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FULL PAPER

Optimization of enzymatic hydrolysis of boso fish (*Oxyeleotris marmorata*) protein based on the degree of hydrolysis and the physical properties of the resultant hydrolysates

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Abstract

Fish protein hydrolysates (FPHs) were produced from boso fish (*Oxyeleotris marmorata*) through enzymatic hydrolysis using papain as the hydrolysis agent. Papain concentration (0.2–0.4%), temperature (45–55°C), and time (4–12 hrs) were chosen as the independent factors while the degree of hydrolysis (DH), viscosity, and turbidity of the resultant FPHs were recorded as the responses. This study aimed to optimize the enzymatic hydrolysis of boso fish protein using the Box-Behnken Response Surface Methodology (RSM) design. The optimum condition for the boso fish protein hydrolysis based on the DH was predicted at 0.2% of papain concentration, 55°C, and 12 hrs, which would give a DH of 18.63% and an R^2 of 90.90%. The FPHs prepared with moderate hydrolysis conditions (0.3% of papain concentration at 50°C for 8 hrs) were spray-dried with the yield ranging from 5.37% to 16.70% (w/w). The spray-dried FPH displayed a globular structure with a diameter of 1–7 μm as observed under the scanning electron microscope (SEM). The total colour difference of the dried FPH was 10.15 compared to the standard white tile. The particle size analysis in distilled water exhibited some distinct peaks which were distributed within 200–1,100 nm with an intensity of 18.1%. This study showed the potential of boso fish for FPH production by using the papain enzyme.

1. Introduction

Oxyeleotris marmorata, a member of *Eleotrida* family, is locally known as betutu or boso fish. This fish can be found in tropical and subtropical oceans. The distribution of this species covers the Mekong Delta and the Chao Phraya Basin, Malaysia, Indochina, Thailand, Cambodia, Vietnam, Singapore, Philippines, and Indonesia (Lestari *et al*., 2019). Boso fish species are abundant and cheap that can be found on the North Beach of the Java Sea, Indonesia (Priatni *et al.* 2017). Marine fishes are the common materials for producing FPHs through hydrolysis, which results in short-chained and easily digested peptides. In order to increase the efficiency of fish processing, by-products or underutilized fish can be used for producing FPHs. A good strategy is needed to increase the economic value of this low-quality fish, particularly with the consideration to process these by-products into high-value products (Wisuthiphaet and Kongruang, 2015). Fish protein hydrolysate is one of the fish products that have much attention by food technologists because of the availability sustainability of raw material with high protein content and containing amino acids essential and bioactive peptides (Khora S., 2013).

FPHs can be produced through either chemical or enzymatic hydrolysis. The enzymatic hydrolysis is more efficient and cheaper and the resultant product contains more essential amino acids compared to the counterpart produced through the chemical hydrolysis (Bernadeta *et al*., 2012). Several techniques have been developed for the production of FPHs, i.e., enzymatic hydrolysis, autolysis, and thermal hydrolysis. Enzymatic hydrolysis is the common method with various enzymes to be chosen (Halim *et al.*, 2016). The enzymatic protein hydrolysis is influenced by some factors, e.g., enzyme concentration, pH, time, and temperature (Halim *et al.*, 2016). Hassan *et al.* (2019) reported the properties of spray-dried visceral protein hydrolysates which were prepared through either enzymatic or chemical hydrolysis of *Pangasianodon hypophthalmus*. Spray-

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dried hydrolysates obtained through enzymatic hydrolysis with either pepsin or papain displayed higher yield (5.6–5.8%), DH (61.00–65.16%), protein content (78.14–82.55%), and lightness (64.82–78.62) compared to those obtained through chemical hydrolysis with either acid or alkali (yield = 3.3–4.76 %, DH = 15.38–60.84%, protein content = 57.86–69.13%, lightness = 57.62–58.94).

DH is a parameter to evaluate the hydrolyzation process and is correlated with the functional properties of protein hydrolysates (Wisuthiphaet and Kongruang, 2015). There are several approaches for determining the DH of protein hydrolysates, e.g., formol titration, measurement of soluble protein content, osmometry, and ortophtalaldehyde assay (Morais *et al.*, 2013). In addition, RSM was often used as a tool to optimize hydrolysis conditions (Zhang *et al.*, 2017, Hema *et al.*, 2017). This study aimed to optimize the enzymatic hydrolysis of boso fish protein based on the DH and the physical properties of the resultant FPHs. The optimization of hydrolysis conditions was done using Box-Behnken RSM design. This method is used to generate higher-order response surfaces, it requires fewer runs compared to the normal factorial technique (Rao and Kumar, 2012).

2. Material and methods

Boso fish was obtained from a fish market at the north beach of Indramayu City, West Java, Indonesia. Boso fish was frozen at -20°C until further usage. A commercial papain enzyme (CAS no. 2323.627-2, Xian Arisun Chem Pharm Co. Ltd, Shaanxi, China) was purchased through an online market. All chemicals used in this study were analytical grade.

2.2 Protein hydrolysis

The hydrolysis of boso fish was performed following a method previously described in Priatni *et al*. (2017). The frozen boso fish was thawed, mixed with distilled water with a ratio of 1:4, and homogenized by using a blender. The pH of the fish-water mixture was adjusted to 6.0 using 1 N HCl solution. The hydrolysis process was performed using papain enzyme at a concentration of 0.2–0.4% and temperature of 45–55°C for 4–12 hrs in a water bath. The enzymatic hydrolysis was stopped by heating the hydrolysate at 85°C for 15 mins. Protein hydrolysate was vacuum-filtered and the filtrate was stored at -20°C. The DH, turbidity, and viscosity assessments were performed on liquid hydrolysates. Particle size measurement and morphology analysis were done on spray-dried hydrolysates. For these purposes, the filtrate was spray-dried (Model 290 Mini Spray Dryer, Buchi, Postfach, Flawil, Switzerland) at 180°C (inlet) and 95°C (outlet).

2.3 Optimization of protein hydrolysis through Box-Behnken RSM

The optimization of protein hydrolysis was conducted following a method by Unnikrishnan *et al*. (2020) with a modification. Hydrolysis variables were optimized using RSM and the Box-Behnken design was employed in this regard. The range and centre point values of three independent variables were presented in Table 1. First-order regression models were fitted to the responses as a function of input variables using Equation 1.

$$Y = \beta_o + \beta_i X_i + \beta_{ij} X_i X_{ij}\,; i \neq j = 1, 2, 3 \quad (1)$$

Where Y = response, βo = offset term, βi, βij = regression coefficients, and Xi, Xj = the levels of independent variables

Table 1. The independent variables of the boso fish hydrolysis and the experimental design levels for the response surface analysis

Design levels	Independent variables		
	Temperature, °C (X_1)	Time, hrs (X_2)	Papain concentration, % (X_3)
-1	45	4	0.2
0	50	8	0.3
1	55	12	0.4

Minitab 19 software (Minitab LLC, State College, PA, USA) was used for the experimental design. The significance of regression coefficients was set at a 5% level.

After the optimum condition was obtained, it was used to run a hydrolysis experiment and the responses were recorded. The observed and the predicted values of the responses were later compared.

2.4 Degree of hydrolysis

The DH of the fish hydrolysate was determined according to the method of Morais *et al*. (2013) with a modification. Hydrolysate samples (500 µL) were mixed with 500 µL of 20% trichloroacetic acid (TCA) solution to obtain the soluble and insoluble fractions in 10% TCA. After 30 mins of rest, the mixture was centrifuged for 15 mins at 3,000×*g*. The soluble protein content of the supernatant was analyzed using the Lowry method with bovine serum albumin (BSA) as the standard. DH was calculated using the relationship between soluble protein and total protein content (Equation 2).

$$\mathrm{DH}(\%) = \frac{\text{soluble protein content in 10\% TCA (mg)}}{\text{total protein content (mg)}} \times 100 \quad (2)$$

2.5 Viscosity measurement

The viscosity of the liquid FSH was determined

according to the Rosli and Sarbon method (2015) with a modification using a viscometer (DV1 Digital Viscometer, Brookfield Ametek, Middleboro, MA, USA) which was equipped with a spindle (S61) at 100 rpm. The viscosity was determined at room temperature and the reading was taken in triplicate.

2.6 Turbidity measurement

The turbidity of the liquid FSH was determined according to the method of Hassan *et al*. (2019) with a modification. For that purpose, the absorbance of the sample at 410 nm was recorded using a UV-Vis spectrophotometer (U-2800, Hitachi High Tech Science Corporation, Minato-ku, Tokyo, Japan). The turbidity was calculated following Equation 3.

$$T = \frac{2.303\,A}{l} \quad (3)$$

Where T = turbidity, A = absorbance, l = path length of the cuvette

2.7 Microstructure Analysis

The microstructure of the spray-dried FSH was analyzed using a scanning electron microscope (SEM, JSM-IT30, Jeol Ltd., Akhishima, Tokyo, Japan). The sample was placed in a specimen holder and then coated with a thin layer of gold (10 nm). The analyses were conducted using an accelerating voltage of 20 kV.

2.8 Colour measurement

The colour of the spray-dried FPH was measured by using a chromameter (CR 410, Minolta Corp., Osaka City, Osaka, Japan) with the standard white tile. The colour was recorded as the CIELab tristimulus colour coordinates, i.e., L^* (lightness), a^* (green to red coordinate), and b^* (blue to yellow coordinate). Calculations were done to obtain the total colour difference (Pathare *et al.*, 2013).

$$\Delta E^* = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \quad (4)$$

Where ΔE^* = total color difference, ΔL^* = difference in lightness, Δa^* = difference in green-red coordinate, and Δb^* = difference in blue-yellow coordinate

2.9 Particle size distribution

The particle size distribution of the spray-dried FPH was determined using a particle size analyzer (PSA, Zetasizer Nano ZS, Malvern PANalytical, Worcestershire, UK). The sample was dispersed (1% w/v) (John *et al.*, 2018) in either deionized water or 0.9% NaCl solution and centrifuged for 30 mins at 2,300×*g*.

3. Results and discussion

3.1 Optimization of enzymatic hydrolysis condition by response surface methodology

RSM was used to optimize the enzymatic hydrolysis conditions in the production of boso FPH. A total of 15 runs were executed based on Box-Behnken RSM design. The hydrolysis conditions and their responses were presented in Table 2. The DH of the FPHs ranged from 5.09% to 16.67%. The DH was possibly affected by the fish part, the fish species, and the protease enzymes used in hydrolysis (Amiza *et al*., 2011). The viscosity of FPHs ranged from 2.58 cP to 4.62 cP and the turbidity ranged from 0.316 T to 0.479 T. The hydrolysis condition may affect the molecular structure and the functional properties of the resultant peptides (Moscoco *et al*., 2015). Hoa *et al*. (2015) reported that a temperature increase from 45°C to 55°C during enzymatic hydrolysis resulted in a viscosity decrease of the hydrolysate from 3.187 cP to 2.383 cP. The viscosity, however, increased when the hydrolysis was conducted above 55°C.

The result of the analysis of variances (ANOVA) of the DH data from 15 runs was presented in Table 3. The result showed that both linear and square time (X_2 and X_2 X_2, respectively) significantly (p<0.05) affected the DH of the resultant FPHs. Based on the DH, the optimum condition was reached at 55°C for 12 hrs with a papain concentration of 0.2%. The R^2 value was 0.9090 with a predicted DH of 18.63%. DH is an important parameter for understanding the influence of the hydrolysis conditions in relationship to proteolytic activity and peptide properties (Morais *et al*., 2013). The degree of hydrolysis is the percentage ratio of the number of broken peptide bonds to the number of peptide bonds of protein (Halim and Sarbon, 2017). The optimum conditions for protein hydrolysis were influenced by raw materials. The boso FPH in this study displayed a higher DH than the one prepared from silver carp by-products (max. 4.9%) (Fallah *et al*. 2015). Wisuthiphaet and Kongruang (2015) reported that DH increased with increasing hydrolysis time and was influenced by enzyme concentration. However, they observed that hydrolysis for 15 hrs resulted in DH of 20–24% with no significant effect on enzyme concentrations (2–6%). The 3-dimensional graph showed that the DH generally increased with increasing either temperature or hydrolysis time (Figure 1a). In a previous study, an optimization of FPH production from swamp eel using papain was performed. The previous optimum condition was achieved by using 0.49% of papain concentration at 45°C for 9 hrs with a predicted DH of 7.96% (Priatni *et al.*, 2020). Thus, the optimum boso fish hydrolysis required a smaller papain concentration, a higher temperature, and a longer time, compared to the

Table 2. The hydrolysis conditions and their responses (DH, viscosity, and turbidity)

Run	Hydrolysis conditions			Responses		
	Temperature (°C)	Time (hrs)	Papain Concentration (%)	DH (%)	Viscosity (cP)	Turbidity (T)
1	50	8	0.3	5.38	3.65	0.426
2	45	8	0.4	5.09	3.54	0.426
3	50	8	0.3	7.46	3.66	0.329
4	45	12	0.3	16.67	2.58	0.449
5	55	8	0.4	7.62	3.54	0.327
6	50	4	0.4	6.93	6.66	0.325
7	50	12	0.2	16.10	4.62	0.474
8	50	8	0.3	6.60	3.96	0.345
9	45	8	0.2	10.01	3.78	0.447
10	50	12	0.4	15.03	2.82	0.479
11	55	12	0.3	14.56	3.00	0.463
12	55	4	0.3	8.95	5.22	0.394
13	45	4	0.3	8.88	3.84	0.316
14	50	4	0.2	7.93	4.02	0.336
15	55	8	0.2	13.39	4.14	0.385

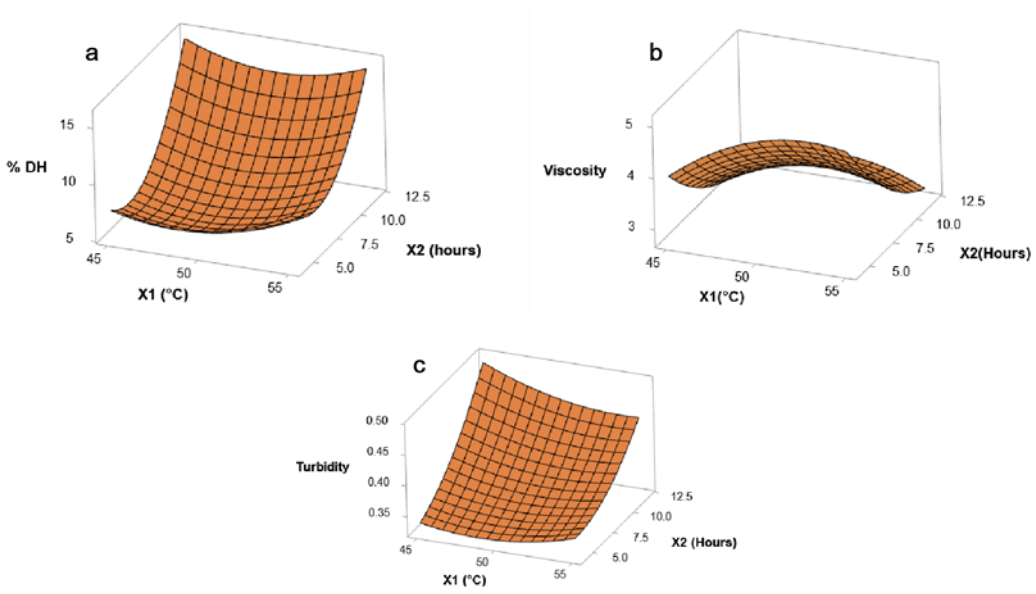

Figure 1. The 3-dimensional surface plot of the interactions between temperature (X_1) and time (X_2) for each response, i.e., DH (a), viscosity (b), and turbidity (c) of the boso FPH.

optimum condition for the swamp eel hydrolysis. Based on Table 3, the final equation (Equation 5) which shows the correlation between factors to the DH could be generated.

$$DH(\%) = 172 - 6.2\,X_1 - 1.83\,X_2 - 48\,X_3 + 0.0664\,{X_1}^2 + 0.2581\,{X_2}^2 + 89\,{X_3}^2 - 0.0272\,X_1X_2 - 0.42\,X_1X_3 - 0.04\,X_2X_3 \quad (5)$$

Phuong *et al*. (2017) reported a 10–25% increase in DH when the formic acid was increased from 3% to 6% w/w during the 6–30 hrs hydrolysis of black tiger shrimp heads. This proved that organic acids cut the peptide bonds during protein hydrolysis.

The viscosity of FPH is an important dependent variable for monitoring hydrolysis. The ANOVA showed that time (X_2) and interaction ($X_2\,X_3$) between time and papain concentration affected the viscosity significantly (p<0.05) (Table 4). Figure 1b showed that the viscosity of the boso FPH decreased after 12 hrs of enzymatic hydrolysis. The optimal condition in this study was at 0.4% papain concentration and 55°C for 12 hrs with the predicted viscosity of 2.08 cP. The fitted models displayed an excellent determination coefficient (R^2 = 0.9515) for the DH. A model with an R^2 greater than 0.80 is considered a well-fitted model with an adequate data variation (Rasimi *et al*., 2020). During hydrolysis, papain cleaved large peptides into smaller peptides and changed the hydrophobic and hydrophilic properties of proteins. Therefore, enzymatic hydrolysis could decrease the apparent viscosity of the resultant hydrolysate which affected its functional properties (Bajaj *et al*., 2017). According to Jeewanthi *et al.* (2015), the functional properties of protein hydrolysate will depend on the

Table 3. Analysis of variance (ANOVA) of the DH data

Source	DF	Adj SS	Adj MS	F-Value	*p*-Value	
Model	9	204.136	22.682	5.55	0.037	significant
Linear	3	132.290	44.097	10.79	0.013	
X_1 (°C)	1	1.863	1.863	0.46	0.530	
X_2 (h)	1	110.069	110.069	26.94	0.003	
X_3 (%)	1	20.358	20.358	4.98	0.076	
Square	3	70.484	23.495	5.75	0.045	
X_1 (°C) X_1 (°C)	1	10.177	10.177	2.49	0.175	
X_2 (h) X_2 (h)	1	62.947	62.947	15.41	0.011	
X_3 (%) X_3 (%)	1	2.915	2.915	0.71	0.437	
2-Way Interaction	3	1.361	0.454	0.11	0.950	
X_1 (°C) X_2 (h)	1	1.183	1.183	0.29	0.614	
X_1 (°C) X_3 (%)	1	0.178	0.178	0.04	0.843	
X_2 (h) X_3 (%)	1	0.001	0.001	0.00	0.987	
Error	5	20.430	4.086			
Lack-of-Fit	3	18.233	6.078	5.53	0.157	not significant
Pure Error	2	2.196	1.098			
Total	14	224.565				

Table 4. Analysis of variance (ANOVA) of the viscosity data

Source	DF	Adj SS	Adj MS	F-Value	*p*-Value	
Model	9	13.3701	1.48557	10.90	0.009	significant
Linear	3	6.2280	2.07600	15.24	0.006	
X_1 (°C)	1	0.5832	0.58320	4.28	0.093	
X_2 (h)	1	5.6448	5.64480	41.43	0.001	
X_3 (%)	1	0.0000	0.00000	0.00	1.000	
Square	3	1.9509	0.65030	4.77	0.063	
X_1 (°C) X_1 (°C)	1	0.7094	0.70943	5.21	0.071	
X_2 (h) X_2 (h)	1	0.4310	0.43103	3.16	0.135	
X_3 (%) X_3(%)	1	0.6880	0.68801	5.05	0.075	
2-Way Interaction	3	5.1912	1.73040	12.70	0.009	
X_1 (°C) X_2 (h)	1	0.2304	0.23040	1.69	0.250	
X_1 (°C) X_3 (%)	1	0.0324	0.03240	0.24	0.646	
X_2 (h) X_3 (%)	1	4.9284	4.92840	36.17	0.002	
Error	5	0.6813	0.13625			
Lack-of-Fit	3	0.6192	0.20640	6.65	0.133	not significant
Pure Error	2	0.0621	0.03103			
Total	14	14.0514				

Table 5. Analysis of variance (ANOVA) of the turbidity data

Source	DF	Adj SS	Adj MS	F-Value	*p*-Value	
Model	9	0.036577	0.004064	1.35	0.389	not significant
Linear	3	0.032003	0.010668	3.54	0.104	
X_1 (°C)	1	0.000595	0.000595	0.20	0.676	
X_2 (h)	1	0.030504	0.030504	10.11	0.025	
X_3 (%)	1	0.000903	0.000903	0.30	0.608	
Square	3	0.003144	0.001048	0.35	0.793	
X_1 (°C) X_1 (°C)	1	0.000921	0.000921	0.31	0.604	
X_2 (h) X_2 (h)	1	0.001960	0.001960	0.65	0.457	
X_3 (%) X_3(%)	1	0.000702	0.000702	0.23	0.650	
2-Way Interaction	3	0.001430	0.000477	0.16	0.920	
X_1 (°C) X_2 (h)	1	0.001024	0.001024	0.34	0.585	
X_1 (°C) X_3 (%)	1	0.000342	0.000342	0.11	0.750	
X_2 (h) X_3 (%)	1	0.000064	0.000064	0.02	0.890	
Error	5	0.015088	0.003018			
Lack-of-Fit	3	0.009680	0.003227	1.19	0.486	not significant
Pure Error	2	0.005409	0.002704			
Total	14	0.051665				

enzyme specificity and selectivity, the hydrolysis conditions, i.e. temperature, pH, and time, and also the nature of the protein. According to the data in Table 2, we proposed that papain concentration was important in fish protein hydrolysis as indicated by a decrease in the DH and the viscosity of the resultant FPH as the papain concentration increased. The equation that shows the correlation between independent factors and viscosity is presented (Equation 6).

$$Viscosity\ (cP) = -50 + 1.957\ X_1 + 0.881\ X_2 + 5.3\ X_3 - 0.01753\ X_1{}^2 + 0.0214\ X_2{}^2 + 43.2\ X_3{}^2 - 0.012\ X_1\ X_2 - 0.018\ X_1\ X_3 - 2.775\ X_2 X_3 \quad (6)$$

Turbidity measurement is commonly used in monitoring the anti-aggregation of protein. In a previous study by Rifai *et al.* (2020), the monitoring of turbidity, however, was used to follow the aggregation of BSA as induced by dithiothreitol (DTT). The turbidity of the BSA samples increased as the DTT-induced aggregation continued (Rifai *et al*., 2020). The ANOVA results for the current turbidity data are presented in Table 5. Although the model was not significant (p>0.05), time (X_2) was significantly correlated with turbidity (p<0.05) with an R^2 of 0.7080. Data analysis by RSM showed that the optimum condition of boso FPH production was obtained by using 0.35% of papain concentration and 50°C for 4 hrs, which gave predicted turbidity of 0.324. Figure 1c also show that the turbidity increased after 12 hrs of enzymatic hydrolysis. The turbidity of boso FPH increased after 4 hrs of hydrolysis and this was anticipated due to the aggregation during the hydrolysis. Protein aggregation occurred due to chemical modification of protein including hydrolysis, deamidation, isomerization, and oxidation (Mahler *et al*., 2009). The correlation between independent factors and turbidity was presented (Equation 7).

$$Turbidity\ (T) = 1.58 - 0.053\ X_1 + 0.0294\ X_2 - 0.09\ X_3 + 0.00063\ X_1{}^2 + 0.00144\ X_2{}^2 + 1.38\ X_3{}^2 - 0.0008\ X_1\ X_2 - 0.0185\ X_1\ X_3 + 0.01\ X_2 X_3 \quad (7)$$

The optimum hydrolysis conditions based on each response and their desirability as obtained by RSM are presented in Table 6. According to the desirability, the recommended hydrolysis condition for the production of boso FPH was 0.2% of papain concentration and 55°C for 12 hrs. The verification was later conducted experimentally using this optimized condition. The observed DH value was 19.26%, which was slightly higher than the predicted DH (18.63%).

3.2 The yield of the spray-dried boso fish protein hydrolysates

The spray-dried FPH in our study were prepared from Run 1, 3, and 8 which followed the same hydrolysis conditions (0.3% of papain concentration and 50°C for 8 hrs). Their yields ranged from 5.37% to 16.70% w/w. Hassan *et al*. (2019) observed the yield of the dried protein hydrolysate from *Pangasius viscera* which was prepared through enzymatic hydrolysis. A higher yield (5.8%) was obtained using 1% w/w papain, or 0.2% higher than the one produced with 1% w/w pepsin. The yield of protein hydrolysates was also affected by enzyme concentration. A higher enzyme concentration implied more enzyme molecules interact with fish proteins and thus there were more protein molecules released into the system. The drying method also contributed to the yield. Freeze-drying offered a higher yield than spray-drying since almost all components are sublimated in the freeze-drying method and a very small quantity was left as residual in the drying machine (Hau *et al*., 2018).

3.3. Morphology, colour, and particle size distribution of the spray-dried hydrolysates

The morphology of powdered protein hydrolysate is important for determining its functional properties. The spray-dried boso FPH showed predominantly globular structures ranging between 1–7 µm (Figure 2). This result was different from a previous study on spray-dried FPH from yellowfin tuna by Unninkrisnan *et al.* (2020). The microstructure of spray-dried FPH from yellowfin tuna had mainly rupture flake-like structures in the range of 5–12 µm. Protein hydrolysis resulted in low molecular weight peptides that were difficult to form a homogenous powder structure through spray drying. Moreover, particle size would be affected by the inlet and outlet temperatures of the spray drying process (Unninkrisnan *et al.*, 2020). Morphology of the dried protein hydrolysate was consistent with the DH. The DH was influenced by hydrolysis conditions, e.g., protein sources, time, temperature, and enzyme. Therefore, the particle size variations might be due to the protease activity during the hydrolysis process (Arias-Moscoso *et al*., 2015).

The ΔE_{ab} of the spray-dried product was 10.15± 1.04, meaning that the colour of the boso FPH was

Table 6. The recommended optimized conditions based on each responsible for the production of boso FPH

Response base	Hydrolysis conditions			Desirability	Decision
	Temperature (°C)	Time (hrs)	Papain (%)		
DH	55	12	0.2	1	selected
Viscosity	55	12	0.4	1	
Turbidity	49.55	4	0.35	0.95	

distinctively different from that of the standard white tile. According to Pathare *et al.* (2013), the ΔE_{ab} exceeding three is perceived differently and analytically classified as very distinct. In our study, the major contributor of the ΔE_{ab} was the Δb^*. The b^* of the spray-dried hydrolysates was 13.07±1.08 while that of the standard white tile was 3.10±0.00. This implies that the hydrolysates were yellower than the standard white tile. Their differences in a^* and L^* were small enough, 0.91 and 0.21, respectively.

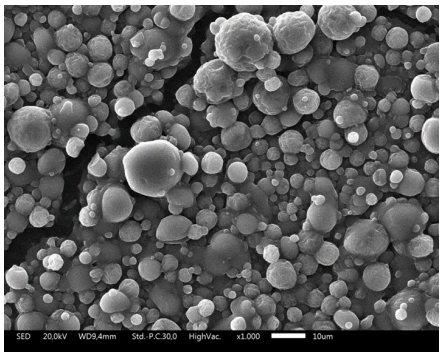

Figure 2. The morphology and the microstructure of the spray-dried boso FPH.

Raw materials and hydrolysis conditions affected the colour of the resultant hydrolysates. The colour of dried hydrolysates is important for industrial applications. Colour affects the overall acceptability of food products and it is influenced by some factors such as treatments, raw materials, and process conditions (Hassan *et al.*, 2019). Enzymatic hydrolysis at a temperature range of 50–60°C obtained the darker colour of the protein hydrolysate. The colour of protein hydrolysates was also related to the pigments and the composition of the raw materials (Sinthusamran *et al.*, 2020).

Particle size and particle distribution are important properties that affect the behaviour and functionality of powder. The observed spray-dried boso FPH displayed two peaks in the particle distribution when it was dispersed in distilled water (Figure 3a). The highest peak ranged from 200 to 1,100 nm with an intensity of 18.1%. Meanwhile, spray-dried boso FPH which was dispersed in 0.90% of NaCl solution exhibited three peaks. Two of them were distinctive with similar intensities between 7% and 7.7% and distributed within 6–105 nm. The particle size of protein was influenced by material sources, protein preparation, and drying methods. Particle size distribution is important information in food protein formulation (John *et al.*, 2018). Beliciu and Moraru (2009) investigated the effect of solvent on the determination of micelle particle size by dynamic light scattering (DLS). The study showed the unsuitability of water as a solvent. A significant dissociation of casein micelles occurred when water was used as the solvent. The study suggested using a solvent with a chemical composition as close as possible to that of the studied protein hydrolysate.

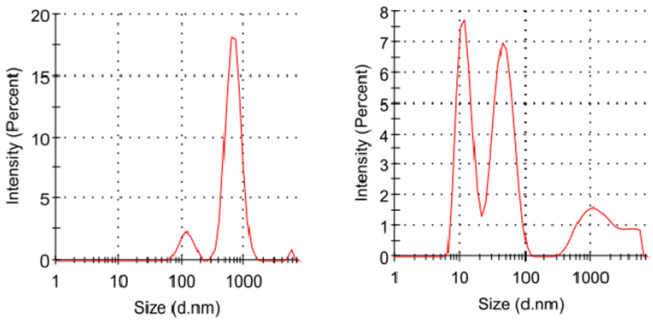

Figure 3. The particle distributions of the spray-dried boso FPH in distilled water (a) and 0.90 % NaCl (b).

4. Conclusion

Boso fish shows its potential as a raw material for FPH production through enzymatic hydrolysis with a low concentration of papain enzyme (0.2%). The DH of boso FPH was influenced by the hydrolysis conditions and significantly (p<0.05) affected by hydrolysis time. The optimization of boso FPH production using Box Behnken RSM showed that the DH correlated with the viscosity of FPH. This study suggested the optimum hydrolysis condition for boso FPH production was 0.2% of papain concentration and 55°C for 12 hrs. The spray-dried boso FPH displayed predominantly globular structures and the size ranged from 1 µm to 7 µm while the total colour difference was 10.15±1.08 compared to the standard white tile.

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