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Characteristics of peptones from grouper (*Epinephelus fuscoguttatus*) and parrotfish (*Scarus javanicus*) head by-products as bacterial culture media

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Peptones were extracted from the head by-products of grouper (*Epinephelus fuscoguttatus*) and parrotfish (*Scarus javanicus*) with different acid combinations. The peptones showed significant differences on yield, solubility, color, bacterial growth profile, and biomass production (P < 0.05). The yield of parrotfish peptone (PFP) and grouper fish peptone (GFP) ranged from 3.27% to 3.45% and 4.61% to 5.70%, respectively. The major component of both peptones was protein varied between 83.80% and 86.67%. The whiteness of peptone samples was in the range of 33.56% to 60.06% with the highest in GFP by adding 1.5% (v/m) of propionic/formic acid (1:2, v/v). Although the solubility of peptone samples was slightly lower than the commercial peptone, both PFP and GFP samples exhibited better performance in the growth of bacteria (*Escherichia coli* and *Staphylococcus aureus*). The biomass production of PFP and GFP increased significantly when compared that to commercial peptone. The PFP and GFP samples contained high values of the amino acids (glycine, glutamic acid, proline, and alanine). The results revealed that grouper and parrotfish head by-product are potential material for bacterial peptone.

Keywords: grouper; parrotfish; head by-products; hydrolysate; fish peptone; bacterial culture media.

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Introduction

The global fish production reached about 171 million tons in 2016, of which 53% representing in capture and 47% in aquaculture as reported by FAO [1]. About 88% of total fish production was utilized for human consumption and the rest of them was utilized for non-food uses including fish meal, animal feed, fertilizer, and so forth. Generally, this non-food material of fish contains head, skin, viscera, scales, bones, and fins or by-products. These by-products provide value-added products, which are rich in protein, fat, and mineral [2].

Indonesia is one of the major fish producer countries. Indonesia produced 23.26 million tons of fish in 2017 [3]. Among them, marine capture contributes 6.04 million tons, representing 35% of total fish production. Several marine fish species are considered economically important species, such as grouper and parrotfish. Their production in 2016 reached 15,645 tons and 2,998 tons, respectively [4]. Commercially, these

marine species are processed as fish fillets and exported to other regions, such as Hongkong, Taiwan, and Singapore. Around 30% of the total fish processing industry are by-products [5]. These by-products are potential material to utilize for peptone production, which is more profitable and marketable than other products. On the other hand, utilizing fish waste could minimize serious environmental problems [6].

Peptone is defined as protein hydrolysates extracted from materials that contain high protein [7]. Moreover, peptone contains water-soluble proteins, especially rich in an amino acid composition that does not coagulate by heat. It is widely used in biological and biotechnological applications. Peptone is commonly produced from land animals like bovine, porcine, and their derivatives [8]. Nevertheless, bovine peptones are suspected in the risk of outbreaks of diseases, such as mad cow disease (MCD). Meanwhile, porcine peptones are unacceptable to those Muslim and Jewish-majority countries because of religious beliefs. Therefore, there is considerable attention in replacing animal peptones to fish materials.

Fish peptone is capable to be extracted with enzymatic or acidic hydrolysis. For enzymatic hydrolysis, the protein can be cleaved into smaller amino acids, but with disadvantages of slow reaction rate and high cost [9]. On the other hand, acidic hydrolysis has advantages of low cost, short hydrolysis period, simple operation, and applicable to industrial scales [10]. Benites *et al*. stated that the selection of an acidifying agent is based on three factors: cost, availability, and bactericidal action [11]. Also, acid solution is mostly categorized into positive list compounds in terms of halal or kosher viewpoint.

Peptone is an essential source of nitrogen for microbial growth and biomass production due to the high polypeptides and amino acids. Aspmo *et al*. stated that an important component in microbial growth media is a nitrogen compound [12]. Some studies reported that peptone extracted from cowtail ray (*Trygon sephen*) viscera was capable of supporting microbial growth rate in the cultures of *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* [13]. Furthermore, Shirahigue *et al*. reported peptones hydrolyzed from tilapia and cobia waste with different acid had a significant growth rate and biomass production in the culture of *Escherichia coli* and *Staphylococcus aureus* compared to commercial peptone [2]. These findings also in accordance with the peptone isolated from Atlantic cod stomach with formic and phosphoric acid reported by Gildberg *et al*. [14]. As mentioned above, fish peptones provide better microbial growth, as well as higher biomass production due to the high content of soluble protein compounds, particularly nitrogen compounds. They might be used as substitute for commercial peptones derived from land animals. In addition, fish peptones are acceptable for all religions and do not relate to dangerous diseases.

There are few reports on peptone hydrolyzed from grouper and parrotfish head by-products using a different combination of acids in supporting bacterial growth and biomass production. This research aimed to characterize peptone from grouper (*Epinephelus fuscoguttatus*) and parrotfish (*Scarus javanicus*) head by-products using different acid combinations as bacterial growth media in comparison with commercial peptone.

Materials and Methods

Materials

The heads of grouper (*Epinephelus fuscoguttatus*) and parrotfish (*Scarus javanicus*) were obtained from a fishery processing industry (PT. Alam Jaya) located in Surabaya, East Java, Indonesia. The mean weight of grouper and parrotfish heads was 1.1 and 0.7 kg, respectively. The head by-products were packed in polyethylene plastic and set at low temperatures around 4°C during transporting to laboratory (approximately 2 hours). The fish heads were washed using tap water then ground using a

Table 1. Treatment of acid mixtures used to prepare parrotfish peptone (PFP) and grouper fish peptone (GFP).

Treatment	Description
PFP1	1.5% (v/m) of propionic acid/formic acid (1:2, v/v)
PFP2	1.5% (v/m) of propionic acid/formic acid (1:3, v/v)
PFP3	1.5% (v/m) of propionic acid/formic acid (1:4, v/v)
GFP1	1.5% (v/m) of propionic acid/formic acid (1:2, v/v)
GFP2	1.5% (v/m) of propionic acid/formic acid (1:3, v/v)
GFP3	1.5% (v/m) of propionic acid/formic acid (1:4, v/v)

milling machine (MHW-80, Indonesia). The ground sample were placed into polyethylene plastic and stored in the freezer at a temperature of -20°C for up to 7 days. The bacteria used in this study were *Escherichia coli* and *Streptococcus aureus* strains, which were obtained from InaCC LIPI, Indonesia. All other chemicals used were of analytical grades.

Preparation of parrotfish and grouper head by-product hydrolysate

Hydrolysate of parrotfish and grouper head by-products were performed according to the method reported by Shirahigue *et al*. [2] with some modifications. About 900 g ground fish head was added into 10% of distilled water with pH around 4.2 at each treatment. Both parrotfish and grouper head by-products treated with the three combinations of propionic acid:formic acid (1:2, 1:3, and 1:4, v/v). 1.5% of each acid combination was added into samples (v/w) as presented in Table 1. The treated samples were incubated at room temperature (24-26°C) for 7 days to hydrolyze fish protein by activating endogenous enzymes. After the incubation period, the hydrolyzed samples were treated at 85°C for 20 minutes to inactive endogenous enzymes followed by centrifugation at 5,000 rpm for 10 minutes to separate samples into three fractions (solid phase, liquid phase, and oil phase). The liquid phase material was spray-dried at an inlet temperature of 160°C and an outlet temperature of 90°C. The obtained peptones were stored at a low temperature until further use.

Characterization of peptone samples

(1) Proximate composition measurement

The proximate composition of peptone isolated from parrotfish and grouper head by-product was carried out as follows.

Moisture content:

The measurement of moisture content was conducted by the gravimetric method [15]. Moisture content calculated with the formula:

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W} \times 100\,\%$$

Where W_1 is weight of cup before dried, W_2 is weight of cup after dried, and W is weight of sample.

Protein content:

The measurement of protein content was performed by using Kjeldahl method [15]. Protein content was calculated with the formula:

$$N(\%) = \frac{(\text{ml HCl-ml blanko}) \times 14.007 \times N\ \text{HCl}}{W} \times 100\,\%$$

$$\text{Protein (\%)} = N(\%) \times 6.25$$

Fat content:

Fat content was determined by using Soxhlet method [15]. Fat content was calculated using the followed formula:

$$\text{Fat (\%)} = \frac{(T1 - T2)}{W} \times 100\,\%$$

Where T1 is weight of flask after dried and T2 is weight of flask before dried.

Ash content:
Ash content was conducted by using gravimetric method [15]. Samples (2 g) were placed into a porcelain cup, then heated in a furnace with a temperature of 550°C for 5 hours until the color is white. The treated samples were put into the desiccator and calculated with formula below:

$$\text{Ash (\%)} = \frac{\text{W1-W2}}{\text{W}} \text{ x } 100\ \%$$

Where W1 is weight of cup before dried, W2 is weight of cup after dried, and W is weight of sample.

(2) Yield of peptone
The yield of peptone extracted from head by-products of PFP and GFP was determined using the following equation:

$$\text{Yield(\%)} = \frac{\text{M}}{\text{M}_0} \times 100\%$$

Where M is the weight of peptone (g) obtained after drying, and M_0 is the weight of raw materials of grouper and parrotfish (g).

(3) Measurement of solubility
The solubility of PFP and GFP samples was carried out by the gravimetric method [16]. The filter paper was dried using an oven at 105°C for 3 hours. 1 g sample was dissolved in 150 mL of distilled water, then homogenized and filtered using the Whatman paper. Solubility was calculated by the formula:

$$\text{Solubility in water} = \frac{100-(a-b)}{(100-KA)\times c} \text{ x } 100\ \%$$

Where a is weight of filter paper with residue, b is weight of initial filter paper, KA is value of moisture content, and c is weight of sample.

(4) Measurement of color parameter
The color parameter was determined by using a Konica Minolta chroma meter CR-400 (Japan) by calibrating the instrument first. The peptone sample was flattened in a sample container and the color parameter was measured. The color parameter of peptone samples was expressed by *L**, *a**, *b**, color intensity, and whiteness.

(5) Measurement of amino acid composition
Identification of amino acid composition was carried out according to the method of Nollet and Fidel [17]. The sample (0.1 g) was added with 5 mL of 6N HCl and hydrolyzed at 110°C for 22 hours. The hydrolyzed sample was then transferred into flask and distilled water was added. The sample was filtered with a 0.45 µm filter. 500 µL of filtrate were mixed with 40 µL ABBA and 460 µL aquabidest. Then, 10 µL of sample solution was added with 70 µL AccQ Fluorine Borate and 20 µL of fluorine A reagent before homogenization was performed for 1 minute. Subsequently, the homogenized sample was incubated at 55°C for 10 minutes. Finally, the solution was injected into the Ultra-Performance Liquid Chromatogram (UPLC) system.

Bacterial Growth Analysis
(1) Preparation of culture media
Bacterial growth media compositions consisted of peptone (either parrotfish peptone or grouper peptone or commercial peptone), NaCl, and yeast extract which were equivalent to the composition of Luria Bertani (LB) broth media (Table 2). The treated growth media were sterilized using autoclaves at 121°C for 15 minutes. The bacteria were cultured in 250 mL volume Erlenmeyer flasks containing 150 mL of different medium in triplicate and incubated at 30°C and 150 rpm using an incubator [18].

Table 2. Composition of culture media used in the bacterial tests (g/L).

Ingredient	PFP	GFP	LB media
Commercial peptone	-	-	10.00
Fish peptone	10.00	10.00	-
Yeast extract	5.00	5.00	5.00
Sodium chloride	10.00	10.00	10.00

(2) Bacterial growth measurement
The optical density (OD) of the media was analyzed to determine the growth of *Escherichia*

coli and *Staphylococcus aureus* at a wavelength of 600 nm using a spectrophotometer [2].

(3) Biomass production

Biomass was measured at 24-hour incubation by transferring 25 mL of samples containing bacteria and media into a sterile falcon tube, then centrifuged for 20 minutes at 5,000 rpm. The precipitate was separated and then 5 mL of 0.85% NaCl solution (w/v) was added followed by centrifugation at 5,000 rpm for 20 minutes. The precipitate was then transferred to a petri dish and dried for 24 hours at 105°C [13].

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by a Tukey's post-hoc test using SPSS 25.0 software. Statistical significance was defined as $P < 0.05$ value.

Results and discussion

Proximate composition of head by-products and peptones

The proximate composition of raw materials and peptones, which were isolated from grouper fish peptone (GFP) and parrotfish peptone (PFP) and prepared with the different acid combinations, was determined and the results were tabulated in Table 3. In general, the head by-products of parrotfish (*Scarus javanicus*) contained moisture, protein, fat, and ash content of 75.41%, 16.30%, 2.30%, and 4.19%, respectively, while grouper (*Epinephelus fuscoguttatus*) contained moisture, protein, fat, and ash content of 75.25%, 16.65%, 2.37%, and 4.26%, respectively. The waste of Pacific ocean perch (*Sebastes alutus*) composed of 77.7% moisture, 14.9% protein, 9.3% fat, and 6.7% ash (wet weight basis) [19], while Atlantic cod (*Gadus morhua*) by-product contained 79.4% moisture, 15.0% protein, 0.15% fat, and 6.8% ash (wet weight basis) [20]. In addition, Khoddami *et al*. reported the proximate composition of tuna (*Euthynnus affinis*) head contained 68.79% moisture, 19.30% protein, 7.01% fat, and 4.77% ash [21]. Comparing to the literature review, both parrotfish (*Scarus javanicus*) and grouper (*Epinephelus fuscoguttatus*) by-product had higher protein content than that of Pacific Ocean perch and Atlantic cod, but lower protein content than tuna. Moreover, the protein content of Parrotfish was slightly higher than that of grouper fish. However, parrotfish had similar fat and ash content with those of grouper. The parrotfish had slightly lower in moisture content than the grouper.

For proximate composition of peptones, generally, the major component of peptone was protein, which varied between 83.80% and 86.67% of PFP and GFP, respectively. Gildberg *et al*. reported protein content of 75.45% and 83.6% peptone extracted from the Atlantic cod by-product with different formic and propionic acid, respectively [14]. The peptone from the cowtail ray by-product also contained 79.4% protein when extracted at 3% (w/v) mixture of propionic and formic acids (1:1, v/v) [13]. The highest protein content was obtained from the PFP1 sample but showed no statistically significant difference to GFP1 accounted for 86.67% and 86.43%, respectively. Furthermore, the protein content of the peptone is in line with the nitrogen compound since nitrogen is an essential source in the microbial growth substrates [2]. This study could provide potential materials to produce medium for microorganisms. Other components such as moisture, fat, and ash of the extracted peptone samples at different conditions ranged from 5% to 6.34%, 1.15% to 1.95%, and 1.78% to 3.56%, respectively. These results were similar to Poernomo and Buckle [13] who observed that peptone extracted from cowtail ray fish wastes at different conditions of the ratio of acids. Theoretically, when the protein content in the food product increases, the moisture content decreases. Data above indicated the chemical composition of both parrotfish and grouper peptones, particularly protein content, is suitable for supporting microbial growth culture.

Yield of peptone

Peptone yield is determined as gram (g) of peptone per 100 g of liquid phase of hydrolysate.

Table 3. Proximate composition of the head by-products, parrotfish peptone (PFP) and grouper fish peptone (GFP).

Sample		Components			
		Moisture	Protein	Fat	Ash
By-products	Parrotfish	75.25±0.15	16.65±0.25	2.37±0.05	4.26±0.19
	Grouper fish	75.41±0.25	16.30±0.29	2.30±0.21	4.19±0.13
Peptones	PFP1	5.00±0.12^{a}	86.67±0.24^{d}	1.45±0.05^{abc}	1.89±0.2^{a}
	PFP2	5.94±0.1^{bc}	84.23±0.29^{ab}	1.95±0.05^{c}	2.89±0.16^{c}
	PFP3	5.74±0.65^{abc}	83.80±0.66^{a}	1.92±0.08^{c}	3.56±0.2^{d}
	GFP1	5.47±0.12^{ab}	86.43±0.02^{d}	1.34±0.29a^{b}	1.78±0.2^{ab}
	GFP2	5.67±0.12^{abc}	85.39±0.02^{c}	1.84±0.29^{bc}	2.12±0.2^{ab}
	GFP3	6.34±0.24^{c}	85.08±0.37^{bc}	1.15±0.25^{a}	2.45±0.2^{bc}

Data were reported as mean ± standard deviation. Different lowercase letters within the same column indicate significant differences (P < 0.05).

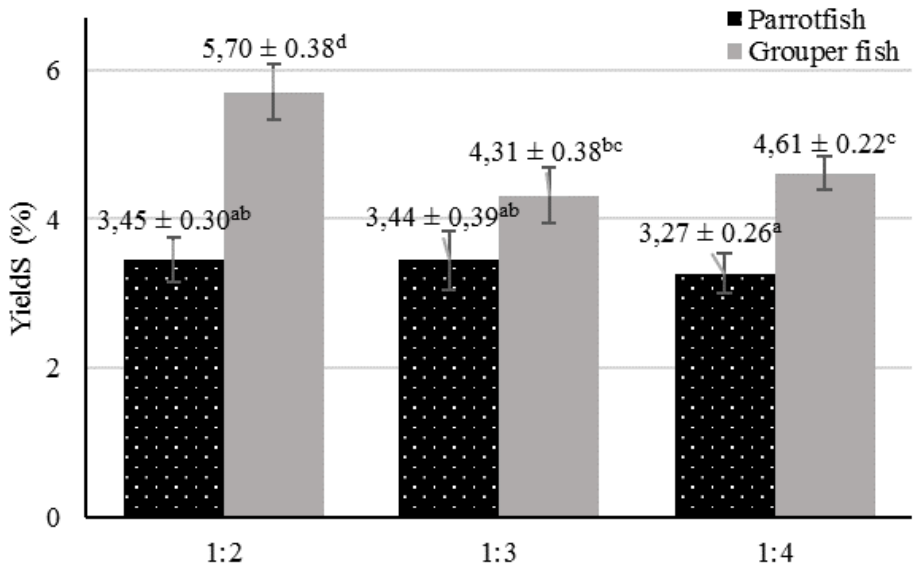

Figure 1. The yield of parrotfish peptone (PFP) and grouper fish peptone (GFP) extracted from the head by-products.

The yield of parrotfish peptone (PFP) and grouper fish peptone (GFP) obtained from the head by-products with the different combinations of propionic and formic acid was observed and the results were shown in Figure 1. The yield of peptone extracted from both parrotfish and grouper head by-products showed a significant difference (P < 0.05). Yields of GFP and PFP ranged from 3.27% to 3.45% and 4.61% to 5.70% based on a dry weight basis, respectively. The highest yield from fish waste peptone (5.70%) was obtained when the extraction was conducted from grouper head by-product with 1.5% (v/m) of a propionic:formic acid ratio of 1:2 (v/v) (GFP1). In contrast, the lowest yield of peptone presented in the peptone isolated from parrotfish with the ratio of 1:4 (v/v) (PFP3). The results suggested that, when the higher formic acid in the combination of propionic acid, the yield of peptone from both samples decreased. It is related to action of formic acid in extraction process, in which formic acid can inhibit endogenous bacteria in terms of lactic acid bacteria during hydrolyzing [22]. Several studies have reported that the peptone extracted from yellowstripe sead fish and tuna viscera were 5.23% and 5.54%, respectively [23, 24]. The difference of peptone yields in these samples

Table 4. Color parameter and solubility of the heads of parrotfish peptone (PFP) and grouper fish peptone (GFP).

Sample	*L**	*a**	*b**	Color intensity	Whiteness	Solubility (%)
PFP1	86.05±0.06^{c}	-1.45±0.06^{a}	32.72±0.04^{d}	64.38±0.06^{c}	33.56±0.05^{c}	96.43±0.03^{b}
PFP2	84.22±0.03^{b}	-1.15±0.03^{b}	35.54±0.05^{e}	61.12±0.05^{b}	27.96±0.04^{b}	96.28±0.05^{ab}
PFP3	82.13±0.02^{a}	-1.03±0.01^{b}	37.61±0.02^{f}	59.04±0.01^{a}	22.73±0.03^{a}	96.19±0.08^{a}
GFP1	91.37±0.08^{f}	-0.69±0.08^{d}	17.63±0.02^{a}	80.35±0.06^{f}	60.06±0.09^{f}	99.67±0.02^{e}
GFP2	90.35±0.01^{d}	-0.84±0.05^{c}	22.94±0.03^{c}	75.15±0.07^{d}	51.71±0.04^{d}	99.20±0.06^{d}
GFP3	90.61±0.04^{e}	-0.77±0.05^{cd}	21.81±0.04^{b}	76.24±0.05^{e}	53.46±0.05^{e}	99.02±0.03^{c}

Data were reported as mean ± standard deviation. Different lowercase letters within the same column indicate significant differences ($P < 0.05$).

indicated that the peptone yields might be associated to the raw material of fish by-product and extraction methods since peptone was liquid soluble fraction obtained after centrifugation of treated samples. The mentioned data also indicated that the added value of fish by-products might be improved by GFP and PEP isolation.

Color parameter of peptone

The parameter of color in the extracted peptone at different two species and three different acids was observed, and the data presenting *L**, *a**, *b**, intensity, and whiteness are tabulated in Table 4. In general, the peptone samples extracted from parrotfish and grouper head by-products had a significant difference ($P < 0.05$) on the color parameter, as well as from extraction condition tested in this study. In both species by-products, the *L** values of peptone samples slightly decreased with the increasing concentration of formic acid. For redness (*a** values), an increase was observed in the GFP samples compared to the PFP samples ($P < 0.05$). When a higher concentration of formic acid applied in the peptone extraction, the redness of samples increased. However, the GFP samples showed more yellowness as indicated in *b** values compared to the PFP samples ($P < 0.05$). The most yellowness in both peptone samples were due to the higher concentration of formic acid. As a result, the color intensity and whiteness in the GFP was higher than PFP ($P < 0.05$). It can be suggested that peptone extracted from grouper head by-product was whiter in color than that extracted from parrotfish head by-product. In addition, an increased in formic acid during extraction will decrease the whiteness of peptone. The color of peptone is affected by the raw materials used and the extraction method. Previous studies have reported that peptone extracted from marine fish by-products (multi-species) contained 52.64%, 2.50%, 7.99%, and 51.44% of *L**, *a**, *b**, and whiteness values, respectively [23]. Barokah *et al*. [25] observed in the microencapsulated peptone from the marine by-products (mackerel, yellowstripe scad, round scad, white sardinella, large head hairtail, and cowtail ray) performed the *L**, *a**, *b**, and whiteness values, accounted for 60.01%, 1.70%, 10.33%, and 57.44%, respectively. These reported data are comparable to those from parrotfish and grouper head by-products and resulting in both PFP and GFP had higher in the lightness and whiteness than those from multi-species peptones.

The solubility of peptone products

The solubility of peptone is an indicator to determine the quality of peptone, as stated by Khalil [9]. Peptone is protein hydrolysates that are soluble in water and does not coagulate by heat. Therefore, the solubility of peptone obtained is expressed in Table 4. Generally, the peptones were significantly different on the solubility with the values varied between 96.19% and 99.67% ($P < 0.05$). In the context of GFP samples, the solubility values showed significantly higher than those in the PFP samples ($P < 0.05$). In both PFP and GFP, the solubility slightly increased with decreasing concentration of formic acid. It indicated that the peptone samples extracted from grouper head by-products were more effective to be bacterial

Table 5. Amino acid composition of the heads of parrotfish peptone (PFP), grouper fish peptone (GFP) and commercial peptone (Bactopeptone).

Non-essential amino acid	PFP1	PFP2	PFP3	GFP1	GFP2	GFP3	Bactopeptone*
Alanine	8.12	8.97	8.12	9.70	10.05	10.26	9.20
Aspartic acid	7.21	7.76	6.24	6.39	7.45	7.60	5.00
Glutamic acid	9.11	10.12	9.36	9.97	10.67	10.44	8.10
Glycine	21.34	20.68	21.28	18.67	18.67	21.48	15.90
Serine	6.30	5.79	5.81	4.20	3.95	5.12	1.50
Tyrosine	1.39	1.07	1.87	1.40	1.09	1.40	0.60
Essential amino acid	**PFP1**	**PFP2**	**PFP3**	**GFP1**	**GFP2**	**GFP3**	**Bactopeptone***
Arginine	5.86	5.58	9.28	7.59	6.29	8.16	3.80
Histidine	1.90	1.58	2.10	2.24	1.79	1.93	0.80
Isoleucine	3.49	3.46	2.96	4.07	4.15	3.45	2.10
Leucine	6.54	6.52	5.58	7.32	7.38	3.43	3.80
Lysine	2.68	3.09	2.87	3.73	4.37	4.87	3.40
Phenylalanine	6.73	6.12	6.08	6.96	5.75	3.54	2.80
Proline	8.70	8.94	8.92	7.64	8.22	8.99	8.80
Threonine	5.68	5.27	5.15	4.86	4.60	4.09	1.10
Valine	4.95	5.05	4.37	5.26	5.56	5.25	2.80

*Barokah *et al*. [24].

culture media since their solubility closed to commercial peptone (100%). The solubility of PFP samples in accordance with the solubility of yellowstripe sead fish peptone reported by Saputra and Nurhayati *et al*. [23]. However, the GFP samples have slightly higher values than that of mentioned in yellowstripe sead fish. The high solubility value of peptone product is due to the presence of a hydroxy group in the mackerel peptone that interacts with water molecules. High solubility value in protein hydrolysate is caused by the reaction of protein breakdown into simpler peptides [25].

Amino acid composition

The amino acid composition of the peptones from PFP and GFP is tabulated in Table 5. The PFP and GFP samples contained high values of the amino acids composed of glycine, glutamic acid, proline, and alanine, whereas tyrosine, histidine, lysine, and isoleucine showed low concentration. However, both PFP and GFP samples had higher total amino acid compositions than that of commercial peptone. These results were in accordance with peptones extracted from the by-product of marine fish species, such as cowtail ray [13], Atlantic cod fish [14], and peptone from bolti fish [9]. The highest amino acid composition in the PFP samples was glycine with the range of 20.68 g to 21.34 g based on 100 g sample. The highest and lowest amino acid composition of GFP was in the line with the PFP samples, which ranged from 18.67 g to 20.48 g and 1.09 g to 1.40 g of glycine and tyrosine, respectively. The essential amino acid was detected in both PFP and GFP with the major composition in proline and the minor in histidine, which were calculated in the range of 7.64 g to 8.94 g and 1.79 g to 2.10 g, respectively. However, acid hydrolysis will destroy tryptophan as reported by Poernomo and Buckle [13]. With the presence of varying amino acids obtained from treated fish head by-products in this study, it can be concluded that these findings will be a favorable substrate for growth of microorganisms as reported by Aspmo *et al*. [12] who indicated that leucine, valine, and isoleucine in microbial growth substrate could support the growth rate.

Growth curve analysis

In the present study, two types of bacteria, *Escherichia coli* representing Gram-positive and *Staphylococcus aureus* representing Gram-negative, were selected to determine the growth curves in the Luria-Bertani (LB) broth with slight modifications. The growth curves of the

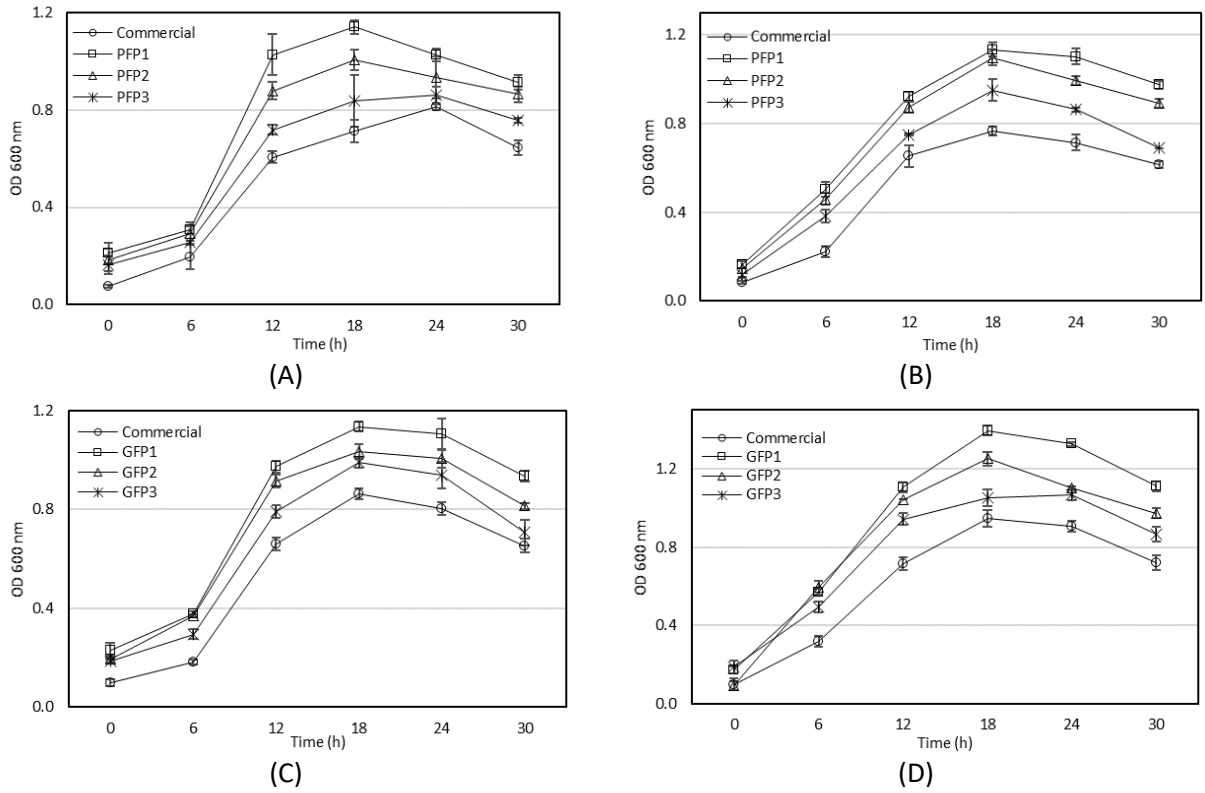

Figure 2. Bacterial growth profiles measured as optical density in 600 nm. (A) and (C): *Escherichia coli*. (B) and (D): *Staphylococcus aureus*.

extracted peptones and commercial peptone are depicted in Figure 2. Both bacteria grew properly in the LB broth supplemented with the peptone samples extracted at different marine species (PFP and GFP) as well as the commercial peptone. Generally, all bacterial growth rates with supplemented peptone samples exhibited significant different ($P < 0.05$) compared to the commercial peptone. For the PFP samples, the growth of both *Escherichia coli* and *Staphylococcus aureus* showed significantly increase ($P < 0.05$) in the medium supplemented by the peptone samples in the ratio of propionic acid/formic acid (1:2, v/v) or in the PFP1, followed by PFP2 and PFP3, while the commercial peptone showed the lowest growth curve. In addition, the GFP samples showed the similar trend of growth rate to the medium supplemented by the PFP samples. This may suggest that the peptone isolated from both parrotfish and grouper head by-product is more effective for supporting the growth of bacteria than the commercial peptone. Previous studies have investigated that peptones extracted from different fish species, such as cod, salmon, tuna, and unspecified fish showed higher microbial growth rate than that of a casein peptone [26]. In addition, peptones isolated from different fish species performed better bacterial growth profile than that of commercial [27, 28]. Nevertheless, Najim *et al*. reported that the fish waste peptones had lower performance when compared to commercial peptone for *Pseudomonas aeruginosa*, *Lactobacillus acidophilus*, and *Saccharomyces cerevisiae* [29].

Biomass production

The bacterial biomass production of peptone produced from different species of fish head by-product and different acids was observed and the results were illustrated in Figure 3. The biomass production of both bacterial cultures consisted of *Staphylococcus aureus* and *Escherichia coli* in the medium containing PFP and GFP samples ranged

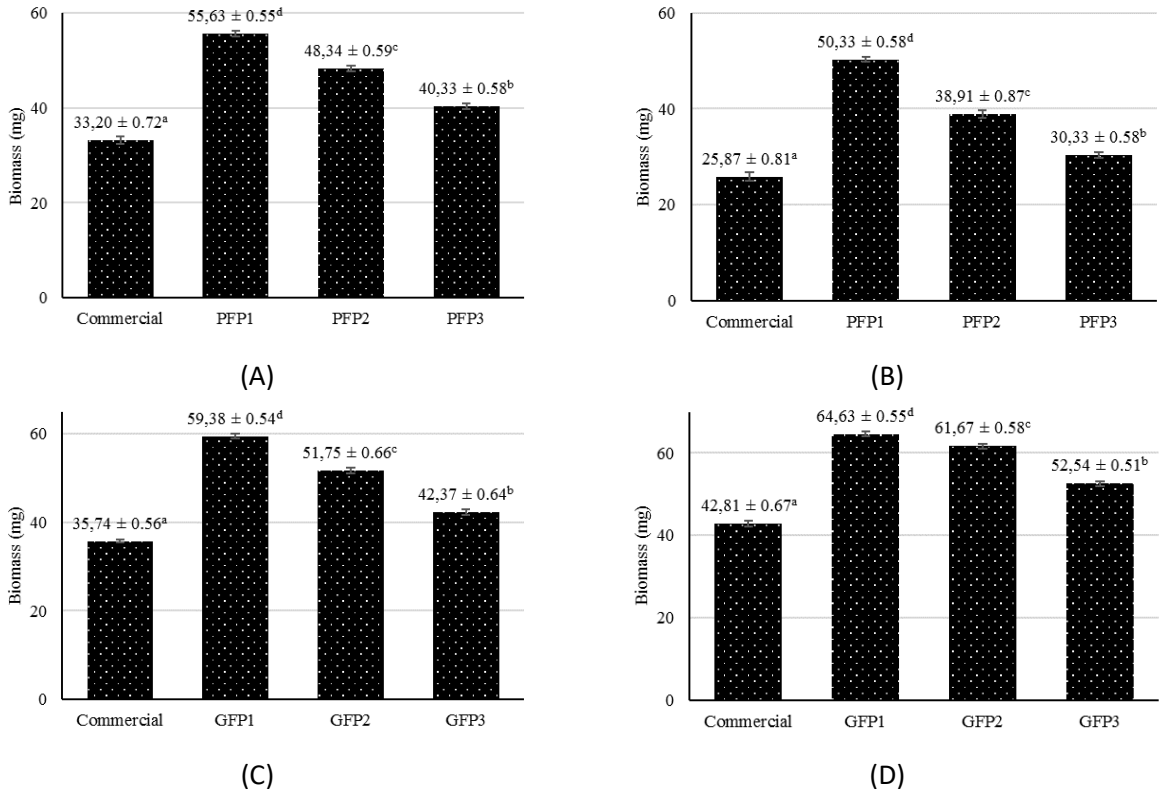

Figure 3. Biomass production supplemented the heads of parrotfish peptone (PFP) and the grouper fish peptone (GFP) in the growth of *Staphylococcus aureus* (A and C) and *Escherichia coli* (B and D).

from 40.33 to 65.43, 30.33 to 50.33, 42.37 to 59.38, and 52.54 to 64.33 (in mg per 100 mL), respectively. In general, the culture of *Staphylococcus aureus* and *Escherichia coli* inoculated with the media containing PFP samples and GFP samples showed significant increase of biomass ($P < 0.05$) in comparison to the commercial peptone. The highest production of *Staphylococcus aureus* biomass in the medium supplemented both PFP and GFP samples was observed in PFP1 and GFP1 ($P < 0.05$). Typically, the trend of biomass production of *Escherichia coli* either in GFP or PFP samples was similar to the *Staphylococcus aureus*. However, all commercial peptones applied in all treatments showed the lowest biomass production. This indicates that the peptones produced from both fish head by-product were more effective in supporting bacterial growth in terms of biomass by providing the appropriate sources of nitrogen. Poernomo and Buckle stated that the high biomass production provided adequate nutrients in supporting the growth rate of microorganisms [13]. In addition, the higher biomass production obtained, the more effective the growth rate of microorganism. These findings are in line with some studies which revealed that peptones produced from different fish wastes, such as *Panulirus argus*, *Panulirus laevicauda*, and *Macrobrachium amazonicum*, showed a significant biomass production of *Escherichia coli* compared to commercial peptone (OXOID) [23]. Moreover, Poernomo and Buckle [13] and Shirahigue *et al*. [2] reported the higher biomass production of five microorganisms in Cowtail ray peptones and by two bacteria (*Escherichia coli*, *Staphylococcus aureus*) in both tilapia (*Oreochromis niloticus*) and cobia (*Rachycentron canadum*), respectively.

Conclusion

Peptone from parrotfish and grouper head by-products was successfully extracted using different combination of acids. The highest protein content was 86.67% and 86.43% in PFP1 and GFP1, respectively. For yield, solubility, and whiteness of peptone samples, GFP1 showed the highest value than that of other treatments. In addition, amino acid composition of peptone samples (PFP and GFP) was rich in glycine, glutamic acid, proline, and alanine, but low in tyrosine, histidine, lysine, and isoleucine. For bacterial growth analysis, both parrotfish and grouper fish peptones exhibited better growth rate than that of commercial peptone. Moreover, the biomass production performed higher in the PFP and GFP than that in the commercial peptone. For both growth rate and biomass production, PFP1 and GFP1 treated with 1:2 (v/v) of propionic and formic acids showed the highest values in growth rate and biomass yield. This research indicated that the best treatment of acid combinations in the peptone extracted from parrotfish and grouper fish heads was the ratio of 1:2 (v/v) of propionic and formic acids. These head by-products can be alternative sources as bacterial cultural media.

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