla být zpracována na další výrobky, by představovalo ideální způsob řešení tohoto problému. Vedlejší produkty mohou obsahovat velké množství bílkovin, zejména kolagenu, které jsou široce využívány v potravinářství a dalších průmyslových odvětvích. Mezi vedlejší produkty, které obahují vysoký podíl kolagenu, patří např. drůbeží kůže, kterou lze zpracovat postupem zahrnujícím kontrolované odbourávání nežádoucích složek, konkrétně tuků, rozpustných nekolagenních proteinů a pigmentů. To, co bylo dříve považováno za odpad, se tak změní v cennou surovinu bohatou na kolagen a další zpracováním na rozpustný kolagen a hydrolyzát kolagenu. Například kuřecí kůže obsahuje vysoký podíl tuků (84 %), avšak může být účinně odtučněna třepáním rozemleté suroviny ve směsi rozpouštědel po určitou dobu; výsledkem je surovina s obsahem zbytkového tuku přibližně 14 %, pokud se použije směs petroletheru a ethanolu. Použití acetonu znamená podobný výsledek (18 %). Naopak použití roztoku $NaHCO_3$ však nevede k přijatelnému výsledku (více jak 80 %). Bylo také testováno třepání suroviny ve vodném roztoku s lipolytickými enzymy. Pro tento účel byly použity tři typy enzymů v různých koncentracích, ale obsah zbytkových tuků byl také mnohem vyšší ve srovnání s chemickými rozpouštědly (48 – 69 % zbytkového tuku).Výsledkem studie bylo rovněž navržení možností dalšího zpracování suroviny na výrobky s potenciálem využití např. v potravinářském, nebo jiném průmyslu.

Klíčová slova: *hydrolyzát; kolagen; kuře; kůže; lipolytický enzym, materiály III. kategorie, vedlejší produkty; zpracování*