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Optimization of collagen extraction from chicken feet by papain hydrolysis and synthesis of chicken feet collagen based biopolymeric fibres

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ABSTRACT

The extraction of collagen from chicken feet by papain hydrolysis was optimized at various temperatures, time and solid to solvent ratios. The optimum extraction condition (with highest yield, 32.16%, w/w) was found after 28 h of enzymatic hydrolysis at 30 °C. The chicken feet collagen was found rich in glycine (16.30%), hydroxyproline (14.15%) and proline (8.70%). The molecular weight distribution of isolated collagen was found in the range of 25–150 kDa. The functional characteristics of chicken feet collagen such as solubility, emulsification, foaming, water and oil holding capacity indicate its potential application in food, pharmaceuticals and cosmetics products. The fibres were also developed by electrospinning from chicken feet extracted collagen and polycaprolactone. Scanning electron micrographs demonstrated the interconnected network of porous structure of collagen and its bio-polymeric fibres. The slight shifting and sharp absorption of peaks obtained from biopolymeric collagen presented the evidence of interactions, occurred between the polycapralactone and the collagen.

1. Introduction

The food processing industries generate variety of waste products and disposals. The food waste valorisation leads to the production of high value macromolecules including proteins, peptides, polysaccharides, starch, fat and various other bioactive molecules such as minerals, vitamins, nutrients etc. The biological instability, oxidizing nature, high moisture content and highly prone to microbial spoilage are the major constraints in the valorisation of food waste (Jayathilakan, Sultana, Radhakrishna, & Bawa, 2012).

Poultry processing generates variety of by-products, among which liver, gizzard and chicken feet are predominant. Chicken feet are often discarded without any treatment, that causes environmental hazards. Approximately 3.9 million metric tons/year of chicken feet are produced by poultry processing industries (Chakka, Muhammed, Sakhare, & Bhaskar, 2017). The poultry by-products have been reported to be rich in protein contents such as, chicken feather (85–99% w/w), blood (60–80% w/w), heads and feet (16% w/w) (Huang & Liu, 2010; Okanović, Ristić, Kormanjoš, Filipović, & Živković, 2009). Several efforts have been made to develop chicken skin based value-added products to use poultry waste effectively (Onuh, Girgih, Aluko, & Aliani, 2014). Being rich in collagen, chicken feet are mainly used in animal feed and low-end meat products. The economic values of chicken feet are increased by the extraction and characterization of collagen (Zhou et al., 2016). Chicken feet collagen is extracted for various applications in food and pharmaceutical industries using acidic, alkaline and enzymatic hydrolysis methods (Delgado, Shologu, Fuller, & Zeugolis, 2017). Collagen is a biodegradable and biocompatible polymer, used for seeding and attachment of cells (Kim et al., 2016). Chicken feet collagen has unique physical characteristics, such as tensile strength, uniformity, flexibility, biocompatibility and biodegradability (Oechsle et al., 2016). Due to rapid rise in cost of natural, intestinal-derived sausage casings, collagen has become predominant as biopolymeric based natural casings in various applications in food industry. As a result, chicken collagen can be the best suited alternate to meet the need, due to ease of availability from by-products of poultry. Moreover, fibril forming collagens are predestined to produce edible sausage casings (Oechsle et al., 2016).

Biopolymeric fibres produced from collagen are applied in drug delivery, enzyme immobilization and advanced biomedical applications (Nagai, Tanoue, Kai, & Suzuki, 2015). The coating of a collagen with polycapralactone resulted in biopolymeric fibres with good cell

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adhesion, spreadability and the dimensional stability (Truong, Glattauer, Briggs, Zappe, & Ramshaw, 2012).

In this study, the effects of hydrolysis time, temperature and solid to solvent ratio were studied to optimize the collagen extraction from chicken feet. The extracted collagen was used with polycapralactone to produce biopolymeric fibres to improve their mechanical properties. The aim of this study was to valorise the chicken feet for maximum yield of collagen and development of biopolymeric fibres that can be used in food, cosmetics and pharmaceutical industries.

2. Materials and methods

2.1. Materials

The samples of chicken feet were obtained from Charoen Pokphand Foods (CPF) Ltd., Thailand and stored in freezer (− 20 °C) at the Bioprocess Technology laboratory of the Asian Institute of Technology (AIT), Thailand. The samples were subjected to thawing at 4 °C for 24 h and cut into smaller pieces after removal of claws. The samples were grounded by means of a mechanical grinder (Philips Co. Ltd., China), followed by storage at − 20 °C, till further analysis. All chemicals and reagents used for this study were of analytical grade.

2.2. Proximate analysis

Proximate analysis of chicken feet samples was carried out for moisture content (AOAC official method no. 934.01), crude fats (AOAC, official method no. 920.39), ash (AOAC, official method no. 942.05) and protein content (AOAC official method no. 981.10) following the AOAC standard methods (AOAC, 2002).

2.3. Experimental design for collagen extraction

Papain enzyme (EC 3.4.22.2; 30,000 USP units/mg, Merck, Germany) was used to extract collagen from ground chicken feet followed by the method of Muralidharan, Shakila, Sukumar, and Jeyasekaran (2013) with slight modifications. Chicken feet were rinsed with tap water to remove the surface contaminants followed by soaking in 0.8 M sodium chloride (NaCl, 1:6, w/v) for 20 min. Endogenous proteases were further inactivated and non-collagenous proteins were removed by treating the samples with 0.1 M sodium hydroxide (1:10, w/v) for 24 h. Enzymatic hydrolysis with papain enzyme (1%, w/v) was conducted at different temperatures (4, 30 and 56 °C) and different extraction time (20, 24 and 28 h) followed by the salting out of extracted collagen using various volumes (150, 200 and 250 ml) of 2 M NaCl solution (Cheng, Hsu, Chang, Lin, & Sakata, 2009). The sample was subjected to centrifugation (Centrikon T-324, Germany) at 10,000 × *g* for an hour. The resultant supernatant was dialyzed for 24 h in cellulose dialysis bag (Sigma, Aldrich, 25 mm, MWCO-14 kDa). The dialyzed supernatant was lyophilized by vacuum freeze drying (Scanvac Cool Safe 55-4, Denmark).

The overall experimental design to optimize the extraction of collagen from chicken feet was carried out by the design expert software (Version, 9.0.03, Stat-Ease inc, Minneapolis, MN, USA). Box Behnken design was used for the evaluation of effects of extraction time (X1), temperature (X2) and solid to solvent ratio (X3) for extraction of collagen from chicken feet. The total of 15 runs were performed with three replicates at the central point. The following quadratic regression model was used for the analysis of data.

$$Y = \beta 0 + \sum \beta i\, Xi + \sum \beta ii Xi^2 + \sum \beta ij\, Xi\, Xj \quad (1)$$

Where, Y is the response variable that represents the yield of collagen, β_0, β_i, β_{ii} and β_{ij} are the regression coefficients and X_i, X_j and Xi^2 represent the individual, interactive and quadratic effects of the variables on the response, respectively.

2.4. Collagen characterization

2.4.1. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectrometer (FTIR) (Perkin Elmer, USA) with a middle range infrared light source (4000–400 cm^{-1} wavenumbers) was used to analyse the chemical fingerprinting of the collagen. The collagen sample was ground with potassium bromide (KBr) to a fine powder, placed under high pressure with compression dye up to the formation of a pellet and then examined by FTIR (Sadiq, Hanpithakpong, Tarning, & Anal, 2015).

2.4.2. Morphological analysis of collagen

The surface morphology of the collagen sample was visualized by scanning electron microscopy (SEM, Hitachi S-3400N, Japan). The sample was mounted on SEM specimen stubs and sputter coated with gold particles under vacuum. After coating, sample was examined at 20 kV acceleration potential.

2.4.3. Molecular weight of extracted collagen

The molecular weight of collagen sample was analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) by using pre-cast gels (Mini-PRO-TEAN®,TGX™, 12%, Biorad, California, USA) at the electrical power of 200 V for 45 min. The gel was stained by using 1% (w/v) of Coomassie brilliant blue R-250 staining solution and molecular weights were determined by using pre-stained molecular weight marker (Biorad, USA).

2.4.4. Amino acid composition

The amino acid composition analysis was conducted following the method of Carnicer et al. (2009) with slight modifications. The collagen sample (50 mg) was dispersed in 6 N hydrochloric acid (HCl) at 110 °C for 24 h. The mixture sample was subjected to rotary evaporator (Büchi rotavapor R-144, Switzerland) to evaporate HCl. The concentrated sample was dissolved in 10 ml of 0.2 M sodium citrate buffer (pH 2.2). The sample was filtered through a 0.45 mm membrane filter (Titan, Switzerland) and injected in to an amino acid analyser (Biochrom 30, Cambridge, UK) using ninhydrin as colour reactant and on a single ion-exchange resin column.

2.5. Functional properties of extracted collagen

2.5.1. Water holding capacity of chicken feet collagen

Water holding capacity (WHC) of the collagen was determined by the method described by Nasrin, Noomhorm and Anal (2015) with slight modifications. Protein samples (1 g) were dispersed in 25 ml distilled water in capped test tubes by vortexing for 5 min at 25 °C. The samples were then centrifuged (Centrikon T-324, Germany) at 5000 × *g* for 30 min. The supernatant was eluted out and the water absorbed was weighed. WHC was determined as the weight of water absorbed per g of sample.

2.5.2. Oil holding capacity (OHC) of chicken feet collagen

Oil holding capacity (OHC) was determined following the method of Li, Jia, and Yao (2009) with slight modifications. Sample (100 mg) was dispersed in 10 ml of soybean oil and properly mixed by vortexing for a min at 25 °C. It was then centrifuged at 2500 × *g* for 30 min. The supernatant was removed, and the oil absorbed aliquot sample was weighed. Similarly, OHC was determined as the weight of oil absorbed per g of sample.

2.5.3. Emulsifying properties

Emulsifying activity index (EAI) and emulsifying stability (ES) were determined by the method as described by Jain and Anal (2016) with slight modifications. The collagen solution (50 ml of 10 g/L) and 50 ml of soybean oil were homogenized at 2000 rad/min for 2 min using a homogeniser (IKA RW-20, Stafen, Germany). The emulsion (50 μl) was

pipetted out from the bottom initially and then after 10 min. It was further diluted with 5 ml of 1 g/L SDS solution. The absorbance of the solution was taken at 500 nm using a UV–Vis spectrophotometer (UV-1800, Shimazdu). Emulsifying activity index and emulsion stability were calculated as follows:

$$EAI(m^2/g) = [2 \times 2.303 \times A_0/0.25 \times \text{Protein Concentration}] \quad (2)$$

$$ES(min) = [A_0 \times \Delta t/\Delta A] \quad (3)$$

Where, A_0 = absorbance at 0 min A_{10} = absorbance at 10 min
$\Delta A = A_0 - A_1$ and Δt = 10 min

2.5.4. Foaming properties

Foaming capacity and foaming stability of the collagen were determined according to the method described by Jain and Anal (2016) with slight modifications. Collagen (5 g/L) was mixed with 20 ml of distilled water and homogenized at 2000 rad/min for 10 min with a homogeniser. It was then transferred to 50 ml measuring cylinder and total volume was recorded after 30 min. The foaming capacity was calculated as follows:

$$\text{Foaming capacity}(\%) = [V - V_0]/V_0 \times 100 \quad (4)$$

Where, V_0 = Volume before whipping (ml), V = Volume after whipping (ml)

The whipped samples were kept stand still at 20 °C for 30 min. The total volume was recorded and foam stability was calculated by following equations:

$$\text{Foaming stability}(\%) = [V1 - V_0]/V_0 \times 100 \quad (5)$$

Where, V_1 is the volume after standing (ml)

2.6. Electrospinning and coating

The polycapralactone (PCL, 45% w/v) solution was made by dissolving in dimethyl formide (DMF). It was placed into a 20 ml syringe with the needle of 20 gauge for the preparation of electrospinning process (Chakrapani, Gnanamani, Giridev, Madhusoothanan, & Sekaran, 2012). The high voltage was supplied with the help of electrode (3.2–3.5 kV) and the gravity flow was maintained. A piece of aluminium foil (12 × 12 cm^2) was placed at a 10 mm distance from needle tip for generating the biopolymeric fibres. Furthermore, the PCL biopolymeric fibres were subjected to coating layer-by-layer (LBL) with two different concentrations (10 mg/ml and 20 mg/ml) of collagen in distilled water for 12 h (Ghosal, Thomas, Kalarikkal, & Gnanamani, 2014).

2.7. Characterization of biopolymeric fibres

2.7.1. Mechanical properties

The mechanical properties of biopolymeric fibres were determined by following the method of Elango et al. (2016). The mechanical properties (tensile strength and elongation) were measured by Texture Analyser (TA.XT Plus, UK) at a speed of 1 mm/min using a load of 50 N.

2.7.2. FTIR analysis of biopolymeric fibres

Fourier transform infrared (FTIR) spectroscopy analysis was carried out to determine the chemical fingerprinting of biopolymeric fibres, by using the method as described by Sadiq et al. (2015). The overall changes in secondary structure of biopolymeric fibre were analysed compared to the control (collagen).

2.7.3. Scanning electron microscopy

The surface morphology of the biopolymeric fibres was visualized by scanning electron microscopy (SEM, Hitachi S-3400N, Japan). The sample was mounted on SEM specimen stubs and sputter coated with gold particles under vacuum. After coating sample was examined at 15 kV acceleration potential.

2.8. Data analysis

All experiments were carried out in triplicates and results are expressed as mean values with standard deviation (± SD) of three replicates. One-way analysis of variance (ANOVA) and Tukey's HSD tests were carried out to determine significant group differences ($p < 0.05$) between samples by using SPSS statistical software package (SPSS, version 22.0, USA).

3. Results and discussion

3.1. Proximate analysis of chicken feet

Protein was found to be the major component in chicken feet, representing 21.58% (w/w), whereas, fat and ash contents were 4.44% and 14.28% on dry matter basis, respectively. Hashim, Ridzwan, and Bakar (2014) also reported similar results and found 20.16% protein, 3.90% fat and 8.16% ash in proximate composition of chicken feet.

3.2. Fitting of model

Response Surface Methodology (RSM) was used to optimize the enzymatic extraction of collagen from chicken feet (Jain & Anal, 2016). RSM has been used for optimization of extraction conditions to increase the yield of bioactive compounds from various natural sources (Hiranrangsee, Kumaree, Sadiq, & Anal, 2016). The effects of extraction time, temperature and solid to extraction solvent ratio in response to the collagen yield were observed by using a Box-Behnken design (Table 1). The regression analysis was conducted to model fitting using RSM for the responses and all linear and quadratic terms of independent variables. Furthermore, a multiple regression equation was obtained, representing an empirical equation between the independent variables and responses as shown in the following equation.

Table 1
Enzymatic hydrolysis of chicken feet: Box behnken experimental design with predicted and experimental response of yield of collagen.

Independent variables				Responses variable		
Run order	Time (h) (A)	Temp (°C) (B)	Solid to solvent ratio, (g/ml) (C)	Collagen Yield (%)		
				Experiment	Predict	RD
1	24	30	1:20	22.98 ± 0.51[a]	27.34	−18.97
2	24	56	1:15	9.10 ± 0.48[b]	12.99	−42.75
3	20	30	1:25	30.51 ± 0.38[c]	30.94	−1.41
4	28	4	1:20	12.48 ± 0.14[e]	18.48	−48.08
5	24	4	1:15	21.91 ± 0.38[a]	16.34	25.42
6	20	56	1:20	20.83 ± 1.01[a]	14.83	28.80
7	24	30	1:20	32.08 ± 1.17[h]	27.34	14.78
8	24	56	1:25	18.93 ± 0.38[f]	24.51	−29.48
9	20	4	1:20	9.41 ± 0.14[b]	12.87	−36.77
10	28	56	1:20	14.71 ± 0.38[d]	11.25	23.52
11	20	30	1:15	16.11 ± 0.38[d]	18.22	−13.10
12	24	4	1:25	30.34 ± 0.28[c]	26.45	12.82
13	28	30	1:15	21.57 ± 0.14[a]	21.14	1.99
14	24	30	1:20	26.95 ± 0.25[g]	27.34	−1.45
15	28	30	1:25	32.16 ± 0.25[h]	30.05	6.56

Average ± SD of triplicates. Model parameters: p-value for model = 0.35, lack of fit tests = 0.25, lack of fit F-value = 3.18, coefficient of correlation (R) = 0.854.
Relative deviation (RD) = [(experimental value-predicted value)/experimental value]*100.
Superscripts a, b, c, d, e, f, g and h above the response values indicate the significant differences ($P < 0.05$).

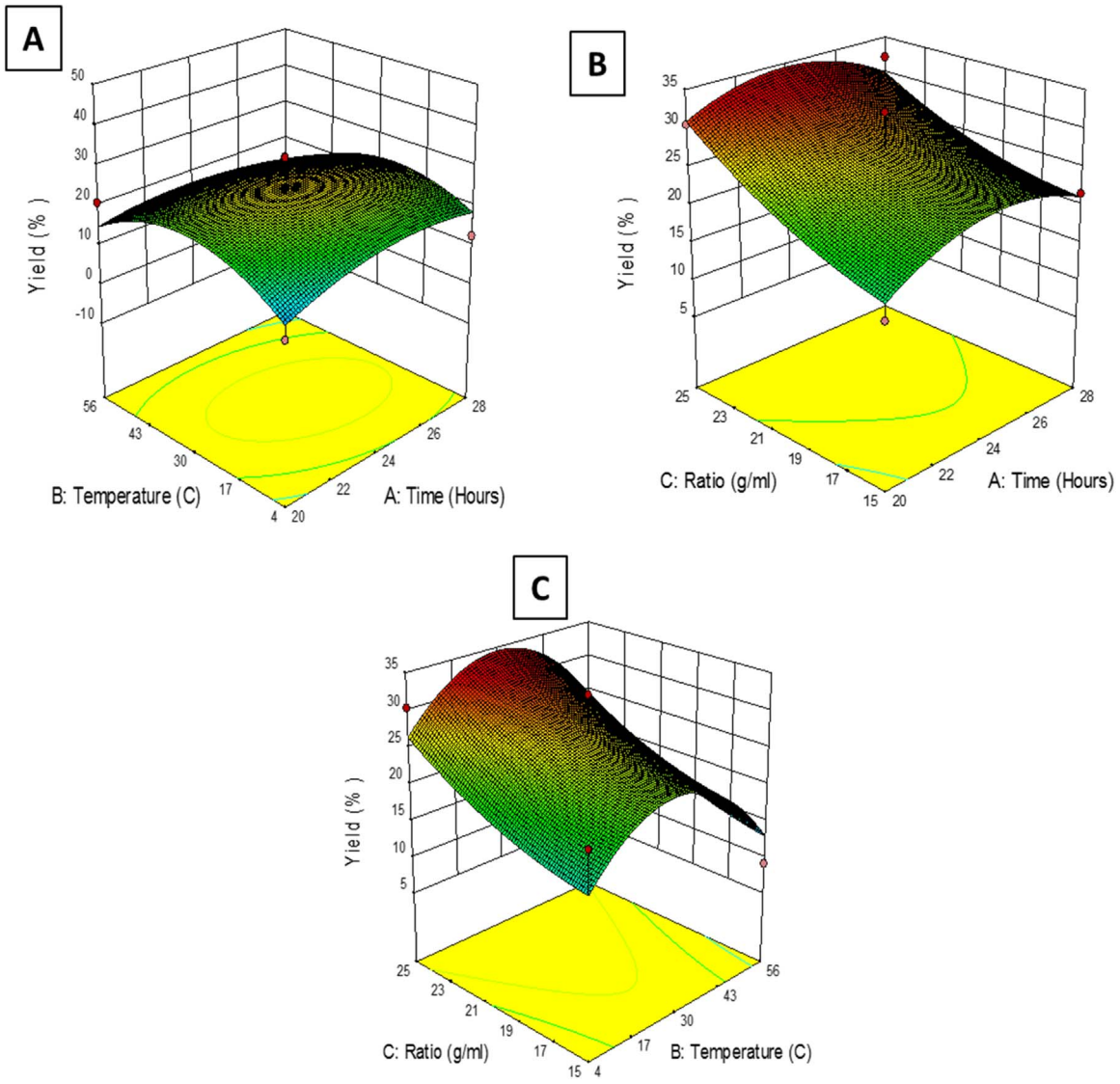

Fig. 1. Enzymatic hydrolysis of chicken feet: Response surface plots (A, B and C) showed the effects of extraction time, temperature and solid to solvent ratio, on the collagen yield.

$$Y = 27.34 + 0.51A - 1.32B + 5.41C - 3.98A^2 - 9B^2 + 1.73C^2 - 2.30AB - 0.95AC + 0.35BC \quad (6)$$

Where, Y represents the yield of collagen, A, B and C are the values of independent variables.

F-test was carried out to determine the significant effect of each variable on the yield of collagen (response variable). The p-value for model (0.35) and lack of fit tests (0.25) indicated no significance. Furthermore, coefficient of correlation ($R = 0.854$) and variance explained were used to validate the fitness of model, which indicated that model explained 73% of the experimental data whereas, 27% of the variation in the experimental data cannot be explained by the model. As the value of R approaches closer to 1, it indicates the model is well adjusted (Sitthiya, Devkota, Sadiq, & Anal, 2018). The lack of fit F-value of 3.18 is not significant as the p-value is > 0.05. The non-significance lack-of-fit showed that the model was valid for the present work (Mourabet, El Rhilassi, El Boujaady, Bennani-Ziatni, & Taitai, 2014).

The effects of independent variables on the response were presented by the series of three-dimensional response surface graph (Fig. 1). The optimum extraction conditions were found to be; 30 °C for 28 h of enzymatic hydrolysis followed by the salting out using 1:25 (g/ml) of 2 M NaCl solution, resulting in highest collagen yield of 30.05%. Experimentally, the yield of collagen was 32.16% which was found to be similar to the predicted values generated by the RSM.

3.3. Collagen characterization

3.3.1. Amino acid composition

The glycine (16.30%), hydroxyproline (14.15%) and proline (8.70%) were the prominent amino acids found in chicken feet collagen (Table 2). The results were in accordance with the previous reports

Table 2
Amino acid composition of chicken feet collagen.

Amino Acid Profiles	Content (%)	Amino Acid Profiles	Content (%)
Alanine	7.35	**Leucine**	5.46
Arginine	7.22	**Lysine**	6.10
Aspartic acid	7.75	**Methionine**	1.90
Cystine	Not Detected	**Phenylalanine**	3.30
Glutamic acid	8.0	**Proline**	8.70
Glycine	16.30	**Serine**	3.25
Histidine	0.50	**Threonine**	2.70
Hydroxylysine	2.0	**Tryptophan**	Not Detected
Hydroxyproline	14.15	**Tyrosine**	0.82
Isoleucine	2.0	**Valine**	2.50

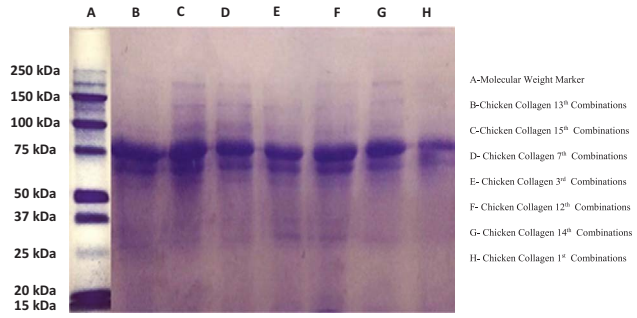

Fig. 2. SDS-PAGE profile of chicken feet collagen.

indicating that glycine is the major amino acid present in chicken feet collagen (Chakka et al., 2017; Zhou et al., 2016). The amino acid profile of chicken feet collagen was found similar to yak bone and pig skin collagens, reported by Li, Jia & Yao (2009). However, tryptophan and cysteine were not detected. The higher amount of glycine and hydroxyproline imparts the important functional properties (Chakka et al., 2017) to collagen by stabilising the triple helix (Tabarestani, Maghsoudlou, Motamedzadegan, Mahoonak, & Rostamzad, 2012).

3.3.2. Molecular weights of chicken feet collagen

Fig. 2 illustrates the electrophoretic patterns in terms of molecular weights of chicken feet collagen extracted by papain hydrolysis under different treatments. All the collagen samples showed two distinct bands of α chains (α1 and α2) which was in agreement with the previous research report (Fernandes-Silva et al., 2012). This finding indicated that the collagen I is the main component of the chicken feet (Zhou et al., 2016). The papain hydrolysis showed the presence of dispersed molecular weight bands which was due to cleavage of telopeptide regions by enzyme, leading to slightly lower molecular weight components of collagen (Chuaychan, Benjakul, & Kishimura, 2015). Lin and Liu (2006) reported the electrophoretic pattern of collagen with molecular weight distribution in the range of 25–150 kDa.

3.3.3. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of both (standard and chicken feet) collagen exhibited the specific peaks of amides of A, B, I, II and III as shown in the Fig. 3. It was found that the collagen from the chicken feet and the standard collagen exhibited the peaks for amide A at 3464.31 cm^{-1} to 3433.48 cm^{-1} and 3291.72 cm^{-1} respectively. Absorption characteristic of amide A are commonly associated with N-H stretching vibration (Zhou et al., 2016). The amide B peaks of chicken feet and standard collagen were observed at 2927.47 cm^{-1} to 2852.74 cm^{-1} and 3077.83 cm^{-1} to 2944.65 cm^{-1} respectively, representing the asymmetrical stretch of CH_2. The absorptions of amide I of chicken feet and

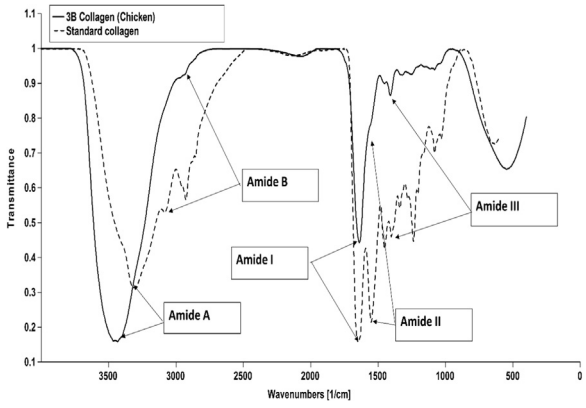

Fig. 3. Fourier Transform Infrared (FTIR) spectra of chicken feet collagen and standard collagen.

the standard collagen were found to be 1639.42 cm^{-1} and 1656 cm^{-1} respectively, which were in the range of 1625–1690 cm^{-1} as reported by Duan, Zhang, Du, Yao, and Konno (2009). It was observed that amide I bands for chicken feet collagen were slightly lower than the standard collagen, indicating a lower degree of molecular distribution for chicken feet collagen. Subsequently, the secondary structure might be slightly affected by papain hydrolysis during extraction process (Zhou et al., 2016). The characteristic frequencies (1600–1700 cm^{-1}) of amide I band are associated with the stretching vibrations of the carbonyl group (C=O bond) along the polypeptide backbone and contribute to the peptide secondary structure (Nagai et al., 2015).

The amide II peak position was observed in the range of 1555.06 cm^{-1} to 1451.95 cm^{-1} and 1543.03 cm^{-1} to 1450.85 cm^{-1} for chicken feet collagen and standard collagen respectively. The higher sequence of collagen structure is directly related to the triple helical structure and its amount of H-bonds. However, the difference in molecular structure within collagen is attributed by the band absorptions and its differences. The spectrum in the range of 1200 cm^{-1} and 1300 cm^{-1} (amide III) is related to C-N stretching and N-H bending and it is associated with the triple helix structure of collagen (Krishnamoorthi, Ramasamy, Shanmugam, & Shanmugam, 2017). Furthermore, the stretching of N-H bands at 3300 cm^{-1} was observed indicating the partial denaturation of collagen.

3.3.4. Surface morphology of collagen from chicken feet (Scanning Electron Microscopy)

Fig. 4 illustrates the SEM micrographs of the interconnected network of porous structure of collagen. The molecular interactions at the core and the surface of fibrils are the contributing factors for the interlinked network between the fibrils which lead to more covalent bonds and delicate network of chicken collagen (Oechsle et al., 2016).

3.4. Functional properties

3.4.1. Water holding capacity

The water holding capacity of chicken feet collagen was found to be 1.9 ± 0.1 g/g (Table 3). Water holding capacity of proteins could be affected by various environmental factors including the ionic strength and concentration of proteins (Li et al., 2009). The water holding capacity of chicken feet collagen was found higher than previously reported for yak bone (0.29 ± 0.03 g/g) and pigskin (0.21 ± 0.03 g/g) collagen polypeptides (Li et al., 2009). The high water holding capacity of collagen corresponds to desired rheological and textural characteristics and reduction in the dehydration process during storage (Simões et al., 2014).

3.4.2. Oil holding capacity

The oil holding capacity is an important functional property of collagen, used in the meat and confectionery products (Souissi, Bougatef, Triki-Ellouz, & Nasri, 2007). It determines the capacity of protein to hold the fat by the non-polar chains. The OHC of chicken feet collagen was found to be 5.3 ± 0.3 g/g. The oil holding capacity of chicken feet collagen was found higher than the previously reported for yak bone (1.67 ± 0.04 ml/g) collagen polypeptides (Li et al., 2009)

3.4.3. Emulsifying properties

The surface characteristics of collagens are related to the emulsifying capacities and emulsion stability in the food products. The charged molecules binds the fat globules and lowers the interfacial energy between oil and water phase by mutual repulsion and prevents the partial coalescence of fat particles in emulsified products (Sarkar, Goh, & Singh, 2010). Emulsion activity index (EAI) and Emulsion stability (ES) are used to describe the emulsifying properties of collagens.

The emulsifying activity index and emulsion stability of collagen obtained from chicken feet were 71.4 ± 1.3 m^2/g and 18.7 ± 0.8 min, respectively. The emulsion activity index of chicken feet collagen was

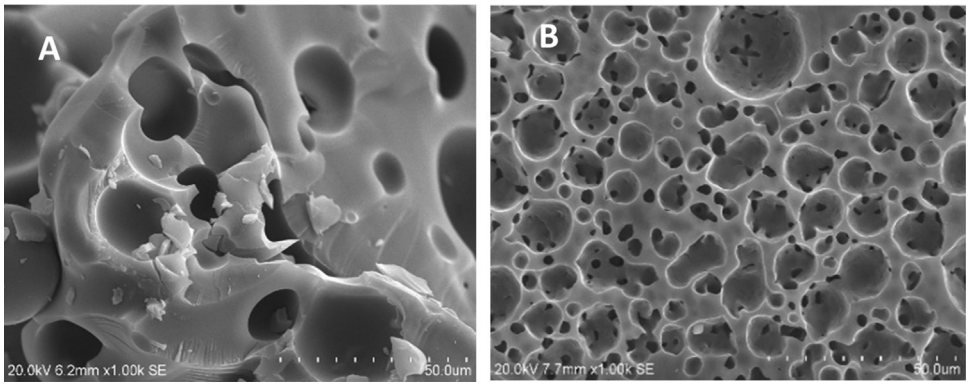

Fig. 4. Scanning electron micrographs (A = high magnification and B = low magnification) of chicken feet collagen.

found higher than previously reported for different concentrations of gelatin (1–3%) isolated from the skin of splendid squid (Nagarajan, Benjakul, Prodpran, Songtipya, & Kishimura, 2012).

3.4.4. Foaming properties

The ability of collagens to form a stable foam is one of the great functional properties in food applications especially in baking, beverages and toppings. Unlike the emulsion, the interfacial tension between gas and water phase is lowered by the gas droplets, present inside the aqueous film of proteins and thus resulting in the foam formation (Chi et al., 2014). The whipping ability of protein is closely related to the capacity of protein to build a foam. The foaming capacity and foaming stability of chicken feet collagen were 16.7 ± 2.9% and 11.7 ± 2.9% respectively. Foaming capacity of collagen is related to the concentration of soluble proteins as, it helps to reduce the surface tension and resulting interaction leads to the formation of multilayer film with increased interfacial flexibility (Wani, Sogi, & Gill, 2015).

3.5. Characterization of biopolymeric fibres

3.5.1. Mechanical properties

The tensile strength and elongation of collagen samples were 1.69 ± 0.03 MPa and 57.09 ± 0.09%, respectively. Samples containing more collagen content showed the less tensile strength. The increased tensile strength of biopolymeric fibre (3.2 ± 0.23 MPa) was assigned to the increase in the PCL content of the biopolymeric fibre (Elango et al., 2016).

3.5.2. FTIR analysis of biopolymeric fibres

The FTIR spectra of both the PCL-collagen biopolymeric fibre and chicken feet collagen are presented in Fig. 5. Distinct shifts of amides (A, B, I, II, III) peaks as compared to chicken feet collagen were observed in PCL-collagen fibres. It was found that the PCL-collagen biopolymeric fibre and the chicken feet collagen exhibited the absorption peaks for amide A at 3439.33 cm^{-1} and 3364 cm^{-1} respectively, that corroborates with the previous research report, indicating the peak for the amide A of PCL-collagen at 3273 cm^{-1} (Ghosal et al., 2014). The amide B peaks of PCL-collagen biopolymeric fibre and chicken feet collagen were found at 2950 cm^{-1} and 2927 cm^{-1} respectively, which represented the asymmetrical stretch of CH2. The peaks of amide I of PCL-collagen and chicken feet collagen were found at 1730–1638 cm^{-1}

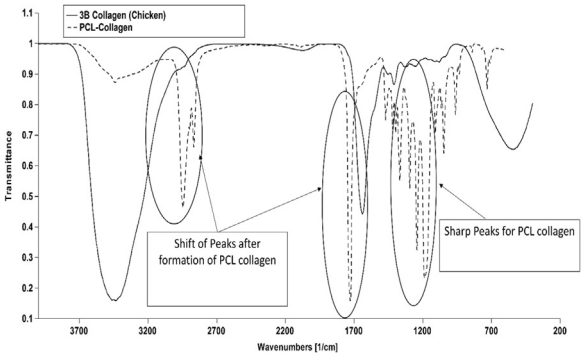

Fig. 5. FT-IR spectra of PCL-Collagen biopolymeric fibre and chicken feet collagen.

which were almost in the same range (1625–1690 cm^{-1}) as reported by Duan, Zhang, Du, Yao & Konno (2009). The amide II peak of PCL collagen was found in the range of 1639.88 cm^{-1} to 1559.86 cm^{-1}. The slight shifting of all the peaks in PCL collagen as compared to chicken feet collagen presented the evidence of interactions, occurred between the PCL and the collagen. However, the amide groups present in PCL-collagen biopolymeric fibres scaffolds are responsible for the cell attachment and the proliferation of materials in different applications.

3.5.3. Scanning electron microscopy (SEM)

The SEM micrographs clearly showed the presence of fibrils with beads in biopolymeric fibres (Fig. 6). The entanglement or the cross-linking between the molecules of blends indicated the formation of biopolymeric fibres. Moreover, the structure of PCL-collagen biopolymeric fibres was different from the native collagen (chicken feet collagen).

4. Conclusion

The chicken feet are one of the major waste products of poultry industries. The collagen was extracted from chicken feet by optimizing the extraction conditions. The extracted collagen was found to exhibit functional properties such as water and oil holding capacities, emulsifying and foaming properties. The functional characteristics of chicken feet collagen indicated its potential to be used in food, pharmaceutical

Table 3
Water holding capacity (WHC), oil holding capacity (OHC), emulsifying activity index (EAI), emulsion stability (ES), foaming capacity (FC) and foaming stability (FS) of chicken feet collagen.

Sample	WHC (g/g)	OHC (g/g)	EAI (m^2/g)	ES (min)	FC (%)	FS (%)
Chicken feet collagen	1.9 ± 0.1	5.3 ± 0.3	71.4 ± 1.3	18.7 ± 0.8	16.7 ± 2.9	11.7 ± 2.9

Results expressed as means of three replicates ± SD (standard deviation).

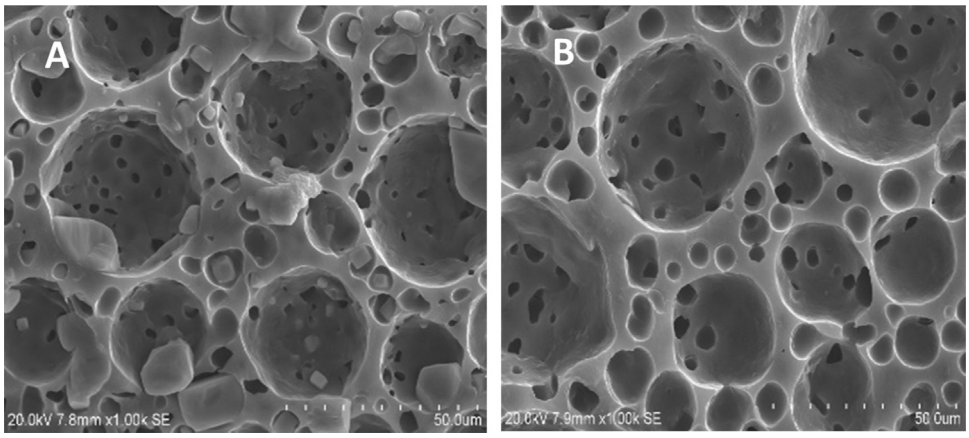

Fig. 6. SEM image of collagen coated in PCL biopolymeric fibres, (A) 10 mg collagen; (B) 20 mg collagen.

and cosmetic industries. Biopolymeric fibres were produced from chicken feet collagen, hence chicken feet collagen can be applied in drug delivery, enzyme immobilization and advanced biomedical applications

Conflict of interest

The authors declare no conflict of interest.

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