Effect of egg white on physicochemical properties of mixed fish sausage

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Abstract. Fish sausage can be produced using a mixture of multiple species of fish, but such products are not generally well-received by customers due to their fishy flavor and soft texture. The objective of this research was to develop fish sausage using three types of fish (salmon, spotted knife fish and seven-striped carp). In addition, the effect of the addition of egg white on the chemical and physical properties of the fish sausage was also studied. Results showed that the formula FS7, which contains salmon, spotted knife fish, and seven-striped carp at a ratio of 0.33:0.33:0.33% (w/w), had a moisture content of 68.19%, emulsion stability of 97.15%, water holding capacity of 86.37%, and cooking loss of 4.63%. Formula FS7 was, therefore, chosen for improvement of its texture by adding fresh egg white. The effect of egg white at all levels had an impact on the color of the mixed fish sausage. Results show that increasing egg white from 1% to 3% in the mixed fish sausage formula FS7 slightly increased both L* and h°. However, increasing egg white did not affect the hardness of the mixed fish sausage. The addition of egg white at all levels increased the emulsion stability, but not the water holding capacity. Based on the results of this experiment, the use of 1% egg white helps to increase the lightness of mixed fish sausage but decreases the percentage of the water holding capacity.

1. Introduction
Salmon (Salmo salar), a marine fish from the family of Salmonidae, has high levels of eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), which are known for their anti-thrombogenic and anti-inflammatory properties [1-2]. Spotted knife fish (Notopterus chitala) and seven-striped carp (Probarbus julliet), which are freshwater fish in the family of Notopteridae and Cyprinidae, are used in Thai cooking recipes...
such as Thai steamed fish curry in banana leaves, deep fried fish cakes, fish balls, and fish sausage [3]. In general, commercial sausages are prepared using chicken, pork, or beef emulsified with fat compounds from various sources. During mixing and blending, bundles of protein fibers, myofibrils, and filaments are broken down and the size of fatty tissues is gradually reduced. Myofibrillar proteins become soluble and migrate to the fat globule surface, concentrate, and form a protein matrix at the fat/water interface when salt is added [4]. Apart from the use of these main ingredients, phosphates are added to increase water holding capacity (WHC) of animal proteins during processing and storage. Nitrites are used as a preservative for inhibiting the growth of *Clostridium botulinum*. Flavors and flavor enhancers such as salt, monosodium glutamate (MSG) [5], guanosine monophosphate (GMP) [6] or 5'-ribonucleotide salts [3] are also used to increase palatability and consumer acceptance. Juntachote (2018) reported that low-fat chicken sausages containing 1.5 and 2% carrageenan enhanced the quality characteristics of low-fat chicken sausages [7]. However, the consumption of these chicken, pork, or beef sausages, which are high in saturate fat and cholesterol, could lead to high blood cholesterol, hypertension, heart disease, or other health problems. Fish sausage is an alternative choice for good health that has low saturate fat and cholesterol, but high EPA or DHA if marine fish like salmon is used [2]. The drawback of fish sausage products is the soft texture of fish. The objective of this research was to develop fish sausage by varying the ratio of the three types of fish (salmon, spotted knife fish, and seven-striped carp). The addition of fresh egg white on the chemical and physical properties of the mixed-fish sausage was also investigated.

2. Material and methods

2.1. Preparation of fish sausage

The fillets of salmon (*Salmo salar*), spotted knife fish (*Notopterus chitala*), and seven-striped carp (*Probarbus jullieni*), along with back-fat pork, soy sauce, salt, fish sauce, and sugar were purchased from a retail store in Nonthaburi, Thailand. Sodium tripolyphosphate and sodium nitrite were purchased from Sigma-Aldrich (USA). The fish samples were stored on ice for 1 hour during transportation to the laboratory. As described by Pakoawnoy (2014) [8], the fish sausage was prepared with some modification. The control formula of the fish sausage consisted of each type of fish fillets (salmon, spotted knife fish or seven-striped carp) (71.22%), back-fat pork (17.81%), ice (7.33%), soy sauce (1.07%), salt (0.85%), fish sauce (0.71%), sugar (0.71%), and sodium tripolyphosphate (0.29%) w/w. Sodium nitrite was also added at a concentration of 125 ppm. The preparation of the sausages started with cleaning of the fish fillets (salmon, spotted knife fish and seven-striped carp) with tap water and drained for 5 minutes, then cut into small pieces and blended in a mixer at high speed for 2 min. Then all other ingredients were added and mixed for another 10 minutes. The temperature was maintained at 12±3ºC throughout the preparation. The resulting batter was then stuffed into a collagen casing (25 mm in diameter), baked at 70±2ºC for 60 minutes, boiled at 80±2ºC for 60 min, and cooled in cold water (15ºC). All fish sausage samples were packed individually in polyethylene bags and kept frozen at -18ºC.

2.2. Development of mixed-fish sausage (FS) formulations

The ratio of salmon (X1), spotted knife fish (X2) and seven-striped carp (X3) for producing mixed-fish sausage in this experiment was varied as shown in the mixture design chart in Table 1. Ten mixed-fish formulations of fish sausage were produced as described in 2.1 above. In addition, three single-fish formulations were prepared in FS as controls (FS1- FS3).
Table 1. The formula of fish sausage having different ratios of fish fillet.

| Sample | Ratio of flesh fish (%) | Quantity (g/100 g) |
|--------|-------------------------|--------------------|
|        | X1 | X2 | X3 | X1 | X2 |
| FS 1   | 1  | 0  | 0  | 71.22 | 0 |
| FS 2   | 0  | 1  | 0  | 0 | 71.22 |
| FS 3   | 0  | 0  | 1  | 0 | 0 |
| FS 4   | 0.5 | 0.5 | 0 | 35.61 | 35.61 |
| FS 5   | 0.5 | 0 | 0.5 | 35.61 | 0 |
| *FS 6  | 0 | 0.5 | 0.5 | 0 | 35.61 |
| FS 7   | 0.33 | 0.33 | 0.33 | 23.74 | 23.74 |
| FS 8   | 0.66 | 0.16 | 0.16 | 47.48 | 11.87 |
| FS 9   | 0.16 | 0.66 | 0.16 | 11.87 | 47.48 |
| FS 10  | 0.16 | 0.16 | 0.66 | 11.87 | 11.87 |

*FS 6 is a control.

2.3. Effect of egg white addition on the chemical and physical properties of mixed-fish sausage

Chicken eggs were purchased from a retail store in Nonthaburi, Thailand. The eggs were cleaned with tap water and separated into egg yolk and egg white. Only the fresh egg white was used in this experiment. The FS (fish sausage) formula having the highest results in terms of emulsion stability and water holding capacity (as described in 2.2 above) was selected for further study of the effect of addition of egg white to the sausage. Three concentrations (1%, 2%, and 3% (w/w)) of fresh egg white were added and labeled as EW1, EW2 and EW3, as shown in Table 2.

Table 2. Quantity of all ingredients used in mixed-fish sausage with egg white.

| Ingredient                      | Quantity (g/100 g) | Control | EW1 | EW2 | EW3 |
|---------------------------------|--------------------|---------|-----|-----|-----|
| Egg white                       | 0                  | 2       | 1   | 2   | 3   |
| Salmon                          | 23.74              | 23.50   | 23.27 | 23.03 |
| Spotted knife fish              | 23.74              | 23.50   | 23.27 | 23.03 |
| Seven stripped carp             | 23.74              | 23.50   | 23.27 | 23.03 |
| Pork back-fat                   | 17.81              | 17.63   | 17.45 | 17.28 |
| Ice                             | 7.33               | 7.26    | 7.18  | 7.11  |
| Fermented soy sauce             | 1.07               | 1.06    | 1.05  | 1.04  |
| Salt                            | 0.85               | 0.84    | 0.83  | 0.82  |
| Fish sauce                      | 0.71               | 0.70    | 0.69  | 0.68  |
| Sugar                           | 0.71               | 0.70    | 0.69  | 0.68  |
| Sodium Tripolyphosphate         | 0.29               | 0.29    | 0.28  | 0.28  |
| Sodium nitrite                  | 0.00125            | 0.00125 | 0.00125 | 0.00125 |

2.4. Physicochemical determination
The color of fish sausage was determined using the CIE system and reported as L* value (lightness), a* value (redness/greenness), and b* value (yellowness/blueness), using a Hunter Lab colorimeter [9]. Texture profile analysis (TPA) was performed using a TA-XT2 texture analyzer (TA.XT2 Stable Micro System, UK), as described by Zakaria (2018) [10] with some modification. Sausage samples were cut into a cylindrical shape (20 mm height × 25 mm diameter) in order to fit on the platform. A compression probe was used (P/6). Calibration of the load cell was also performed. The TPA textural parameters were as follows: pre-test speed 1 mm/s; test speed of 1 mm/s; and post test speed of 1 mm/s. The settings for probe height calibration were as follows: 30 mm distance speed; 8 mm/s return speed; and 5 g contact force. The samples were then placed on the center of the platform and the analysis was run with 75% of strain. The texture profile results in terms of hardness, springiness, cohesiveness, gumminess and chewiness were monitored. The emulsion stability (ES) was determined by centrifugation of samples obtained after the emulsification step at 4,000 rpm for 3 minutes at 25 °C and was calculated as described by Huang et al. (2001) [10]. Cooking loss was measured according to the method of Gnanasambanbam and Zayas (1992) [11], as follows: 10 g of fish sausage was placed in a centrifuge tube and heated at 80°C for 30 min; after cooling to room temperature, the weight of the drained sample was measured; finally, the percentage of cooking loss was calculated from the weight difference between the unheated and heated sample. Water holding capacity (WHC) of samples was measured using the method of Motohiro (1981) [12], as follows: 10 g of fish sausage sample was placed in a centrifuge tube and centrifuged at 10,500 rpm for 15 minutes at 15 °C; the WHC was then expressed as percentage differences of the weight calculated from the sample before and after centrifugation.

2.5. Statistical analyses
All results were expressed as mean ± standard deviation. Results were analyzed by one-way ANOVA. Mean values were tested with Duncan’s test for paired comparison, with a significance level of α = 0.05, using the SPSS statistics software version 22.

3. Result and discussions
3.1. Quality of mixed-fish sausages
The fish sausage can be produced by mixing together different fish species (salmon, spotted knife fish, and seven-striped carp). Color results of all mixed fish sausages had the value of L* (68.42-74.66), a* (1.64-8.70), b* (12.97-18.82), hº (63.14-83.59), and chroma (13.64-20.94) as shown in Table 3.

Table 3. Effect of different fish types and ratio in mixed-fish sausages on color.

| Samples | Lightness (L*) | Redness (a*) | Yellowness (b*) | Hue angle (hº) | Chroma (c*) |
|---------|---------------|--------------|----------------|--------------|-------------|
| FS 1    | 74.66±0.76a   | 8.70±0.54abc | 18.62±0.50b    | 65.91±0.34de | 20.94±0.41a |
| FS 2    | 69.05±0.62c   | 6.13±0.41e   | 12.81±0.68c    | 65.37±0.81c  | 14.69±0.40f |
| FS 3    | 66.93±0.38f   | 1.64±0.15f   | 15.37±0.38c    | 83.59±0.36a  | 15.71±0.14e |
| FS 4    | 72.86±0.69bc  | 8.27±0.19a   | 16.45±0.14b    | 63.14±0.14f  | 18.54±0.09b |
| FS 5    | 72.80±0.69bc  | 6.69±0.20b   | 16.35±0.15b    | 66.59±0.15d  | 17.78±0.08c |
| FS 6    | 71.68±0.78d   | 4.20±0.09e   | 12.97±0.69c    | 72.25±0.11b  | 13.64±0.30d |
| FS 7    | 72.05±0.85ed  | 5.28±0.14d   | 14.60±0.26d    | 70.44±0.02c  | 15.47±0.07e |
The results in terms of hardness, springiness, cohesiveness, gumminess, and chewiness of all mixed-fish sausages were in the range of 105.11-200.06 g, 0.93-0.98 sec, 0.47-0.59, 45.8-109.73 g, and 42.97-106.12 g\cdot sec, respectively. The highest value of all texture characteristics was found in the FS7 formulation, as shown in Table 4.

The emulsion stability (ES) of all FSs did not show any significant differences (p<0.05) (Table 5), with values of ES in the range of 96.26-97.57%. The water holding capacity determines the protein quality that can bind or retain inherent water in the structure. The formulation FS 7 (salmon, spotted knife fish and seven-striped carp in the ratio of 0.33, 0.33, 0.33% (w/w)) showed the highest water holding capacity at 86.37±0.38%. After cooking, the quality of sausage emulsion was determined by the cooking loss. The eating quality is related to cooking loss; in other words, it should be reduced by the high percentage of cooking loss, thereby reducing product satisfaction. Results showed the values of cooking loss of all samples were significantly different (p<0.05). However, the formula FS7 had the lowest percentage of cooking loss. According to the results of WHC and cooking loss compared to all of the developed formulation, the FS7 formulation was chosen for improving the texture of mixed-fish sausage, as shown in Table 5.

3.2. Effect of egg white addition on the chemical and physical properties of mixed-fish sausage

Egg white is generally used as a binder in food products. The addition of egg white changes the physico-chemical properties of mixed-fish sausage. The moisture content of egg white was 87.72 ± 0.22%. Due to this high-water content in the fresh egg white, increasing egg white from 1% to 3% in the mixed-fish sausage

| Samples | Hardness (g) | Springiness (sec) | Cohesiveness | Gumminess (g) |
|---------|--------------|------------------|--------------|---------------|
| FS 1    | 163.39±0.40d | 0.95±0.00c       | 0.53±0.01d   | 95.77±0.14d   |
| FS 2    | 179.29±0.80c | 0.98±0.01ab      | 0.55±0.01ab  | 102.39±0.98c  |
| FS 3    | 128.90±0.56b | 0.99±0.00a       | 0.54±0.02d   | 54.94±0.27b   |
| FS 4    | 196.69±0.59b | 0.97±0.01bc      | 0.55±0.01cd  | 103.56±0.56b  |
| FS 5    | 146.01±0.60f | 0.98±0.01ab      | 0.58±0.01ab  | 78.30±0.85f   |
| FS 6    | 200.06±0.69a | 0.93±0.03d       | 0.59±0.03a   | 109.73±0.56a  |
| FS 8    | 137.72±0.86f | 0.97±0.00abc     | 0.48±0.01e   | 69.01±0.24f   |
| FS 9    | 155.78±0.77c | 0.98±0.00ab      | 0.57±0.01abc | 88.56±0.86c   |
| FS 10   | 105.11±0.47l | 0.97±0.02abc     | 0.47±0.01e   | 45.84±0.61l   |

*FS6 is a control.
*Values with the same superscript (a-i) within columns are not significantly different (p>0.05).
FS7 formulation slightly increased both L* and h°, as shown in Table 6, but did not affect the hardness values of the mixed-fish sausage. However, the texture of EW1 showed the highest values for hardness (265.92 g), springiness (0.95 sec), cohesiveness (0.59), gumminess (95.60 g), and chewiness (98.47 g•sec), as shown in Table 7.

Table 5. Effect of different fish types and ratio in mixed-fish sausages on moisture contents, emulsion stability, water holding capacity, cooking loss, and pH value.

| Samples | Moisture (%) | Emulsion stability (%) | Water holding capacity (%) | Cooking loss (%) | pH Value |
|---------|--------------|------------------------|---------------------------|------------------|-----------|
| Control | 74.19±0.53a  | 97.04±0.55             | 77.39±0.87d              | 9.62±0.11c       | 6.86±0.00b |
| FS 1    | 58.32±0.74f  | 96.81±0.92             | 79.74±0.34c              | 8.26±0.79d       | 6.54±0.01h |
| FS 2    | 71.48±0.87b  | 97.42±0.99             | 82.34±0.92b              | 7.11±0.74c       | 6.74±0.15s |
| FS 3    | 67.03±0.73d  | 97.38±0.64             | 78.79±0.40c              | 8.78±0.54c       | 6.82±0.10d |
| FS 4    | 67.51±0.62d  | 96.30±0.95             | 81.95±0.36b              | 6.85±0.65b       | 6.81±0.00c |
| FS 5    | 63.78±0.40c  | 96.26±0.67             | 82.02±0.41b              | 4.92±0.64a       | 6.83±0.00c |
| FS 6    | 74.19±0.53a  | 97.04±0.55             | 77.39±0.87d              | 9.62±0.11c       | 6.86±0.00b |
| FS 7    | 68.19±0.67d  | 97.15±0.60             | 86.37±0.38ab             | 4.63±0.37a       | 6.82±0.00d |
| FS 8    | 67.14±0.86f  | 97.51±0.27             | 78.19±0.09cd             | 9.29±0.57bc      | 6.79±0.00f |
| FS 9    | 67.75±0.52d  | 96.56±0.68             | 83.40±0.40b              | 8.31±0.74cd      | 6.78±0.01f |
| FS 10   | 69.53±0.86c  | 96.50±0.66             | 77.14±0.97d              | 9.00±0.59bc      | 6.87±0.00a |

*FS6 is a control.
*Values with the same superscript (a-f) within columns are not significantly different (p>0.05).

Table 6. Effect of egg white in mixed-fish sausages on color.

| Samples | Lightness (L°) | Redness (a°) | Yellowness (b°) | Hue angle (h°) | Chroma (c°) |
|---------|----------------|--------------|-----------------|----------------|-------------|
| Control | 67.79±0.59d    | 5.87±0.31d   | 14.71±0.46d     | 68.26±0.60a    | 15.84±0.53d |
| EW 1    | 68.60±0.79c    | 10.97±0.20a  | 17.38±0.39a     | 57.70±0.43b    | 20.56±0.41a |
| EW 2    | 70.81±0.36b    | 10.36±0.48b  | 16.35±0.36a     | 57.67±0.70b    | 19.36±0.56b |
| EW 3    | 71.97±0.38a    | 9.71±0.37a   | 16.20±0.48a     | 58.96±0.46a    | 18.73±0.37a |

*Values with the same superscript (a-d) within columns are not significantly different (p>0.05).

Table 7. Effect of egg white ratio in mixed fish sausages on texture profile.

| Samples | Hardness (g) | Springiness (sec) | Cohesiveness | Gumminess (g) |
|---------|--------------|------------------|--------------|---------------|
| Control | 201.02±0.55b | 0.93±0.09a       | 0.44±0.05b   | 89.59±0.93c   |
| EW 1    | 265.92±0.52a | 0.95±0.02a       | 0.59±0.05a   | 95.60±0.94a   |
| EW 2    | 265.49±0.93a | 0.93±0.02a       | 0.54±0.04a   | 93.06±0.81b   |
| EW 3    | 265.40±0.71a | 0.90±0.03a       | 0.54±0.02a   | 93.34±0.85b   |

*Values with the same superscript (a-d) within columns are not significantly different (p>0.05).
The addition of egg white at all levels increased the emulsion stability. Results of WHC of mixed-fish sausage gradually decreased with increased amounts of added of egg white. Increased egg white amounts changed the percentage of cooking loss. When the ovalbumin protein and fish protein were heated, sulfur containing amino acid formed a disulfide bond and conjugated with the hydrophilic group of fish protein, turning into a three-dimensional structure. Thus, this phenomenon helps to stabilize the protein structure [13]. During heating, gelation of egg white and mixed-fish emulsion occurred, resulting in adhesion between the egg white protein and the fish protein. As a result, the gelation of the sausage became brittle and easily breakable. The higher quantity of egg white reduced the appearance and softened the texture of fish sausage, due to the fresh egg white having a higher moisture content than fish fillet. Based on the results in terms of hardness, emulsion stability, cooking loss, and water holding capacity, the EW1 formulation, with 1% egg white in mixed-fish sausage, showed the proper characteristics of fish sausage as shown in Table 8.

Table 8. Effect of egg white in mixed fish sausages on physicochemical properties.

| Samples | Moisture (%) | Emulsion stability (%) | Water holding capacity (%) | Cooking loss (%) | pH Value |
|---------|--------------|------------------------|---------------------------|-----------------|----------|
| Control | 67.81±0.39c  | 99.79±0.05b            | 84.30±0.48a               | 1.96±0.19a      | 6.89±0.00b |
| EW 1    | 68.55±0.39b  | 99.90±0.04a            | 81.97±0.86b               | 1.42±0.45b      | 6.87±0.00d |
| EW 2    | 68.47±0.69b  | 99.85±0.05ab           | 81.10±0.81bc              | 1.49±0.26b      | 6.89±0.00e |
| EW 3    | 69.37±0.20a  | 99.83±0.07ab           | 80.53±0.12c               | 1.52±0.24b      | 6.91±0.00a |

*Values with the same superscript (a-d) within columns are not significantly different (p>0.05).

4. Conclusions
The mixed-fish sausages of formulation FS7, with salmon, spotted knife fish and seven striped carp at the ratio of 0.33:0.33:0.33% (w/w), had the highest water holding capacity (86.37%), but lowest cooking loss (4.63%). Egg white was chosen to improve the texture property of FS7 mixed-fish sausage. The addition of egg white at 1% to FS7 fish sausage increased the moisture content but reduced the water holding capacity and cooking loss. Therefore, in this experiment, the maximum usage of egg white in mixed-fish sausage was not greater than 1%. Dried egg white could be further studied for its effect on the texture properties of mixed-fish sausage.

Acknowledgement
The research was funded by Thailand institute of scientific and technology research (/6116301010 and graduate college, King Mongkut’s University of Technology North Bangkok. The authors would like to thank Mr. Peter Blumenfeld for proof reading.

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