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Formulation of Flavor Enhancer from Common Barb (*Rasbora jacobsoni*) Protein Hydrolysate

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Abstract. Natural flavor enhancers based on common burb (*Rasbora jacobsoni*) fish protein hydrolysate which is allegedly in accordance with human health needs have been investigated, namely by formulating them using ingredients such as sugar, salt, garlic powder, carboxyl methylcellulose (CMC) and sodium tripolyphosphate (STPP). The purpose of this study was to obtain the best flavor enhancing formulation from the protein hydrolysate from common burb. The best formulations were based on sensory characteristics, followed by testing its physical and chemical characteristics. Each formula consists of the composition of the protein hydrolysate and other ingredients consisting of: P1 (100% protein hydrolysate), P2 (80% protein hydrolysate: 20% ingredients), P3 (60% protein hydrolysate: 40% ingredients), P4 (50% protein hydrolysate: 50% ingredients), P5 (40% protein hydrolysate: 60% ingredients), P6 (20% protein hydrolysates: 80% ingredients) and P7 (100% ingredients). The results showed that the best formula in making flavor enhancers by the common burb protein hydrolysates was obtained from the treatment of P4 (50% protein hydrolysate: 50% ingredients), which had a moisture content of 6.03%, 19.76% ash content, fat content of 2.43%, protein content 11.14%, carbohydrate content of 67.44%, reducing sugar of 30.90%, emulsion capacity of 20.70 m2/g, emulsion stability of 141.20 minutes, and water soluble index of 0.059 g/ml.

INTRODUCTION

Consumption of MSG that exceed safe limits of consumption can cause harmful health effects. The level of MSG excessive consumption can lead to oxidative stress in the liver [1]. The feeding of a dose of 4 mg MSG / g BW / day in male mice for 30 days resulted in a change in the structure of hepatocytes (liver cells) in the form of parenchymatosa degeneration, hydropic degeneration, and necrosis [2]. One of the local alternative materials that can be used as a flavor is flavor enhancer the result of enzymatic hydrolysis derived from freshwater fish.

Freshwater fish that can be used as a raw material in the manufacture of the protein hydrolysate from common barb fish (*Rasbora jacobsoni*) [3]. This is due to have a glutamic acid content at the most that are equal to 12.72%, which can be used as a flavor savory precursor in the manufacture of flavor enhancer. Flavor enhancers made from raw fish generally have a taste that is less ready to be used directly because it has a bitter taste [4]

These constraints can be covered with an addition of other ingredients such as sugar, garlic powder, STPP (sodium tripoliphospat), CMC (carboxymethyl cellulose) and salt to enhance the savory flavor of the product. The addition of sugar to 15% can improve maillard product then formed the taste of umami [5]. While, the addition of salt to 15% can improve the taste and flavor in a food product [6]. It is required the addition of a garlic powder component containing dialil sulfide to reduce the fishy-smelling aroma or smell of fish that stings. The addition of CMC (carboxymethyl cellulose) also aims to establish a stable viscosity and a homogeneous suspension of hydrolyzed protein [7]. The addition of STPP (sodium tripoliphospat) intended as water-binding when formulating a flavor enhancer [8]. This study is an effort to get the best flavor enhancer formula of common barb fish protein hydrolysate that can be accepted by consumers through the preference test. The best treatment of flavor enhancer from common barb fish protein hydrolysate Determined by additional of ingredient in order to manufacture the chemical characteristics of the product.

MATERIALS AND METHODS

Materials and Tools

Common barb fish (*Rasbora jocobsoni*) fresh obtained from Jember Traditional Market. The protease extracted from crown flower and papaya by Witono *et al.*'s (2009) Method. Ingredients have been used such as:

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cysteine, gelatin, sugar, salt, garlic powder, STPP (*sodium tripolyphosphate)* and CMC (*carboxymethyl cellulose*). As well as chemical agent consist of 0.1 N NaOH, 0.1 N HCl, phosphate buffer of pH 7, *trichloroacetic acid* (TCA), Follin, *soluble casein*, benzene, SDS, H2SO4, selenium, boric acid (H_3BO_3), *methyl red* and *methyl blue* indicator, 0.02 N HCl, NaOH (PA), DNS (*dinitrosalisilic acid*), K-Na Tartar, glucose and ethanol.

While the tools which have been used in the research included stainless steel blender (Phillip), centrifuges (Yenaco models-YC-1180) and its tube, spectrophotometer (Shimadzu) and its cuvette, pH meter (Jen Way Type 3320, Germany), vortex (Thermolyne type 16700), refrigerator, water bath (GFL 1083), Ohaus analytical balance, electrical heating (Maspion) and glassware (Pyrex and Duran).

Production of Flavor Enhancer Made From Common Barb Fish

This research was conducted in two stages, namely the manufacture of the wet enzymatic hydrolysate of common barb fish and flavor enhancers. Common barb fish was steamed for 10 minutes and then destroyed by adding distilled water 2:1 (v/w). Suspension of common barb fish has added the combination of enzymes of 3% v/b (63.4% of crown flower enzyme and 36.6% of papain enzyme), 0.58% of cysteine and 1.33% of gelatin. It is conducted hydrolysis at 55°C for 3 hours. Furthermore, the enzyme inactivated at a temperature of 100ºC for 10 minutes. After all the hydrolysis process was obtained by wet hydrolysate of common barb protein.

The Manufacture of flavor enhancer was conducted by adding the formulation of ingredients (sugar, garlic powder, STPP (*sodium tripolyphosphate*), CMC (*carboxymethyl cellulose*) and salts in the wet hydrolysate that have been made. The ingredients are sugar 25%, salt 25%, garlic powder 5%, 5% STPP, CMC 40%. Each formula consisted of protein hydrolysates and the other ingredients of P1 (100% protein hydrolysates), P2 (80% protein hydrolysates: 20% ingredient), P3 (60% protein hydrolysates: 80% ingredient) and P7 (100% ingredient). Analysis of chemical composition and partial functional properties of the following best three formulas:

P4= 50% protein hydrolysate: 50% ingredient
P5= 40% protein hydrolysate: 60% ingredient
P6= 20% protein hydrolysate: 80% ingredient

Flavor enhancer has been analyzed the organoleptic preference [9]. The best treatment base on the organoleptic preference by using effectiveness test [10], moisture, ash, fat, protein, carbohydrates content [11], reducing sugars [12], emulsion capacity and stability [13], and water-soluble index [14].

RESULTS AND DISCUSSION

Flavor Organoleptic Preference Value

The criteria of dislike to moderately like that can be seen in Fig. 1 Glutamate acid contained in the protein hydrolysate is a factor that affects the taste of the flavor enhancer. Glutamate acid can be umami flavor to the food [15]. The sample most liked by panelists is P6 because the use of ingredients with the addition of garlic powder has the characteristics of both savory and strong taste. Savory and strong flavor of garlic powder is affected by the presence ofallicin [16]

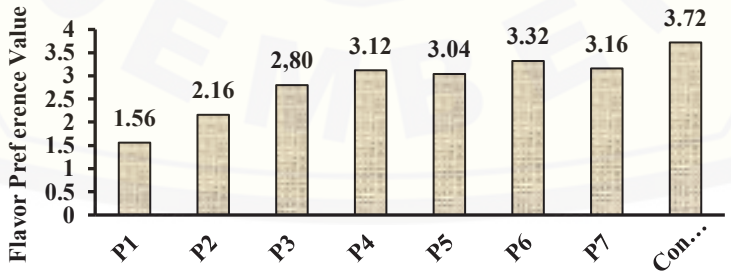

FIGURE 1. Flavor enhancer preference values of common barb fish protein hydrolysate in various treatments**.**

The addition of sugar aims to improve flavor so that sweet taste arises can improve the taste of the product [17]. The salt is a flavored enhancer in food products [18]. CMC is a cellulose derivative which has a 69% level of sweetness resembling sugar so that with the increase of CMC, make preference level of panelists to the flavor increase more [19].

Color Organoleptic Preference Value

Organoleptic Test. Criteria of dislike to like level can be seen in Fig. 2 A level of color in the flavor enhancer associated with the maillard products. Maillard reaction is a reaction between the carbonyl group and the primary amine group which involves the condensation reaction [20].

Fig. 2 shows that the flavor enhancer color that is most preferred by the panelists that is the P3 treatment. The use of ingredients such as CMC, STPP, sugar and garlic powder causing the color lighter flavor enhancer. This is because the fourth ingredients in powder form in a bright color, so the result of flavor enhancer will be brightly colored. CMC is a cellulose ether carboxylic acid derivative which is white [21].The calcium content in the powder [22].

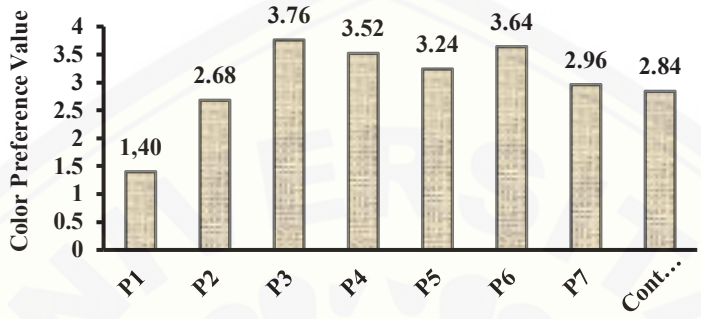

FIGURE 2. Color flavor enhancer preference values of common barb fish protein hydrolysate in various treatments.

Aroma Organoleptic Preference Value

The criteria of dislike to moderately like can be seen in Fig. 3 the highest aroma value is P4 treatment.

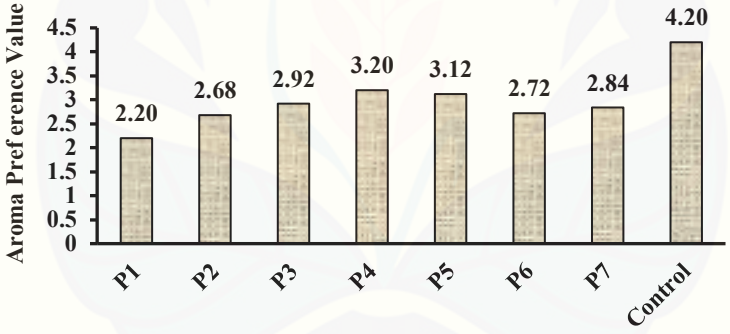

FIGURE 3. Aroma flavor enhancer preference values of common barb fish protein hydrolysate in various treatments.

Ingredient usage by 50% and 50% protein hydrolysate can produce a balanced maillard reaction. This is because the amount of protein and sugar is balanced so that formed a mallard reaction. The Maillard reaction that occurs in food products can produce taste and flavor [23]. The aroma is also affected by the interaction between the Crown flower enzyme and papain that can result in volatile fatty acids. This might cause a distinctive aroma of fish hydrolysate [15].

Overall Organoleptic Preference Value

The average value of overall organoleptic flavor enhancers can be seen in Figure 4. The most preferred treatment is P6. This is caused by the amount of protein hydrolysate of common barb fish that used a little more, so the aroma posed no fishy smell, light brown color. Ingredients Added that inhibit the Maillard products to produce a lighter color than other treatments. From the parameters of flavors, flavor enhancers panelists taste the flavor that is not too bitter because with the use of a protein hydrolysate less the bitter taste caused by the presence of short-chain peptide compounds can be reduced.

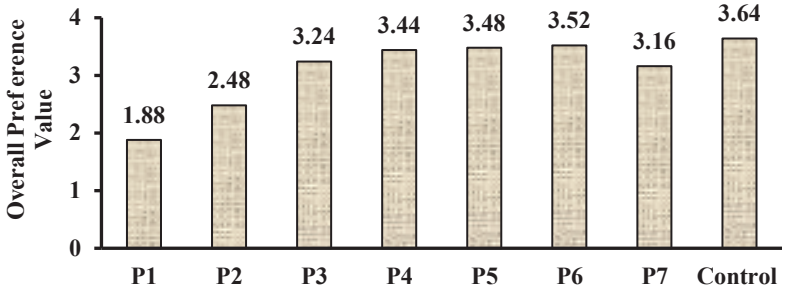

FIGURE 4. Overall flavor enhancer preference values of common barb fish protein hydrolysate in various treatments.

The Best Flavor Enhancer of Common Barb Protein Hydrolysate Formula Based on Organoleptic Preference Test

Based on the results of the organoleptic test covering favorite color, aroma, taste and overall made the selection of the three best treatment using effectiveness test. The test results on the effectiveness of the flavor enhancer of protein hydrolysate of common barb fish can be seen in Table 1.

TABLE 1. The Effectiveness of Flavor enhancer from Common barb Fish (*Rasbora jacobsoni*) Protein Hydrolysate

Fish (*Rasbora jacobsoni*) Protein Hydrolyzate

Treatments	Effectiveness
P1	0,00
P2	0,11
P3	0,20
P4	**0,24**
P5	**0,22**
P6	**0,21**
P7	0,19
Control	0,32

Table 1 show that the three best treatments are treatment P4, P5, P6. Three treatments flavor enhancer that has been the subsequent chemical analysis.

Chemical Characteristics of Flavor Enhancers from Common Barb Fish Protein Hydrolysate

Parameter chemical testing was conducted on the water content, ash content, fat content, protein content, carbohydrate content, reducing sugar content, water solubility index, emulsions capacity and stability.

Water Content

The observation of the water content of flavor enhancers can be seen in Figure 5. The lowest water content obtained on P6 treatment with a value of 4.184 ± 0.113%, while the highest water content in treatment P4 with a value of 6.025 ± 0.477%. The fewer the number of fish used resulting in lower levels of the dissolved protein hydration levels will be so low that the water content is lower flavor enhancer [4].

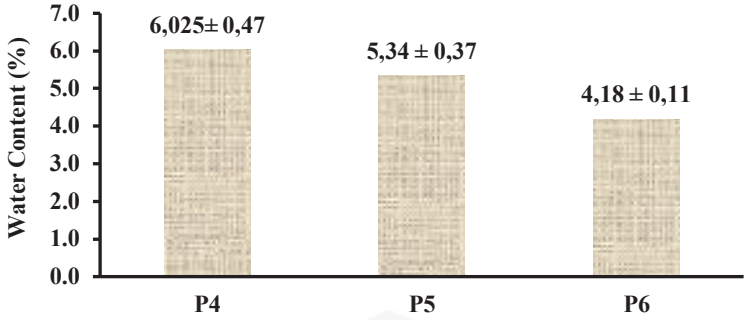

FIGURE 5. Water Content of Flavor Enhancer from Common Barb Fish Protein Hydrolysate

CMC can increase the viscosity so that the surface area of the dried material is getting bigger and higher drying speed. STPP Polar groups are hydrophilic (water-loving ions) so that the phosphate fraction able to bind the water that causes the water by protein binding ability becomes higher [24]. The water content decreases with increasing concentration of sugar. A decrease in water activity of foodstuffs due to the effect of the addition of sugar in high concentrations (at least 40% dissolved solids) [25].

Ash Content

The observation of ash content on flavor enhancers can be seen in Figure 6**.** The highest ash content is P6 treatment of 27.826 ± 0.632%. The use of ingredients affects the ash content of flavor enhancers. The provision of salt can cause the amount of minerals increased (especially sodium) so that the ash content increases [26]. Salts containing NaCl of 97% (SNI No.01-3556.2-1999) that can influence the increasing of ash content. The addition of STPP can increase the levels of ash for their phosphate mineral that is included in the inorganic salts [27]. Garlic powder also affects the ash content of flavor enhancers because they contain calcium, magnesium, and minerals [16].

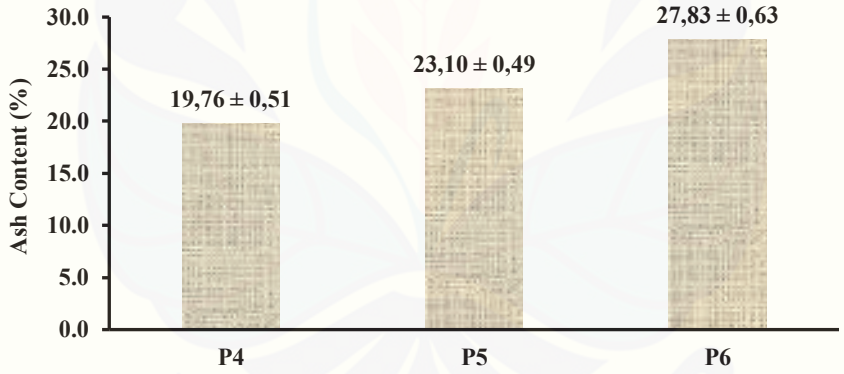

FIGURE 6. Ash Content of Flavor Enhancer from Common Barb Fish Protein Hydrolysate

Protein Content

The observation of the levels of protein flavor enhancers can be seen in Figure 7. The highest protein content is P4 treatment. The fewer fish raw material used in the manufacture of protein hydrolysates, the levels of protein produced in the end product would be lower [28]. According to the Indonesian National Standard No. 01-4273-1996 seasonings flavor has a protein content of at least 7%. This means that the treatment that best meets the National Standards of Indonesia as a spice of flavorings. But the results obtained lower than the protein content in commercial fish protein hydrolysate in the amount 84.00% (International Quality Ingredients, 2005).

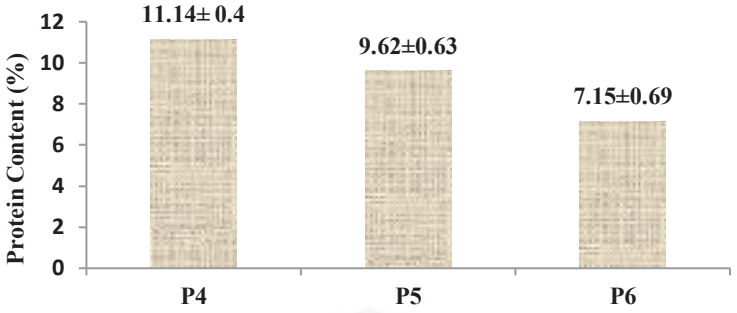

FIGURE 7. Protein Content of Flavor Enhancer Made from Common Barb Fish Protein Hydrolysate

Fat Content

The observation of the fat content can be seen in Figure 8. The highest fat value in treatment P4 is equal to 2.428 ± 0.302%. From these observations can be seen that the lower protein hydrolysate is used, the fat content of the resulting decreases. The higher levels of salt added flavor enhancer fat decreases [29]. Fat loss is due to the influence of the concentration of salt, this is because the salt can act as a catalyst in the oxidation of fatty fish. STPP is added to a food product is binding water. In the hydrolysis reaction, oils or fats will be converted into free fatty acids and glycerol. The damage of fat or oil can be caused by the presence amount of water that is still contained in the flavor enhancer [30].

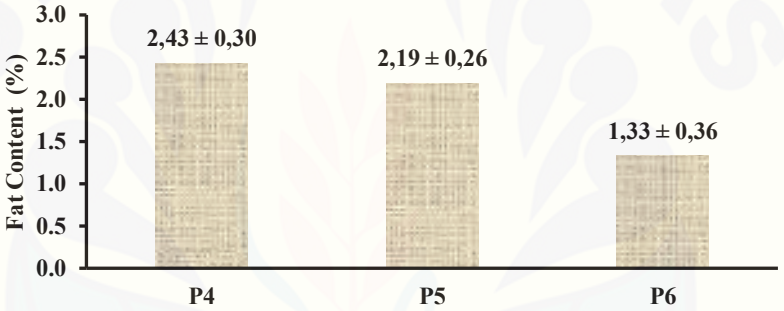

FIGURE 8. Fat Content of Flavor Enhancer from Common Barb Fish Protein Hydrolysate

Carbohydrate Content

Observations carbohydrate values can be seen in Fig. 9 The higher use of ingredients as well as the increasingly low use of the protein hydrolysate carbohydrate content the higher the flavor enhancer. The highest carbohydrate levels in P6 treatment of 63.02 ± 2.12%. The higher sugar concentration addition can lead the increasing of carbohydrates levels due to sugar is sucrose which included as the types of carbohydrates [23].

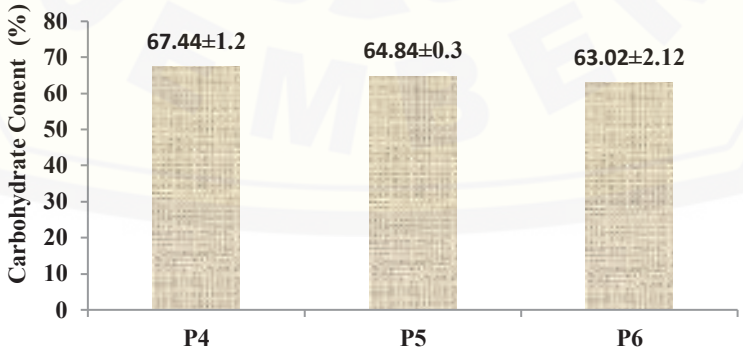

FIGURE 9. Carbohydrate Content of Flavor Enhancer from Common Barb Fish Protein Hydrolysate

The use of CMC with high concentrations can increase carbohydrate levels for CMC including cellulose derivatives containing hydrocolloid gum and are easily hydrolyzed polysaccharides [31]. Garlic powder of carbohydrate composition of 23.1 to 24.6 g / 100gram can affect the end product use garlic powder as an

ingredient of the product [32].

Reducing Sugar Content

The observation of reducing sugar content can be seen in Figure 10. The highest reducing sugar levels in P6 treatment of 47.282 ± 0.372%. Increased reducing sugar is caused by the addition of STPP on materials binding water. The presence of reactive free OH groups can cause reduced sucrose into invert sugar (glucose and fructose). During heating, sucrose will invert sugar is hydrolyzed into fructose and glucose is a reducing sugar. Increased reducing sugar also caused by the use of CMC.

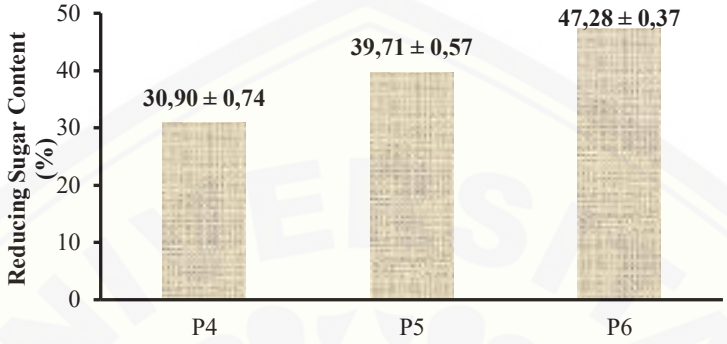

FIGURE 10. Reducing Sugar Content of Flavor Enhancer from Common Barb Fish Protein Hydrolysate

Cellulose is hydrolyzed to produce glucose which will be included into a reducing sugar [33]. Garlic powder containing carbohydrates are included into glucose. Therefore, the more the concentration of garlic powder is used, the higher the levels of reducing sugars in the flavor enhancer.

Fuctional Characteristics Of Flavor Enhancers From Common Barb Fish Protein Hydrolysate

Physical properties can be known through a series of tests that power emulsions and emulsion stability using the method performed [13], as well as water solubility index by [14].

Emulsion Capacity and Stability

The observation of the emulsion capacity can be seen in Figure 11. The highest value of emulsion capacity is P4 treatment equal to 20.700 ± 0.508. It is known that the higher the protein hydrolysate emulsion higher power. The presence of NH^{3+} and COO^{-} groups on amino acids and short peptide chain so formed can increase the solubility. Proteins spread between surfaces can result in the accumulation of oil and water more evenly [34].

Salt used can reduce the amount of protein because of NaCl weaken the interactions among groups of different proteins charge [35]. The amount of fat and protein that are not proportional to the amount of CMC and STPP which serves as an emulsifier will lead to the ability to cloak emulsifier or fat will bind to the increasingly weak. This can cause the emulsion flavor enhancer power will decrease [8].

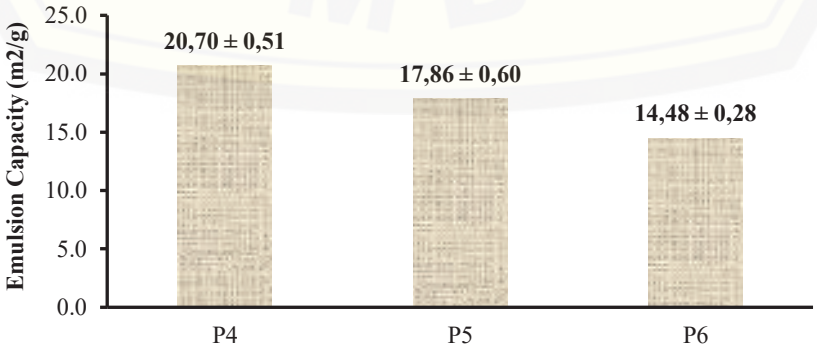

FIGURE 11. Power Emulsion of Flavor Enhancer from Common Barb Fish Protein Hydrolysate

The observation of the stability of the emulsion can be seen in Fig. 12 The higher the protein hydrolysate is used then the value of ESI (emulsion stability) will increase [36]. Emulsion stability is affected by oil content and protein in the emulsion system. Stabilizer of emulsion can be played by the protein [37]. The amount of protein which is not comparable with the amount of CMC and STPP which serves as an emulsifier will lead to the ability to cloak emulsifier or fat will bind to the increasingly weak. This can cause the emulsion stability will decrease or stable emulsion will not be formed. An emulsion is stable if there is no separation between the phases, the three main components, namely the formation of the dispersed phase emulsion (fat), dispersing phase (water) and emulsifiers.

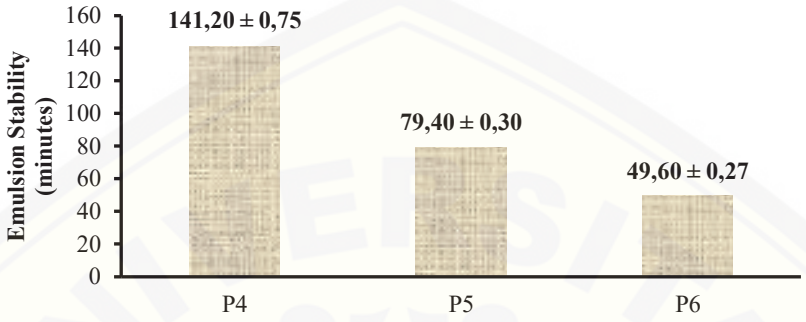

FIGURE 12. Stability Emulsion of Flavor Enhancer from Common Barb Fish Protein Hydrolysate

Water Solubility Index

The observation of water solubility index can be seen in Fig. 13 The highest water solubility index value in treatment P4 of 0.0586 ± 0.0012%. Protein solubility in water due to the ability of a protein associated with water. Increasingly least the protein content of the material, the material's ability to dissolve in water will decrease. The level of water solubility may decrease caused the use of ingredients. The higher use of CMC can reduce the level of solubility due to the deposition of the CMC in the solution, so it is not soluble in water [38]. The index drop of water solubility may be due to the agitation in the manufacture of flavor enhancers. The longer the agitation can cause the cellulose structure in the CMC increasingly expand and enlarge the distance between one group with the other group, the distance lead bonding process termination and replacement of the more difficult groups. If substitution is not the case then the water will not be bound and led to a water-insoluble CMC [39].

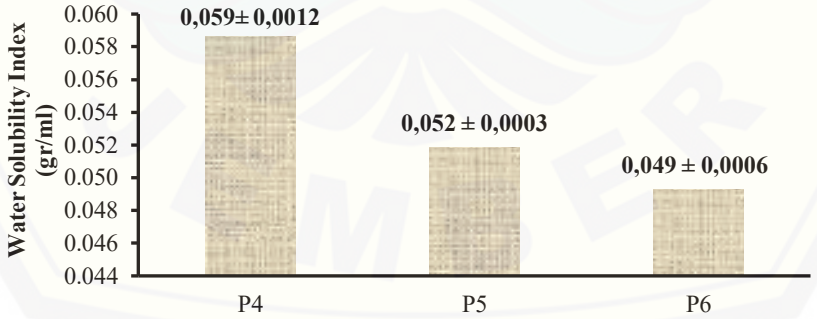

FIGURE 13. Water Solubility Index of Flavor Enhancer Made from Common Barb Fish Protein Hydrolysate

The Best Fuctional and Chemical Test Result of Flavor Enhancer

Based on the results of the physical and chemical tests which include water content, ash content, fat content, protein content, carbohydrate content, water solubility index, emulsion capacity and stability and reducing sugar content, the best using a selection of three treatment effectiveness test. The test results on the effectiveness of the flavor enhancer common barb fish protein hydrolysate can be seen in Table 2.

Table 2 showed that of the three treatments (P4, P5, and P6) that has been conducted functional and chemical testing, it was found that each of its effectiveness values of 0.07, 0.06, and 0.04. The highest

effectiveness values is in treatment P4 of 0.07, while the lowest value in effectiveness treatment is P6 of 0.04. P4 treatment (50% Protein hydrolysate: 50% ingredient)

The characteristics which include of water content of 6.03%; ash content of 19.76%; protein content of 11.14%; fat content of 2.43%; carbohydrate content of 67.44%; reducing sugar content of 30.90%; emulsion capacity of 20.70 m^2/g; the emulsion stability of 141.20 minutes; as well as water solubility index of 0.059 g/ml. The effectiveness of treatment with the highest value is the value of the best treatment because the value obtained by considering all the variables that play a role in determining the quality of the product [40].

TABLE 2. Effectiveness of Chemical and Physical Characteristics Flavor Enhancer from Common barb Fish (Rasbora jacobsoni) Protein Hydrolysate.

Treatments	Effectiveness
P4	0.07
P5	0.06
P6	0.04

CONCLUSION

In the organoleptic and effectiveness test, there are three treatments that obtained the best among common barb fish protein hydrolysate comparison with ingredient (CMC, garlic powder, sugar, and salt STPP) and the treatments are treatment P4 (50% hydrolysate Protein: 50% ingredient), P5 (40 Protein hydrolysate%: 60% ingredient), P6 (20% hydrolysate Protein: 80% ingredient).

The treatment which is right on the manufacture of flavor enhancers from common barb fish protein hydrolysate is treated P4 (50% hydrolysate Protein: 50% ingredient) with the results of chemical characteristics include to water content of 6.03%; ash content of 19.76%; protein content of 11.14%; fat content of 2.43%; carbohydrate content of 67.44%; reducing sugar content of 30.90%; emulsion capacity of 20.70 m^2/g; emulsion stability of 141.20 minutes; and Water solubility index of 0.059 g/ml.

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