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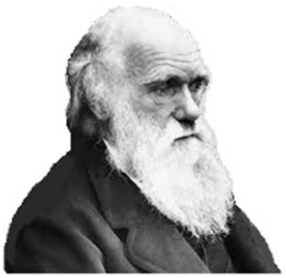

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The Influence of Concentration of Acetic Acid and Pepsin Enzyme in Nilem Fish Skin Collagen Extractionto the Amount of Rendement Produced

Junianto[1,*], **Iskandar**[1], **Achmad Rizal**[1] and **Windi Damayanti**[2]

[1]Department of Fishery, Universitas Padjadjaran, West Java, Indonesia

[2]Laboratory of Fishery Products Processing, Univesitas Padjadjaran, West Java, Indonesia

*E-mail corresponden: anto_lisc@yahoo.com

ABSTRACT

The added value of nilem fish skin needs to be increased. The purpose of this study is to determine the concentration of acetic acid solution combined with the pepsin enzyme in the extraction of collagen from nilem fish skin that is necessary to obtain the highest yield/renderment. The study employed an experimental research method that used a completely randomized factorial design. The first treatment is the concentration of acetic acid solution. This consists of three levels, namely 0.5M, 0.7M and 0.9M. The second treatment is the concentration of the enzyme pepsin. This in turn consists of three levels, namely 0.5 %, 1.0 % and 1.5% (weight / weight). The parameters observed were collagen renderment. The results showed that the combination treatment concentration of 0.7 M solution of acetic acid by the pepsin enzyme at 1.0%, in the extraction of collagen from fish skin, produce the highest yield compared to other combinations. The renderment yield is 6.18%.

***Keywords*:** Enzyme pepsin, skin, nilemfish, collagen, extraction, *Osteochilus hasselti*

1. INTRODUCTION

Fish nilem (*Osteochilus hasselti,* CV) is a freshwater fish farming which has some comparative advantage. These advantages include high egg production, disease resistance andperyphiton eaters. In addition, nilem fishis an Indonesia local fish wich is easily cultivated.

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According to Junianto et al. (2015), nilem fish eggs taste very delicious because it contains high amino acid glutamate, so it is very popular by the public and has the opportunity as an export commodity (Photo 1).

Photo 1. *Osteochilus hasselti*, Menon, 1954.

Comparative advantage of nilem fish as referred to above must be followed by competitive advantage is through post-harvest technology. The synergy between the two advantages will have a positive impact on economic independence and poverty alleviation, especially for people who are engaged in fishery fish nilem in WestJava and generally Indonesian fisheries nationally.

According to Junanto (2015) the activities of the fisheries processing industry as part of post-harvest technology is a medium to transform comperative advantage into a competitive advantage in the fishery field.

Processing activities on traditional nilem fish have been done many of them processed into *nilem pepes, nilem eggpepes*, and jerky. Suseno et al (2004) has utilized nilem meat as a supplementation material in the making of simping, a signature food from Purwakarta West Java. Junianto et al (2016) have done a produced to nilemeggs become a typical crispysnack. Various processing of meat and eggs of fish nilem that have been done, produces waste such as skin of nilem fish. Fish skin can be used as a raw material source of collagen (Liu et al 2015). According to PK Bhagwat and Dandge PB (2016), collagen is a product that has a high added value because it has a very wide use ranging from the food industry, pharmaceuticals, biomedical to cosmetic.

One method for extracting collagen from fish skin is a method of pepsin soluble collagen (Aberoumand 2012). This method uses the enzyme pepsin in acetic acid solution to increase the rendement of collagen. According to Siddiqui *et al.* (2013), many variables that determine to get quantity of collagen from enzymatic extraction results. The concentration of acetic acid and enzymes are several variables that gave the effect.

This study aims to determined combined treatment solution concentration of acetic acid with the pepsinenzyme in the extraction of collagen from fish skin nilem to get the highest rendement.

2. RESEARCH METHODS

The method used was experimental with completely randomized factorial design. The first treatment is the concentration of acetic acid solution which consists of three levels, namely 0.5M, 0.7M and 0.9M. The second treatment is the concentration of the enzyme pepsin which consists of three levels, namely 0.5 %, 1.0 % and 1.5% (weight / weight). The total combination of treatments was 9, each treatment combination was repeated 3 times.The research stages are carried out as follows:

2. 1. Preparation of fish and fish skin samples

Nilem fish obtained from fishcultivators in Cianjur, West Java. Nilem fish are transported alive to Fishery Products Processing Laboratory, University of Padjadjaran, Jatinangor-Sumedang, West Java, Indonesia. Then fish get preparation to take the skin. Nilem fish skin is cleaned from blood and remain of flesh stuck with cold water (<4 ºC). Furthermore, cut with scissors, the size of the pieces of fish skin nilem approximately ≤ 0.5 cm^2.

Based on the method of Kiew and Don (2013) modified, the preparation nilem fish skin sample before the extraction process is done is as follows: Samples of nilemfish skin soaked in a solution of 0.1 M NaOH with a sample rate and volume of a solution of 1: 20 (w/v) for 6 hours. During the immersion, the solution are stirred continuously.

The NaOH solution is changed by every 2 hours. Then after soaking is complete, the sample pieces of fish skin is washed with distilled water nilem cold (<4 ºC) until the pH of distilled water washing former neutral or slightly alkaline (pH = 7.0 to 8.0). Then the sample is packed in plastic and stored on the frezeer until ready for use in the next stage.

2. 2. Collagen Extraction Process

Nilem leather fish samples from thawing frezeer (thawing) then weighed as much as 100 grams and then inserted into a 1000 ml glass beaker. Then into a glass beaker which already contains fish skin samples included acetic acid solution with a concentration in accordance with the level of treatment. The amount of solutionis 1000 ml. Pepsin enzyme was put after,as the amount as the treatment. Then stirred the mixtureuntil homogeneous and allowed to stand for 24 hours. Finished soaking, the mixture is sentrifuged with a speed of 10,000 rpm for 20 min at 4 ºC viscous liquid phase (supernatant) is collected and disposed of the solid phase. Then the viscous liquid is precipitated by adding NaCl until the final concentration is 0.8M.

The precipitation is carried out for 24 hours in a refrigerator at a cold temperature (5-8 ºC). The precipitate was then separated by disentrifius with a speed of 10,000 rpm for 20 min at 4 ºC. Then the precipitate was dissolved again with 0.5 M acetic acid to dissolve. The solution was dialyzed with 0.1 acetic acid and aquadest. The process dialyzed for each type of solution is done in 2 stages. Each stage is done for 3 hours. Next, the solution is filtered with a filter cloth. The filtered collagen solution was then dried using a freeze dryer. Collagen obtained is called soluble collagen pepsin (ULC)

2. 3. Observation

Observations were made to the yield. The calculation of rendement is carried out as follows:

$$\text{Collagen Rendement (\%)} = \frac{\text{collagen weight}}{\text{fish skin weight}}$$

2. 4. Data analysis

Collagenrendement data obtained were analyzed statistically using F test, if the results obtained were significant then the analysis was continued with Duncan Multiple Range Test, each test was done at 95% confidence level.

3. RESULTS AND DISCUSSION

Table 1. Mean of Collagen rendemenextracted from nilemfish skin from various combinations of treatment between the concentrations of acetic acid by the enzyme pepsin (%)

The concentration of acetic acid (M)	Deuteronomy	The concentration of pepsin enzyme (%)		
		0.5	1.0	1.5
0.5	1	4 1 8	4.4 7	5.06
	2	4.26	4.3 1	5.18
	3	4.32	4.3 5	5.25
	Average	4.25 ± 0, 07	4.38 ± 0.0 8	5.16 ± 0, 10
0.7	1	5.06	6.28	5.98
	2	5.35	6.11	5.85
	3	5.11	6.15	5.90
	Average	5.17 ± 0.16	6.18 ± 0.0 9	5.91 ± 0.0 7
0.9	1	5.68	5.84	5.70
	2	5.57	5.73	5.62
	3	5.60	5.65	5, 81
	Average	5.62 ± 0.06	5.74 ± 0.09	5.71 ± 0.09

The yield of collagen produced from each treatment combination between acetic acid concentration and pepsin enzyme is presented in Table 1. The highest yield of collagen was obtained from a combination of 0.7 M acetic acid concentration treatment with 1.0% pepsin enzyme concentration. Percentage of rendement yielded is 6.18%.

Based on a statistical analisis variance test (test F) (Appendix 1) states that the yield of collagen is strongly influenced by the interaction between the concentration of acetic acid and

pepsin enzyme concentrations were added. The combined treatment of 0.7 M acetic acid solution with the addition of 1.0% enzyme pepsin yields the highest yield. The value of collagen rendement obtained from the treatment was significantly different from other treatments.

The higher the concentration of acetic acid to the limit of 0.7 M in the addition of 1.0% pepsin enzyme, the higher yield of collagen is higher. Similarly, at a concentration of 0.7 M acetic acid, the higher the addition of enzymes to the extent of 1.0%, the yield of collagen produced even greater. According Veeruraj et al (2015) addition of enzyme pepsin extraction of collagen is intended to increase the solubility of collagen in acetic acid. Bama et al (2010), describes that enzym specifically pepsin can break the ties of telopeptida without damaging the integrity of the triple helixon collagen found in fish skin.According to Zhang et al (2010), the rate of addition of pepsin enzyme concentration in the extraction of collagen with highly acidic solvents depending on the type of fish and the composition and configuration of collagen.

Value yield of collagen extracted from skin nilem (6.18%) were produced of the best treatment is higher than the catla fish skin (3.9%) and mrigala (3.2%) (Bhagwat PK and Dandge PB. 2016) and black tilapia fish (5.97%) (Sahubawa and Son, 2011). If the results of this nilem fish skin yield compared to the yield of tuna fish skin collagen (13.97%) and sharkskin (8.96%) (Hema et al., 2013) and bigeye snapper fish skin (10.94%) (Kittiphattanabawon et al., 2005) the result is smaller. Differences in yield of collagen this is due to the difference in content protein in the fish skin, nilemfish skin which used to containlower than the protein sharkskin, tuna and bigeye snapper (Hema et al. 2013; Kittiphattanabawon et al. 2005). Differences species, habitats, pre-treatment and the extraction method is f actors that can cause differences in the results of rendement.

4. CONCLUSION

Based on the results of the study concluded that the combination treatment concentration of 0.7 M acetic acid solution with the pepsin enzyme 1.0% in the extraction of collagen from fish skin produce the highest yield compared to other combinations. The rendement yield is 6.18%.

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Attachment

The list of variations of the effect of the combination of treatments on the recovery yield of nilem skincollagen

Source variety	DB	JK	KT	F.hit	Ft.0,05
Treatment	8	10,71403	1.339254	132.99	2.59
The concentration of pepsin enzyme (E)	2	1.611763	0.805882	80.02	3.63
The concentration of acetic acid (AA)	2	7,598096	3.799048	377.24	3.63
Interaction (E vs AA)	4	1.50417	0.376043	37.34	3.01
Error	16	0.16113	0.010071		
Total	26	10,87516			

Duncan's Multiple Range Test Interactions Effect of each treatment rate enzin Pepsin and Concentration Concentration Acetic Acid on Extraction Yield of Collagen from fish skin Nilem

Acetic acid concentration level (M)	The level of pepsin enzyme concentration (%, w / w)		
	0.5	1.0	1.5
0.5	A 4.25 a	B 4.38 a	C 5.16 a
0.7	A 5.17 b	B 6.18 b	C 5.91 b
0.9	A 5.62 c	B 5.74 c	B 5,71 c

Description: The same uppercase letters show no significant difference according to Duncan's multiple range test at the 95% confidence level
The same small letters toward the rows show no significant differences according to Duncan's multiple range test at a 95% confidence level