Preparation of collagen concentrate from chicken feet

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Abstract

The meat-processing industry produces a huge and ever greater amount of involuntary by-products at slaughterhouses, which increase significantly the waste amount. In terms of poultry, these comprise feet, heads, viscera and skin - items that are rich in protein, especially collagen. The latter can be gained through a procedure that involves grinding and defatting raw material, since poultry by-products from meat-processing industry contain a wide range of fat (15-70%). Indeed, it is also necessary to extract other non-collagenic matter, such as pigment and water-soluble proteins. In this study, non-collagen components were removed by treatment of chicken feet in 0.1% NaOH. Three methods of defatting of raw material (applying $NaHCO_3$, lipolytic enzyme and 10 different solvent systems) were tested. The use of solvent mixture of petroleum ether and ethanol proved to be the most efficient method of defatting chicken feet with a residual fat content of approximately 5%. Collagen concentrate prepared this way possesses the potential to be utilized by commercial sectors, such as the food or pharmaceutical industry.

Keywords: *by-products, defatting, protein, poultry, slaughterhouse*

Introduction

The food industry has stimulated a growth through promoting flavourful items and provide a balance of protein from animal sources. Such food substances benefit consumers and producers, but also give rise to a large volume of by-products, often accounting 25-30% of total production. These animal-sourced by-products could be utilized by the meat-processing industry to ensure competitiveness against suppliers of plant-based proteins[1].

According to data from the US Department of Agriculture, 8,909,014,000 chickens, 232,389,000 turkeys and 27,749.000 ducks were slaughtered in 2016. In live weight this equalled 24.5 million tons of chicken, 3.2 million tons of turkey, 86,200 tons of duck and 1,700 tons of other poultry, a total of 27.8 million tons[2].

The meat-processing industry generates unwanted by-products as a result of the trade, such as blood, bones, surplus pieces cut from meat, skin, fatty tissue, horns, hooves, limbs and guts. Ecological disposal of such waste is very expensive. Although some by-products are eaten in certain countries, in accordance with the particular national customs, elsewhere they may be considered inedible. However, such by-products possess a high content of essential amino acids, minerals and vitamins, and it would be economical to extract these where possible. For example, by-products of the animal slaughter trade sometimes find application as a component in foodsubstance, as feed for livestock or pets, and fertilizers. In addition, as a raw material, they can be utilized in the production of pharmaceuticals, biomolecules (such as protein hydrolysates, enzymes, extracts with functional properties and bioactive peptides) and chemicals[3].

Bianchi et al. studied the potential energy output of poultry by-products, which could be harnessed to produce electricity, especially in deregulated energy markets. Although a small-scale indirect steam turbine would represent a viable competitor to any of such system, it would need fossil fuels to operate. Choosing the capacity of an applicable device in relation to the corresponding amount of energy would depend on the given price of electricity and natural gas. For example, if the price is 0.06 $ / kWh, a 10 MW device is recommended, without the need of fossil fuels. If the cost of electricity rises and natural gas drops in price, then equipment rated at 50 MW or more is recommended[4].

By-products originating from the poultry processing industry (such as edible tissues, bones, blood, fat, shells, unhatched eggs, discarded chickens, feathers, heads and legs) account for 22-30% of total meat production[1,5]. Notably, bedding for poultry is a by-product that itself arises through processing poultry. Mechanical separation of meat from bones results in a great deal of poultry residue, which on average contains 17% proteins (especially collagen). According to data from the FAO (Food and Agricultural Organization) from 2012, the consumption of poultry had risen by 3.6% per year. Indeed, between the years 2000 - 2009, the quantity of chicken consumed per person per 12-month period went up from 30 to 38 kg. In 2010, around 78 million tonnes of poultry were eaten worldwide. In first-world countries, poultry by-products are incorporated into pet food (for cats and dogs), feed additives for livestock and compost for the agricultural sector; whereas, in developing countries, they are simply dumped on refuse sites[1,6].

In this context, major nutrients for animals comprise the following by-products: meat; bone and blood meal; ground plasma; hydrolysate from feathers; tallow or lard containing proteins, fats, minerals and trace elements; forms of vitamin B; and some fat-soluble vitamins[7].

To date, literature presents various procedures for removing water-soluble pigments and proteins from poultry feet. Du et al. (2013) applied the following: chicken feet were mixed with 0.5M NaOH at a ratio of 1:10 (v/v) and shaken for 6 h at 4 °C; alkaline solution was changed every 2 h[5]. In contrast, Almeida and Lannes (2013) used 4% acetic acid for 16 h at room temperature[8]. Huda et al. (2013) studying duck feet employed 5% solution of lactic acid at the ratio of 1: 8 (v/v) for 24 h at 4-7 °C[9].

Poultry skins contain approximately 70% of fat[10], chicken bones contain around 20% of fat[11] and chicken feet contain about 15% of fat[12]. If these by-products valuable in the food or cosmetic industry, such as gelatine, are supposed to be processed further, it is desirable that fat content of these tissues is as small as possible as fat is an undesirable ingredient in gelatine. That is why, this study concerns also fat separation from chicken feet.

Since fat content of chicken feet is generally very high, it is necessary to defat them. A few studies have described deffating chicken feet or other poultry-based tissues. According to Du et al. (2013) technique for the processing of poultry heads was as follows: pure samples were mixed with 15 mM $NaHCO_3$ at a ratio of 1:4 (v/v) and mixed for 1 h at 4 °C, then centrifuged at 10,000 x g for 10 min. at 4 °C. This step was repeated 3 times until no fat was observed in the sludge[5]. Soxhlet method was applied in the study of chicken skins by Sarbon et al. (2012)[13]. However, due to the complexity of the process, this method is inappropriate for practical application. Huda et al. (2013) removed the fat from the top layer by spoon at the end of the alkaline treatment of duck feet[9]. This method was found to be very inefficient.

The aims of the paper

The purpose is to process chicken feet, which are a significant source of collagen, into collagen concentrate. To achieve this, several methods of defatting of a raw material have been examined. During the process, undesirable water-soluble proteins and pigments need to be removed as well.

Materials and methods

Chicken feet

Chicken feet rich in protein are hence a suitable by-product of the poultry industry. For this experiment, they were supplied by RACIOLA Uherský Brod, s.r.o. The content of dry matter was determined by drying the feet at 103°C until constant weight had been achieved. The proteins present were determined by Kjeldahl method[14]. The amount of collagen was calculated from the quantity of hydroxyproline (by multiplying the value by the coefficient 8[15]); the level of fat was determined by extraction according to Soxhlet, applying a two stage process with two solvents (chloroform and ethanol)[16]; lastly, the mineral content was determined by burning the sample and annealing at 650°C for at least 1 h in a muffle furnace[16]. Each test was perfrormed in three replications; results are presented as an arithmetic mean with standard deviation.

Appliances, tools and chemicals

These comprises: a SPAR Mixer SP-100AD-B meat cutter (TH Industry RD, Taiwan), Memmert ULP 400 drying devices (Memmert GmbH + Co. KG, Germany), an LT 43 shaker (Nedform, Czech Republic), a Kern 440 - 47 electronic scale, a Kern 770 electronic analytical balance (Kern, Germany), a desiccator, a Samsung fridge-freezer (Samsung, South Korea), a metal filter sieve (seize of pores 200 μm), a PA filter cloth (pore size 200 μm), a Nabertherm muffle furnace (Nabertherm GmbH, Germany), Parnas-Wagner distillation apparatus, $NaHCO_3$, petroleum ether, ethanol, chloroform, acetone, butyl alcohol, diethyl ether, penthane, and hexane (Verkon, Czech Republic). Lipolase 100 T (lipase from *Thermomyces lanuginosus*) was produced by submerged fermentation of the genetically modified microorganism *Aspergillus oryzae* (Novozymes, Denmark), with 100 KLU/g (kilo lipase unit/g) as declared enzyme activity.

Process to convert chicken feet into a product rich in collagen

Processing chicken feet into a product boasting a high amount of collagen involves the steps shown in Figure 1 below.

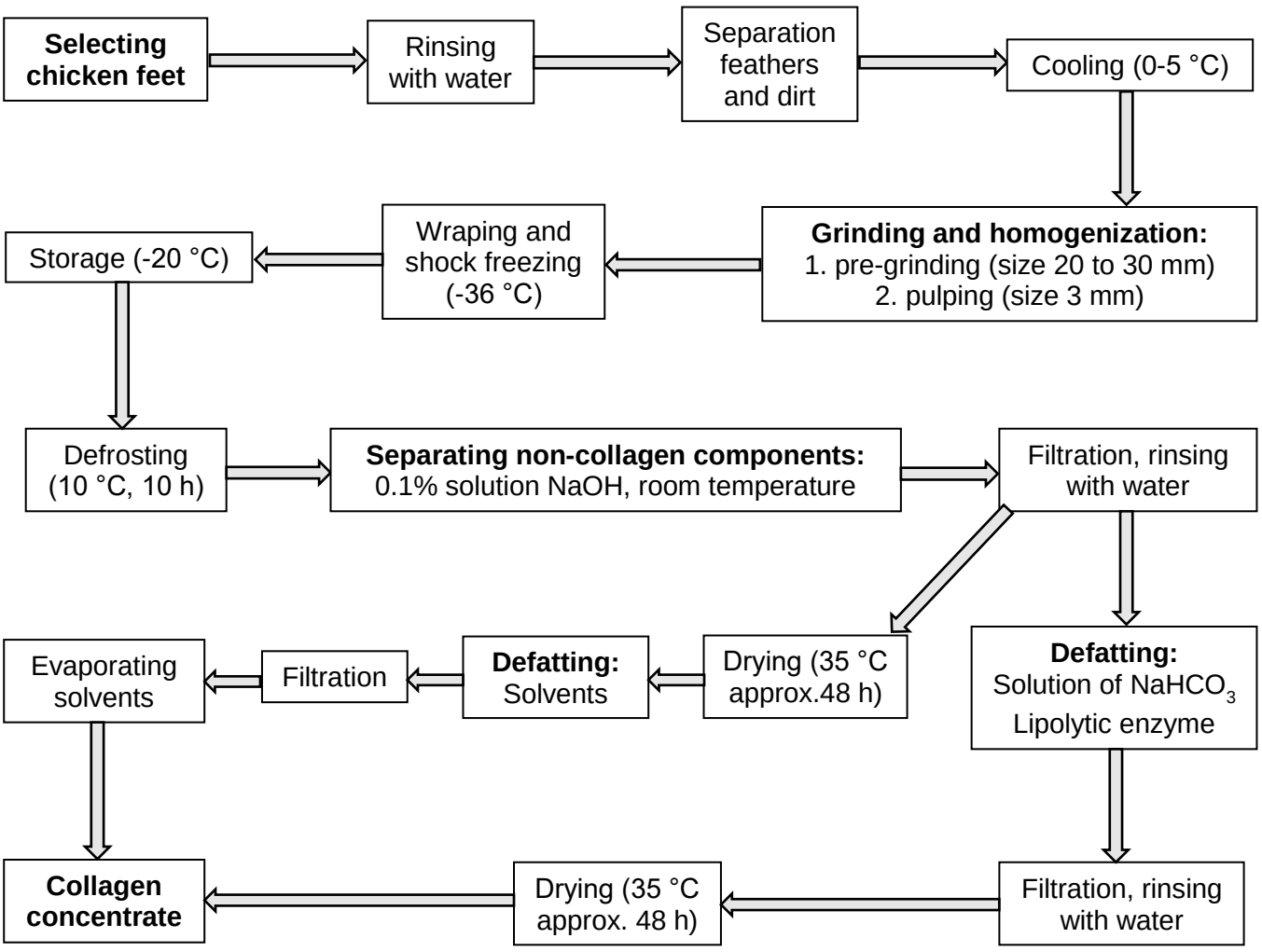

Figure 1: Flow chart of processing chicken feet into collagen concentrate

I. Grinding and homogenization of the raw material

The preparatory stage of preparing the raw material, i.e. grinding it to the desired structure for further processing, is very important. Proper conditions have to be set in order to prevent denaturation of the collagen protein during the entire purification process. Key to creating a high quality and hygienically safe product is ensuring basic hygiene conditions when obtaining the feet. A crucial aspect is to rinse said raw material in water immediately after selecting the feet intended for use. The slaughterhouse had a cleaning system in place for carrying the feet away via a pipe, in which the raw material was initial cleaned and cooled.

After purification and allowing excess water to drip off, the feet were placed in storage containers. These were then moved to chilled areas to prevent unwanted microbial growth and degradation of the feedstock, wherein the feet were stored at 0-5°C for no more than 36 hours after slaughter. Homogenization, i.e. grinding up whole feet, was conducted as soon as possible after slaughter, so that the temperature of the raw material did not exceed 12°C. Due to rise in the temperature of the raw material during homogenization, it was necessary to slightly freeze it at -2°C to -5°C prior to the operation. Grinding required two phases, utilizing an industrial meat grinder equipped with a four-arm knife for both phases as shown in Figure 2. The initial pre-grinding stage required a kidney-shaped cutting head of size 20 to 30 mm, whereas a different cutting head, at the size of 3 mm, was employed in the second stage (pulping). Under these conditions, the final temperature of the homogenized material increased by a maximum of 3°C. The ground and homogenized raw material was put into PE bags with walls of 150 μm in thickness. The packed raw material was then deep frozen (shock frozen) at -36°C ± 2°C. Said frozen raw material was then stored in the freezer at -20°C ± 2°C. Prior to the experiments, the raw material was thawed out in the refrigerator at 10°C ± 2°C over a 12 h period.

Figure 2: Details of cutting heads (kidney-shaped head on the left, cutting head of the size of 3 mm on the right)

II. Separation of undesirable non-collagenous components from the raw material

The procedure described by Du et al. (2013) with a slight modification to remove non-collagenous components was used. Once thawed, material was mixed with 0.1% NaOH solution at a ratio of 1:8 and shaken on a shaker at room temperature for 45 min. It was then filtered through a filter sieve lined with a single layer of PA cloth and rinsed with tap water. The whole process was repeated 4 times. The raw material was partially defatted during this process.

III. Removal of fat from the raw material

Three potential methods of removing fat from the raw material have been suggested herein, i.e. a use of $NaHCO_3$ solution, lipolytic enzyme or solvents. The residual fat content was determined by Soxhlet method, requiring two steps for extracting the same; the fat was extracted with chloroform for 8 h and then

with ethanol for the same length of time. Each experiment was performed twice; the results are presented as an arithmetic mean.

1. Deffating with $NaHCO_3$ solution

The method described by Du et al. (2013) with a slight modification was applied. Once thawed, the chicken feet were mixed with 150 mM $NaHCO_3$ at a ratio of 1:5 (v/v) in an Erlenmeyer flask and shaken for 1 h at room temperature. Afterwards, the material was filtered through a filter sieve; the entire procedure was repeated 4 times. The resultant material was then dried for about 24 h at 35°C.

2. Deffating with a lipolytic enzyme

To this end, the authors utilized the lipolytic enzyme Lipolase 100 T. Defatting efficiency was studied by the factorial experiments 2^2 and the central experiment; the factors monitored comprised adding the enzyme and the duration of defatting. 100 g of chicken feet were mixed in an Erlenmeyer flask with 500 mL of distilled water, into which the lipolytic enzyme Lipolase 100 T was added at the amount according to the scheme of the experiments (factor A; see Table 2). The pH was adjusted to 7.0 ± 0.3. The flask was sealed and shaken in an incubator at 12°C; shaking continued for a determined period of time, according the aforementioned scheme (factor B; see Table 2). After 1, 2 and 5 h of shaking, the pH was checked and adjusted to the prescribed value. The product (collagen concentrate) was filtered through a sieve lined with three layers of PA cloth and dried at 35°C for 24 h.

3. Deffating with solvents

Experiments were carried out with 8 types of solvents, in addition to which a combination of 2 types of solvent was tested.

The chicken feet were dried at 35°C in an air-circulation oven for about 48 h prior to the defatting process. Afterwards, they were mixed with the solvent or mixture of solvents at a ratio of 1:10 (v/v) in an Erlenmeyer flask; the solvent mixture was prepared at a ratio of 1:1 (v/v) of its components. The flask was sealed and shaken for 32 h at room temperature in four stages (at 3, 6, 8 and 15 h). After each period, the solvent was exchanged. The defatted material was left in the hood for 30 min. to allow the residual solvent to evaporate.

Results and discussion

Table 1 shows the composition of the chicken feet.

Table 1: Composition of the chicken feet

	Dry matter [%]	**Proteins** [%] *	**Collagen** [%] *#	**Fat** [%] *	**Minerals** [%] *
Chicken feet	35.5 ±3.0	48.3 ±0.4	82.8 ±0.7	34.8 ±0.8	16.1 ±0.2

*based on the dry weight of the raw material #from total protein content

Results of deffating:

1. Deffating with $NaHCO_3$ solution

The residual fat content of the raw material, as defatted by 15 mM $NaHCO_3$ solution, exceeded 25%, hence was inefficient for further use of the raw material.

2. Enzyme method

The results of the experiments are shown in Table 2. The residual fat content ranged from 24% to 28%, which was very high value in relation to the original fat content of the raw material (approximately 35%). The amount of enzyme added and the processing time did exert any significant effect on the efficiency of the process. Notably, Lipolase 100 T exhibited very poor defatting efficiency.

Table 2: Schedule of the experiments and deffating results

Exp. No.	**Addition of enzyme** (factor A) [%]*	**Period of deffating** (factor B) [h]	**Residual fat** [%]
1	1.0	18	26.5
2	1.0	48	28.5
3	2.5	18	26.1
4	2.5	48	26.0
5	1.75	33	23.8

* based on the dry weight of the raw material

3. Solvent method

Table 3 shows the results of the experiments. The highest defatting efficiency of the raw material was achieved using a 1:1 (v/v) mixture of petroleum ether and ethanol, since only 5% residual fat remained in defatted raw material. A synergistic effect was evident for mentioned solvent mixture; in contrast, the efficiency of pure petroleum ether was approximately 1.5 times lower (almost 8% residual fat content), while the figure for pure ethanol was around 4 times lower (≈ 21% residual fat).

Table 3: Chemicals applied in the deffating process and their resultant efficiency

Solvent (mixture)	**Residual fat** [%]	**Solvent**	**Residual fat** [%]
Petroleum ether+ethanol	4.97	Butylalkohol	7.66
Petroleum ether+acetone	6.41	Acetone	7.74
Pentane	6.67	Petrolether	7.93
Hexane	6.95	Choroform	8.42
Diethyl ether	7.56	Ethanol	22.2

The final product (collagen concentrate) defatted with solvent mixture petroleum ether+ethanol contains 75.0% of collagen, 14.6% minerals, 5.4% of non-collagenous organic matter and 5.0% of residual fat (based on the dry matter).

Conclusion

Chicken feet containing a large amount of collagen were cleaned, chilled, ground and homogenized. Once water-soluble proteins and pigments were removed, it was necessary to defat the raw material due to the high fat content (approximately 35%), which involved testing various methods to discern which was the most effective. Defatting with $NaHCO_3$ solution and lipolytic enzyme both proved unsuitable because the residual fat content remaining in the raw material exceeded 25% and 26%, respectively. Defatting with a mixture of petroleum ether and ethanol for 32 h (the mixture was exchanged 3 times) at room temperature was the most efficient method for defatting the material, wherein the residual fat content equalled approximately 5%; the resultant product described herein is very rich in collagen. It is worth noting that the prices for solvents represent a crucial aspect for processing at an industrial scale. The current retail costs per a litre of solvents are: butyl alcohol: 17.9 EUR; diethyl ether: 10.6 EUR; pentane: 8.0 EUR; hexane: 5.9 EUR; petroleum ether: 5.5 EUR; chloroform: 5.5 EUR; acetone: 3.9 EUR; and ethanol: 2.3 EUR (source: www.verkon.cz). Since ethanol and petroleum ether rank as ones of the cheapest available, a blend of petroleum ether and ethanol would constitute the most efficient and economically viable system

for defatting poultry tissues. Collagen concentrate (75.0% of collagen) prepared from chicken feet could potentially be utilized in the food industry (as food supplements or nutrients) or in cosmetics (as moisturising agents).

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Příprava kolagenního koncentrátu z kuřecích běháků

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Souhrn

Maso-zpracující průmysl produkuje velké množství nežádoucích vedlejších jatečných produktů, které mají jen velmi malé využití, a množství těchto vedlejších produktů se neustále zvyšuje. Příkladem takového typu vedlejších produktů jsou drůbeží běháky, hlavy, vnitřnosti a kůže, které jsou bohaté na proteiny, zejména kolagen. Kolagen je získán v několika krocích, během kterých je materiál podroben procesu mletí a odtučnění, jelikož drůbeží vedlejší produkty obsahují 15-70 % tuku. Dále je nezbytné ostranit nekolagenní složky (pigmenty a proteiny rozpustné ve vodě). Nekolagenní složky byly odstraněny opracováním v 0,1% NaOH. Dále byly otestovány 3 způsoby odtučnění suroviny (použití $NaHCO_3$, lipolytického enzymu a 10 různých rozpouštědlových systémů). Použití směsi rozpouštědel petrolether a ethanol se ukázalo jako nejefektivbnější metoda odtučnění kuřecích běháků (zbytkový obsah tuku přibližně 5 %). Připravený kolagenní koncentrát lze využít např. v potravinářském nebo farmaceutickém průmyslu.

Klíčová slova: vedlejší produkty, odtučnění, bílkovina, drůbež, jatka