Indonesian Food Science and Technology Journal
IFSTJ : Vol 7 No 2, July 2024 (PP: 172-179)
ISSN : 2615-367X

INDONESIAN FOOD SCIENCE AND TECHNOLOGY JOURNAL (IFSTJ)

Journal homepage : online-journal.unja.ac.id/ifstj/issue/archive

Characteristics of *Sardinella* Smart Flavor based on Enzymatic Hydrolysis Using a Calotropin and Papain Combination

Yuli Witono#, Ardiyan Dwi Masahid, Maria Belgis, Ahmad Nafi', Lailatul Azkiyah, Livia Wahyuni, Nur Ika Okta Majid

Study Program of Agricultural Products Technology, Faculty of Agricultural Technology, University of Jember, Jalan Kalimantan 1 No 1, Jember, 68121, Indonesia

#Corresponding author: E-mail: yuliwitono.ftp@unej.ac.id

***Abstract*— *Sardinella lemuru* is one type of fish with the highest population and the most widely caught fish in Indonesia. The protein content is very high, namely 17.8-20% contains various amino acids including glutamic acid, which can potentially be a smart flavor precursor. The research focuses on enzymatic smart flavor production by examining the effect of concentration and hydrolysis time of calotropin and papain combination on characteristics of the smart flavor produced and determining the best treatment to produce smart flavor. This research used two factors: the concentration of enzyme (3%, 4%, and 6% with a ratio of papain and calotropin of 70%: 30%) and hydrolysis time (1, 2, and 3 hours). The research showed that yield ranged from 50.15-50.69%; brightness 89.72-91.57; water 6.91-9.51%; soluble protein 1.13-1.56 mg/ml; antioxidant activity 48.40-54.47%; emulsion power 0.16-0.18 m^2/g, emulsion stability 17.27-24.33 minutes; water holding capacity 83.44-89.27% and effectiveness 1.00 (enzyme 6% and time of hydrolysis 3 hours). The best treatment with effectiveness was *Sardinella lemuru* smart flavor (6% enzyme and 3 hours hydrolysis) with a yield 50.54%, brightness 89.72, water 6.91%, soluble protein 1.56 mg/ml, antioxidant activity 54.47%, emulsion power and stability 0.18 m^2/g and 24.33 minutes, and water holding capacity 89.27%.**

***Keywords*— *Sardinella lemuru*, enzyme, hydrolysis, smart flavor**

Manuscript received March 18, 2024; revised June 27, 2024; accepted July 21, 2024. Available online July 31, 2024

I. INTRODUCTION

Sardinella lemuru production in 2017 reached 23.267,55 tonnes based on data from the Ministry of Maritime Affairs and Fisheries (KKP). The price of *Sardinella lemuru* is quite economical, namely around Rp. 6.000/kg. *Sardinella lemuru* contains high protein, between 17.8-20% [1] and essential fatty acids, especially omega-3, as much as 6.56% [2]. In addition, *Sardinella lemuru* contains glutamic acid and can be used as a precursor for natural savory flavors in food [3]. MSG (*Monosodium Glutamate*) is usually added to create a savory taste in food. MSG is a synthetic flavoring that is added to food as L-glutamic acid. Basic Health Research (2018), 77,6% of the population in Indonesia consumes MSG more than once per day. MSG production in Indonesia reaches 254.900 tons/year, with an average annual consumption increase of around 24,1%. According to WHO (World Health Organization), the safe limit for consuming MSG is 120 mg/body weight per day. [4], continuous use of MSG in the long term will cause side effects such as oxidative stress in the liver [5].

Natural flavoring can be made from safe food ingredients such as *Sardinella lemuru* in Indonesia. Natural flavoring is made by hydrolyzing *Sardinella lemuru* protein. The hydrolysis process results in protein hydrolyzate, the basic ingredient for making smart flavors. Fish protein hydrolyzate contains natural antioxidants which are suitable for the body. Patin fish hydrolyzate has an antioxidant inhibition percentage ranging from 37.86%-67.62% [6], tuna bone protein has an inhibition of 36.72% [7], and yellowfin fish protein has an inhibitory up to 70% [8]. Fish protein hydrolyzate shows potential as an

antioxidant through its ability to trap free radicals (free radical scavenging) and proton donors and bind metal ions [9].

Enzymatic hydrolysis of *Sardinella lemuru* uses the protease enzymes papain and calotropin. Papain is an endopeptidase type, and calotropin is an exopeptidase type. Endopeptidase breaks peptide bonds inside [10], and exopeptidase breaks protein polypeptides on the outside. Using papain and calotropin enzymes in a ratio of 70:30 can produce the highest soluble protein and Maillard products and reduce rancidity in inferior fish [11]. The research focuses on enzymatic smart flavor production by examining the effect of concentration and hydrolysis time of the calotropin and papain combination on the characteristics of the smart flavor produced and determining the best treatment to produce smart flavor.

II. MATERIAL AND METHODS

A. Material

The materials used are *Sardinella lemuru* from TPI (Fish Auction Place) Puger, calotropin from biduri sap extraction and papain from papaya sap, garlic powder, sugar, salt, CMC (*Carboxy Methyl Cellulose*) and STTP (*Sodium Tripolyphosphate*). Chemicals such as 0.5 M phosphate buffer pH 7 (Na_2HPO_4 and NaH_2PO_4), Aquades, Lowry's reagent (50 ml 2% Na_2CO_3 + 0.1 N NaOH with 1 ml of 1% $CuSO_4$ solution + 1% Sodium potassium tatrate in water), DPPH (*2,2- diphenyl-1-picrylhydrazyl*), Follin, ethanol p.a, 70% ethanol, and SDS (*Sodium dodecyl sulfate*).

B. Sample preparation

Making protein hydrolyzate [12]

The first step is filleting *Sardinella lemuru* by removing the head, bones, scales and tail. *Sardinella lemuru* meat is weighed, and distilled water is added in a 1:2 (w/v) weight of the fish meat and then blended. The *Sardinella lemuru* suspension was adjusted to pH seven, and enzymes were added with a papain:calotropin ratio of 70%:30% (3%, 4% and 6%). Next, hydrolysis was carried out using a water bath 55^{0}C for 1, 2 and 3 hours. Next, enzyme inactivation was carried out at 90^{0}C for 15 minutes. The hydrolyzate is then cooled and filtered.

Making Sardinella lemuru smart flavor

Making *Sardinella lemuru* smart flavor refers to research by [12]. Add *Sardinella lemuru* hydrolyzate, 5% garlic powder, 25% sugar, 25% salt, 40% CMC (*Carboxy Methyl Cellulose*), and 5% STTP (*Sodium Tripolyphosphate*). Next, dry it using a 60^{0}C oven for 24 hours and reduce the size.

C. Parameters

Yield

Yield is the percentage of product produced from the final weight. The way to calculate the yield is to weigh and calculate the final weight of the product resulting from the process using the formula:

$$\text{Yield (\%)} = \frac{\text{Weight after processing (g)}}{\text{Wet weight of initial sample (g)}} \text{ x100\%}$$

Color [13]

Color measurement using a color reader. Measurement of the sample by placing a color reader on the sample surface three times by taking at different points. The L* (lightness) shows the brightness value with a range of 0-100. The formula obtains the L* value:

$$\text{L*} = \frac{94{,}35}{\text{Standart L}} \text{ x L sample}$$

Water [14]

Measurement of water begins by drying the weighing bottle using an oven for 2 hours and drying it. The bottle is weighed to determine its weight (a) g. The sample was weighed as much as 2 g using a weighing bottle (b) g. The bottle containing the sample was placed in the oven for 24 hours at 102-105^{0}C, dried for 15 minutes, then weighed. This stage is repeated several times until a constant weight (c) g is obtained. Next, calculations are carried out using the formula:

$$\text{Water (\%)} = \frac{b-c}{b-a} \text{ x100\%}$$

Soluble protein content

The first is making a standard curve using various Bovine Serum Albumin (BSA) concentrations. The next is testing the smart flavor by dissolving a 0.1 g sample in 10 ml distilled water. The solution was centrifuged for 5 minutes, 0.125 ml was taken and reacted with Mix-Lowry reagent. Leave it for 10 minutes in the dark and add 0.25 ml follin solution. Let stand for 30 minutes in the dark and add distilled water until the volume reaches 5 ml, then measure with a wavelength (λ) of 750 nm.

Antioxidant activity [15]

This analysis begins by preparing 0.1 mM DPPH solution by diluting 0.00195 g into ethanol p.a. The sample was diluted by dissolving 0.01 g of *Sardinella lemuru* smart flavor in 10 ml of 70% ethanol. Next, 1.5 ml of sample was mixed with 1.5 ml of DPPH solution. The sample mixture and DPPH were then vortexed and left for 30 minutes at 37^{0}C in the dark. The solution was left to stand and then absorbed at a wavelength of 515 nm to measure the reduction of DPPH (*2,2- diphenyl-1-picrylhydrazyl*) radicals. The same treatment was carried out on the blank, but distilled water replaced the sample. The formula obtains antioxidant activity:

$$\text{Antioxidant (\%)} = \frac{\text{(A blank – A sample)}}{\text{A blank}} \text{ x 100}$$

Emulsion power and stability

The measurement of emulsion power begins by weighing 0.09 g of smart flavor and adding 45 ml of 0.05 M phosphate buffer pH 7. The smart flavor is added with 15 ml of cooking oil and homogenized using a stirrer, then centrifuged at 10.000 rpm for 1 minute. Take 1 ml of the bottom and add 5 ml of SDS (*Sodium dodecyl sulfate*). After that, absorbance with a wavelength of 500 nm. The emulsifying power measurement is calculated using the Emulsifying Activity Index (EAI) value. Calculation of emulsion power using the formula:

$$EAI\ (m^2/g) = \frac{2 \times 2{,}303}{c \times (1-\emptyset) \times 10} \times abs \times dilution$$

C = protein concentration (g/ml)
∅ = oil volume fraction (ml/ml)
Abs = absorbance
dilution = solution fraction (SDS + emulsion)
EAI = *Emulsifying Activity Index*

The stability was the same as the emulsion power test, but after adding SDS, the sample was left for 30 minutes. Next, absorbance was carried out using a spectrophotometer with a wavelength of 500 nm. Calculation of emulsion stability can be calculated using the formula:

$$ESI\ (minute) = \frac{T \times \Delta t}{\Delta T}$$

Note :
T = absorbance at 0 minutes
Δt = time difference which will be 30 minutes
ΔT = difference between absorbance at time 0 minutes and absorbance at the time to be calculated
ESI = Emulsifying Stability Index

Water Holding Capacity

Water holding capacity testing uses chicken meat as a medium to determine the meat's ability to bind free water. The meat is crushed using a food processor and then weighed 5 g. 1.5 ml of distilled water and 0.05 g of smart flavor samples were added to the meat. The samples were left in the freezer for 30 minutes, then in a water bath for 10 minutes at a temperature of 95^{0}C. Then the sample is run through cold water, and the final weight is weighed and calculated using the formula:

$$Missing\ water = \frac{Initial\ weight - final\ weight}{Initial\ weight} \times 100$$

$$WHC\% = 100 - missing\ water\ \%$$

Effectiveness

Effectiveness begins with determining the weight value of each parameter, determining the best and worst value for each parameter, then determining the weight of the variable and calculating the effectiveness value using the formula:

$$NE = \frac{sample\ value - worst\ value}{best\ value - worst\ value}$$

Next, calculate the NH values for all parameters using the formula:
NH= Effectiveness value (NE) x normal weight of parameters

D. Data analysis

The data obtained was analyzed statistically using Microsoft Excel software with the Analysis of Variance (ANOVA) method. The analysis was continued with Duncan's Multiple Range Test (DMRT) at the test level α = 0.05 if there were significant differences in each treatment. The results of the analysis data are presented in the diagram.

III. RESULT AND DISCUSSION

A. Yield

Yield is the comparison of the product's weight with the weight of the raw materials used [16], yield values in **Figure 1**.

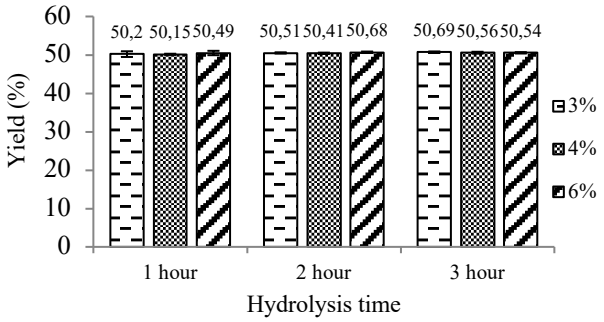

Fig 1. Yield of *Sardinella lemuru* smart flavor

Based on ANOVA analysis with a significance level of 5% (α<0.05), addition enzyme concentration and hydrolysis time did not significantly affect the yield of *Sardinella lemuru* smart flavor. The highest was 50.69% at an enzyme concentration of 3% and a hydrolysis time of 3 hours. The lowest was 50.15% in an enzyme concentration of 4% and a hydrolysis time of 1 hour. Factors influencing the yield value include reducing some volatile components and other components, such as water. The liquid in the wet hydrolyzate raw material experiences evaporation during drying. An increase in hydrolysis components such as dissolved solids, amino acids, and others, accompanied by a decrease in water content, causes a decrease in yield during the drying process [17].

B. Color (Brightness)

Brightness analysis of the *Sardinella lemuru* smart flavor using a color reader. *Sardinella lemuru* smart flavor brightness is in **Figure 2**.

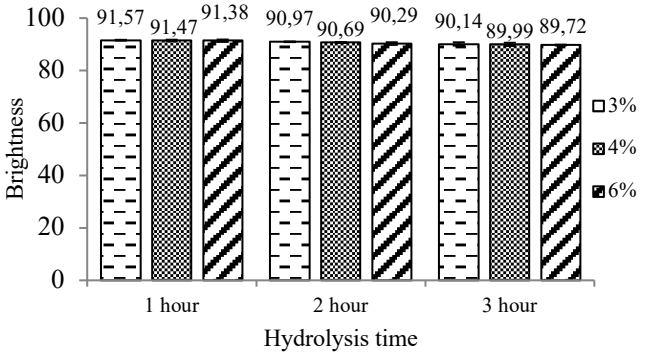

Fig. 2. Brightness of *Sardinella lemuru* smart flavor

Based on ANOVA analysis with a significance level of 5% ($\alpha<0.05$), additional enzyme concentration and hydrolysis time did not significantly affect the brightness value of *Sardinella lemuru* smart flavor. The highest brightness of *Sardinella lemuru* smart flavor was 91.57 at an enzyme concentration of 3% and a hydrolysis time of 1 hour. The lowest brightness of *Sardinella lemuru* smart flavor was 89.72 at an enzyme concentration of 6% and a hydrolysis time of 3 hours. The brightness of smart flavors is caused by several factors, such as ingredients, enzymes, and hydrolysis conditions [18]. In addition, the enzymatic hydrolysis process tends to reduce the color brightness of the hydrolyzate [19]. Hydrolysis will break the protease enzyme peptide bonds and produce an amine group. The amine group acts as a precursor to the Maillard reaction, which reacts with reducing sugars. According to [20], the longer the hydrolysis time, the more the brightness decreases. Brightness decreases are caused by amino acid groups and reducing sugars, which continuously react with each other during hydrolysis, causing the formation of melanoid compounds due to the Maillard process.

C. Water

Water plays an important role in food spoilage. Winarno [21] states that water is important in food ingredients because it can affect their texture, appearance, and taste. The water value of the smart flavor *Sardinella lemuru* is in **Figure 3**.

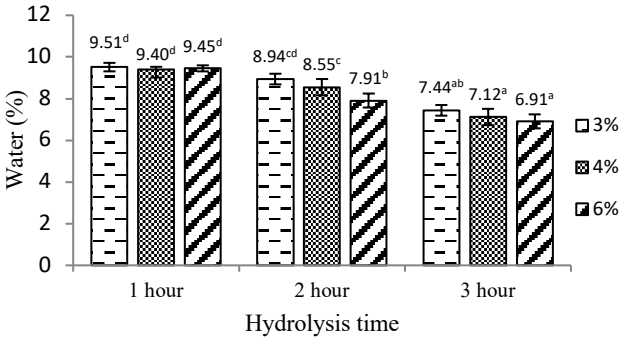

Fig. 3. Water of *Sardinella lemuru* smart flavor

Based on ANOVA analysis with a significance level of 5% ($\alpha<0.05$), the addition of enzyme concentration and hydrolysis time significantly affected the water of *Sardinella lemuru* smart flavor. The highest water of *Sardinella lemuru* smart flavor was 9.51% at an enzyme concentration of 3% and a hydrolysis time of 1 hour. The lowest water of *Sardinella lemuru* smart flavor was 6.91% at an enzyme concentration of 6% and a hydrolysis time of 3 hours. The water of *Sardinella lemuru* smart flavor decreased with increasing enzyme concentration and hydrolysis time. According to Pirena [22], the higher the enzyme concentration and longer hydrolysis time, the longer the contact between enzyme and substrate so that it can break more polypeptide bonds into simple peptides. This process results in a large amount of the protein matrix breaking down so that the hydrophilic properties of the protein weaken to bind water molecules, and the water of the material decreases [23]. Based on the quality standard for spice powder (powder stock) [24], the average water of *Sardinella lemuru* smart flavor meets the requirements. The average water of the smart flavor *Sardinella lemuru* is 6.91-9.51%, while the maximum water limit of SNI 01-3709 of 1995 is 12%. Based on SNI 01-3709-1995, powder quality standards include a maximum water content of 12%, a maximum ash content of 7% and a maximum water insoluble ash content of 1%.

D. Soluble protein content

Soluble protein content shows the amount of water-soluble protein found in food and is easily digested because it is in the form of oligopeptides [25]. The soluble protein content of *Sardinella lemuru* smart flavor is in **Figure 4**.

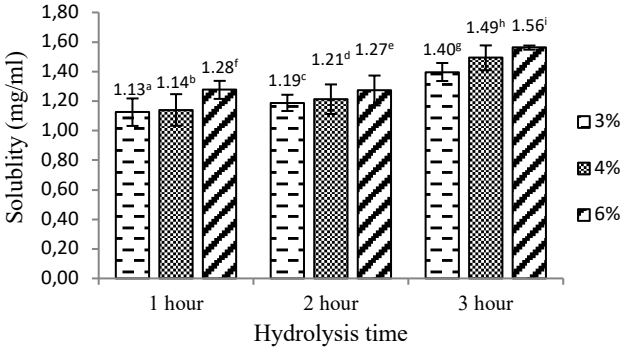

Fig. 4. Soluble protein of *Sardinella lemuru* smart flavor

Based on ANOVA analysis with a significance level of 5% ($\alpha<0.05$), the addition of enzyme concentration and hydrolysis time significantly affected the soluble protein content of *Sardinella lemuru* smart flavor. The highest soluble protein content of *Sardinella lemuru* smart flavor was 1.56 mg/ml at an enzyme concentration of 6% and a hydrolysis time of 3 hours. The lowest soluble protein content of *Sardinella lemuru* smart flavor was 1.13 mg/ml at an enzyme concentration of 3% and a hydrolysis time of 1 hour. Soluble protein content increased with increasing enzyme concentration and hydrolysis time. The increase in dissolved protein levels is caused by the

performance of protease enzymes in hydrolyzing proteins into short-chain peptides with a small molecular weight and a high solubility value [26]—the more breakdown of the protein structure, the more significant the percentage of soluble protein. In addition, the longer the hydrolysis time, the more extensive protein degradation, resulting in higher degrees of hydrolysis and soluble protein levels [27].

E. Antioxidant activity

The DPPH method works by decolating free electrons in a molecule so it is not reactive. Antioxidant activity is indicated by percent inhibition. The antioxidant activity of *Sardinella lemuru* smart flavor is in **Figure 5**.

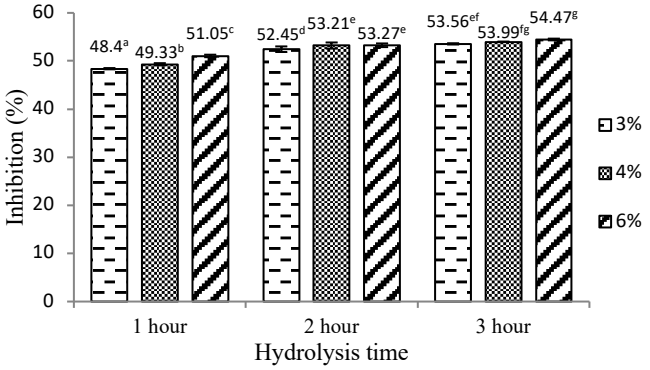

Fig. 5. Antioxidant activity of *Sardinella lemuru* smart flavor

Based on ANOVA analysis with a significance level of 5% ($\alpha<0.05$), the addition of enzyme concentration and hydrolysis time significantly affected the antioxidant activity of *Sardinella lemuru* smart flavor. The highest antioxidant activity of *Sardinella lemuru* smart flavor was 54.47% at an enzyme concentration of 6% and a hydrolysis time of 3 hours. The lowest antioxidant activity of 48.40% was found at an enzyme concentration of 3% and a hydrolysis time of 1 hour. The antioxidant activity of *Sardinella lemuru* smart flavor increased with increasing enzyme concentration and hydrolysis time. [28], state that enzyme hydrolysis can increase soluble protein and is directly proportional to increased antioxidant activity. The amino acid composition influences the antioxidant activity [29]. Hydrophobic amino acids such as tryptophan, phenylalanine, proline, and valine have potent free radical scavenging activity because they contain an imidazole ring as a proton donor [9]. [30], the amino acid content in *Sardinella lemuru* is dominated by leucine, alanine, tryptophan, proline, phenylalanine, glycine, and cysteine.

F. Emulsion power and stability

Emulsifying power is the ability of proteins to form emulsions and maintain emulsion stability. The level of protein solubility can influence emulsify power [12]. The emulsion power value of *Sardinella lemuru* smart flavor is in **Figure 6.**

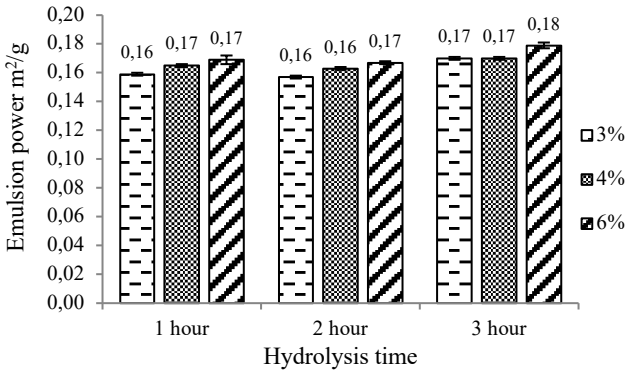

Fig. 6. Emulsion power of *Sardinella lemuru* smart flavor

Based on ANOVA analysis with a significance level of 5% ($\alpha<0.05$), addition enzyme concentration and hydrolysis time did not significantly affect the emulsion of the *Sardinella lemuru* smart flavor. The highest emulsion power of *Sardinella lemuru* smart flavor was 0.179 m^2/g at an enzyme concentration of 6% and a hydrolysis time of 3 hours. The lowest emulsion power of *Sardinella lemuru* smart flavor was 0.157 m^2/g at an enzyme concentration of 3% and a hydrolysis time of 2 hours. According to [31], emulsion power is influenced by the amount of protein contained in the ingredients, especially in fish hydrolysates. [12], states that high emulsifying power is due to short peptides and amino acids forming during hydrolysis. This process can increase solubility so that proteins are evenly distributed and accumulated between the surface of oil and water.

Emulsion stability is the ability to maintain the emulsion that has been formed. The nature of emulsion stability is influenced by protein and the level of protein solubility. The emulsion stability of the *Sardinella lemuru* smart flavor is in **Figure 7**.

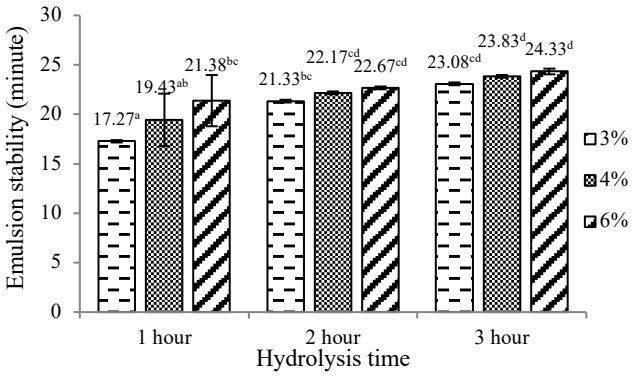

Fig. 7. Emulsion stability of *Sardinella lemuru* smart flavor

Based on ANOVA analysis with a significance level of 5% ($\alpha<0.05$), the addition of enzyme concentration and hydrolysis time significantly affected the stability of the *Sardinella lemuru* smart flavor emulsion. The stability of the *Sardinella lemuru* smart flavor emulsion was the highest, with a value of 24.33 minutes at an enzyme concentration of 6% and a hydrolysis time of 3 hours. The stability of the *Sardinella lemuru* smart flavor emulsion was the lowest, with a value of 17.27 minutes

at an enzyme concentration of 3% and a hydrolysis time of 1 hour. The EAI value is influenced by the number of short peptide chains and amino acids formed after hydrolysis using enzymes. The high protein concentration in the hydrolyzate can increase the capacity and stability of the emulsion and easily bind with oil [32]. [33], state that the power and stability values of the emulsion are influenced by the characteristics of the peptide molecules and the length of the peptide chain produced.

G. Water Holding Capacity

The water holding capacity of *Sardinella lemuru* smart flavor with the addition of different enzyme concentrations and hydrolysis times ranged from 83.44-89.27%. The water holding capacity value of *Sardinella lemuru* smart flavor is in **Figure 8**.

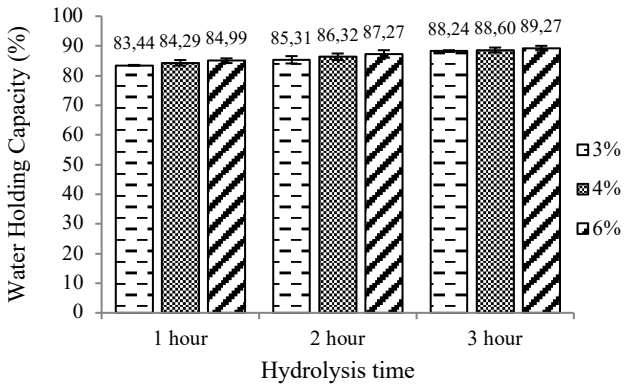

Fig. 8. Water holding capacity of *Sardinella lemuru* smart flavor

Based on ANOVA analysis with a significance level of 5% ($\alpha<0.05$), the addition of enzyme concentration and hydrolysis time did not significantly affect the water-holding capacity of *Sardinella lemuru* smart flavor. The water-holding capacity of *Sardinella lemuru* smart flavor was the highest with a value of 89.27% at an enzyme concentration of 6% and a hydrolysis time of 3 hours. The lowest water-holding capacity of *Sardinella lemuru* smart flavor was 83.44% at an enzyme concentration of 3% and a hydrolysis time of 1 hour. The water holding capacity of each sample was higher than the blank, namely 65.73%. According to [17], the high water-holding capacity can retain components in the meat, so cooking loss is slight. In addition, the molecular weight of the peptide can influence water holding capacity. Low molecular weight is more effective in increasing water-holding capacity because it has hydrophilic properties. [33], a high hydrolysis process can break down COOH and NH_2 (carboxylic and amino acids), which are polar, so they tend to have high water-holding capacity.

H. Effectiveness

The effectiveness is determined by assessing the weight of each parameter. The results of the effectiveness test of *Sardinella lemuru* smart flavor with the addition of different enzyme concentrations and hydrolysis times ranged from 0.00-1.00. Effectiveness test results are in **Table 1**.

TABLE I
EFFECTIVENESS OF SMART FLAVOR

Sample	Value
Enzyme 3%, hydrolysis 1 hour	0.00
Enzyme 4%, hydrolysis 1 hour	0,14
Enzyme 6%, hydrolysis 1 hour	0,38
Enzyme 3%, hydrolysis 2 hours	0,44
Enzyme 4%, hydrolysis 2 hours	0,55
Enzyme 6%, hydrolysis 2 hours	0,64
Enzyme 3%, hydrolysis 3 hours	0,78
Enzyme 4%, hydrolysis 3 hours	0,90
Enzyme 6%, hydrolysis 3 hours	**1,00**

Based on the *Sardinella lemuru* smart flavor effectiveness results, the highest parameter value (1.00) was at an enzyme concentration of 6% with a hydrolysis time of 3 hours. The treatment had a brightness value of 89.72, a yield value of 50.54%, water of 6.91%, soluble protein of 1.56 mg/ml, antioxidant activity of 54.47%, emulsion power and stability of 0.18 m^2/g and 24.33 minutes, and the water holding capacity value is 89.27%. A value of 1 indicates antioxidant activity, soluble protein, emulsion stability and water content of the enzyme 6%, hydrolysis 3 hours has the highest value compared to the others. Meanwhile, the enzyme 3% hydrolysis 1 hour obtained a value of 0 because in terms of antioxidant activity, soluble protein, emulsion stability and water content it had the lowest value compared to other treatments.

IV. CONCLUSION

The research showed that yield ranged from 50.15-50.69%; brightness 89.72-91.57; water 6.91-9.51%; soluble protein content 1.13-1.56 mg/ml; antioxidant activity 48.40-54.47%; emulsion power 0.16-0.18 m^2/g, emulsion stability 17.27-24.33 minutes; water holding capacity 83.44-89.27% and effectiveness 1.00 (enzyme 6% and time of hydrolysis 3 hours). The best treatment with effectiveness was *Sardinella lemuru* smart flavor (6% enzyme and 3 hours hydrolysis) with a yield of 50.54%, brightness 89.72, water 6.91%, soluble protein content of 1.56 mg/ml, antioxidant activity of 54.47%, emulsion power and stability 0.18 m^2/g and 24.33 minutes, and water holding capacity 89.27%.

ACKNOWLEDGMENT

This research was carried out thanks to support from the Professor Productivity Grant Program of University of Jember 2020-2021.

CONFLICT OF INTEREST

Authors declare no conflict of interest to disclose.

REFERENCES

[1] Wahyuningsih, W. Optimizing production of lemuru fish (*Sardinella longiceps*) with high fatty-acid omega-3 using liquid smoked ginger flavor process. *Journal of Coastal Zone Management*. 2005, 8. 97.

[2] Arifan, F., Deddy K. W. Optimasi Produksi Ikan Lemuru (*Sardinella Longiceps*) Tinggi Asam Lemak Omega-3 Dengan Proses Fermentasi Oleh Bakteri Asam Laktat. *Jurnal Sains dan teknologi*. 2011, 1. 15.

[3] Suseno, S.H., Ali, S., Fannany, A. Kandungan Zat Gizi dan Potensi Antibakteri Ikan Laut dalam di Selatan Jawa. *Jurnal Perikanan.* 2004, 757.

[4] Alatas, S. S. S. Status Gizi Anak Usia Sekolah (7-12 Tahun) dan Hubungannya dengan Tingkat Asupan Kalsium Harian di Yayasan Kampung kids Pejaten. *Skripsi*. Fakultas Kedokteran Universitas Indonesia. 2011.

[5] Anindita, R. Soeprobowati T. R., Suprapti N. Potensi Teh Hijau (*Camelia Sintesis L*) dalam Perbaikan Fungsi Hepar pada Mencit yang diinduksi *Monosodium Glutamat* (MSG). Buletin Anatomi dan Fisiologi. Magister Biologi Universitas Diponegoro. 2012.

[6] Baehaki, A. 2015. Hidrolisat Protein Ikan Patin Menggunakan Enzim Papain dan Aktivitas Antioksidan Hidrolisatnya. *Jurnal Pengolahan Hasil Perikanan Indonesia.* 18. 108.

[7] Je JY, Qian ZJ, Lee SH, Byun HG, Kim SK. Purification and Characterization of an antioxidant peptide obtained from backbone tuna protein by enzymatic hydrolysis. *Process Biochemistry.* 2007, 42. 840.

[8] Jun SY, Park PJ, Jung WK, Kim SK. Purification and characterization of an antioxidative peptide from enzymatic hydrolysate of Yellowfin sole (*Limanda aspera*) frame protein. *European Journal of Food Research and Techology.* 2004, 219. 20.

[9] Samaranayaka AGP dan Li-Chan ECY. Food-derived peptidic antioxidants: A review of their production, assessment, and potential applications. *Journal of Functional Food*. 2011, 3. 229.

[10] Cahyaningati, K. Aktivitas antioksidan hasil hidrolisis enzimatis protein ikan wader *(Rasbora joacibsoni)* menggunakan kombinasi calotropin dan papain. *Skripsi*. Jember: Universitas Jember. 2019.

[11] Witono, Y., Iwan, T., Wiwik, S. W., Amelia, R. Hidrolisis Ikan Bernilai Ekonomi Rendah Secara Enzimatis Menggunakan Protease Biduri. *Jurnal Teknologi dan Industri Pangan*. 25. 2014.

[12] Wijayanti,. R. P. Formulasi Flavor Enhacer dari Hidrolisat Protein Ikan Wader *(Rasbonara jacobsoni)*. *Skripsi.* Jurusan Teknologi Hasil Pertanian Fakultas Teknologi Pertanian Universitas Jember. 2016.

[13] Fardiaz, S. Mikrobiologi Pangan I. Jakarta. Gramedia Pustaka Utama. 1992.

[14] AOAC. Official Methods Analysis of The Association of Official Analytical Chemist. Washington DC. 1995.

[15] Brand, W., Cuvelier, M. E., dan Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. U. Technol. 28. 25. 1995.

[16] Sani, R.N., Fithri C.N., Ria D.A., dan Jaya M.M. Analisis Rendemen dan Skrining Fitokimia Ekstrak Etanol Mikroalga Laut Tetraselmis chuii. *Jurnal Pangan dan Agroindustri*. 2014, 2. 121.

[17] Witono, Y., Maryanto., Iwan, T. , Ardiyan, D., Kinanti, C. Aktivitas antioksidan hasil hidrolisis enzimatis protein ikan wader (*Rasbora joacibsoni)* menggunakan kombinasi calotropin dan papain. Jurnal Agroteknologi. 2020, 14. 51.

[18] Harada-Padermo, S. dos S., Dias-Faceto, L. S., Selani, M. M., Alvim, I. D., Floh, E. I. S., Macedo, A. F., Bogusz, S., Dias, C. T. dos S., Conti Silva, A. C., dan Vieira, T. M. F. de S. Umami Ingredient: Flavor enhancer from shiitake (*Lentinula edodes*) byproducts. *Food Research International*. 2020, 137.

[19] Cotlar, C., Ponce, A., dan Roura, S. Improvement of functional and antimicrobial properties of brewery by product hydrolyzed enzymatically. *LWT Food Sci Technol*. 2013, 50**.** 378.

[20] Anggraini, A., Yunianta. Pengaruh Suhu Dan Lama Hidrolisis Enzim Papain Terhadap Sifat Kimia, Fisik, Dan Organoleptik Sari Edamame. *Jurnal Pangan dan Agroindustri*. 2015, 3. 1015.

[21] Winarno, F. G. Kimia Pangan dan Gizi. Bogor. Mbrio Press. 2008.

[22] Pirena, M., Hidrolisis Daging Ikan Patin (*Pangasius hypophtalmus*) dengan Protease Biduri *(Calotropis gigantea)* Sebagai Bahan Baku Pembuatan Flavor Enhacer. *Skripsi*. Jember: Universitas Jember. 2015.

[23] Novian, U. Karakteristik Miofibril Kering Ikan Kuniran *(Upeneus Sp)* Diekstrak Menggunakan Enzim Papain dengan Metode Press. *Skripsi*. Jember: Universitas Jember. 2005.

[24] Badan Stardardisasi Nasional. SNI 01-3709-1995 -1995: Standar mutu bubuk rempah-rempah (kaldu bubuk). Jakarta: Badan Stardardisasi Nasional. 1995.

[25] Purwoko, Tj., Handajani. Kandungan Protein Kecap Manis Tanpa Fermentasi Moromi Hasil Fermentasi Rhyzopus oryzae dan R. oligosporus. *Biodiversitas.* 2007, 8. 223.

[26] Haurowitz, F., & Koshland, D.E. Hydration of Proteins. Diakses 10 juni 2022. https://www.britannica.com/science/protein/Hydration-of-proteins. 2020.

[27] Hasnaliza H, Maskat MY, wan Aida WM, Mamot S. The effects of enzyme concentration, temperature and incubation time on nitrogen content and degree of hydrolysis of protein precipitate from cockle (*Anadara granosa*) meat wash water. *Int Food Res J.* 2010, 17. 147.

[28] Bamad, F., Wu, J., Chen, L. Effects of Enzymatic Hydrolysis on Moleculer Structure and Antioksidant Activity of Barley Hordein. *Journal of Cereal Science*. 2011, 54. 20.

[29] Arcan, I., Yemeniciog. A. Effects of Controlled Pepsin Hydrolysis on Antioxidant Potential and Fractional Changes of Chickpea Proteins. *Journal Food Research International.* . 2010, 43. 140.
[30] Panjaitan, F. C. A., Sunarti S., Filli P., Raini P., Gressty S. Identifikasi Potensi Kandungan Peptida Bioaktif Ace Inhibitor pada Lemuru (*Sardinella lemuru*) dengan Teknik in Silico. *Prosiding Seminar Nasional Ikan XI*. 207. 2022.
[31] Ragap, C., Guthrie, A., Picciano, F.M. *Human Nutrition*. USA : Mosby-Yearbook. inc. 2004.
[32] Suwarno, M. Potensi Kacang Komak (*Lablab pupureus (L) sweet*) Sebagai Bahan Baku Isolat Protein. *Skripsi*. Bogor : Fakultas Petanian. Institus Pertanian Bogor. 2003.
[33] Kristinsson, H. G. and Rasco, B. A. Fish protein hydrolysates: production, biochemical, and functional properties. *Critical Review of Food Science and Nutrition*. 2000, 40**.** 43.