**Food Research 8 (1) : 148 - 159 (February 2024)**
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

# Formulation and organoleptic characteristics of flavor enhancer from shrimp head protein hydrolysate

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***Article history:***
Received: 20 June 2022
Received in revised form: 29 July 2022
Accepted: 28 December 2023
Available Online: 25 January 2024

***Keywords:***
Protein,
Hydrolysate,
Flavor enhancer,
Shrimp head

***DOI:***
https://doi.org/10.26656/fr.2017.8(1).331

**Abstract**

Protein hydrolysate can be produced from by-products of the fishing industry, such as shrimp heads. This product is made by enzyme hydrolysis through the breakdown of shrimp head protein into short-chain peptides and amino acids. Enzymatic hydrolysis produces protein hydrolysate, which has savory, umami, and water-soluble characteristics. The savory and umami taste results from the high content of glutamic acid and other free amino acids. This study aimed to determine the best formulation and organoleptic characteristics of the shrimp head protein hydrolysate flavor enhancer. Production of shrimp head protein hydrolysate (SPH) using alkalase enzyme at a temperature of 55°C with an enzyme concentration of 20,000 units/kg substrate for 7 hrs. Parameters tested were yield, proximate and amino acid analysis, and organoleptic test for SPH flavor enhancer. SPH has an amino acid composition dominated by arginine, alanine, glycine, glutamic acid, leucine and lysine. The flavor enhancer with 40% SPH composition was chosen as the best formula because it has sensory characteristics close to P0 commercial flavoring. The dominant sensory attributes were spicy, salty, umami and sweet. Overall, protein hydrolysate-based flavor enhancers' solubility and color properties are close to commercial flavor enhancers. The flavor enhancer made from SPH has the potential to be used as a substitute for commercial flavor enhancers which are rich in essential amino acids.

## 1. Introduction

The processing of fresh shrimp produces commercial products and by-products. Processed products from fresh shrimp are a leading commodity in Indonesia and have a high selling value for seafood globally. The fresh shrimp processing industry produces waste that can be utilized (by-products). The by-products in shrimp heads, carapace, and shrimp tails are around 35-70% (Mirzah and Filawati, 2013). Shrimp heads have a high protein content in addition to chitin 50% (Cahú *et al.,* 2012). The by-product of shrimp usually used is the carapace, but the protein part is discarded. The protein content of raw materials and shrimp head protein hydrolysate (SPH) was 10.52±0.08% and 3.71±0.08%, respectively. Shrimp heads' raw material and protein hydrolysate contain amino acids 21.12% and 3.33% wt, dominated by non-essential amino acids such as glutamic acid (0.5% w/w) and essential amino acids leucine (0.30% w/w) and lysine (0.24% w/w) (Yuniarti *et al.,* 2021). This composition makes the by-product of shrimp processing potentially exploitable for its protein content. So far, the utilization of by-products of processing fresh shrimp is for the components of chitin, carotenoids and glycosaminoglycans. These by-products can also be

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eISSN: 2550-2166 
Published by Rynnye Lyan Resources

utilized for their protein content into protein hydrolysate products simultaneously. The fresh shrimp processing industry can potentially be zero waste (Santos *et al.,* 2012). These products are organic ingredients with bioactive abilities that can be used in the food and health industries (Kandra *et al.,* 2012).

One of the uses of protein content in shrimp is to make SPH. Protein hydrolysate from fisheries is a product of fishery raw materials, fish meat, and by-products of the fishing industry. This product is made by hydrolysis, breaking fish meat protein into short-chain peptides and amino acids. Protein can still be used for both food and functional food. Protein is a material that is needed as a building material. In addition, recent research showed that proteins in the form of simple peptides have bioactive abilities such as antioxidants and antimicrobials. High-quality peptides and free amino acids are produced from the protein hydrolysis process. Fish protein hydrolysis products are made from enzymatic and chemical hydrolysis.

Hydrolysis is carried out chemically using acids or bases and enzymatically. The form of the hydrolysate product is liquid and solid (powder) (Petrova *et al.,* 2018). Although protein hydrolysate using acid is more promising and economical, it produces products containing chemical residues. The resulting protein hydrolysate is more appropriate for animal feed (Wisuthiphaet and Kongruang, 2015). The production of fish protein hydrolysate enzymatically produces protein hydrolysate, which has better nutritional properties than acid, making it more appropriate for industry (Wisuthiphaet *et al.,* 2016). SPH has a high biological value, is easy to digest, increases muscle mass, and increases growth (da Silva *et al.,* 2017). Protein hydrolysate as a food ingredient or food additive has wide applications. It has the antioxidant capacity of DPPH, ABTS radical scavenging activity and Fe metal chelating activity, emulsifier, ability to form foam and high protein solubility (91% more) at a wide pH range (3 -9) (Nalinanon *et al.,* 2011).

The production of protein hydrolysate requires the ability of enzymes to hydrolyze a high protein, called the degree of hydrolysis (DH). Various enzymes make protein hydrolysate from the by-product of processing fresh shrimp. Protease enzymes from microbes such as Alcalase, Neutrase, Protamex, and Flavorzyme, produce different degrees of hydrolysis. Alkalase enzymes produce the highest degree of hydrolysis (Dey and Dora, 2014). Several studies have been conducted on the manufacture of laboratory-scale shrimp head hydrolysate (Limam *et al.,* 2008; Cao *et al.,* 2009). However, few studies describe the yield of protein hydrolysate produced from shrimp heads. Production of protein hydrolysate on a larger scale than shrimp heads requires optimization of the best hydrolysis time to make the best degree of hydrolysis.

Enzymatic hydrolysis produces protein hydrolysate, which has savory, umami, and water-soluble characteristics. The high content of glutamic acid and other free amino acids has a synergistic effect giving a high umami sensation. This property is used to make natural flavorings, has an umami character, is high in protein, is easy to apply and is liked by consumers. Nowadays, consumers are considering the selection of flavorings from natural ingredients such as fermented products. Utilization of SPH as an ingredient in flavor enhancers can increase the added value of shrimp by-products and introduce glutamic acids to natural flavors. This study aims to determine the formulation and characteristics of flavoring enhancers' organoleptic from protein hydrolysate shrimp heads.

## 2. Materials and methods

### *2.1 Materials*

The materials used include fresh shrimp heads from the frozen shrimp processing industry, PT. First Marine in Muara Baru, North Jakarta, Indonesia. The enzyme used is a protease enzyme (Alcalase® 2.4 L FG, Novozyme) with an activity of 2.4 AU-A/g. Other materials used to analyze the degree of hydrolysis (DH) were disodium tetraborate decahydrate, o-phthaldialdehyde (OPA) 97%, and sodium dodecyl sulfate (SDS), ethanol, dithiothreitol (DTT) and 6.25% trichloroacetate (Merck). Measurement of amino acids using the Association of Official Analytical Chemists (AOAC) method (AOAC, 2005). The materials used for testing the proximate content were HCl $K_2SO_4$, $H_2SO_4$, NaOH, and diethyl ether (Merck). The tools used for the manufacture of SPH are a meat bone separator, food processor, 60-liter hydrolysis tank complete with temperature control and automatic stirrer, 50-liter micro and ultrafiltration tank full with two membranes with a pore size of 0.5 and 0, 1 m, spinner with 300 and 600 mesh pore filtration bags, spray dryer.

### *2.2 Production of protein hydrolysate powder*

SPH using a modified method (Yuniarti *et al.,* 2021). Shrimp heads were mashed using a meat bone separator and then put into a hydrolysis tank filled with water in a 1:1 (w/v) ratio and homogenized. The temperature is controlled between 55-60°C. Alkalase enzyme (20,000 units/kg substrate) was mixed when the temperature reached 55°C. The hydrolysis process was carried out for 7 hrs. For every hr of hydrolysis, measure the degree of hydrolysis (DH). Enzyme inactivation process by raising the temperature to 90°C for 20 mins. The hydrolysate

https://doi.org/10.26656/fr.2017.8(1).331 

formed was allowed to stand to create two separate fractions. The filtrate fraction and the resulting residue were separated by filtration using a spinner with a filter bag with a pore size of 300 and 600 mesh. The filtrate was then filtered using a micro and ultrafiltration machine with a pore size of 0.5 and 0.1 m to separate the clear-colored protein hydrolysate filtrate and the residue. This clear-colored filtrate is a protein hydrolysate of shrimp heads whose chemical profile will be determined. Protein hydrolysate liquid plus maltodextrin 20% of the total volume of hydrolysate liquid and dried using a spray dryer.

### *2.3 Shrimp head protein hydrolysate flavoring formulation*

The formulation in this study was carried out by mixing the main ingredients of SPH and additional ingredients, as shown in Table 1. Formulas with a suitable composition can be used effectively for diversification, increasing nutritional value, and consumer acceptance. It also aims to obtain the same type and intensity of attributes at the same concentration, preventing differences in taste, aroma, and color from affecting sensory testing results. After the ingredients are evenly mixed, they are dried in the oven for±3 h at a temperature of 60°C.

### *2.4 Analysis of the amino acid composition of shrimp head protein hydrolysate powder*

The amino acid composition was analyzed using HPLC (AOAC, 2005). HPLC operating conditions: Temperature: 27°C (room temperature), Type of column HPLC: Ultra tech sphere (Colom C-18), eluent flow rate: 1 mL/min, Pressure: 3,000 Psi, Mobile phase: Na-Acetate buffer and methanol 95%, Detector: Fluorescence, Wavelength: 350-450 nm. The amino acid analysis consists of 4 stages: the stage of making protein hydrolysate, drying stage, derivatization stage, injection stage, and amino acid analysis. The hydrolysate sample was dried using a rotary evaporator for 15-30 mins. The dry sample was added with 5 mL of 0.01 N HCl and filtered using millipore filter paper. The derivatization step is to add 30 µL of the derivatization solution to the dried sample. The derivatization solution consisted of a potassium borate buffer solution with a 1:1 sample and then mixed with a 5:1 Ophthaldialdehyde (OPA) solution with a sample. The mixture was filtered using Whatman filter paper. The filtered solution of 5 µL was injected into the HPLC. The separation of all the amino acids has waited until it is complete. It takes about 25 mins. The concentration of amino acids present in the material is calculated by making standard chromatograms using standard amino acids. The formula can calculate the content of amino acids in the ingredients:

$$Amino\ acid\ (\%) = \frac{sample\ area \times C \times Fp \times BM \times 100\%}{standard\ area \times sample\ weight}$$

Where C = Standard concentration of amino acids (0.5 moL/mL), FP = dilution factor (5 mL) and BM = Molecular weight of each amino acid (g/mol).

### *2.5 Proximate analysis*

The water content was analyzed by drying the sample at 105°C for 24 hrs (AOAC, 2005). Ash content was analyzed by drying the sample at 600°C for 6 hrs (AOAC, 2005). The fat content was analyzed using the Soxhlet-based method (AOAC, 2005) by extracting the sample for 4-6 hrs, then further heated in an oven at 60° C for 24 hrs. Proteins were analyzed by the Kjeldahl-based method (AOAC, 2005).

### *2.6 Analysis organoleptic of flavor enhancer from shrimp head protein hydrolysate*

#### *2.6.1 Consumer acceptance test*

The consumer acceptance test was carried out using the rate all that apply (RATA) method. This method can eliminate the need for trained panelists and a longer test time. With the use of untrained panelists, consumers can provide direct feedback to provide product descriptions that are close to consumer desires. In addition to binary data, this method produces intensity data in values that indicate the magnitude of each attribute perceived by the panelists (Ares *et al.,* 2014). The RATA method is carried out by giving an intensity rating by consumers on description attributes that are considered to be able to describe the product (Alexi *et al.,* 2018) and accompanied by a hedonic rating test (Jaeger and Ares, 2015) to see consumer preferences for the product being tested. The hedonic rating test is one of the affective tests that aim to assess the overall acceptance of the product (Meilgaard, 2007). Although the work consists of two

Table 1. The formulation of additional ingredients in the production of flavor-enhancing products.

| Treatment | Formulation | | | | | | |
|---|---|---|---|---|---|---|---|
| | Commercial flavor enhancer (%) | Shrimp Head Protein Hydrolysate (%) | Salt (%) | Sugar (%) | Onion (%) | Pepper (%) | Ginger (%) |
| P0 | 100 | - | - | - | - | - | - |
| PKU 100% | - | 100 | - | - | - | - | - |
| PKU 60% | - | 60 | 18.4 | 8 | 10 | 2 | 1.6 |
| PKU 50% | - | 50 | 23 | 10 | 12.5 | 2.5 | 2 |
| PKU 40% | - | 40 | 27.6 | 12 | 15 | 3 | 2.4 |

https://doi.org/10.26656/fr.2017.8(1).331

stages (giving a checklist and a rating), the RATA method does not provide significant difficulties to consumers (Ares *et al.,* 2014). Research using the RATA method was carried out in several stages.

*2.6.2 The focus group discussion stage*

The stage aims to explain the standard equivalent terms of sensory attributes written on the questionnaire and will be used in sample testing. This stage is carried out because the panel examines consumers who are generally less familiar with the terms used as sensory attributes. The quality attributes in Table 2 are explained by involving more than 100 consumer panelists drawn from the Fisheries Business Expert Polytechnic environment in Bogor and Jakarta. The selected panelists are students familiar with flavor enhancer products and do not have allergies or certain diseases directly caused by flavoring products. The reference material used to discuss the attributes in Table 2 is shown in Table 3. The reference material is dissolved in 30 mL warm water per sample dose at 50 - 60$^{o}$C.

Table 2. Product quality attributes flavor enhancer.

| Descriptive attributes | Notes |
|---|---|
| Burnt | Aroma associated with burnt spices |
| Fishy | The smell of fresh fish mixed with spices |
| Bitter | Basic taste, a little bit bitter |
| Salty | Has a dominant salty taste due to the addition of salt |
| Umami | MSG base taste |
| Sweet | Sugar base taste |
| Spicy | Hot in the oral cavity due to spices |
| Color intensity | Appearance of the color of the solution |

Table 3. Reference materials used in the discussion of product quality attributes.

| Reference materials | Serving size (g/30 mL) (% b/v) |
|---|---|
| Fishy | 0.18 – 0.27 |
| Salty | 0.07 – 0.10 |
| Sweet | 0.04 – 0.05 |
| Spicy | 0.02 – 0.03 |

*2.6.3 Panelist screening stage*

This was carried out to obtain background information on panelists. The panelists who pass this stage are the panelists who are the target needs for flavor -enhancing products. Generally, the screening stage is carried out before the testing stage, but if the screening stage is not possible to do first, then the screening stage and the testing stage can be carried out simultaneously. This study carried out the screening stage after the panelists had finished testing the sensory samples. Panelists who do not pass the data screening stage will be excluded and not analyzed. The screening stage is carried out by filling out questionnaires by prospective panelists. The questionnaire was designed to obtain background information from the panelists, including gender, age and medical history.

*2.6.4 The sample testing stage*

Fifteen grams of flavor enhancer powder was dissolved in 500 mL (w/v) warm water at a temperature ranging from 50 - 60$^{o}$C, then served as much as 30 mL of each sample in a clear glass at random. This presentation is to avoid comparisons between samples. To prevent bias, samples were coded with three-digit random numbers and presented in random order. Details regarding the type of sample and serving size are shown in Table 4.

Table 4. Coding and serving instructions on sample testing.

| Sample Name | Sample Code | Serving size |
|---|---|---|
| P0 | 98 | 15 g/500 mL water |
| PKU 100% | 768 | 15 g/500 mL water |
| PKU 60% | 267 691 721 | 15 g/500 mL water |
| PKU 50% | 397 555 234 | 15 g/500 mL water |
| PKU 40% | 482 111 109 | 15 g/500 mL water |

The test begins with neutralizing the panelists' sense of taste by drinking mineral water. The panelists tasted the samples presented one by one and assessed the sample without comparing it with other samples. The first test conducted by the panelists was the hedonic rating test. The hedonic test is done by tasting each sample without comparing between samples. The hedonic test is a preference test for attributes by concluding and giving points representing the conclusion of the overall preference. Panelists evaluate each sample with a 6-point preference scale (1 = strongly dislike to 6 = very much like), as shown in Table 5. The 6-point hedonic scale is used to prevent panelists from giving a neutral score. Neutral values in product development are undesirable because it cannot be known whether the panelists tend to like the product or not (Everitt, 2009).

The output of the hedonic test is a Preference mapping graph and a Contour plot. Preference mapping is used to understand the relationship between product characteristics and consumer preferences in two dimensions (Yenket *et al.,* 2011). Consumer preference is defined as a person's choice of liking or disliking the product being consumed (Ghose and Lowengart, 2013). Preference theory is used to analyze the level of satisfaction for consumers. This study will guide the development of new products, product characteristics or features, prices, and other marketing mixes. The

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Table 5. The scale used for the liking test.

| Score | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Hedonic scale | Strongly disagree | Disagree | Neutral | Somewhat agree | Agree | Strongly agree |

Table 6. Intensity rating for scoring of the quality attribute.

| Score | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Intensity rating | Very low | Moderately low | Moderate | Moderately high | Very high |

preference mapping method is widely applied to sensory science. It is used to map the consumer grouping of various products or the quality and characteristics of food flavors from multiple products (external preference mapping).

The second test carried out is the intensity test. The intensity test is carried out by placing a checkmark on the question table for each attribute that is considered to describe the sample being tested. The order of removing the samples between the panelists will be randomized, the panelists will not get the same sample order format. This is done to avoid bias. The attributes selected by the panelists are then given an intensity rating according to the panelists' perceptions. The intensity rating is based on the five intensity levels presented in Table 6.

The product attribute intensity test output is the Principal Component Analysis (PCA) graph and spiderweb. PCA is a multi-variable analysis to determine correlations and similarities between related observations. The output of this analysis is a biplot graph that describes the sensory profile of the flavor enhancer product (Abdi and Williams, 2010). The spiderweb graph shows the distribution of data values to see the quality of SPH products from eight key attributes in two-dimensional form. This graph has eight radii showing variable values starting from the same point.

*2.7 Principal component analysis biplot chart*

Sensory data AVERAGE SPH samples were processed by multivariate principal component analysis (PCA) analysis using XLSTAT software to determine the relationship between SPH samples and their sensory attributes. PCA analysis is also used to determine the dominant sensory characteristics in each sample tested and classify the test samples based on the similarity of the sensory qualities attached to the sample. PCA analysis using XLSTAT software produces eigenvalue data, scree plot graphs, score plots, loading plots, and biplots.

*2.8 Statistical analysis*

Proximate analysis of flavor enhancer products was carried out with two replications. The data is calculated to find the mean and standard deviation. Proximate measurement data were analyzed with the Multivariate Analysis of Variance (MANOVA) significance test, followed by the Tests of Between-Subjects Effects test and Duncan's further test using the IBM version of SPSS v.25 X86 - X64. RATA and hedonic data were analyzed using XLSTAT 2019 software and Preference Mapping on Sensory Data Analysis. Color and solubility tests were carried out with two replications. The data is calculated to find the average value and standard deviation. Measurement data were analyzed by ANOVA test and Duncan's further test using SPSS v.25 X86 - X64 IBM version.

## 3. Results and discussion

*3.1 Determination of the yield of shrimp head protein hydrolysate for flavor enhancer*

Protein hydrolysate production requires yield data at each stage of production. Yield data is needed to determine the effectiveness of hydrolysate production both economically and biochemically. SPH in this study was in liquid form due to externally added water required during the enzymatic hydrolysis process. The final amount of SPH solution was 49.20 kg or 79.20% (Table 7). Protein hydrolysate can be in liquid or solid form, depending on the making of the hydrolysate. The solid-state has the advantage that it has a longer shelf life and does not require space and cooling treatment. This yield was higher than the hydrolysate product in the form of a paste produced from the hydrolysis reaction of shrimp head using the alcalase enzyme (12 AU/kg) for 1 hr at 40°C resulting in a yield of 45.1% (Mizani *et al.,* 2005). While the protein hydrolysate produced from the hydrolysis process (protease/peptidase enzyme activity 20-50 Units/mg, pH 8.0, temperature 60°C for 2 hrs) on

Table 7. Determination of the yield of shrimp head protein hydrolysate at each stage of food flavor production.

| Materials | Shrimp head (amounts) |
|---|---|
| Frozen raw material | 33 kg |
| Soft meat | 28 kg |
| Water | 28 L |
| Enzymes | 1.12 L |
| SPH liquid | 56 L |
| Spinner residue | Shell, walking legs 2.2 kg |
| Filtration residue | Hydrolysate 4.1 kg |
| SPH filtration result | 49.20 kg |
| Maltodextrin (20%) | 9.84 kg |
| SPH powder | 10.18 kg |

https://doi.org/10.26656/fr.2017.8(1).331

Northern pink shrimp heads was 7.07%, Endeavor shrimp was 7.38% and Black tiger shrimp was 6.46% (Ruttanapornvareesakul *et al.*, 2005).

However, the solid protein hydrolysate form requires more time and costs and a more complicated drying process. Short-chain peptides are sensitive to temperature, which affects their functional properties (Mackay and Chilkoti, 2008), so they require a proper drying process. Several drying methods for protein hydrolysate include the freeze-drying technique, the use of foam-mat dry technology (Sukkhown *et al.,* 2018), the centrifuge technique (Seniman *et al*., 2014), and spray dry technology (Dhanabalan *et al.,* 2020). Protein hydrolysate in liquid form is usually an intermediate product used for further processing. Liquid protein hydrolysate can be used as an emulsifier (Chai *et al.,* 2020), as fertilizer for patchouli and mung bean (*Vigna radiata*) (Nurdiawati *et al*., 2019), biostimulant (Madende and Hayes, 2020).

### *3.2 Amino acid composition in shrimp head protein hydrolysate flavoring products*

The analysis results for determining the amino acid composition of SPH powder as raw material for flavor enhancers using HPLC can be seen in Table 8. The SPH contains 19.55 w/w or 195.5 mg/L amino acids. These amino acids consist of non-essential amino acids and essential amino acids. SPH contains non-essential amino acids of 3.26% w/w, where the glutamic acid content is the highest among other non-essential amino acids. Hydrolysis of shrimp by-product *Penaeus chinensis* using the dispase enzyme (2%), at pH 6.5, hydrolysis temperature 57°C, hydrolysis time of 3 hrs, and the ratio of raw materials (substrate): water of 1:10, degree of hydrolysis (DH) of 57.65% produced a liquid hydrolysate with a free amino acid composition of 29.67 mg/mL consisting of essential amino acids of 11.30 mg/mL and non-essential amino acids of 18.37 mg/mL (Guo *et al.,* 2009). The amino acid composition is dominated by arginine, alanine, glycine, glutamic acid, leucine and lysine.

The hydrolysate products are short-chain amino acids and peptides. Free amino acids are the main component of taste in seafood products. The flavoring of SPH is rich in the amino acids arginine, alanine, glycine, glutamic acid, leucine and lysine. The amino acid arginine produces a slightly bitter and sweet taste; serine has a sweet and sour taste like monosodium L-glutamate (MSG). Glutamic acid combined with sour taste has an umami taste like MSG. Alanine has a slightly savory/umami flavor like MSG. MSG is the main ingredient used as a food flavoring ingredient (Kirimura *et al.,* 1969). Short-chain peptides from protein hydrolysis have the potential to be used as dietary supplements for athletes' diets. These supplements should be consumed before and after training as a "strength-power diet" food. Such high-quality protein should contain mainly di- and tripeptides. The proportion of di- and tripeptide absorption kinetics is higher than free amino acids (Manninen, 2009).

Table 8. Amino acid composition in SPH flavoring products.

| Amino acid | Amino acid content |
|---|---|
| Aspartic acid | 1.36% |
| Threonine | 0.57% |
| Serin | 0.48% |
| Glutamate | 3.26% |
| Proline | 1.19% |
| Glycine | 2.07% |
| Alanine | 1.61% |
| Cystein | 0.75% |
| Valine | 0.89% |
| Methionine | 0.18% |
| Isoleucine | 0.58% |
| Leucine | 1.13% |
| Tyrosine | 0.29% |
| Phenylalanine | 0.66% |
| Histidine | 0.43% |
| Lysine | 1.46% |
| Arginine | 2.63% |
| Tryptophane | 0.04% |
| Total asam amino | 19.55% |

### *3.3 The nutritional composition of flavoring enhancers with the addition of head shrimp head protein hydrolysate*

The flavoring enhancers made from head SPH (PKU 100-40%) have a higher water content than commercial flavoring (P0) (Table 9). This happens because protein hydrolysate is a dry material with lower humidity than the surrounding air, so it quickly absorbs water or has hygroscopic properties (Hogan and O'Callaghan, 2013). The addition of maltodextrin in the manufacture of fish protein hydrolysate is an effort to stabilize the protein hydrolysate against water absorption by encapsulation (Wang and Selomulya, 2020). Water absorption in protein hydrolysate products causes changes in the quality characteristics of protein hydrolysates, such as becoming sticky and brown (non-enzymatic browning) (Klompong *et al.,* 2012). Several ingredients can be added to dry head SPH, including maltodextrin and gum arabic (Kurozawa *et al.,* 2009). Hygroscopic food storage is better if additional materials such as silica gel with a porosity diameter of 7.5 nm can reduce water absorption in dry products (Zheng *et al.,* 2014).

Commercial flavoring enhancers (P0) have the

https://doi.org/10.26656/fr.2017.8(1).331 

highest ash (mineral) content. Commercial flavorings enhancers are food additives containing ingredients permitted by each user country by the competent authority. These ingredients include glutamic acid, monosodium glutamate, monopotassium glutamate, calcium diglutamate, and monoammonium glutamate, which contain high salt (Khodjaeva *et al.,* 2013). The high salt content is probably the cause of the increased mineral content in commercial flavorings. Pure protein Hydrolysate (100% PKU) has the lowest mineral content because it is not mixed with other ingredients like salt and other seasonings. However, PKU 100% had the highest carbohydrate content because PKU 100% only consisted of 2 components, SPH and maltodextrin. Maltodextrin is carbohydrate/starch enzymatically and chemically hydrolyzed, dextrose equivalent (DE) > 20. Maltodextrin is widely applied in the food industry for bulking, gelling, crystallization prevention, promotion of dispersibility, freezing control and binding (Chronakis, 1998). The fat content of all flavorings is not too different, but the fat content of commercial flavoring enhancers (PO) is almost twofold. The high-fat content is due to the composition of commercial flavoring ingredients containing fat.

### *3.4 Hedonic value and preference map for shrimp head protein hydrolysate flavoring products*

The percentage of panelists' preferences for each sample is shown in Table 10. The results of the preference mapping analysis in this study are shown in Figure 1. In Table 9, the highest level of preference for panels is commercial flavoring (P0) at 100%, and flavoring enhancers contain 40% SPH (PKU 40%). The next level of preference is 60% SPH (PKU 60%), 50% SPH (PKU 50%), and the last option is the flavoring enhancers that contain 100% SPH (PKU 100%). The panelist's choice of commercial flavoring may be due to the commercial flavoring factor, which is more appropriate and famous in the community (Ghose and Lowengart, 2013).

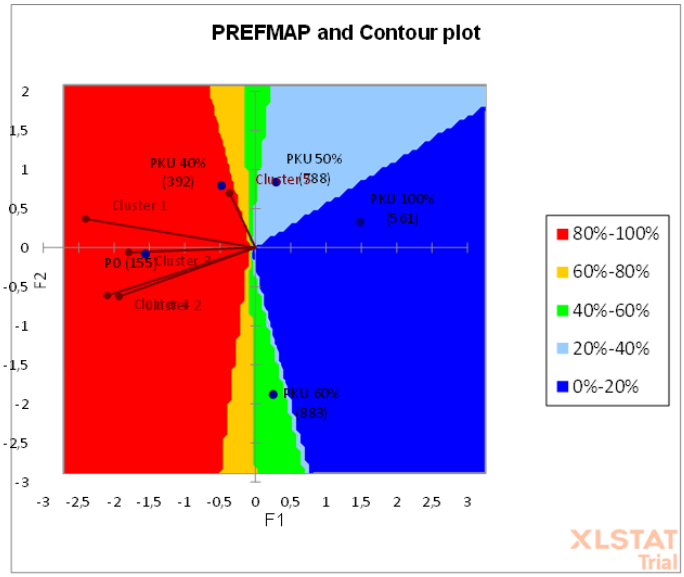


Figure 1. Mapping of panelists' preference level on shrimp flavor enhancer samples.

Table 10. The percentage of panelist's preference for the sample.

| Sample | Panelist's preference (%) |
|---|---|
| P0 | 100% |
| PKU 100% | 0% |
| PKU 60% | 40% |
| PKU 50% | 20% |
| PKU 40% | 100% |

### *3.5 Analysis of sensory attribute components and attribute characteristics*

Figure 2 explains the diversity of data on the main component of F1 of 67.79% and the main component of F3 of 0.73%. The cumulative total percentage of variance on the eigenvalues is 99.27%. Figure 2 can explain 99.27% of the actual data variance. The resulting interpretation can explain the relationship between the product sample and its various sensory attributes.

### *3.6 The closeness of the observed object*

The results from the PCA Biplot chart show that the SPH samples are divided into four groups/quadrants. In Quadrant I, there is a sample of PKU 100%, quadrant II has a sample of PKU 50%and PKU 60%, quadrant III has a sample of P0, and quadrant IV has a sample of PKU 40%. The closeness of the relationship is known by comparing the proximity of the point distances between

Table 9. Proximate content of commercial flavoring, SPH powder and flavor enhancers.

| Samples | Water (%) | Ash (%) | Protein (%) | Fat (%) | Carbohydrate (%) |
|---|---|---|---|---|---|
| P0 | 1.16±0.32$^{d}$ | 67.63±0.15$^{a}$ 0.89$^{a}$ | 9.74±0.35$^{c}$ | 1.30±0.01$^{a}$ | 20.17$^{e}$ |
| PKU 100% | 5.15±0.26$^{cb}$ | 1.32±0.22$^{e}$ 0.220.10$^{e}$ | 18.54±0.48$^{a}$ | 0.35±0.04$^{d}$ | 74.64$^{a}$ |
| PKU 60% | 5.57±0.44$^{b}$ | 20.75±0.14$^{c}$ | 12.73±0.08$^{b}$ | 0.69±0.21$^{bc}$ | 60.25$^{d}$ |
| PKU 50% | 6.20±0.39$^{a}$ | 16.86±0.37$^{d}$ | 8.96±0.26$^{d}$ | 0.51±0.03$^{c}$ | 67.48$^{b}$ |
| PKU 40% | 4.23±0.29$^{c}$ | 23.68±0.12$^{b}$ | 7.68±0.07$^{e}$ | 0.75±0.01$^{b}$ | 63.66$^{c}$ |

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p \leq 0.05$).

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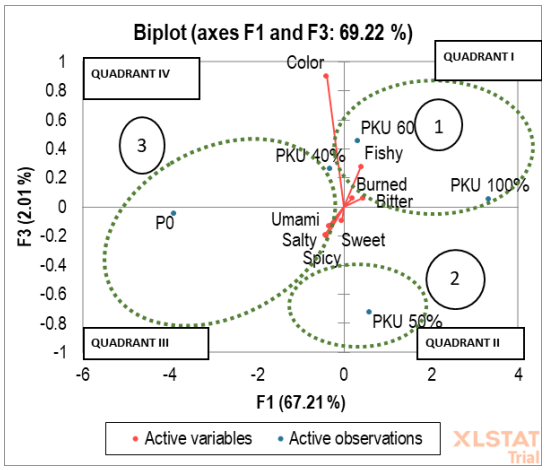


Figure 2. The results of the analysis of the main components of the sensory attributes of the taste enhancer sample Biplot PCA (F1:F3).

samples in the same quadrant. The quadrant shows the close relationship between samples that have dominant attribute characteristics in each sample. PKU of 50% and 60% have closeness caused by the similarity of their attributes, consumer perceptions assess that the burned feature is the most dominant in the two samples. The burned characteristic can be caused by the heating process after the formulation (mixing) process, thus giving a slightly burnt (scorched) aroma.

### *3.7 Correlation between variables*

Correlation can be known from the large angle formed between the vector variables. Suppose the angle formed from the two vector variables is close to $0^{o}$ or is getting narrower and has the same direction. In that case, the variable has a positive correlation. At the same time, if the two vector variables are opposite in direction and form a wide angle, then the variable has a negative correlation. Meanwhile, the uncorrelated variables are represented by two lines forming an angle close to $90^{o}$. Group 1 shows that the attributes of fishy and bitter have closeness based on the angle of the vector formed. This attribute only has similarities to the 100% PKU sample. The 100% PKU sample is a non-treated control sample in the form of pure SPH powder without being mixed with ingredients, so consumer perceptions of the sample predominantly consist of fishy and bitter attributes. Group 2 showed that 50% and 60% of PKU samples had dominant attributes: burned, color, and sweet taste. The reason is that the two samples during the heating process, which was carried out after the mixing process, gave a burned aroma to the sample. The attributes of color and sweetness arise from consumer perceptions but do not have an evident closeness. It is shown that the attributes of color and sweetness are in different quadrants. Sample P0 closely relates color, sweetness, and umami attributes. Still, consumer perception appears that the sample P0 only positively correlates with umami attributes because it is in the positive quadrant. Sample P0 is a non-treated control that is a commercial flavoring, so the fishy, burned, and bitter attributes negatively correlate with the sample. The PKU 40%sample in group 3 has a more precise perception from consumers; the umami, salty and spicy attributes have a close relationship with the sample. The PKU 40% undergoes a 60% ingredient mixing process, with more salt added, contributing to the increase in the umami attribute. Salt and glutamate have a synergistic effect in the mixing process.

Based on Figure 3, it can be seen that sample P0 which is a control treatment consisting of 100% commercial flavorings, has a high value on the intensity of the sensory attributes of salty taste and umami taste, with an average intensity value of 3.93 and 3.82, respectively. The color sensory characteristics of sample P0 also have a high intensity of 3.44. Based on Figure 3, the PKU 40% sample has the intensity of the sensory attributes closest to the sensory intensity of the P0 sample. For PKU 40%, the intensity value of the salty taste is 2.68, the umami taste is 2.71, and the color is 2.88 (Table 11). The fishy sensory attribute has the highest intensity value in the PKU 100% sample. The spicy sensory feature has the highest intensity in the 40% PKU sample, with a value of 2.33 (Table 11).

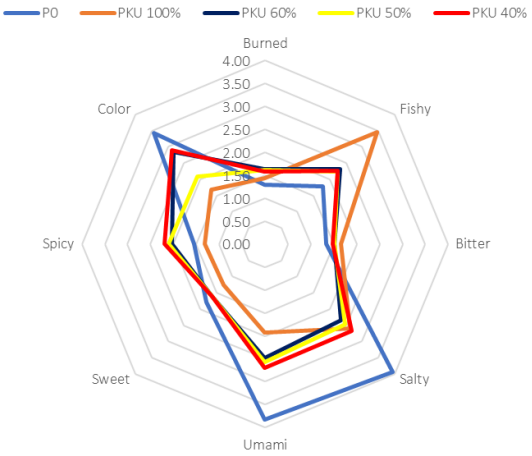


Figure 3. Sensory characteristics of shrimp head flavor enhancer samples.

### *3.8 Solubility and color of shrimp head protein hydrolysate and commercial flavor enhancers*

The physical characteristics of SPH and commercial flavor enhancers were investigated based on the degree of solubility (Table 12) and color (Table 13). The highest solubility level was PKU 100%, followed by 60%, 50% and 40% solubility, which were not significantly different, and the lowest solubility level was commercial flavoring (P0). The PKU 100% has a protein hydrolysate and maltodextrin composition. Maltodextrin is a food ingredient widely used in food, cosmetic and pharmaceutical products. Maltodextrin contains d-glucose, which binds alpha (1–4) glucosidic to form the

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Table 11. The mean value of the intensity of the sensory attributes of the shrimp head flavor enhancer sample.

| Sample | Burned | Fishy | Bitter | Salty | Umami | Sweet | Spicy | Color |
|---|---|---|---|---|---|---|---|---|
| P0 | 1.30 | 1.78 | 1.34 | 3.93 | 3.82 | 1.80 | 1.55 | 3.44 |
| PKU 100% | 1.44 | 3.45 | 1.65 | 2.61 | 1.93 | 1.27 | 1.32 | 1.67 |
| PKU 60% | 1.64 | 2.32 | 1.51 | 2.35 | 2.49 | 1.62 | 2.02 | 2.83 |
| PKU 50% | 1.62 | 2.24 | 1.52 | 2.47 | 2.57 | 1.62 | 2.10 | 2.10 |
| PKU 40% | 1.59 | 2.25 | 1.49 | 2.68 | 2.71 | 1.62 | 2.20 | 2.88 |

Table 12. Solubility values of 40, 50, 60%, 100% SPH and commercial flavor enhancers.

| Sample | Solubility (%) |
|---|---|
| P0 | $99.87\pm0.01^{c}$ |
| PKU 100% | $99.98\pm0.01^{a}$ |
| PKU 60% | $99.95\pm0.02^{b}$ |
| PKU 50% | $99.95\pm0.01^{b}$ |
| PKU 40% | $99.93\pm0.01^{b}$ |

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p \leq 0.05$).

Table 13. Color test values of 40, 50, 60%, 100% SPH and commercial flavor enhancers.

| Samples | L* | a* | b* |
|---|---|---|---|
| P0 | $92.68\pm0.18^{b}$ | $1.15\pm0.05^{d}$ | $22.55\pm0.10^{a}$ |
| PKU 100% | $95.62\pm0.02^{a}$ | $1.17\pm0.02^{d}$ | $19.63\pm0.01^{b}$ |
| PKU 60% | $86.69\pm0.16^{c}$ | $1.30\pm0.01^{c}$ | $18.77\pm0.12^{c}$ |
| PKU 50% | $84.62\pm0.02^{f}$ | $1.36\pm0.00^{b}$ | $17.63\pm0.01^{d}$ |
| PKU 40% | $81.80\pm0.15^{d}$ | $1.77\pm0.02^{a}$ | $15.34\pm0.01^{e}$ |

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p \leq 0.05$).

D-glucose polymer, a product of starch hydrolysis. Maltodextrin is an odorless, colorless, tasteless encapsulating agent with high water solubility (Castro *et al.*, 2016). Other ingredients affect solubilities, such as salt and natural powders (Hu *et al.*, 2021).

The results of the ANOVA test showed P-value < 0.05, so there was a difference in the average color value based on the concentration of the formula. The results of color analysis on the product include the value of L*, the value of a*, and the value of b*. The L* value indicates a light-dark spectrum with a range of 0 (dark) to 100 (white), so the more significant the L value, the brighter the color. The highest L* value in this test was in the PKU 100% product with 95.62±0.02 and the lowest value was in the PKU 40% sample with 81.80±0.15. Duncan's further test showed that the PKU 100% was significantly different from the letters indicated.

The addition of more ingredients will cause the brightness value to decrease. The a* value indicates a red -green color spectrum with a range of -60 (green) to +60 (red). Based on Duncan's further test, 40% CAR had the highest a* value of 1.84±0.03 indicated by the letter a. Commercial flavor enhancers have the lowest a* value of 1.15±0.05 indicated by the letter e. It was concluded that the higher the addition of the ingredient, the higher the a* value, and vice versa. The b* value indicates a blue-yellow color spectrum with a range of - 60 (blue) to + 60 (yellow). Based on Duncan's further test, commercial flavor enhancers have the highest b* value among others indicated by the letter a, while the PKU 40% has the lowest b* value indicated by the F value. Commercial flavor enhancers have a high b* value while L* values are lower than the PKU 100%. In conclusion, commercial flavor enhancers have a higher brightness color than the 100% PKU but have light yellow color.

The hydrolysis, drying, and heating processes at the formulation stage are the stages that can cause color changes in the product. The occurrence of color changes in PKU products of 40, 50, 60, and 100% can be caused by the content of peptides and free amino acids, which are more involved in the Maillard reaction, they have a high correlation to color changes in the product (Zhang *et al.,* 2015). The Maillard reaction is a non-enzymatic browning reaction between the carbonyl groups of carbohydrates and amino groups of amino acids, peptides, or proteins that occurs very quickly at high temperatures to produce a brown pigment called melanoidin (Horvat and Roščić, 2010). Maillard reactions play an important role in food processing and storage by producing various Maillard reaction products (MRPs) that contribute to food taste, color, aroma and bioactivity (Fu *et al.,* 2019). The carbonyl compounds produced during the Maillard reaction can be significant precursors in developing many heterocyclic compounds, polymers and product flavors (Mottram, 2007).

## 4. Conclusion

Protein hydrolysate from shrimp heads has an amino acid composition dominated by arginine, alanine, glycine, glutamic acid, leucine, and lysine. SPH can be used for making alternative flavor enhancers. The higher the composition of the protein hydrolysate added to the flavoring enhancers, the stronger the fishy attribute, so it is not the choice of the panelists. The flavor enhancer with the lowest composition of 40% SPH already has sensory characteristics approaching the commercial flavoring enhancers P0. The dominant sensory attributes are spicy, salty, umami, and sweet. Overall, the physical

https://doi.org/10.26656/fr.2017.8(1).331 

attributes of the solubility and color of the flavoring enhancers made from protein hydrolysate are close to commercial flavor enhancers. Flavors made from SPH as a by-product of shrimp processing have the potential to be used as a substitute for commercial flavor enhancers.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgments

This work was supported by Jakarta Technical University of Fishery through APBN 2019 which provides research funding.

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