Effect of duck feet collagen addition on physicochemical properties of surimi


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Abstract
Duck feet collagen was added to threadfin bream and sardine surimi to study its effect on physicochemical properties such as folding test, gel strength, cook loss, water holding capacity, expressible moisture, texture profile analysis and colour measurement. As compared to commercial fish scale collagen and cow collagen, the addition of duck feet collagen resulted in a significant improvement in the quality of the sardine surimi. Duck feet collagen was able to improve the folding test score of sardine surimi from 3.00 to 5.00; gel strength was increased from 275.70 g.mm to 2682.70 g.mm and hardness of gel was increased from 1.12kg to 6.00kg. Addition of duck feet collagen improved the gel strength of threadfin bream surimi from 1696.70 g.mm to 5579.40 g.mm and hardness of gel was increased from 4.55kg to 10.32kg. Colour of threadfin bream and sardine surimi also improve with the addition of duck feet collagen. The lightness was increased from 66.47 to 66.89 (threadfin bream) and from 62.32 to 63.60 for sardine. The results suggest that duck feet collagen has potential as an alternative source of protein additive for the improvement of the physicochemical properties of low grade surimi.

Introduction
Malaysia is the third most widely produced duck meat country in the world after China and France (FAO, 2012). Duck population in Malaysia increased from 766.55 metric tons in 2001 to 1334.47 metric tons in 2001 (Dept. of Veterinary Services Malaysia, 2012). More production of duck meat means more by product (including duck feet) is likely to be produced, which can be used as a source of raw material to produce collagen. Collagen is formed mainly from connective tissue of animals. It has a specific secondary structure known as triple helix, which confers strength to the connective tissue matrix. This includes all the myofibril cells allowing coordinated action of movement (Hernandez-Briones et al., 2009). Both collagen film and collagen powder as a medical material for wound exudates control can be extracted from collagen of poultry feet (Pachence, 1992; Li, 1993). Liu et al. (2001) conducted a study on extraction of collagen from broiler chicken feet using four types of acid treatment and found that the best condition was chicken feet soaked in 5% lactic acid.

Surimi was known as the Japanese term for minced fish. The water soluble components including sarcoplasmic proteins have been removed by leaching with potable water. Generally, the fish used for the production of surimi has been underutilized, has high functional properties (gel forming ability), white flesh with subtle odour and flavour, low in fat and abundance in nature (permit mass production with consistent quality). The manufacturing and development of surimi has achieved a great success in industry. Surimi production is increasing according to the variety of fish product application and functionality of fish surimi (Gwinn, 1992). Good quality surimi can be produced by utilizing the low-value of white flesh fish species which have excellent gelling ability. Nonetheless, the dark flesh fish species also has potential for the production of surimi. In order to improve physicochemical properties of surimi, the addition of collagen or gel strength enhancers like starch, egg white, whey protein or soybean protein is generally required to maintain good textural characteristics (Zayas, 1997).

Collagen proteins function to stabilize shrinkage and promote increased cooking yields because of

Keywords
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their gelling and water binding properties (Prabhu and Doersher, 2000; Schilling et al., 2003; Prabhu et al., 2004). Santana et al. (2012) reported that the addition of fish gelatin could improve the gel strength, lower the expressive moisture, and can improve the texture properties of surimi powder gel. Besides that, the gel forming ability of fish mince could also be substantially increased by addition of gelatin at 0.5% level (Binsi et al., 2009). This study was done to determine the effect of duck feet collagen addition on physicochemical properties of surimi prepared from white flesh fish (threadfin bream) and dark-flesh fish (sardine).

**Materials and Methods**

**Preparation of duck feet collagen**

Duck feet were purchased from local duck food industries, Perak Duck Food Industries Sdn. Bhd. which is located in Northern part of Peninsular Malaysia and stored at -20°C for further analysis. Both commercial fish scale collagen (CFC) and cow collagen (CCC) were purchased from local suppliers (Euro Chemo-PharmaSdn.Bhd). Duck feet were thawed in a chiller at 4-7°C for 24 hours. The claws of duck feet were disposed off; duck feet were cut into small pieces and then ground twice using a 10mm plate mechanical mincer (Model E VE/ALL-12, Rheninghaus, Torino, Italy). To extract the collagen, the ground duck feet were mixed with 5% lactic acid solution by w/v (duck feet/solution=1/8) and soaked for 24 hours at 4-7°C. At the end of soaking, the layer of fat on the surface of the solution was disposed off. Treated duck feet suspended solutions were homogenized using a blender (Panasonic, MX-799) for 5 min (15sec work and 5 sec rest) and then filtered using double gauze to discard bone residues. The suspended solutions were further neutralized to pH 7 with 1.0N NaOH. The neutral solutions were centrifuged with a high speed centrifuge (Model Union 5KR, Korea) at 5000g for 15 min (15sec work and 5 sec rest) and then filtered using double gauze to discard bone residues. The suspended solutions were further neutralized to pH 7 with 1.0N NaOH. The neutral solutions were centrifuged with a high speed centrifuge (Model Union 5KR, Korea) at 5000g for 15 min at 10°C. The supernatant was discarded and the precipitate was lyophilized by a freeze dryer (LD53, Kingston, New York) to obtain dry collagen.

**Surimi processing**

The preparation of white flesh fish (threadfin bream) and dark-flesh fish (sardine) surimi was done according to the method of Babji and Gna (1994). Fish head, viscera and scale were removed and the fish was washed using chilled water. Beheaded fish was deboned by using fish bone separator and the flesh collected from the perforation drums. The minced fish flesh was washed three times using chilled water with 5:1 water to meat ratio for 2 min and then allowed to settle for 5 min. The water layer was removed and the residue was filtered by using commercial sieve. The remaining water in the washed product was removed by squeezing out the water using a cotton cloth and hand press machine. Then, the leached meat was passed through the strainer with the mesh size of 1.0-1.5mm to remove the remaining scales, connective tissues, membrane and small bone from the leached meat. The raw surimi was mixed with 3% sucrose, 3% sorbitol and 0.3% sodium pyrophosphate using a mixer. After that, the surimi was packaged and stored at a temperature of -20°C or below.

**Preparations of surimi gel**

The preparation of surimi gel was done according to the method of Babji and Gna (1994). The 2% duck feet collagen was mixed with 3% salt and 95% of each surimi for 2 min in a cutter mixer (Robot Coupe®, Model Blixer®, 3B, France) and stuffed into cellulose casing of 25mm diameter. The stuffed samples were then cooked in warm water at 36°C for 30 min for low temperature setting, followed by high temperature setting at 90°C for another 10 min in two separate water baths (Model WB-22, Korea). After cooking, all gels were immediately cooled in iced water for 30min and stored at 4°C overnight prior to analysis.

**Folding test**

Folding test was done according to the procedures of Lanier (1992). Samples were cut into 3mm thick portions. The slices were held between the thumb and the forefinger folded to observe the way they broke. The scale used was as follows: (1=breaks by finger pressure, 2=cracks immediately when folded in half, 3=cracks gradually when folded in half, 4=no cracks showing after folding in half, 5=no cracks showing after folding twice).

**Gel strength**

Textural analysis of gels was done by using a computer-assisted TA.XT Plus (Stable Micro Systems, Godalming, UK) according to the method of Benjakul et al. (2001). The gel samples were cut into cylindrical shapes with 2.5cm in length. The breaking force (g) and deformation (mm) were measured by using the texture analyzer equipped with a spherical plunger with a diameter of 0.25 in. 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 cell load capacity of the texture analyzer was 30kg and the return distance
was 35mm. Gel strength of the gels was the product of the breaking force and deformation.

\[
\text{Gel strength} = \text{Breaking force (g)} \times \text{Deformation (mm)}
\]

Cook loss

Cook loss was calculated using the method of Pietrasik (1999). Following overnight storage, each chilled gel was removed from the plastic tube, blotted dry with a paper towel and weighed for cook yield. Overall cook loss was calculated as a percentage based on the raw stuffed weight.

Water holding capacity (WHC)

WHC was measured according to Pietrasik (1999). Gels (25 x 15mm) of known weight were placed in the tubes and centrifuged (Sorvall RC 5B Plus, Du Pont) at 365 x g for 20 min at 4°C. WHC was expressed as the ratio of gel weight after centrifugation to the initial gel sample weight.

Expressible moisture (EM)

EM was measured according to the method of Benjakul et al. (2001). Gel samples were cut into a thickness of 5mm, weighed and placed between two pieces of Whatman paper No.41 at the bottom and top of the sample. The standard weight (5kg) was placed on top and held for 2 min. The samples were then weighed again after 2 min. The formula for calculating EM is as follow:

\[
\text{EM (g)} = \frac{\text{Weight of pre-pressed sample (g)} - \text{Weight of pressed sample (g)}}{\text{Weight of pre-pressed sample (g)}} \times 100
\]

Texture profile analysis (TPA)

TPA was done according to the method of Bourne et al. (1978) with slight modifications. Textural characteristics of gels were analyzed by using Texture Analyzer TA-XT2 (Stable Micro Systems, Godalming, UK). Compression Platen (SMS P/75) with a heavy duty platform and the following settings: cell load, 30kg; speed, 1.0mm/sec; test speed, 1.0mm/sec; post-test speed, 1.0mm/sec; distance, 15mm; time before second compression, 2sec; trigger force, 5g. The following parameters were determined: Hardness (kg): The area of the curve (in mm²) during the first down stroke, which is proportional to the work performed by the probe on the sample during the first compression or the work performed during the first bite. Cohesiveness (ratio): The ratio (dimensionless) of positive force during the second to that of the first compression cycle (downward strokes only). The strength of the internal bonds makes up the body of the sample. Springiness (mm): The force at maximum compression during the second compression cycle. It represents the hardness of the sample at second bite. Gumminess (kg): The force necessary to disintegrate a semisolid sample for swallowing (Hardness x cohesiveness). Chewiness (kg mm): The energy required to chew a solid sample to a steady state of swallowing (gumminess x springiness).

Colour measurement

A colorimeter (Model Minolta Spectrophotometer CM-3500D, Osaka, Japan) was used to measure the colour of surimi gel sample based on CIE Lab Scale. The instrument was calibrated with zero calibration (CM-A100) and followed by white calibration plate (CM-A120). The parameters determined were L*, a*, b* values. L* represents the lightness (L* = 100 is the lightest and L* = 0 is the darkest, a* represents the redness (red +60 to green -60), while b* represents the yellowness (yellow +60 to blue -60).

Statistical analysis

All data were analyzed using the statistical one-way analysis of variance (ANOVA), followed by Duncan multiple range test of Statistical Package for Social Science version 16.0 (SPSS inc., Chicago, Illinois, U.S.A). Statistical significance was established at 0.05 levels.

Results and Discussions

Folding test

Folding test is a simple and quick method used to determine the quality of gel springiness (Nowsad et al., 2000). High quality surimi is indicated as no fracture shown in the sample test (Ramirez et al., 2011). Folding test of threadfin bream and sardine surimi gel with or without addition of collagen is shown in Table 1. There is no significant difference (p>0.05) between threadfin bream with or without the addition of collagen. This indicates that the quality of threadfin bream surimi gel is good although with the addition of protein additives. Santana et al. (2012) also reported the folding test of surimi powder gel still had good visual gelling pattern alone without addition of hydrocolloids. However, sardine (Sardinapilchardus) surimi gel was observed to be of significantly lower grade (p<0.05) than sardine surimi gel added with duck feet collagen, fish collagen and cow collagen. Sardine surimi gel control which cracked gradually when folded (score 3.0) in half showed no cracks when folding twice (5.00) after addition of collagen whether duck, fish or cow collagen. This showed that collagen improved the folding test score of low quality surimi. Folding test is very subjective and can be considered as preliminary test to differentiate high and low grade surimi but lacks of sensitivity to
distinguish different functional properties of surimi samples such as gel strength (Reppond et al., 1987).

Gel strength

The gel strength of both threadfin bream and sardine surimi gel with or without addition of duck feet collagen, fish collagen and cow collagen, after overnight chilling at 6-8°C is also shown in Table 1. Both surimi gel exhibited similar trend where gel strength of surimi gel added with duck feet collagen was significantly higher (p<0.05) than surimi gel added with fish collagen and cow collagen while surimi gel without addition of collagen showed the lowest gel strength.

The salt soluble protein, myofibrillar protein is crucial in gel formation (Ensoy et al., 2004; Hultin et al., 2005; Park and Lin, 2005). Gelling ability of white muscles myofibrillar protein was better when compared to those from red muscles under similar processing conditions (Sun and Holley, 2011). This explains the lower gel strength of sardine surimi gel than threadfin bream surimi gel. Gel strength was found to be inversely proportional to the expressible moisture of cooked gel which is in agreement with the findings of Ng and Huda (2011). Water retained inside the gels is associated with the strength of the network formed where high expressible moisture in gels indicates poor gelling ability.

Gel strength of surimi gel added with fish collagen showed lower gel strength improvement, which may be due to the less hydroxyproline content in fish collagen compared to mammal’s collagen (Montero et al., 2002). Fish collagen could improve the gel strength of surimi powder gel by 22.05% (Santana et al., 2012). Differing from animal collagen, fish collagen contains low hydroxyproline and proline or amino acid content (Morrissey et al., 2000). Lin and Liu (2006) also reported that bird feet collagen contained higher amino acids content and displayed higher thermal stability compared to aquatic animal collagen. Higher amino acids content also indicated higher gel forming ability of collagen.

Cooking loss

To predict the behaviour of the meat products during cooking due to non-meat ingredients or other factors in the meat industry, cooking loss is considered as the most important test (Pietrasik and Li-Chan, 2002). Both threadfin bream and sardine surimi gel showed the same trend for cooking loss, indicating the ability of collagen to decrease cooking loss. The cooking loss of the control surimi gel was significantly higher (p<0.05) than surimi gel added with collagens. These results are similar to those Prabhu et al. (2004) which showed that addition of collagen compared with the control caused significantly higher cooked yields. However, there is no significant difference between sample with duck feet collagen compared with fish and cow collagen. The value shown that, the addition of duck feet collagen to reduce cooking loss was highest for threadfin bream (3.84%) and sardine surimi (4.77%) gels.

Lower cooking loss typically represents good quality products. Gelling and water binding ability of functional collagen protein could lead to an increase in cook yields at low levels (Schilling et al., 2003; Prabhu et al., 2004). Prabhu et al. (2004) found the addition of pork collagen in frankfurters at 1% and above caused significantly higher cooking yields. Water can be retained chemically through the physical form of protein matrix where collagen fibers swell on contact with water besides preventing the exits of moisture and fat from the system, thus reducing cooking loss (Pereira et al., 2011).

Water holding capacity (WHC)

Results as shown in Table 1 indicate that, the matrix formed in those gels had a greater capacity to entrap water. Percentage of WHC for threadfin bream surimi gel is relatively higher than sardine surimi gel. It was found that WHC of both threadfin bream and sardine surimi gel with addition of duck feet collagen showed highest percentage because collagen is able to increase WHC in processed products (Prabhu et al., 2004). Food industries emphasize the importance of higher water holding capacity due to its positive correlation in minimizing the decrease of the weight of final product during storage time (Huda et al., 2011).

The addition of fish gelatin in surimi powder gel could improve the water holding capacity (Araceli et al., 2009; Santana et al., 2012). From Table 1, WHC for sardine with and without collagen did not tally with EM due to the addition of cryoprotectant that influenced the WHC (Suharyanto et al., 2009) during surimi processing. Gomez-Guillen and Montero (1996) also concluded that the addition of egg white and soy protein at level of 2% increased water holding capacity of sardine mince gel. Schilling et al. (2003) reported contradictory result that pork collagen was found to improve the water-holding capacity and texture of PSE pork in restructured boneless deli rolls through increasing protein functionality.

Expressible moisture (EM)

EM is indicated by the amount of liquid squeezed from a protein system by the force applied (Jauregui et al., 1981). The effects of adding collagen on EM
of surimi gel is shown in Table 1. Niwa (1992) suggested EM to be inversely associated to WHC. Both controls of threadfin bream and sardine surimi gels showed the highest EM of 12.87% and 24.90% respectively. EM of threadfin bream surimi gel added with duck feet collagen (5.62%) was significantly lower (p<0.05) than threadfin bream surimi gel added with fish collagen (8.88%), cow collagen (9.02%) and control threadfin bream surimi gel. There was significant difference (p<0.05) between EM of raw sardine surimi gel (24.90%) and sardine surimi gel added with collagen but EM of sardine surimi gels added with duck feet collagen (10.56%) did not differ significantly (p>0.05) from sardine surimi gels added with fish collagen (12.72%) and cow collagen (12.40%).

EM is found to increase as the amount of entrapped water decreased (Ramirez et al., 2007). Addition of duck feet collagen, fish collagen and cow collagen into threadfin bream surimi gel resulted in better prevention of water loss from protein system compared to sardine surimi gel. Schilling et al. (2003) reported consistently lower expressible moisture in treatments containing pork collagen compared with those without pork collagen in boneless cure hams. WHC is directly correlated with the myofibrillar protein content (Smith, 1991), in which the myofibrillar protein contributed to the juiciness in meat and hence a higher WHC and lesser EM (Kinsella, 1982). Duck feet collagen added into surimi gel showed the lowest expressible moisture, so the ability to reducing water loss is better than fish and cow collagen.

**Texture profile of surimi gel**

Table 2 shows the textural properties analysis (TPA) results of threadfin bream surimi and sardine surimi with and without addition of collagen. Santana et al. (2012) showed that samples added with hydrocolloid including fish gelatin had higher TPA value than surimi powder without hydrocolloid. Texture profile analysis with compression method produced five TPA parameters, that is, hardness, cohesiveness, springiness, gumminess and chewiness. Lee and Chung (1989) reported that the uses of these five TPA parameters are better for assessing the overall binding properties of surimi gel with or without added ingredients. However, those five parameters with higher values do not necessary indicate better quality (Yu and Yeang, 1993).

Addition of duck feet collagen into surimi caused the hardness, gumminess and chewiness of surimi to be significantly higher (p<0.05) than those added with fish collagen, cow collagen and without addition of collagen. There was no significant difference (p>0.05) in springiness of all the three surimi with or without collagen addition while cohesiveness of surimi added with either duck feet collagen, fish collagen or cow collagen was significantly higher (p<0.05) than the surimi without added collagen.

Low hardness value of sardine control is consistent with low folding test score and as a good quality of surimi, threadfin bream showed higher hardness compared with sardine surimi gel. The ability of duck feet collagen in increasing the hardness attribute is higher than that of fish collagen, fish collagen or cow collagen was significantly higher (p<0.05) than the surimi without added collagen.

Properties of interest to the meat processing industry are water binding ability and gel hardness as hardness determines the commercial value of a meat (Park et al., 1996; Huda et al., 2010).

The quantity of added collagen increased the hardness of meat formulation by entrapping water chemically through the protein matrix and swelling when on contact with water (Pereira et al., 2011). 2% of collagen is incorporated into surimi gel as its effect to texture of product is not significant and thus is a compatible ingredient in a processed meat system regarding flavor and functionality (Prabhu et al., 2004).

**Colour of surimi gel**

Colour of surimi gel with or without treatment with collagen is shown in Table 3. Colour is one of the main factors that consumers take into consideration in evaluating product quality (Grunert, 1997). A
more intense lightness is shown by higher L* value which is a desirable attribute and has high consumer acceptance (Resurreccion, 2003; Huda et al., 2010). The difference in colour characteristics is actually contributed by the addition of collagen: duck feet collagen, fish collagen and cow collagen. Lightness value of surimi gel decreased when the levels of gelatin increased (Kaewudom and Benjakul, 2011).

Lightness of threadfin bream surimi (66.47) which is white muscle is higher than sardine surimi (62.32). Addition of fish collagen into threadfin bream surimi resulted in its lightness (67.04) being significantly higher (p<0.05) than those with addition of duck feet collagen (66.89) and cow collagen (66.55). Similar trend can be seen in lightness when sardine surimi was added with fish collagen (63.80), duck feet collagen (63.60) and cow collagen (63.49). The change in colour of surimi is mainly imparted by colour of duck feet collagen and fish collagen. Addition of collagen and its interaction with water made it swell and caused an increase in light scattering which lead to the increased value of lightness (Pereira et al., 2011). According to Schilling et al. (2003), the addition of collagen only slightly affected the redness and yellowness of the products that was produced from industry. This colour can be roughly distinguished by naked eyes. However, these differences is rather impractical and possibly be eliminated by low usage of collagen.

**Conclusion**

Addition of duck feet collagen was able to improve the folding test score of sardine surimi from 3 to 5, which is similar to fish collagen and cow collagen. The surimi gel showed no fracture after folding twice. But there was no significant difference between water holding capacity of surimi with or without addition of collagen for both types of surimi. Duck feet collagen also showed better improvement in reducing cooking loss and expressible moisture while increasing gel strength and hardness compared to surimi added with commercial fish and collagen and surimi without collagen. This suggested that the duck feet collagen works better than commercial fish collagen and commercial cow collagen with myofibrillar protein in surimi. Quality of threadfin bream surimi is raised from good to premium and quality of sardine surimi is increased from low-grade to a better grade with the addition of collagen. The addition of duck feet collagen had significant positive effects on the physicochemical properties of surimi. Thus, duck feet collagen could be applied to product development of surimi-like material formulation.

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