

Evaluation of Bouillon Cube Prepared with the Addition of Threadfin Bream (*Nemipterus japonicas*) Hydrolysate

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ABSTRACT

Threadfin bream was hydrolyzed by enzymatic hydrolysis for 120 min (60°C) at pH 8.5, enzyme:substrate ratio 1:3 using Flavourzyme 500 L to produce the threadfin bream hydrolysate (TBH). Bouillon cubes prepared by incorporating TBH or isolated soy protein (ISP) were then analyzed for physicochemical and functional properties, sensory properties and acceptability. Results showed that bouillon cubes added with TBH contain 23.05% protein and 18.53% fat. Solubility, hardness and fracturability of bouillon cubes containing TBH were better than those incorporated with ISP. SDS-PAGE results indicated the presence of short peptides of <20 kDa especially in bouillon cubes with the addition of TBH. The bouillon cubes had bitter and umami taste with intensities of respectively, 9.88 and 11.70 compared to the reference solutions; 12.10 and 12.01. Moreover, functional group analysis showed the existence of amines and carbonyl group peaks. Based on its binding capacity, TBH can be used as partial ingredient in the development of bouillon cube.

Keywords: Bouillon cube, hydrolysate, taste, threadfin bream

INTRODUCTION

Bouillon, commonly described as broth, is made by cooking meat, poultry, fish, or vegetables and other ingredients such as onion, tomatoes, salt and oil in water (Lukmanji et al., 2008). The broth can be further dehydrated and compressed into flavor-concentrated cubes known as bouillon cubes. The addition of bouillon cubes augments food flavor (Akpanyung, 2005). Apart from monosodium glutamate and salt as the main ingredients, bouillon cubes are also prepared with the addition of fat, starch, and spices (Caponio, Gomes, & Bilancia, 2003). Industrially, bouillon cube processing involves mixing of dry

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ingredients with molten fat before it is cooled, shaped, wrapped and packed (Gupta & Bongers, 2011). Bouillon cubes are used during cooking as it is one of the convenient ways to make variety of food such as condiment, gravies and soup. The commercial bouillon cubes available are chicken, mushroom, pork, and seafood broth cubes (Chiang, Yen, & Mau, 2007).

Protein hydrolysate is a product obtained from the hydrolysis of protein-rich sources (Jin et al., 2014; Klompong, Benjakul, Kantachote, & Shahidi, 2007; Mutilangi, Panyam, & Kilara, 1996). Hydrolysate has been produced from soybean, whey protein, fish, shellfish, chicken, and many others. The characteristics of fish and fish byproduct hydrolysates have been well documented (Chalamaiah, Hemalatha, & Jyothirmayi, 2012; Sathivel et al., 2003; Wiriyaphan, Chitsomboon, & Yongsawadigul, 2012). Isolated soy protein (ISP) is a highly refined and concentrated protein fraction produced from soybean (Crockett & Vodovotz, 2011). It is one of the most common ingredients available in commercial bouillon cubes. ISP has also been used as a binder in meat products such as sausages, beef patties, mortadell, meat ball, and corned beef while protein hydrolysate can be used as milk replacers, protein supplements, beverage stabilizers, and flavor enhancers (Li, Youravong, & Aran, 2010).

Incorporating fish protein hydrolysate may improve bouillon cube functionality in terms of solubility, texture and binding capacity. It could also enhance the flavor and nutritional aspect of the bouillon cube.

The role of fish hydrolysate from threadfin bream (*Nemipterus japonicas*) as a binding agent in forming a good texture of bouillon cubes has never been explored. Besides, no study has been done on the incorporation of threadfin bream hydrolysate (TBH) in the form of bouillon cubes to serve as flavor enhancer. This study was carried out to evaluate the physicochemical, functional, and sensory properties of bouillon cubes added with TBH.

MATERIALS AND METHODS

Materials

The fish, threadfin bream (*Nemipterus japonicas*) and red snapper (*Lutjanus erythropterus*) were purchased from a wet market in Selangor, Malaysia. The fish were stored in an ice box filled with ice and immediately transported to the laboratory. Flavourzyme 500 L with a declared activity of 500 Leucine Amino Peptidase Units per gram (LAPU/g) was obtained from Novo Nordisk Industries (A/S, Bagsvaerd, Denmark). Bouillon cube ingredients such as salt, sugar and corn starch were bought from the nearest local hypermarket. Chemicals were purchased from Sigma Aldrich (M) Sdn Bhd. Malaysia.

Preparation of Threadfin Bream Hydrolysate (TBH)

TBH was prepared according to Normah, Jamilah, Saari and Che Man (2005). Minced threadfin bream was hydrolyzed using flavourzyme at the following hydrolysis conditions; 60°C, 120 min, pH 8.5, enzyme substrate ratio 1:3. After hydrolysis and

centrifugation, the supernatant was freeze dried in a freeze drier (SANYO- Biomedical freeze drier).

Preparation of Bouillon Cube

Bouillon cube was prepared according to Jin et al. (2014). The detail of the composition is presented in Table 1. Red snapper was degutted, washed thoroughly under running tap water and filleted to obtain the flesh. The fish was boiled in 2 L water (fish:water, 1:2) for 30 min at 80°C before it was cooked along with the basic ingredients. The fish broth was cooled for 10 minute and then filtered through muslin cloth. The filtrate was collected and cooled to room temperature. Subsequently, the filtrate was divided into three parts, which are control

(as it is), addition of 8.05% ISP (F1) and addition of 8.05% TBH (F2). The mixture was freeze dried until consistent moisture content was obtained. The resulting powder was then moulded into cuboid shape with dimensions of 2.5 cm length, 2.5 cm width and, 2 cm height by using a customized mould tray.

Moisture, Fat and Protein Content

The moisture, fat, and crude protein was determined in triplicate and performed according to the AOAC methods (AOAC, 2005). Moisture content was determined using the oven dried method, fat content by soxhlet extraction and protein by Kjeldahl method.

Table 1
Ingredients used in the formulating of bouillon cube

Ingredients (%)	Control (C)	Formulation 1 (F1)	Formulation 2 (F2)
Red Snapper	71.09	71.09	71.09
Salt	9.48	9.48	9.48
Isolated Soy Protein Powder (ISP)	-	8.05	-
Threadfin Bream Hydrolysate Powder (TBH)	-	-	8.05
Granulated Sugar	7.11	7.11	7.11
Corn Starch	12.32	4.27	4.27
Total	100	100	100

Note: F1 Bouillon cube with the addition of isolated soy protein (ISP); F2 with the addition of threadfin bream hydrolysate (TBH)

Colour

Analysis of bouillon cube colour was determined by using Minolta chroma meter (CR400; Konica Minolta, Japan) based on the CIE system in terms of the L, a*,

and b* to measure brightness, redness, and yellowness, respectively. The white calibration plate was used as reference. Measurement was performed in triplicate.

Water Activity

The water activity of the bouillon cube was measured by using dew point equipment (Aqualab 4TE, Decagon Devices Inc., USA). An amount of 5 g of the sample was placed into a small container with 3 cm diameter before the measurement at ambient temperature (25°C).

Protein Solubility

Protein solubility of the bouillon cube was determined according to the method by Caprita, Caprita and Cretescu (2010) using the potassium hydroxide (KOH) solubility test. Protein solubility was expressed in percentage and calculated as follow:

$$\text{Protein solubility (\%)} = \frac{\text{Protein content of the KOH extracted solution}}{\text{Protein content of the original bouillon cube sample}} \times 100$$

Determination of Cube Hardness

Fracturability and hardness test was performed by using TA.XT2 Texture Analyser (Stable Micro Systems, Surrey UK). The probe used was 0.5” Spherical Probe (P/0.5S) and the measurement was conducted on heavy duty platform (HDP/90) that was set with 10 mm distance from the probe.

Sensory Evaluation

Training of the Panelist and Sample Preparation. Sensory evaluation was carried out in the sensory laboratory. Nine panelists were trained for Quantitative Descriptive Analysis (QDA) to evaluate the bitterness, umami and fishy odor before testing the samples. Panelists were given caffeine solution (0.2-0.6 g/L), monosodium L-glutamate (MSG) solution (1-3 g/L) and fish aqueous solutions. Fish aqueous reference solutions were prepared by homogenizing red snapper flesh with water at the ratio of 1:3, 2:3, 3:3 (water:red snapper flesh). Panelists needed to determine the intensity of each taste and mark on the 15 cm QDA line anchored, with the word ‘weak bitter/umami/fishy odor’ on the left side of the scale and ‘strongly bitter/umami/fishy odor’ on the right side of the scale. The anchor in the center of the line represents the moderate taste intensity. Panelists were trained to identify each taste with the least intensity and memorize the intensity. The solution with the least intensity was used as a reference during the sensory evaluation session.

For sensory evaluation, each panelist was served with 15 mL fish soup. They were asked to mark the intensity of each taste on the QDA line by comparing with the reference solutions which had been identified during the training. Panelists were also given bouillon cubes that were kept in tightly closed bottles. They had to take deep sniffs of the samples and describe the aroma of the bouillon cubes.

Acceptance Test. The acceptability of bouillon cubes was evaluated by thirty panelists. The bouillon cube solution was prepared at the ratio of 1:50 (bouillon cube: water) followed by heating for 10 min. An amount of 15 mL bouillon cube solution prepared was served in transparent containers for the evaluation process. Samples were evaluated for their degree of acceptability for colour, taste, bitterness, aroma, and overall acceptability based on 9-point Hedonic scale (9 = like extremely and 1 = dislike extremely).

Functional Group Analysis of Bouillon Cube. The analysis of the functional group in bouillon cube was performed according to Normah, Siti Hafsa and Nurul Izzaira (2013) by using the Perkin Elmer Spectrum One FTIR spectrometer equipped with a deuterated triglycerine sulphate infrared detector. Perkin Elmer Spectrum Software was used to control the spectrometer and the data was collected over a wavenumber range of 4000-400 cm^{-1} with resolution of 4 cm^{-1} and 16 collections of spectra.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Molecular weight distribution was determined according to Normah and Nur Anati (2015). About 10 μl sample was loaded into the gel comprising of 12% resolving and 4% stacking gel. Benchmark™ protein ladder ranging from 3- 188 kDa was used as a marker. Electrophoresis was run for 50 min at 100-125 mA/gel using the XCell Surelock electrophoresis cell (Consort, Model EV231, Germany).

Statistical Analysis

Data was analyzed by using the Analysis of Variance (ANOVA) to determine significance at 5% level. Duncan Multiple Range Test (DMRT) was used to identify differences between means. The statistical software used was Statistical Analysis System (SAS Institute Inc., 2004).

RESULTS AND DISCUSSION

Moisture, Protein and Fat Content

Moisture, protein and fat content of bouillon cubes are shown in Table 2. Bouillon cube had a low moisture content ranging from 2.03-3.50% with no significant difference ($p > 0.05$) between formulations. Low moisture content will extend shelf life and limit deterioration in quality due to the microbial activity (Lillian, Prisca, Ozioma, Nkechi, & Ifeoma, 2013). It has been suggested that the ideal moisture content for bouillon cube is approximately between 2.25-2.92% (Akpanyung, 2005).

Protein content was significantly higher ($p < 0.05$) in bouillon cube containing isolated soy protein (F1). However, it was only <2% higher than those containing TBH (F2). The high protein content could be due to the addition of isolated soy protein which has been reported to contain approximately 90% protein (Kolar, Richert, Decker, Steinke, & Van der Zanden, 1985). Fish protein hydrolysates with protein content varying between 69-87.69% has the potential to be used as protein supplements (Chalamaiah et al., 2012).

Table 2

Physicochemical properties of bouillon cube incorporated with isolated soy protein (F1) and threadfin bream hydrolysate (F2)

Parameters	Formulation		
	Control	F1	F2
Moisture content (%)	3.50 ± 1.30 ^a	3.30 ± 0.97 ^a	2.03 ± 0.47 ^a
Protein content (%)	17.24 ± 0.16 ^c	24.15 ± 0.43 ^a	23.05 ± 0.27 ^b
Fat content (%)	13.10 ± 0.01 ^b	19.19 ± 0.21 ^a	18.53 ± 1.42 ^a
Colour			
L*	85.34 ± 0.11 ^b	84.79 ± 0.50 ^b	86.32 ± 0.44 ^a
a*	-0.56 ± 0.03 ^c	-0.25 ± 0.04 ^b	-0.09 ± 0.03 ^a
b*	11.95 ± 0.48 ^b	13.65 ± 0.41 ^a	10.65 ± 1.22 ^c
Water activity	0.25 ± 0.01 ^a	0.24 ± 0.01 ^{ab}	0.21 ± 0.03 ^b
Solubility (%)	32.08 ± 5.86 ^b	16.07 ± 4.07 ^c	46.98 ± 7.38 ^a
Hardness (g)	1.73	1.73	2.46
Fracturability (mm)	4.41	3.04	2.41

^{a,b,c} Values with different letters in the same row are significantly different ($p < 0.05$)

Meanwhile, bouillon cubes prepared in the presence of ISP and TBH contained significantly higher fat content ($p < 0.05$) than the control bouillon cube. The increment in fat content could have been derived from ISP and hydrolysates itself, where protein concentrates and isolates contain fat of approximately 1-1.8 % (Mao and Hua, 2012). Hydrolysate also contributes to the increase in fat content as it contains 4-6.1% fat (Chalamaiah, Rao, Rao, & Jyothirmayi, 2010). It was reported that the fat content in 32 samples of bouillon cubes of the most commonly marketed brands ranged between 4.3 and 29.8% (Caponio et al., 2003).

Colour

Bouillon cubes containing TBH had significantly lighter colour ($p < 0.05$) than the others (Table 2). The addition of TBH

affected the colour of the bouillon cubes since the hydrolysate colour was lighter than isolated soy protein. There was a significant difference ($p < 0.05$) in redness (a*) and yellowness (b*) between all the formulations. Most commercial cubes are pale yellow in colour; however it is affected by the material added which will reflect the colour of the cubes (Rodrigues, Pantoja, Soares, Nelson, & Santos, 2016).

Water Activity

Water activity is important in dried food as it will determine the quality of the bouillon cubes regarding spoilage and deterioration by microbes. Bouillon cube should have water activity lower than 0.65 (Smorenburg & Yamson, 2009). All bouillon cubes prepared in this study contain low water activity which varies from 0.21-0.25

(Table 2). Low water activity of bouillon cube indicates good flow-ability of powder (Gupta & Bongers, 2011).

Protein Solubility

Bouillon cubes prepared with the incorporation of TBH contain significantly ($p < 0.05$) higher soluble protein compared to control and F1. Higher protein solubility of F2 is associated with the presence of low molecular weight peptides in the hydrolysate which have been reported to be rich in hydrophilic amino acids (Taheri, Anvar, Ahari, & Fogliano, 2013). In contrary, low solubility of bouillon cube from F1 might be due to the addition of ISP. ISP contains bands with high molecular weight peptides mostly above 20 kDa compared to TBH with bands, most of them being below 20 kDa (Figure 4). Furthermore, ISP is a plant component which is rich in insoluble polysaccharides (Altschul & Wilcke, 2013).

Determination of Bouillon Cube Hardness

Incorporation with TBH resulted in harder bouillon cubes than others (Table 2). In contrary, it has the lowest fracturability reading, compared to others. Higher hardness and lower fracturability indicates good strength of bouillon cubes, as higher fracturability and lower hardness will cause the bouillon cubes to break easily (Paula & Conti-Silva, 2014). The differences in hardness and fracturability were affected by the addition of ISP and TBH. Moreover, hydrolysates have high potential to be used as binding agents (Sathivel et al., 2003).

Sensory Evaluation

Quantitative Descriptive Analysis (QDA).

QDA was carried out by nine panelists and the results are presented in Figures 1 and 2. The umami, bitterness and fishy odor of bouillon cubes and fish soup, prepared from bouillon cubes, was evaluated and the score of reference solution by using 15 cm line scale from weak to strong was 12.01, 12.10, and 10.99 for umami, bitterness, and fishy odor, respectively.

No significant difference ($p > 0.05$) can be seen for intensity of umami between umami reference solution and F2. However, F2 showed significantly higher ($p < 0.05$) intensity than control and F1. According to Rodrigues et al. (2016), hydrolysates with low molecular weight peptides less than 500 Da could contribute to the umami taste.

As for bitterness, F2 was significantly less bitter ($p < 0.05$) than the reference solution but more bitter than F1 and control. F2 was bitter due to the addition of TBH which has bitter taste. According to Bihade, Bankar, Gaikwad and Pawar (2010), as a result of the hydrolysis process, hydrolysate consisted of amine group which could contribute to bitterness. For fishy odor, F2 showed no significant difference ($p > 0.05$) with the reference solution. However, the fishy odor was more significant ($p < 0.05$) than control and F1. The intensities for bitterness and fishy odor of the reference solution were identified by the trained panelists during the training sessions, whereby the set scales refer to the lowest possible intensity that the panelists can identify. With regards to acceptance, these

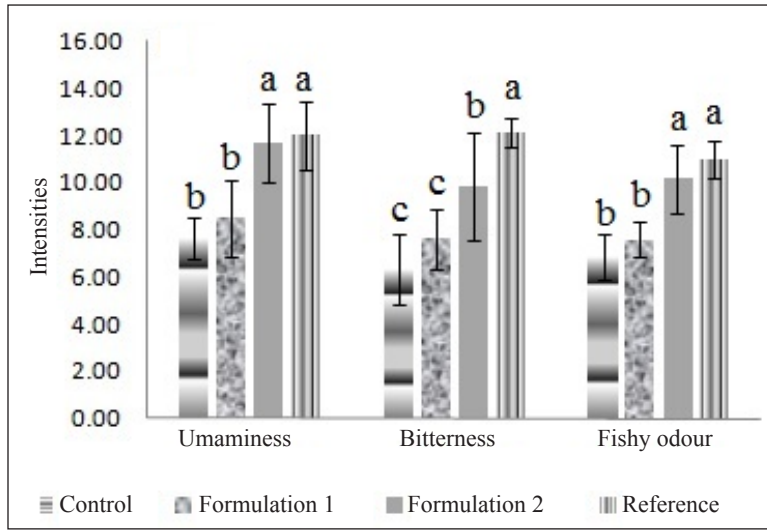


Figure 1. Quantitative descriptive analysis of bouillon cube with different formulations; control, Formulation 1 (incorporated with isolated soy protein) and Formulation 2 (incorporated with threadfin bream hydrolysate) Note: ^{a-c} Values with different letters indicate significant difference at $p < 0.05$

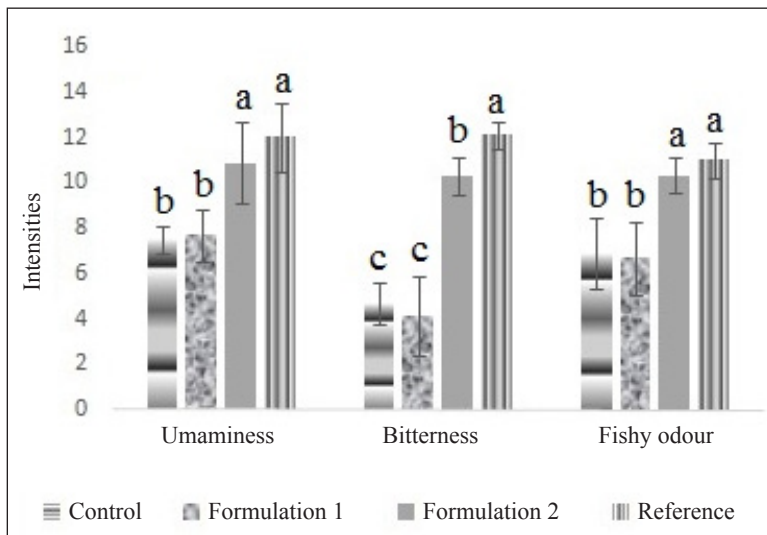


Figure 2. Quantitative descriptive analysis of fish soup with different formulations; control, Formulation 1 (incorporated with isolated soy protein) and Formulation 2 (incorporated with threadfin bream hydrolysate) Note: ^{a,b,c} Values with different letters indicate significant difference at $p < 0.05$.

intensity scales are considered as the accepted values by the panelists. Thus, the result suggested that bitterness and fishy odor of F2 is acceptable.

For fish soup prepared from the bouillon cubes, there was no significant difference (p

> 0.05) between the reference solution and F2 for umami and fishy odor (Figure 2). However, soup prepared from F2 has significantly higher intensity ($p < 0.05$) for both attributes than control and F1. In terms of bitterness, soup from F2 was significantly

less bitter ($p < 0.05$) than the reference solution but more bitter than control and F1. Hydrolysate having low molecular weight peptide contributes to the umami and bitterness (Geisenhof, 2009; Mouritsen, Styrbrik, Johansen, & Mouritsen, 2014). However, the umami in foodstuff is normally mild and of subtle taste, as it is not as intense as bitterness, sweetness and sourness (Hoehl, Schoenberger, & Busch-Stocks, 2014). On the other hand, the aroma,

taste, and colour of food products usually depends on the processing method of the food (Meilgaard, Carr, & Civille, 2006).

Acceptance Test. Overall acceptability results showed that bouillon cubes from F1 were most acceptable (Table 3). However, no significant difference ($p > 0.05$) could be seen for colour and aroma for all samples. For taste, F2 was least accepted, probably due to its bitter taste.

Table 3

Acceptability of bouillon cube incorporated with isolated soy protein (F1) and threadfin bream hydrolysate (F2)

	Colour	Taste	Bitterness	Aroma	Overall acceptability
Control	7.10 ± 1.03 ^a	7.13 ± 1.04 ^b	6.63 ± 1.07 ^b	7.03 ± 1.13 ^a	7.00 ± 0.91 ^b
F1	7.53 ± 1.22 ^a	7.83 ± 1.12 ^a	7.30 ± 1.12 ^a	7.47 ± 1.04 ^a	7.83 ± 0.91 ^a
F2	7.13 ± 1.14 ^a	6.43 ± 1.31 ^c	6.37 ± 1.25 ^b	6.97 ± 1.59 ^a	6.87 ± 1.48 ^b

Values are expressed as means ± standard deviation. Different letters within columns indicate significant difference at $p < 0.05$

Functional Group Analysis in Bouillon Cube. Functional group analysis was conducted by using FTIR spectrum. FTIR peak ranges between 3250 to 3400 cm^{-1} represent the N-H of amine group while the peak ranges between 1760 to 1670 cm^{-1} represent the C=O of carbonyl group (Figure 3). The functional group of F2 showed peaks at 3400 cm^{-1} where it falls within the N-H region, suggesting the presence of bitter group. Amine group is usually present in bitter drugs (Agrawal & Chiddarwar, 2010). On the other hand, F2 also showed peaks at 1650 cm^{-1} where it lies within the carbonyl (C=O) stretch. These bands indicated the presence of strong amide I and II in protein

amide group (-CONH₂) which contribute to bitter taste (Charalambous & Inglett, 2012). Similar prominent peak that lay between N-H stretch and C=O stretch was also found in bitter gourd studies (Ekezie, Jessie-Suneetha, Uma-Maheswari, & Prasad, 2015).

Bouillon cube from control, F1 and F2 showed peaks at 1413.15 cm^{-1} , 1458.50 cm^{-1} , and 1407.45 cm^{-1} , respectively. These peaks have been shown to lie within C-N stretch of aliphatic amines where it is the similar functional group that contributes to the umami (McMurry, 1996). Umami is derived from monosodium glutamate (MSG) but it is only elevated when MSG

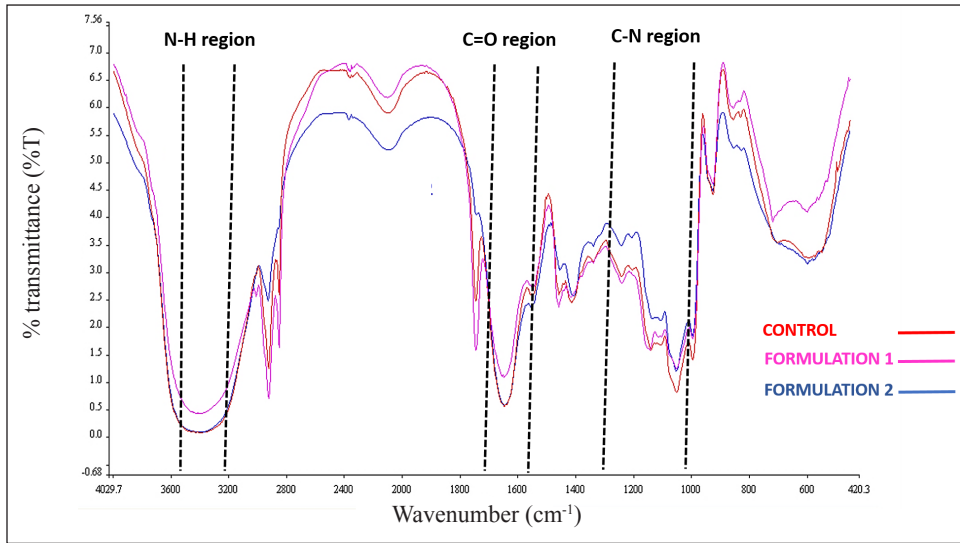


Figure 3. FTIR spectra for bouillon cube incorporated with isolated soy protein (F1) and threadfin bream hydrolysate (F2)

is incorporated with another component (Morini, Bassoli, & Borgonovo, 2011). Sodium chloride, 2,5-dimethylpyrazine, Maillard peptides and glutathionine are examples of components that can enhance and contribute to umami (Hayase, Takahagi, & Watanabe, 2013; Ogasawara, Yamada, & Egi, 2006; Ueda, Yonemitsu, Tsubuku, Sakaguchi, & Miyajima, 1997). Umami is a unique property as it has an apparent tactile component and has a weak, unpalatable taste by itself (Beauchamp, 2009).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The molecular weight distribution patterns are shown in Figure 4. ISP shows an intense band with a wide molecular weight range from 10-70 kDa. Meanwhile, TBH had bands between 10-60 kDa in which bands at 10 and 20 kDa were very intense. The

presence of low molecular weight peptide in TBH was due to the enzymatic hydrolysis (Normah & Nur Anati, 2015). Threadfin bream (TB) and red snapper (RS) flesh showed wide molecular weight peptides which can be visualized ranging from 10-220 kDa. The electrophoresis pattern for control, F1 and F2 showed almost similar pattern with red snapper (RS) flesh, since all the bouillon cubes were derived from red snapper flesh.

A similar band pattern of isolated soy protein (ISP) and F1 was discovered where intense band from 70 kDa and below was visualized as it indicates higher molecular weight compared to control and F2. Meanwhile, F2 showed intense band at lower range less than 13 kDa where these bands were not observed in control and F1. Similar band at less than 12 kDa were also exhibited by TBH. On the other hand, F1 showed very wide and intense

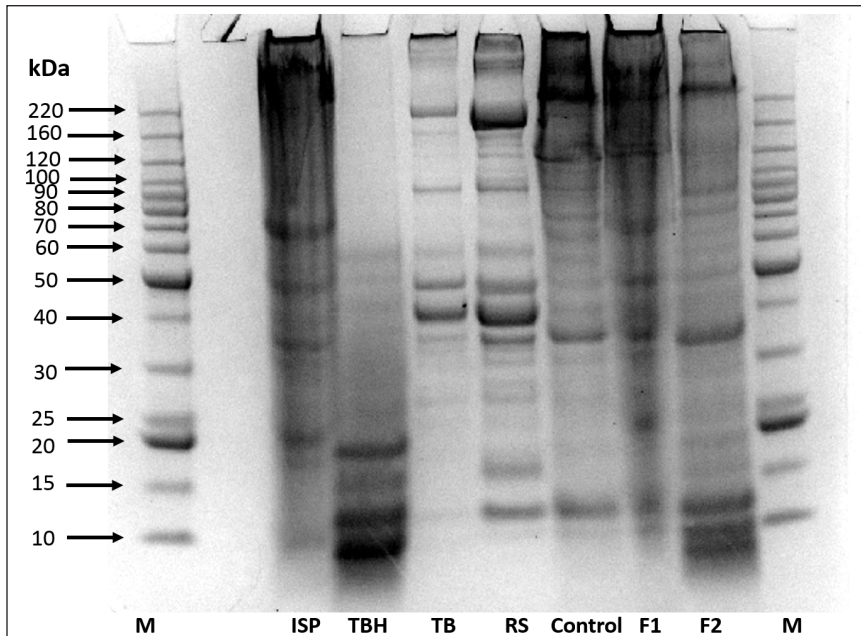


Figure 4. Electrophoresis pattern of bouillon cube incorporated with isolated soy protein and threadfin bream hydrolysate. From left: marker (M), isolated soy protein (ISP), threadfin bream hydrolysate (TBH), threadfin bream flesh (TB), red snapper flesh (RS), control, formulation 1 added with ISP (F1), and formulation 2 added with TBH (F2)

band compared to control and F2. Modified soy protein was reported to contain high molecular weight ranging from 38-80 kDa (Monagle, 2004). It has been stated that molecular weight of protein below 10 kDa will contribute to the bitter flavor (Kristinsson & Rasco, 2000). Thus, the presence of intense band at 13 kDa in F2 suggested that the sample was slightly bitter. Umami flavor has also been studied to have low molecular weight less than 500 Da, however, most umami peptides were weak or even undetectable (Lioe, Takara, & Yasuda, 2006). On the other hand, the presence of low molecular weight band at F2 indicates high solubility properties of bouillon cubes. In contrast, high molecular weight band of F1 shows low solubility

properties of bouillon cubes. The studies indicate that low molecular peptides have potential application in functional food products (Roslan, Yunos, Abdullah, & Kamal, 2014).

CONCLUSION

TBH has the potential to be used as a binding agent as it performed well in texture analysis. Besides, incorporation of TBH into bouillon cubes was considered acceptable as it has good physicochemical and functional properties in terms of solubility and strength. TBH is also a good source of protein and has potential as a flavor enhancer due to the umami properties based on the sensory results.

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