

Physicochemical Properties of Duck Feet Collagen with Different Soaking Time and Its Application in Surimi

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Abstract— Duck feet are considered as waste products because people normally do not consume duck feet. It is highly available in Malaysia and thus has the high potential to be the alternative source to produce collagen other than fish, bovine or porcine. The main objective of this study is to evaluate the physicochemical properties of different treated duck feet collagen and its effect on the quality of sardine surimi. Duck feet was stirred and soaked in 5% lactic acid solution for different time (12, 24, 36 and 48 h) at 4°C. Another treatment for the duck feet is demineralization. Duck feet collagen was demineralized by 0.5M EDTA solution for 24 h prior soaking in lactic acid solution for 24 h. Soaking time 24, 36 and 48 h has successfully decreased fat content of duck feet collagen significantly compared to 12 h. However, no differences in yield were observed for the samples. Prolong soaking time produced duck feet collagen with higher content of imino acid (hydroxyproline + proline). The intensity of lightness (L^*) for the demineralized duck feet collagen is lower compared to duck feet collagen which was not treated by EDTA. Duck feet collagen has the ability to improve the quality of sardine surimi gel by enhancing its lightness, water holding capacity, gel strength and texture with low cooking loss and expressible moisture. The folding test score of sardine surimi gel has been increased from 3.00 to 5.00 by the addition of duck feet collagen. The gel strength for sardine surimi gel with the addition of duck feet collagen is in the range of 2625.90 g.mm to 2681.58 g.mm, which is approximately 9 times higher than the gel strength of sardine surimi without collagen (280.43 g.mm).

Keywords— duck feet; collagen; EDTA; sardine surimi

I. INTRODUCTION

Duck meat can be sorted as poultry meat, and its production is increasing gradually yearly. There is around 10million of ducks are slaughtered every year in Malaysia. Malaysian Government fosters the duck meat production by enforcing National Agriculture policy so that the requirements by the population can be enhanced with time [1]. People mainly do not consume duck feet, normally they are considered as waste material. Duck feet have the potential to be the alternative source of collagen other than porcine and bovine. There are studies which are related to poultry feet collagen. Reference [2] extracted the collagen

containing melanin from silky fowl feet by different acids; Reference [3] pointed out the optimum condition for extraction of collagen from chicken feet. Meanwhile, reference [4] and [5] performed a preliminary study on the physicochemical properties of duck feet collagen and the effect of duck feet collagen addition on the physicochemical properties of surimi.

Collagens are the most abundant proteins in the animal body. There are about 25% of collagen in the animal body [6]. Collagens have been traditionally extracted from the skins or bones of mammals, mainly of bovine and porcine and also from marine sources such as fish collagens (skin, scales, and bones). Poultry feet collagens have been

extracted as medical such as collagen film, and collagen powder for wound exudates control due to the high collagen contents in the poultry feet [7], [8]. In recent years, consumers refuse to use products that are produced from bovine collagen or gelatin due to its potential risks of infectious diseases [9], [10], [11] such as bovine spongiform encephalopathy (BSE). On the other hand, pig collagen is not suitable to be used in certain products due to religious reasons [12].

The special physico-chemical and functional properties of collagen make it highly demanded in the food processing in the food industry [13]. A study performed by reference [14] showed that collagen has high gelling properties, swelling properties, and foaming properties even without gelling. Pork collagen has been used in restructured beef to improve its cooking yields and tensile strength [5]. A strong acid is efficient to increase collagen extraction rate, but too low pH value can cause denaturation and digestion of collagen in extraction [16]. For this reason, lactic acid is desirable for extraction of collagen because it is a weak acid.

Surimi is a Japanese term for minced fish which can be defined as the wet concentrated of fish muscle that is mechanically deboned, minced, washed, mixed with cryoprotectant before freezing to prolonger frozen shelf life storage [17]. Fish muscle can be categorized into white and dark muscle. Dark-fleshed fish like tunas have more dark muscle with haem proteins and lipids compared to white-fleshed fish like cod species and flatfish. Thus, the flavor and colour of surimi made from dark-fleshed fish will be influenced [18]. Studies done by reference [19] have shown that dark flesh contains higher essential amino acids content than the white flesh of oil sardine (*Sardinella longiceps*). Worldwide, there are high catches of dark muscle fish species used for food production, especially for surimi production. Thus, dark muscle fish has the potential to be the alternative raw material for surimi production since there are limited fish resources for surimi production. Sardine has a high content of dark muscle which makes up a high level of lipid and myoglobin [20]. Myoglobin and hemoglobin are associated with the whiteness of surimi [21]. According to reference [22], sardine surimi has a darker colour and poorer gel properties than lean fish surimi. All these factors cause the low quality of sardine surimi. Collagen could be the additives in surimi due to its physicochemical properties which are expected to enhance the quality of surimi products.

The main objectives of this project are to study the effect of different soaking time (12, 24, 36, 48h) during collagen extraction on the physicochemical properties of duck feet collagen, to evaluate the effect of EDTA during collagen extraction on the physicochemical properties of duck feet collagen and to determine the effect of duck feet collagen on the physicochemical properties of sardine surimi.

II. MATERIALS AND METHODS

A. Samples

Duck feet were supplied from Perak Duck Food Industries Sdn. Bhd which is located in Taiping, Perak, Malaysia. The frozen duck feet were stored at -20°C for further extraction and analysis. Lactic acid 88% and sodium hydroxide were used to extract the duck feet collagen.

B. Collagen Extraction

Collagen was extracted by using acid extraction method as described by reference [3]. Duck feet were thawed for 24 h at 6°C. Then, the duck feet's nails were removed, and the duck feet were cut into small pieces. Next, duck feet pieces were ground by using a 10mm plate with mechanical mincer (Model EVE/ALL-12, Rheninghaus, Torino, Italy). Then, the grounded duck feet were decalcified using EDTA solutions by soaking it in 0.5M EDTA solutions (pH 7.5) for 24 hours at 40°C [23]. After that, the ground duck feet were soaked and stirred in 5% lactic acid solution by w/v (duck feet/solution = 1/8) with different soaking time (12, 24, 36 and 48 h) at 4-7°C. The purpose of this step is to isolate the collagen duck feet paste and determine the optimum soaking time for collagen extraction. The layer of fat on the surface of the solution was disposed of. The treated duck feet suspended solutions were homogenized in a blender (Panasonic, MX-799) for 5 min (15 sec work and 5 sec rest) and then filtered by gauze to discard the residues. The suspended solutions were neutralized to pH 7 with 1.0N NaOH. The neutral solutions were centrifuged with a high-speed centrifuge (Model Union 5KR, Korea) for 15 min at 5000g and 10°C. The supernatant was discarded, and the sediment was lyophilized by a freeze dryer (LD53, Kingston, New York) to get dried collagen.

C. Surimi Gel Preparation

The gels were prepared by referring to the method of reference [24]. The frozen surimi samples were thawed at 4°C overnight. The surimi was blended for 2 min with 3% salt and 2% collagen using a high-speed cutter mixer (Robert Coupe®, Model Blixer®, 3B, FRANCE). The mixed surimi was stuffed into 2.5cm diameter casings and heated in a water bath (Model WB-22, Korea) for 30 minutes at 36°C and then cooked for 10 minutes at 90°C. After cooking, all gels were immediately cooled in iced water for 30 minutes and stored overnight at 4°C prior to analysis.

D. Yield, pH, and Swelling Percentage

The yield and swelling percentage of collagen were determined according to the method of reference [3] by using the formula below:

$$Yield (\%) = \frac{\text{Weight of frozen dried crude collagen}}{\text{Weight of ground duck feet}} \times 100 \quad (1)$$

$$Swelling (\%) = \frac{\text{Weight of the super - solid filtered product after soaking}}{\text{Weight of ground duck feet before soaking}} \times 100 \quad (2)$$

while pH values of collagen were measured according to reference [25] by using pH meter (Mettler Toledo Delta 320).

E. Chemical Composition

The chemical composition (moisture, ash, fat, and protein) content of extracted and dried collagen was determined according to the reference [26]

F. Amino Acids Analysis

According to the method of reference [27], the collagen was analyzed in duplicate with three hydrolyses (6N HCl, performic acid + 6N HCl and 4.2N NaOH). Samples were hydrolyzed with 6N HCl to obtain hydrolysates suitable for analysis of all amino acids except cystine+cysteine and tryptophan and methionine. The samples were hydrolyzed at 110°C with widely different sample weights and volumes of acids for 22 hours. Samples oxidized with performic acid were also hydrolyzed with 6N HCl to obtain hydrolysates suitable for the determination of cystine+cysteine as cysteic acid. Then, samples were hydrolyzed with 4.2N NaOH for the determination of tryptophan. The hydrolysates were then applied to an amino analyser (MLC-703); Atto Co., Tokyo, Japan).

G. Determination of Colour

Colour determination of duck feet collagen was done by referring to the method of [4]. Colourimeter Minolta model CM-3500d spectrophotometer (Minolta, Kyoto, Japan) was used for the colour determination. L indicates lightness (perfect black=0; pure white=100), (+) a, (-) a, (+) b and (-) b values indicate redness, greenness, yellowness and blueness respectively.

H. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Based on the method of reference [28], FTIR spectra were obtained from 1mg collagen in approximately 100mg of potassium bromide (KBr). All spectra were obtained from 4000 to 500 cm⁻¹ at a data acquisition rate of 2cm⁻¹ by using the FT-IR spectrophotometer (Nicolet iS10, USA).

I. Folding Test

Folding test was performed according to the method of reference [29]. Cooked samples were cut into slices with 3mm thickness. Then, samples were held between the thumb and forefinger in order to observe the conditions of folded samples. The samples were evaluated by the five points system as follow: (grade AA = No cracks after folding into quarters; grade A = No cracks after folding in half; grade B = cracks gradually when folded into half; grade C = cracks immediately when folded in half; grade D = breaks by finger pressure).

J. Cooking Loss

Cooking loss was determined according to reference [30]. Each chilled gel was removed from the plastic tube, blotted dry with a paper towel and weighed after following overnight storage. Overall cook loss of samples was calculated as a percentage of raw stuffed weight based on the equation below:

$$\text{Cooking loss (\%)} = \frac{\text{Cooked gel weight}}{\text{uncooked gel weight}} \times 100 \quad (3)$$

K. Water Holding Capacity (WHC)

Water holding capacity of the samples was analysed based on the method of reference [30]. Gels (25 x 15mm) of

known weight were put into the tubes and centrifuged (Sorvall RC 5B Plus, Du Pont) at low speed (365g) for 20 mins at 4°C. WHC of the samples were calculated as the equation below:

$$\text{WHC (\%)} = \frac{\text{Weight of gel before centrifuge} - \text{weight of gel after centrifuge}}{\text{Weight of gel before centrifuge}} \times 100 \quad (4)$$

L. Expressible Moisture (EM)

EM was determined according to the method of reference [31]. Samples were cut into a thickness of 5mm and weighed. The samples were placed between two pieces of Whatman paper No.41 at the bottom and top of the sample. A standard weight (5kg) was placed on top of the sample for 2 mins. Then, the samples were weighed again. EM was calculated by using the formula below:

$$\text{EM (\%)} = \frac{\text{Weight of prepressed sample (g)} - \text{weight of pressed sample}}{\text{Weight of prepressed sample (g)}} \times 100 \quad (5)$$

M. Gel Strength

According to reference [32], textural analysis of gels was performed by the computer-assisted TA.XT Plus (Stable Micro System, Godalming, UK). The samples were cut into cylindrical shapes with the length of 2.5cm. The cooked gels were equilibrated and tested at room temperature. The breaking force (g) and deformation (mm) were analyzed by using the texture analyzer equipped with a spherical plunger. The probe (P/0.25S) was pressed into the cut surface of a gel specimen perpendicularly at a constant speed of 1mm/sec for a distance of 15mm. The trigger force used was 5g, with 1mm/sec of pre-test speed and 1mm/sec of post-test speed. The load cell capacity of the texture analyzer was 30kg, and the return distance was 35mm. Gel strength of the samples was calculated by multiply the value of breaking force (g) with deformation (mm).

N. Texture Profile Analysis (TPA)

According to the method of reference [33], texture profile analysis of the gels was performed by using Texture Analyzer Model TA-XT2 (Stable Micro System, Godalming, UK). Before analysis, the gels were equilibrated at room temperature (25-27°C). The samples were then cut into 3cm in length and tested by texture analyser. Compression Platen (SMS P/75) with a heavy-duty platform and the following settings: load cell, 25kg; speed, 3.0mm sec⁻¹; test speed, 1.0mm sec⁻¹; post-test speed, 3.0mm sec⁻¹; prefixed strain, 75%; a time before compression, 2sec. The following texture parameters were determined from the TPA curves: hardness at 25% of deformation, springiness, cohesiveness, gumminess, and chewiness. Hardness was defined by peak force during the first compression cycle. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve. Springiness was defined as the ratio of time recorded between the start of the second area and the second probe reversal to the time recorded between the start of the first area and the first probe reversal. Chewiness was obtained by multiplying hardness and cohesiveness.

O. Colour Analysis of Surimi Gel

Colour analysis of the surimi gel samples was determined according to reference [4] using colourimeter Minolta model CM-3500d spectrophotometer (Minolta Kyoto, Japan).

P. Statistical Analysis

The statistical package used was SPSS 16 (SPSS Inc, Chicago IL) and significance was established at 0.05 levels. All chemical analyses were performed in triplicate.

III. RESULT AND DISCUSSIONS

A. Yield, pH, and Swelling Percentage

The yield, pH and swelling percentage of duck feet collagen treated with different soaking time and EDTA are presented in Table 1. No significant difference ($p > 0.05$) was observed for the yield between the feet collagen extracted by various soaking time. The yield for duck feet collagen extracted by various soaking time was around 28.40% (12 h-28.43%, 24 h-28.32%, 36 h-28.43%, 48 h-28.49%) whereas the yield for EDTA treated duck feet collagen was lower, which was 26.66%. The lower yield of EDTA treated duck feet collagen could be caused by the loss of ash content during demineralization process. According to the results presented by reference [3], the highest yield obtained for lactic acid treated broiler chicken feet collagen was 30.88%.

TABLE I
THE YIELD, pH AND SWELLING PERCENTAGE OF DEMINERALISED DUCK FEET COLLAGEN

Sample	Yield (%)	pH	Swelling percentage (%)
12h	28.43 ± 0.29 ^a	2.74 ± 0.02 ^b	233.10 ± 0.96 ^c
24h	28.32 ± 0.18 ^a	2.68 ± 0.01 ^e	235.43 ± 0.55 ^b
36h	28.43 ± 0.28 ^a	2.70 ± 0.01 ^d	240.71 ± 0.38 ^a
48h	28.49 ± 0.17 ^a	2.71 ± 0.01 ^c	241.31 ± 0.64 ^a
EDTA	26.66 ± 0.27 ^b	2.83 ± 0.01 ^a	232.52 ± 0.44 ^c

Values are mean ± standard deviation.

^{abcde}: Means within the same column with different letters are significantly different ($p < 0.05$).

The pH values for the duck feet collagen were in the range of 2.68 to 2.83, which were similar to the silky fowl feet (2.75) but slightly higher than chicken feet collagen (2.54 to 2.72) [2], [3]. Strong acid helps to enhance collagen extraction efficiency. Nevertheless, pH values lower than 2.0 could cause denaturation and digestion of collagen in the extraction [16]. The swelling percentage increased as the soaking time increased (12 h-233.10%, 24 h-235.43%, 36 h-240.71%, 48 h-241.31%). The swelling percentage of the samples was slightly lower than silky fowl feet (251.2%) and chicken feet (246.4%) [2], [3].

B. Chemical Composition

Table 2 shows the chemical composition of duck feet collagen with various treatments, commercial bovine, and fish collagen. There were no significant differences ($p > 0.05$) in moisture, protein, and fat for the duck feet collagen soaked for 24 h, 36 h, and 48 h. The moisture content (15.87%) of duck feet collagen which was demineralized by EDTA was significantly higher ($p < 0.05$) than duck feet

collagen treated with various soaking time (12 h-6.63%, 24 h-7.36%, 36 h-7.48%, 48 h-7.43%) and commercial collagen (bovine-8.79%, fish-4.57%). For raw duck feet, the high moisture content (58.13%) could be caused by the lack of drying process.

Raw duck feet contained 22.45% of protein, which was higher than the protein content of broiler chicken feet (17.42%) [3]. Duck feet collagen soaked for 12 h shows significantly lower protein content (32.91%) than duck feet collagen soaked in 24 h, 36 h and 48 h (38.57%, 38.48%, and 38.55% respectively). The protein content of commercial bovine (98.96%) and fish collagen (92.10%) was significantly higher ($p < 0.05$) than the duck feet collagen.

TABLE II
CHEMICAL COMPOSITION OF DEMINERALIZED DUCK FEET COLLAGEN

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
12 h	6.63±0.27 ^d	32.91±0.46 ^c	34.16±0.24 ^a	25.36±0.28 ^a
24 h	7.36±0.26 ^c	38.57±0.30 ^c	28.41±0.44 ^c	24.46±0.36 ^{bc}
36 h	7.48±0.29 ^c	38.48±0.23 ^c	28.17±0.77 ^c	24.73±0.37 ^b
48 h	7.43±0.17 ^c	38.55±0.25 ^c	27.95±0.49 ^c	24.36±0.24 ^c
EDTA	15.87±0.22 ^a	37.56±0.19 ^d	29.94±0.39 ^b	14.95±0.47 ^d
Bovine	8.79±0.09 ^b	90.26±0.15 ^a	1.03±0.11 ^d	0.36±0.02 ^f
Fish	4.57±0.18 ^e	87.89±0.53 ^b	0.86±0.08 ^e	7.32±0.16 ^e
Raw Duck Feet	58.13±0.12	22.45±0.26	11.98±0.47	7.31±0.13

Values are mean ± standard deviation.

^{abcde}: Means within the same column with different letters are significantly different ($p < 0.05$).

The fat content (11.98%) of raw duck feet was about the same with broiler chicken feet (12.04%) [3]. The fat content of duck feet collagen decreased with soaking time (12 h-34.16%, 24 h-28.41%, 36 h-28.17%, 48 h-27.95%). Demineralized duck feet collagen contained 29.94% of fat content. The fat content of commercial bovine (1.03%) and fish collagen (0.86%) was significantly lower than duck feet collagen. The high-fat content of duck feet collagen is due to the absence of defatting process. Raw duck feet contained higher ash content (7.31%) than raw broiler chicken feet (5.98%) [3]. The total ash content of duck feet collagen treated with various soaking time (12 h-25.36%, 24 h-24.46%, 36 h-24.73%, 48 h-24.36%) was significantly higher than commercial bovine and fish collagen (0.36% and 7.32% respectively). The ash content in duck feet has been reduced to 14.95% by using 0.5 M EDTA in 24 h; it was significantly lower than the duck feet collagen soaked for a different time. The high ash content of the duck feet collagen could be associated with the high amount of bone residue [3].

C. Amino Acid Composition

The amino composition of different treated duck feet collagen and chicken feet collagen are shown in Table 3. 19 amino acids were detected in the duck feet collagen which has been treated with EDTA and soaked for different time (12 h, 24 h, 36 h and 48 h). Glycine and imino acid are essential for gel strength, especially hydroxyproline and proline which are associated with the gelling attribute. Low level of amino acids represents a poor gelling ability [34].

Glycine (Gly) was the main amino acid detected in all the duck feet collagen samples. Duck feet collagen which had been demineralized in 0.5M of EDTA for 24 h contained higher amount of Gly (61.88 mg/g) compared to duck feet collagen which do not undergo demineralization (12 h-44.60 mg/g, 24 h-53.59 mg/g, 36 h-51.31 mg/g, 48 h-61.88 mg/g). However, the amount of Gly in duck feet collagen was far lower to chicken feet collagen (282.50 mg/g) [3].

The amino acids proline (Pro) and hydroxyproline (HyP) contribute to the rigidity of the collagen structure [35]. From

the result, a higher amount of proline (36.39 mg/g) and hydroxyproline (32.11 mg/g) was found in EDTA treated duck feet collagen compared to duck feet collagen soaked for different time (Pro: 12 h-26.54 mg/g, 24 h-33.50, 36 h-33.30 mg/g, 48 h-31.05 mg/g and HyP: 12 h-22.73 mg/g, 24 h-29.23, 36 h-28.38 mg/g, 48 h-27.09 mg/g). No hydroxyproline was detected, and 107.50 mg/g of proline was found in chicken feet collagen [3].

TABLE III
THE AMINO ACID COMPOSITION OF DEMINERALIZED DUCK FEET COLLAGEN SAMPLES (MG/G)

Amino Acid (mg/g)	12h	24h	36h	48h	EDTA	*Chicken feet Collagen
Histidine	3.40	4.88	4.87	4.81	3.78	10.00
Isoleucine	6.62	7.77	7.43	7.27	7.51	23.00
Leucine	12.23	15.16	14.61	14.22	14.46	44.10
Lysine	11.53	14.84	14.15	14.40	13.51	41.10
Methionine	4.75	10.17	7.18	7.62	5.70	9.50
Phenylalanine	7.40	9.56	9.27	8.88	9.57	21.30
Threonine	6.63	8.93	8.67	8.22	8.81	30.50
Tryptophan	0.78	0.87	0.80	0.80	0.68	ND
Valine	8.46	10.64	10.20	10.00	10.40	42.00
Alanine	20.33	25.63	25.40	24.24	28.84	115.70
Arginine	20.08	25.37	24.73	23.57	26.98	75.30
Aspartic acid	18.40	22.59	21.69	21.04	22.77	81.70
Glutamic acid	29.20	35.65	34.95	33.93	38.15	93.40
Glycine	44.60	53.59	53.36	51.31	61.88	282.50
Proline	26.54	33.50	33.30	31.05	36.39	107.50
Hydroxyproline	22.73	29.23	28.38	27.09	32.11	ND
Serine	8.65	10.88	10.65	10.47	10.58	29.20
Tyrosine	4.36	6.35	6.03	5.88	4.34	6.30
Cysteine	1.26	1.81	1.75	1.70	1.85	1.80
Total	257.95	327.42	317.42	306.50	338.31	1014.90
Imino acids (Hyp+Pro)	49.27	62.73	61.68	58.14	68.50	107.50

ND - not detected. *Chicken Feet: Literature results by Liu *et al.* (2001).

D. Colour Measurement of Collagen

Table 4 shows colour of duck feet collagen treated with EDTA and different soaking time, commercial bovine and fish collagen. The colour intensity of lightness (L*), redness (a*) and yellowness (b*) for duck feet collagen increased with longer soaking time.

This result shows that longer soaking time could cause collagen powder to become lighter, more reddish and yellowish. The EDTA treated collagen possessed significantly lower lightness (79.26), higher redness (2.81) and yellow colour (31.46). Thus, it would not be desirable to improve the lightness colour of sardine surimi. The low intensity of lightness and high intensity of yellowness was due to the high-fat content in collagen samples. The intensity of lightness for bovine and fish collagen was significantly higher ($p < 0.05$) than duck feet collagen whereas the redness and yellowness were significantly lower ($p < 0.05$) than duck feet collagen.

Duck feet collagen was much lighter (79.26-89.82) compare to the lightness (66.85-69.20) of chicken feet [3].

The intensity of redness (0.09-2.81) was lower than chicken feet (2.27-4.47). The a* value (redness) collagen is important as it could affect the quality of the dark flesh surimi. Duck feet collagen with lighter colour could improve the lightness of the dark fresh surimi when added to it, so it can enhance the aesthetic properties of the dark flesh surimi.

TABLE IV
THE COLOUR VALUES OF DEMINERALIZED DUCK FEET COLLAGEN

Sample	Lightness (L*)	Redness (a*)	Yellowness (b*)
12 h	89.82 ± 0.02 ^{bc}	0.09 ± 0.02 ^d	10.18 ± 0.03 ^c
24 h	89.87 ± 0.01 ^{bc}	0.11 ± 0.01 ^c	10.29 ± 0.03 ^f
36 h	89.90 ± 0.02 ^{bc}	0.12 ± 0.01 ^c	10.83 ± 0.03 ^d
48 h	91.26 ± 0.01 ^{ab}	0.36 ± 0.02 ^b	11.57 ± 0.03 ^c
EDTA	79.26 ± 0.01 ^d	2.81 ± 0.01 ^a	31.46 ± 0.03 ^a
Bovine	88.70 ± 0.04 ^c	-0.30 ± 0.01 ^e	14.81 ± 0.01 ^b
Fish	92.55 ± 0.02 ^a	-0.75 ± 0.01 ^f	10.65 ± 0.02 ^e

Values are mean ± standard deviation.

^{abcd}: Means within the same column with different letters are significantly different ($p < 0.05$).

E. Fourier Transform Infrared (FTIR) Spectroscopy

Table 5 shows the FTIR peak location and assignment for the variously treated duck feet collagen, commercial bovine and fish collagen. The amide A bands position were in between 3400 to 3440 cm^{-1} , in which a free N-H stretching vibration occurs. The position of the band will normally move to a lower frequency, 3300 cm^{-1} when there is a hydrogen bonding in the NH group of a peptide [36]. The infrared spectrum shows that the duck feet collagen which was soaked for 12, 24, 36 and 48 h contained a free N-H stretching (12h, 3416 cm^{-1} , 24h, 3419 cm^{-1} , 36h, 3417 cm^{-1} , 48h, 3420 cm^{-1}). Duck feet collagen which has been demineralized by EDTA contained N-H stretching too (3400 cm^{-1}). The amide A band of commercial bovine and fish collagen was found at 3293 cm^{-1} , indicating that there was a hydrogen bonding in the NH group of the peptide.

According to reference [37], the amide B position at 2920 cm^{-1} is related to CH₂ asymmetrical stretching whereas 2853 cm^{-1} is related to CH₂ symmetrical stretching. There was a CH₂ asymmetrical stretching and CH₂ symmetrical stretching occur in all the collagen samples (12h, 2925 cm^{-1} and 2864 cm^{-1} , 24h, 2926 cm^{-1} and 2854 cm^{-1} , 36h, 2926 cm^{-1} and 2854 cm^{-1} , 48h, 2926 cm^{-1} and 2854 cm^{-1} , EDTA, 2926 cm^{-1} and 2854 cm^{-1} , bovine, 2957 cm^{-1} and 2874 cm^{-1} , fish, 2952 cm^{-1} and 2869 cm^{-1}).

The amide I band is associated with C=O stretching and is found in the range of 1600-1690 cm^{-1} . All the amide I band position of collagen samples fitted the range for amide I band position. The amide I band position of duck feet collagen soaked for 12h, 24h, 36h, 48h, treated with EDTA, bovine and fish collagen were observed at 1652 cm^{-1} , 1652 cm^{-1} , 1656 cm^{-1} , 1661 cm^{-1} , 1652 cm^{-1} , 1652 cm^{-1} and 1655 cm^{-1} respectively. The 1655 cm^{-1} peak normally corresponds to the α -helix conformation [38].

The amide II band position of all the duck feet collagen samples were detected around 1592 cm^{-1} (12h, 1591 cm^{-1} , 24h, 1593 cm^{-1} , 36h, 1592 cm^{-1} , 48h, 1592 cm^{-1} , EDTA, 1592 cm^{-1}), fitting well the range of amide II band position [37]. The amide II band position of bovine and fish collagen was found at 1541 cm^{-1} and 1542 cm^{-1} respectively, which has been shifted to a lower frequency, indicating that there was hydrogen bonding in the commercial bovine and fish collagen. There is an involvement of C-N stretching and N-H bending in the collagen samples [38].

The amide III (1229-1301 cm^{-1}) bands are associated with C-N stretching and N-H and related with the triple helical structure of collagen [37]. There were absorption peaks found in the range of 1241 cm^{-1} to 1265 cm^{-1} in all the collagen samples.

F. Folding Test, Cooking Loss, Expressible Moisture (EM) and Water Holding Capacity (WHC)

Folding test has been used to grade the elasticity and gel quality of surimi [39], [40]. The score of folding test for sardine surimi and different collagen (duck feet, bovine, and fish) added to sardine surimi is shown in Table 6. There was

a significant difference ($p < 0.05$) between sardine surimi (3.00) without and with the addition of collagen (5.00). Sardine surimi cracked gradually when folded in half whereas sardine surimi added with collagen do not crack when folded twice. This result indicates that the addition of duck feet collagen was able to improve the poor quality of sardine surimi.

Determination of cooking loss is fundamental for the meat industry to estimate the characteristic of the products during cooking due to non-meat ingredients or other elements [30]. From Table 6, it shows that there was a significant difference ($p < 0.05$) for cooking loss between the different treated duck feet collagen. The cooking loss for sardine surimi was significant higher ($p < 0.05$) than surimi that added with 2% of duck feet collagen, commercial bovine and fish collagen. Sardine surimi added with collagen which soaked for 36 h showed the lowest cooking loss among the surimi samples. This is indicating that duck feet collagen which soaked for 36 h can effectively improve the quality of sardine surimi by reducing the cooking loss and increasing the binding properties. Reference [41] reported that addition of 1% and above of pork collagen in frankfurter could significantly improve the cooking yields of frankfurter.

The WHC normally shows the degree of denaturation of proteins and water content [42]. According to reference [43], WHC of processed products can be improved by collagen. From the result observed, the cooking loss percentage of surimi gels decreased as the percentage of WHC increased indicating cook loss was inversely associated with WHC. The WHC for sardine surimi was significantly lower ($p < 0.05$) than different treated duck feet collagen, commercial bovine, and fish collagen. Sardine surimi added with duck feet collagen showed significant higher ($p < 0.05$) WHC than sardine surimi added with commercial bovine and fish. It is clear that duck feet collagen has higher ability to improve WHC of sardine surimi gels compared with commercial bovine and fish collagen. Sardine surimi added with duck feet collagen soaked for 36 h showed the highest WHC (98.83%) among the samples. A high percentage of WHC indicated that there was high water retention in the surimi gels as the matrix formed in the gels has better capability to entrap water [30].

EM is associated with WHC. High EM represents low WHC [44]. The water retention in the gels was associated with the strength of the network formed. Thus, the high EM in gels indicated that the poor gel-forming ability of the proteins in the samples [45]. The EM sardine surimi added with duck feet, bovine and fish collagen were significantly lower ($p < 0.05$) than the sardine surimi without collagen added. It could be suggested that surimi added with duck feet collagen soaked for 36 h had the highest gel strength as it had lowest EM compared to the other samples. Reference [46] reported that the addition of pork collagen in boneless cured ham resulted in higher EM compared with those without the addition of pork collagen.

TABLE V
FTIR PEAK LOCATION AND ASSIGNMENT OF DEMINERALIZED DUCK FEET COLLAGEN

Peak wavenumber /cm ⁻¹								
Region	12h	24h	36h	48h	EDTA	Bovine	Fish	Assignment
Amide A	3416	3419	3417	3420	3400	3293	3293	NH stretch coupled with hydrogen bond
Amide B	2925	2926	2926	2926	2926	2957	2952	CH ₂ asymmetrical stretch
	2864	2854	2854	2854	2854	2874	2869	CH ₂ symmetrical stretch
Amide I	1652	1652	1656	1661	1652	1652	1655	C=O stretch/ Hydrogen bond coupled with CN stretch
Amide II	1591	1593	1592	1592	1592	1541	1542	NH bend coupled with CN stretch
Amide III	1242	1241	1242	1265	1242	1243	1244	NH bend coupled with CN stretch

TABLE VI
THE SCORE OF FOLDING TEST, COOKING LOSS, EXPRESSIBLE MOISTURE (EM) AND WATER HOLDING CAPACITY (WHC) FOR SARDINE SURIMI WITH AND WITHOUT ADDITION OF COLLAGEN

Samples	Folding Test	Cook Loss (%)	EM (%)	WHC (%)
Sardine surimi	3.00 ± 0.00 ^b	6.59 ± 0.14 ^a	25.34 ± 0.39 ^a	96.58 ± 0.30 ^f
with DFC 12 h	5.00 ± 0.00 ^a	5.46 ± 0.12 ^c	11.68 ± 0.07 ^d	98.16 ± 0.20 ^{bc}
with DFC 24 h	5.00 ± 0.00 ^a	4.59 ± 0.05 ^d	11.41 ± 0.15 ^{de}	98.55 ± 0.70 ^{ab}
with DFC 36 h	5.00 ± 0.00 ^a	4.16 ± 0.04 ^f	11.05 ± 0.07 ^e	98.83 ± 0.06 ^a
with DFC 48h	5.00 ± 0.00 ^a	4.38 ± 0.05 ^e	11.61 ± 0.16 ^d	98.72 ± 0.02 ^{ab}
with DFC (EDTA)	5.00 ± 0.00 ^a	5.38 ± 0.06 ^c	11.63 ± 0.12 ^d	98.42 ± 0.05 ^{bc}
with bovine collagen	5.00 ± 0.00 ^a	5.55 ± 0.10 ^b	13.21 ± 0.43 ^b	97.13 ± 0.20 ^e
with fish collagen	5.00 ± 0.00 ^a	5.48 ± 0.05 ^{bc}	12.33 ± 0.77 ^c	97.84 ± 0.27 ^d

Values are mean ± standard deviation.

^{ab} Means within the same column with different letters are significantly different (p<0.05).

G. Gel Strength Texture Profile Analysis (TPA) of Surimi Gel

Gel strength is the major indicator for the determination of surimi quality. A high level of gel strength is associated with high amount of concentrated myofibrillar proteins and low amount of sarcoplasmic proteins [47]. According to reference [48], the gel-forming ability is a parameter for determination of the quality of fish proteins. The major types of bonds that involve the building of a network structure during gelation including salt linkages, hydrogen bonds, disulphide bonds and hydrophobic interactions. Myosin has an influence on the gel-forming ability of surimi. The gel-forming ability decreases as myosin denaturated [49].

From the result observed (Table 7), duck feet collagen had greater ability to increase the gel strength of sardine surimi significantly compared to commercial bovine and fish collagen. Reference [50] stated that gel strength is associated with WHC. Surimi which has higher WHC will possess

stronger gel strength. Thus, sardine surimi which added with duck feet collagen soaked for 36 h had the highest gel strength (2681.58 g.mm) as it had the highest WHC among the samples.

The TPA of the samples is also shown in Table 7. In general, the addition of duck feet, fish, and bovine collagen gives significant effect on the hardness, springiness, cohesiveness, gumminess, and chewiness of sardine surimi. The protein content of the surimi samples has a significant effect on the hardness of surimi gels whereas its protein quality affects the elasticity of surimi gels [51]. The hardness for sardine surimi added with duck feet collagen which soaked for 36 h was significant higher (p<0.05) than duck feet collagen which soaked for 12 h, 24 h, and 48 h. Sardine surimi added with commercial bovine and fish collagen was significantly lower (p<0.05) in hardness compared to sardine surimi added with duck feet collagen.

TABLE VII
GEL STRENGTH AND TEXTURE PROFILE ANALYSIS FOR SARDINE SURIMI AND SARDINE SURIMI ADDED WITH DEMINERALIZED DUCK FEET COLLAGEN

Sample	Gel Strength (g.mm)	Hardness (kg)	Springiness (mm)	Cohesiveness (ratio)	Gumminess (kg)	Chewiness (kg.mm)
Sardine surimi	280.43 ± 5.13 ^c	1.22 ± 0.12 ^f	14.90 ± 0.06 ^c	0.22 ± 0.02 ^f	0.26 ± 0.03 ^e	3.49 ± 0.12 ^f
with DFC 12 h	2655.00 ± 63.20 ^a	6.07 ± 0.05 ^c	15.00 ± 0.04 ^b	0.50 ± 0.02 ^d	3.09 ± 0.11 ^b	45.27 ± 0.45 ^{bc}
with DFC 24 h	2635.81 ± 107.77 ^a	6.38 ± 0.13 ^b	15.02 ± 0.12 ^b	0.55 ± 0.02 ^b	3.12 ± 0.07 ^b	48.02 ± 0.59 ^a
with DFC 36 h	2681.58 ± 105.22 ^a	6.53 ± 0.07 ^a	15.24 ± 0.11 ^a	0.59 ± 0.02 ^a	3.25 ± 0.11 ^a	48.71 ± 0.84 ^a
with DFC 48 h	2631.04 ± 70.75 ^a	6.39 ± 0.07 ^b	15.00 ± 0.05 ^b	0.54 ± 0.02 ^{bc}	3.05 ± 0.07 ^b	48.72 ± 0.69 ^b
with DFC (EDTA)	2625.90 ± 66.11 ^a	6.50 ± 0.06 ^{ab}	15.01 ± 0.06 ^b	0.53 ± 0.02 ^{cd}	3.05 ± 0.08 ^b	44.53 ± 0.51 ^c
with bovine collagen	1701.20 ± 66.90 ^b	5.21 ± 0.13 ^d	14.99 ± 0.00 ^b	0.46 ± 0.01 ^e	2.35 ± 0.07 ^c	35.32 ± 0.83 ^d
with fish collagen	1625.20 ± 133.80 ^b	4.63 ± 0.14 ^e	15.01 ± 0.04 ^b	0.45 ± 0.00 ^e	2.08 ± 0.06 ^d	31.29 ± 0.83 ^e

Values are mean ± standard deviation.

^{abcdet} Means within the same column with different letters are significantly different (p<0.05).

Cohesiveness is the work done to decompose the internal bone in surimi [52]. The cohesiveness of the sardine surimi gels has the similar trend with the hardness of the samples. The cohesiveness of the samples has an influence on the hardness of the surimi gels. Hardness values will increase as the cohesiveness values of the surimi gels increase [53].

Cohesiveness value near to 1 shows the recovery of the sample after the initial compression. Sardine surimi added with duck feet collagen soaked for 36 h showed the highest cohesiveness (0.59) among the samples. This indicates that it has a better recovery than the other samples. Springiness is the rate of surimi to recover to its initial condition after deformation [52]. Sardine surimi added with duck feet collagen soaked for 36 h had significantly highest ($p < 0.05$) rate of recovery after compression compared to the other samples. Chewiness is referred to the energy needed to chew the surimi until it is ready to swallow. It is a complementary parameter of hardness [53]. Duck feet collagen had higher significant ($p < 0.05$) effect on the chewiness and gumminess on sardine surimi compared to commercial bovine and fish collagen.

The study shows that pork collagen has the ability to enhance protein-protein bind in pale soft and exudative (PSE) pork. This indicates that the addition of pork collagen could provide the essential functional protein that is lost due to protein denaturation in PSE pork [46].

H. Colour of Surimi Gels

Colour is one of the parameter determining the grade of surimi gels other than impurities, moisture content, gel

strength and pH [54]. Colour is also an important quality attributes of foods as it determines the acceptability of the product by consumers. The lightness (L^*), redness (a^*) and yellowness (b^*) of the differently treated surimi are presented in Table 8.

Whiteness is the fundamental element deciding the quality and acceptability of surimi gels [55]. Significant differences ($p < 0.05$) were observed in the sardine surimi with or without collagen added. Sardine surimi with duck feet collagen added exhibited significant ($p < 0.05$) higher intensity in lightness compared to sardine surimi gels added with commercial bovine and fish collagen. The L^* value for sardine surimi added with EDTA treated duck feet collagen was significantly lower ($p < 0.05$) than sardine surimi added with duck feet collagen soaked for a different time. This could be due to the low intensity of lightness for the EDTA treated duck feet collagen powder. This also indicated that EDTA treated duck feet collagen was not effective to improve the colour to dark flesh surimi.

Duck feet collagen soaked for 12 h and 36 h, duck feet collagen treated with EDTA and commercial fish collagen did significantly affect ($p < 0.05$) the redness of sardine surimi whereas the other samples had no significant effect ($p > 0.05$) on the redness of sardine surimi. Since the EDTA treated duck feet collagen powder exhibited much higher b^* (31.36) compare to the other collagen powder which possesses b^* value in the range of 10.18-14.81, it provided the significant higher intensity of yellowness for sardine surimi.

TABLE VIII
THE LIGHTNESS (L^*), REDNESS (A^*) AND YELLOWNESS (B^*) VALUES OF SARDINE SURIMI AND SARDINE SURIMI ADDED WITH DEMINERALIZED DUCK FEET COLLAGEN

Sample	Lightness L^*	Redness a^*	Yellowness b^*
Sardine surimi	61.96 ± 0.47 ^c	-1.04 ± 0.08 ^{ab}	13.17 ± 0.34 ^c
with DFC 12 h	64.39 ± 0.10 ^b	-0.66 ± 0.13 ^d	13.45 ± 0.31 ^{bc}
with DFC 24 h	64.45 ± 0.25 ^b	-0.86 ± 0.11 ^{bc}	13.46 ± 0.49 ^{bc}
with DFC 36 h	64.67 ± 0.18 ^{ab}	-0.79 ± 0.18 ^{cd}	13.33 ± 0.25 ^{bc}
with DFC 48 h	64.85 ± 0.10 ^a	-0.95 ± 0.16 ^{abc}	13.39 ± 0.40 ^{bc}
with DFC (EDTA)	62.08 ± 0.19 ^e	-0.29 ± 0.13 ^e	14.61 ± 0.28 ^a
with bovine collagen	63.58 ± 0.27 ^d	-0.86 ± 0.23 ^{abc}	13.23 ± 0.35 ^{bc}
with fish collagen	63.98 ± 0.48 ^c	-1.07 ± 0.14 ^a	13.62 ± 0.17 ^b

Values are mean ± standard deviation.

^{abcde}: Means within the same column with different letters are significant different ($p < 0.05$).

IV. CONCLUSIONS

Duck feet collagen which was soaked for 24 h, 36 h and 48 h in 5% lactic acid exhibits nearly similar in chemical composition, which is 7.36-7.48% for moisture content, 41.59-41.64% for protein content, 30.19-30.67% for fat content and 24.36-24.73% for ash content. The ash content in duck feet has been reduced by soaking in 0.5 M EDTA for 24 h. The intensity of lightness, redness, and yellowness increases with prolonging the soaking time of duck feet collagen. Duck feet collagen soaked for different time (12 h, 24 h, 36 h and 48 h) has no significant difference in yield whereas the swelling percentage for duck feet collagen soaked for 48 h is significant highest among the samples.

Duck feet collagen has the ability to enhance the quality of low-grade sardine surimi, and its effect is better than commercial bovine and fish collagen. It had significantly increased the gel strength, colour and texture of sardine surimi with the significant lower cooking loss, expressible moisture and higher water holding capacity. Duck feet collagen soaked for 36 h show the greatest effect to improve the quality of sardine surimi.

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