ISSN: 2338-1345 – Vol. 2 (2): 9-16 2014 Journal online http://journal.bakrie.ac.id/index.php/APJSAFE

Asia Pacific Journal of Sustainable Agriculture Food and Energy (APJSAFE)

Effect of Fat Extraction Treatment on The Physicochemical Properties of Duck Feet Collagen and Its Application in Surimi

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Abstract— Duck feet were the byproduct or waste from the duck meat production and the duck feet collagen was evaluated as an alternative Halal collagen to improve the quality of surimi based-product. Duck feet collagen was extracted by treated the duck feet with 5% lactic acid and 36 hours soaking periods. Alcohol (methanol-MDFC, ethanol-EDFC and 1-butanol-BDFC) is used to reduce the fat content in the duck feet collagen. 1-butanol showed the lowest level of fat content than other two alcohol defatted treatments. The yield of BDFC obtained from this treatment was 9.59%. The BDFC was light (90.40) in color. The swelling percentage of BDFC was 216.66%. There were 20 of amino acids detected in all duck feet collagen treated by different alcohol defatted treatments and the BDFC consist of 53.900 mg/g (19.21%) imino acids. Duck feet collagen was added to sardine surimi to study its effect on physicochemical properties. Duck feet collagen was able to improve the folding test score of sardine surimi from 3.00 to 5.00. Sardine surimi added with BDFC has the lowest cooking loss (2.46%), highest gel strength (2601.51 g.mm), and highest hardness (6.98 kg) value. Duck feet collagen has the ability to enhance the quality of low grade sardine surimi and its effect is better than bovine and fish collagen.

***Keywords*— Poultry waste, duck feet, collagen extraction, fat reduction, physicochemical properties**

INTRODUCTION

Duck production in Asia accounts for more than 80% of total world production. The commercial breeds are Pekin, Muscovy, Aylesbury, Khaki Chambell and others. In the year 2010, Malaysia is the third main producer of duck meat after China and France (FAO, 2012). From the statistic result, duck is still very popular and in strong demand in Asia. Recently, many duck cuts, such as breast and legs, have become more familiar for diet-conscious consumers. This was mainly because by high nutritional of duck meat and eggs as it's contain optimal composition of essential amino acids as well as favourable composition of fatty acids (Pingel, 2011).

Other than duck meat and eggs, the duck feet were the byproduct or waste from the duck meat production. Besides the duck meat, duck feet are also popular. Duck feet contain duck skin, bone, and a small amount of meat. They are a great source of glucosamine, chondroitin, and calcium. Duck feet are much tastier than chicken feet. Duck feet are lean and bony and also famous as Chinese cuisine for its partly gelatinous, partly elastic texture. Due to the small size and easy to chew of the duck feet especially the dry roasted duck feet, making the duck feet suitable for pet foods. So, duck feet are expected to provide some functional and nutritional uses when added into the processed meat products.

Schilling *et al.* (2003) stated that quality of meat products can be improved by adding collagen into the meat. Pereira *et al.* (2011) also stated that when collagen fibers are added into the meat products, it was able to reduce the cooking loss and maintain the hardness of product. Collagen is often used to make sausage casings in the tube form (Meena *et al.*, 1999). Rao *et al.* (1981) stated that the color of the bologna had a significant difference when collagen was added as a protein extender and the lightness of the bologna had increased while the redness and yellowness tend to decrease when the collagen level was increased. Besides that, collagen also added into cosmetic for firming purpose. Use of collagen in biomedical application has been growing rapidly because the collagen is highly biocompatibility and safety in use (Lee *et al.*, 2001). Few studied had been conducted to study the collagen from birds' feet such as Liu *et al.* (2001) had discussed the four acid extractions of collagen from chicken feet and Cheng *et al.* (2009) also suggested that variety of acids used to extract collagen from silky fowl feet can obtained a collagen that containing melanin. Huda *et al.* (2012b) stated that duck feet also can use to derive the collagen but the report showed the fat content in duck feet collagen are higher than cow and fish collagen.

This project was carried out achieve several objectives which are: 1) To determine the methanol, ethanol and 1-butanol effectiveness in reducing the fat content in the extracted duck feet collagen. 2) To measure the yield,

swelling percentage, and amino acid composition of reduced fat collagen extracted from duck feet. 3) To study the applications of reduced fat duck feet collagen in gel properties of surimi with commercial collagen, through the analysis of folding test, cooking loss, gel strength and hardness.

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MATERIALS AND METHODS

Source of Duck Feet

Duck feet were obtained from Perak Duck Food Industries Sdn. Bhd., Taiping, Perak. Each foot has an average weight of 50g. The frozen duck feet have been sent directly from the factory plant to Universiti. Then, all the frozen duck feet were kept in a freezer at temperature below -18°C. Lactic acid 88% and sodium hydroxide were used in the extraction of duck feet collagen. Methanol (Qrec (Asia) Sdn Bhd), 99.7% Ethanol (Qrec (Asia) Sdn Bhd) and 1-butanol (Brightchem Sdn Bhd) were used to remove the fat in duck feet.

Extraction of Collagen

Duck feet collagen was prepared by using the method of Liu *et al.* (2001). The frozen duck feet were thawed at 4°C for 24 hours, then cut into small pieces and followed by grinding the duck feet pieces. To extract the collagen from the duck feet was involved many steps which included the preparation of treatment solutions, defatting process, soaking period, homogenization, filtration, neutralization, centrifugation and freeze dry process. To remove the fat, the grinded duck feet were homogenized with various alcohol (methanol-MDFC, ethanol-EDFC and 1-butanol-BDFC). Control without removed fat content labelled as CDFC.

Preparation of Surimi Gel

To prepare the gels, the frozen surimi samples were thawed at 4°C for 24 hours. The surimi is blended for 2 minutes with 3% salt and 2% collagen powder. The mixed surimi was stuffed into casings with 2.5 mm diameter and heated in water bath for 30 minutes in 36°C and the cooked in 90°C for 10 minutes. After cooking, all gels were immediately cooled in iced water for 30 minutes and stored at 4°C overnight prior to analysis.

Analysis of Fat content, Yield, Lightness and Swelling Percentage.

The fat content of the duck feet collagen was determined according to AOAC (2000). The swelling percentage and yield were calculated according to Liu *et al.* (2001). A colourimeter (Minolta model CM-3500d spectrophotometer, Kyoto, Japan) was used to determine the colour (Lightness) of the crude collagen from duck feet.

Amino Acid Composition Analysis

Amino acid composition was determined according to method of Sarwar *et al.*, (1983). Samples were analyzed with three hydrolyses (6N HCl, performic acid + 6N HCl and 4.2 N NaOH). Samples were hydrolyzed with 6N HCl to obtain hydrolysates suitable for analysis of all amino acids except cystine+cysteine and tryptophan and methionine. Samples oxidized with performic acid for the determination of cysteine+cysteine and methionine. Samples hydrolyzed with 4.2N NaOH for the determination of tryptophan. The hydrolysates were then applied to an amino acid analyser (MLC-703; Atto Co., Tokyo, Japan).

Analysis of Folding Test, Cooking Yield, Gel Strength and Hardness

The procedures used to test the folding test were according to Lanier (1992). The method of Serdaroglu (2006) was used to determine the cooking yield. Gel strength was measured according to the method of Nielsen and Pigott (1994). The textural characteristics (hardness) of gels were carried out by using texture analyzer (Stable Micro Systems, Godalming, UK) with probe P/75 compression platen and 30kg load cell according to the method of Bourne (1977).

Statistical Analysis

All the analyses were performed in duplicate experiment and each experiment with triplicate analysis. Then, the data were analyzed using Analyses of Variance (ANOVA). The data was processed using SPSS version 16.0 and significance was defined at $p<0.05$.

RESULTS AND DISCUSSIONS

Fat content, Yield, Lightness and Swelling Percentage

The Fat content, Yield, Lighness and Swelling Percentage control duck feet collagen (unremoved fat), defatted duck feet collagen (by methanol treatment, ethanol treatment and 1-butanol treatment), commercial bovine collagen and commercial fish scale collagen is presented in Table 1.

The fat content of raw duck feet (13.10%) is higher than raw broiler chicken feet (12.06%) (Liu *et al.*, 2001). Fat content of commercial fish scale and bovine collagen (1.93% and 1.57%) are much lower than control duck feet collagen (38.67%). CDFC (38.66%) has higher fat content than skin and bone collagen of bigeye snapper (0.33% and 0.48%) (Kittiphattanabawon *et al.*, 2005). The fat content of the skin collagen of albacore tuna (*Thunnus alalunga*), rohu (*Labeo rohita*), and dog shark (*Scoliodon sorrakowah*) (0.64%, 0.33% and 0.37% respectively) (Hema *et al.*, 2013). The fat content of defatted duck feet collagen (MDFC, EDFC and BDFC) is 13.38%, 14.86% and 2.01% respectively, which have higher fat content than skin and bone collagen of rainbow trout (0.31% and 0.51%) (Shahiri *et al.*, 2012). All the defatted duck feet collagen (MDFC, EDFC and BDFC) have higher fat content than the commercial fish scale collagen (1.93%) and also commercial bovine collagen (1.57%). From this study, showed that 1-butanol was the best alcohol in reducing the fat level in duck feet collagen. 1-butanol able to reduce the fat content in the duck feet collagen from 38.66% to 2.01%, it is approximately 36.65% of fat had removed. This is because smaller alcohols have low

miscibility with triglyceride, while the butanol has better miscibility with the lipid feedstock and contribute to a less pronounced initial mass-transfer-controlled regime (Lotero *et al*., 2005).

The yield of the CDFC is 28.73%, which is lower than the yield of the chicken feet collagen (30.88%) (Liu *et al.*, 2001), the higher yield obtained by treating the chicken feet in 5% lactic acid with 36 hours soaking period. According to Liu *et al.* (2001), if the digestive degree was not controlled well, the high concentration of hydrochloric acid can digest the yielding of amino acid and peptides, resulted in smallest collagen yield. Since, muscle protein can be classified into three groups which differ in function and their solubility in water (Barbut, 2002). The yield is most probably depending on the fractions of different protein in duck feet. MDFC, EDFC and BDFC have the yield percentage with 10.12%, 13.54% and 9.59% respectively. From this study, the 1-butanol treated defatted duck feet collagen showed the lowest yield. This is possible because the defatted duck feet collagen contain almost protein only.

Lactic acid had been reported as a good disrupter for the skin tissues and collagenous structure (Giménez *et al.*, 2005). There is an inverse relationship between swelling of collagen and gel strength of lactic acid-extracted material to the concentration of acid (Liu *et al.*, 2001; Gómez-Guillén and Montero, 2001). During the swelling, the binding ability between collagen interior molecular structure would be weaken and the increase the protein unfolding (Cheng *et al.*, 2008). The swelling percentage of CDFC is 241.47%, but it is lower than the swelling test of chicken feet (248.65%; with 5% lactic acid for 36 hours) (Liu *et al.*, 2001). Acid is used to extract and solubilize the collagen rod while maintaining it triple-helix configuration (Montero and Gómez-Guillén, 2000). There are three factors that greatly influence the swelling properties and solubilization of collagen, which are type of acid used, the ionic strength and the pH that the acid produces (Gómez-Guillén and Montero, 2001). Due to the hydrogen binding power of acid, the nonionized acid acts as a swelling agent to compete with peptide bonds and involved in the intermolecular linking of the protein chain (Asghar and Henrickson, 1982). There is significant difference ($p<0.05$) between all the samples. The swelling percentage of CDFC, MDFC, EDFC and BDFC is 241.47%, 221.80%, 225.36% and 216.66% respectively.

An aspect of the appearance of food is color. Color affects the consumer perception of quality (Grigioni *et al.*, 2007). The L* (lightness) of MDFC, EDFC and BDFC (91.06%, 91.27% and 90.40% respectively) is significantly higher ($p<0.05$) than CDFC (88.27%). The color L* of chicken feet collagen (68.22%) conducted by Liu *et al*. (2001) is much lower than the L* of the CDFC (88.27%). Among the defatted duck feet collagen, EDFC showed the higher L* (lightness) when compare with MDFC and BDFC. Light scattering is caused by particles with larger molecular dimensions whose refractive index differs from the surrounding medium (Watstra and Jenness, 1984). In collagen, these molecules are fat globules.

Amino Acid Composition

According to Asghar and Hendrickson (1982), there are twenty or twenty-one different amino acids are known to be present in different collagen types. Collagen from Nile perch (*Lates niloticus*) had only 18 amino acids reported (Muyonga *et al*., 2004a).The chicken feet collagen had been reported that 21 amino acid (Liu *et al.*, 2001), while duck feet collagen only had 20 amino acids detected.

The physicochemical characteristics of the collagen are affected by amino acid composition in the collagen. Hydroxyproline is an imino acid obtained from proline (Norziah *et al*., 2009). The imino acids (hydroproline and proline) of the CDFC are 30.733 mg/g. The imino acids of the MDFC, EDFC and BDFC are 53.495 mg/g, 50.780 mg/g and 53.900 mg/g respectively. The percentage of imino acids in duck feet collagen (17.963%) is more higher than in the chicken feet collagen (10.592%).

According to Lin *et al*. (2006), difference of collagen denaturation temperatures (thermal stability) is associated to the amount of proline and hydroproline content. The higher the thermal stability of collagen, the higher is the value of proline and hydroxyproline. Imino acid and glycine play an important role in gel strength (Wangtueai and Noomhorm, 2009). Low content of imino acids also indicates poor gelling power (Ward and Courts, 1977). Hydroproline is the mojor determinant in the stabilization of the triple stranded collagen helix due to its hydrogen bonding ability through its –OH group (Burjandze, 1979; Ledward, 1986). According to Singh *et al*. (2011), the pyrrolidine rings of proline and hydroproline also aid in strengthening the triple helix. Besides that, imino acids were also found provide considerable rigidity to the collagen structure and important in maintaining the triple helical structure of collagen (Johnston-Banks, 1990; Ikoma *et al*., 2003; Hwang *et al*., 2007).

Fat content, Yield, Lightness and Swelling Percentage

Folding test is used to measure the quality of the gel springiness (Nowsad *et al*. 2000). This analysis is conducted by folding the gel slices of 5mm thickness slowly in half and half again until cracks (Iso *et al.*, 1985). In this simple test, high quality surimi is indicated as no fracture (Ramírez *et al*., 2011). Gel slides were folded for 5 seconds and evaluated the changes of shape by five stage merit marks (Shaviklo, 2006). Folding test of the Sardine surimi gel with or without addition of collagen is shown in Table 3.

The Sardine surimi gel without addition of collagen is observed to be significantly lower grade than Sardine surimi gel added with duck feet collagen and commercial fish and bovine collagen. Sardine surimi gel without addition collagen was cracked gradually when folded in half but showed no cracks when folding twice after addition of collagen. There is also no significant different between the CDFC, MDFC, EDFC and BDFC. The high content of myofibrillar protein in the dark flesh fish make them not suitable for making surimi, but from the resulted analysis indicated that collagen can improve the quality of low quality surimi. Folding test is very subjective and can be considered as preliminary test to differentiate high and low grade surimi but when used to

distinguish different functional properties of surimi samples such as gel strength folding test is lack of sensitivity (Reppond *et al.*, 1987). According to Ng and Huda (2011), the score for the cooked gel with high gel strength could be same as the cooked gel with lower gel strength, so the gel strength cannot be related to the score of folding test.

According to Pietrasik and Li-Chan (2002), cooking loss is an important test to predict the behavior of the meat product especially during cooking, which is due to non-meat ingredients or other factors. During heating the food the mainly loss is due to the water and fat loss (Alina *et al*., 2012) and these losses depend on the mass transfer process during thermal treatment (Serrano *et al*., 2007; Gerber *et al*., 2009; Vittadini *et al*., 2005). The cooking loss of Sardine surimi gel without addition of collagen is significantly higher than ($p<0.05$) the Sardine surimi gel added with 2% duck feet collagen (control and defatted) and 2% commercial collagen (fish and cow). The cook loss of Sardine surimi gel without addition collagen (6.85%) is slightly higher than the cook loss of Sardine surimi with 2% control duck feet collagen added (3.73%). The surimi gel with added EDFC (higher fat content powder) showed the higher cooking loss. Some studies have reported increased cooking loss as the fat content increases (Liu *et al*., 1991; Berry, 1994). This is low cooking loss value for Sardine surimi gel with added 2% collagen, is due to the functional properties of collagen, which can hold more water in surimi. Functional properties of collagen in food systems include water binding capacity, swelling and strength (Asghar and Hendrickson, 1982). According to Huda *et al*. (2011), increase of water holding capacity will probably decrease the cooking loss. Good quality products typically have low value of cooking loss. Lower cooking loss of meat is beneficial in processed meat products due to the minimization of weight loss (Huda *et al*., 2011; Pereira *et al*., 2011).

When the surimi is solubilized using salt and stored at temperature around 35-40°C an elastic gel is formed, and a cooked gel of greater strength is formed by further heating at >80°C. Gel strength is an important characteristic in surimi and surimi-like materials; it's defined as the breaking force and deformation which reflects the textural and functional properties of cooked gels (Lanier, 1986). To determine the surimi quality, gel strength is considered as the major parameter (Ramadhan *et al*., 2011). According to the result, there is significant difference ($p<0.05$) between the Sardine surimi gel added with duck feet collagen and commercial fish and bovine collagen. However, there is no significant difference for the CDFC, MDFC, EDFC and BDFC. The gel strength of Sardine surimi gel without addition collagen (282.70g.mm) is lower than the Sardine surimi gel added with CDFC (2587.54g.mm). The BFDC (2601.51g.mm) showed the higher gel strength than the CDFC, MDFC and EDFC (2587.54g.mm 2599.95g.mm and 2598.78g.mm respectively). While the gel strength of the Sardine surimi gel added with commercial fish and bovine collagen is 1625.20g.mm and 1701.36g.mm respectively.

Addition of duck feet collagen into Sardine surimi caused the hardness to be significantly higher ($p<0.05$) than the surimi which is added with fish collagen, bovine collagen and without addition of collagen. According to Huda *et al*. (2013), duck feet collagen is able to increase the hardness attribute to a higher value which is higher than of fish collagen and bovine collagen. Different report stated that reduction in fat content resulted in increased the hardness of meat products (Keeton, 1994; Barbut and Mittal, 1996; El-Magoli *et al*., 1996; Mendoza *et al*., 2001). The amount of collagen added influenced the hardness of meat products because the water is chemically entrapped in the protein matrix (Pereira *et al*., 2011).

CONCLUSIONS

This study showed that duck feet collagen which is defatted by using 1-butanol (BDFC) has the lowest fat content than the other two alcohol treatments, that using methanol (MDFC) and ethanol (EDFC). The swelling percentage and pH for the BDFC with lactic acid soaking period is 216.66% and 2.87 respectively. The yield obtained for the BDFC was 9.59%. The duck feet collagen (BDFC) has the highest imino acids content at 53.900 mg/g. The BDFC also has the higher L* (lightness) value, which can affect the lightness of the surimi gel. The score of folding test for Sardine surimi was increased from 3.00 to 5.00 when duck feet collagen is added. There is no significant difference ($p>0.05$) between water holding capacity of surimi with or without addition of collagen. Duck feet collagen with lowest fat content (BDFC) showed better improvement in reducing cooking loss while increasing the strength and hardness of the surimi. This suggested that the BDFC works better than the MDFC and EDFC with myofibrillar protein in surimi. The quality of Sardine surimi is raised from low-grade to high grade with addition of duck feet collagen.

ACKNOWLEDGEMENT

The authors acknowledge with gratitude the support given by Universiti Sains Malaysia (USM) through Short Term Grant 1001/PTEKIND/815081.

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Table 4.1: The Fat content, Yield, Lightness and Swelling Percentage CDFC, MDFC, EDFC, BDFC, bovine collagen and fish collagen

Treatments	Fat (%)	Yield (%)	Lightness*	Swelling percentage (%)
CDFC	38.66±0.37[a]	28.73±0.36[a]	88.27±0.01[f]	241.47±1.38[a]
MDFC	13.38±0.40[c]	10.12±0.37[c]	91.06±0.01[c]	221.80±0.71[c]
EDFC	14.86±0.30[b]	13.54±0.68[b]	91.27±0.01[b]	225.36±0.95[b]
BDFC	2.01±0.10[d]	9.59±0.31[c]	90.40±0.01[d]	216.66±0.55[d]
Bovine collagen	1.57±0.25[e]		88.70±0.04[e]	
Fish Collagen	1.93±0.03[d]		92.55±0.02[a]	

Note: CDFC-Control Duck Feet Collagen, MDFC-Defatted Duck feet Collagen by Methanol, EDFC-Defatted Duck feet Collagen by Ethanol, BDFC-Defatted Duck feet Collagen by 1-butanol

[a,b,c,d,e,f] Values are mean of 6 replicates with ± standard deviation. Different letters in the same column indicate significant differences ($p<0.05$).

Table 2: The amino acid composition of CDFC, MDFC, EDFC, BDFC and chicken feet collagen

Amino Acid (mg/g)	CDFC (%)	MDFC (%)	EDFC (%)	BDFC (%)	Chicken feet Collagen[#] (%)
Essential Amino Acid					
Histidine	1.850(1.081)	3.420(1.262)	2.835(1.113)	2.220(0.815)	10.000(0.985)
Isoleusine	4.415(2.581)	5.810(2.143)	5.530(2.172)	5.775(2.119)	23.000(2.266)
Leucine	8.145(4.761)	11.760(4.338)	11.195(4.396)	12.015(4.410)	44.100(4.345)
Lysine	7.740(4.524)	13.015(4.801)	10.800(4.241)	12.360(4.536)	41.100(4.050)
Methionine	4.600(2.689)	6.170(2.276)	4.500(1.767)	5.135(1.885)	9.500(0.936)
Phenylalanine	5.045(2.949)	7.65(2.822)	7.175(2.818)	7.805(2.864)	21.300(2.099)
Threonine	4.905(2.867)	6.155(2.271)	5.970(2.344)	5.690(2.088)	30.500(3.005)
Tryptophan	0.515(0.301)	0.570(0.210)	0.570(0.224)	0.560(0.206)	ND
Valine	5.755(3.364)	8.245(3.042)	7.790(3.059)	8.210(3.013)	42.000(4.138)
Non Essential Amino Acid					
Alanine	14.155(8.274)	22.490(8.296)	21.495(8.441)	23.510(8.628)	115.7(11.400)
Arginine	13.120(7.669)	21.210(7.824)	19.620(7.705)	21.265(7.804)	75.300(7.419)
Aspartic acid	12.410(7.254)	17.000(6.271)	16.835(6.611)	16.785(6.160)	81.700(8.050)
Glutamic acid	19.66(11.491)	29.935(11.043)	28.155(11.057)	30.260(11.106)	93.400(9.203)
Glycine	28.64(16.740)	51.635(19.048)	49.155(19.304)	55.025(20.195)	282.5(27.835)
Proline	17.030(9.954)	29.575(10.910)	27.605(10.841)	29.665(10.887)	107.5(10.592)
Hydroxyproline	13.703(8.009)	23.920(8.824)	23.175(9.101)	24.235(8.894)	ND
Serine	5.745(3.358)	7.635(2.817)	7.630(2.969)	7.490(2.749)	29.200(2.877)
Tyrosine	2.500(1.461)	3.190(1.177)	3.240(1.272)	3.010(1.105)	6.300(0.621)
Cysteine	1.155(0.675)	1.695(0.625)	1.365(0.536)	1.460(0.536)	1.800(0.177)
Total	171.088	277.780	263.670	280.540	1014.900
Iminoacids (Hyp+Pro)	30.733 (17.963)	53.495 (19.734)	50.780 (19.942)	53.900 (19.782)	107.500 (10.592)

ND - not detected.

Note: CDFC-Control Duck Feet Collagen, MDFC-Defatted Duck feet Collagen by Methanol, EDFC-Defatted Duck feet Collagen by Ethanol, BDFC-Defatted Duck feet Collagen by 1-butanol

[#]Chicken Feet: Literature results by Liu *et al*. (2001)

Table 3: Folding test of the Sardine surimi gel with or without addition of collagen

Treatments	Folding Test	Cooking Loss (%)	Gel Strength (g.mm)	Hardness (kg)
Sardine surimi	3.00±0.00[b]	6.85±0.06[a]	282.70±5.17[c]	1.21±0.10[g]
+CDFC	5.00±0.00[a]	3.73±0.11[d]	2587.54±143.57[a]	6.14±0.08[d]
+MDFC	5.00±0.00[a]	3.00±0.07[e]	2599.95±233.73[a]	6.68±0.00[b]
+EDFC	5.00±0.00[a]	3.08±0.05[e]	2598.78±114.04[a]	6.30±0.02[c]
+BDFC	5.00±0.00[a]	2.46±0.03[f]	2601.51±120.95[a]	6.98±0.06[a]
+Fish Collagen	5.00±0.00[a]	5.79±0.06[b]	1625.20±33.20[b]	4.63±0.14[f]
+Bovine Collgen	5.00±0.00[a]	5.63±0.11[c]	1701.36±66.67[b]	5.22±0.12[e]

Note: CDFC-Control Duck Feet Collagen, MDFC-Defatted Duck feet Collagen by Methanol, EDFC-Defatted Duck feet Collagen by Ethanol, BDFC-Defatted Duck feet Collagen by 1-butanol

[a,b] Values are mean of 6 replicates with ± standard deviation. Different letters in the same column indicate significant differences ($p<0.05$).