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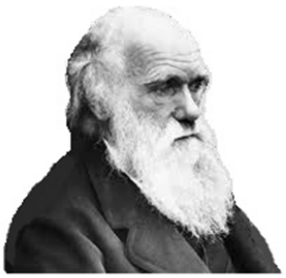

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The Influence of Concentration of Acetic Acid and Pepsin Enzyme in Nilem Fish Skin Collagen Extraction to the Amount of Rendement Produced

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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 0.5 M, 0.7 M, and 0.9 M. 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 advantages. These advantages include a high egg production, disease resistance and peryphiton eaters. In addition, nilem fish is an Indonesian local fish wich is easily cultivated.

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According to Junianto *et al*. (2015), nilem fish eggs taste very delicious because they contain 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 through the 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 the fishery of fish nilem in West Java, and generally Indonesian fisheries nationally.

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

Processing activities on the traditional nilem fish have been done, with 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 produce to nilem eggs become a typical crispy snack.

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 determine getting 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 a combined treatment solution concentration of acetic acid with the pepsin enzyme 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 0.5 M, 0.7 M, and 0.9 M. 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 is obtained from fishcultivators in Cianjur, West Java. Nilem fish is transported alive to Fishery Products Processing Laboratory, University of Padjadjaran, Jatinangor-Sumedang, West Java, Indonesia. Then the fish gets 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 is approximately ≤ 0.5 cm^2.

Based on the method of Kiew and Don (2013) modified, the preparation of nilem fish skin sample before the extraction process is done as follows: Samples of nilem fish 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 is stirred continuously.

The NaOH solution is changed by every 2 hours. Then, after soaking is complete, the sample pieces of fish skin are washed with distilled water nilem cold (<4 ºC) until the pH of distilled water washing is 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 are 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 is included acetic acid solution with a concentration in accordance with the level of treatment. The amount of solution is 1000 mL. Pepsin enzyme was put after, with the amount as the treatment. Then stirred the mixture until it is homogeneous and allowed to stand for 24 hours. Finished soaking, the mixture is centrifuged with a speed of 10,000 rpm for 20 min at 4 ºC till the viscous liquid phase (supernatant) is collected and disposed off the solid phase. Then the viscous liquid is precipitated by adding NaCl until the final concentration is 0.8 M.

The precipitation is carried out for 24 hours in a refrigerator at a low/cold temperature (5-8 ºC). The precipitate is then separated by disentrifius with a speed of 10,000 rpm for 20 min at 4 ºC. Then the precipitate is dissolved again with 0.5 M acetic acid to dissolve. The solution is dialyzed with 0.1 acetic acid and aquadest. Then the process undergoes dialysis for each type of solution and done in 2 stages. Each stage is done for 3 hours. Next, the solution

is filtered with a filter cloth. The filtered collagen solution is then dried using a freeze dryer. Collagen obtained is called the soluble collagen pepsin (ULC).

2. 3. Observation

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

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

2. 4. Data analysis

Collagen rendement 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 rendemen extracted from nilem fish 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 analysis variance test (test F) (Appendix 1) one can see 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 is the yield of collagen. 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 is even greater. According to Veeruraj *et al*. (2015), the addition of enzyme pepsin extraction of collagen is intended to increase the solubility of collagen in acetic acid. Bama *et al*. (2010) describes that enzyme, 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 is dependent on the type of fish and the composition and configuration of collagen.

Value yield of collagen extracted from the skin nilem (6.18%) produced of the best treatment is higher than the catla fish skin (3.9%), 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 the yield of collagen are due to the difference in the content of protein in the fish skin, nilem fish skin which is used to contain lower than the protein shark skin, tuna and bigeye snapper (Hema *et al*., 2013; Kittiphattanabawon *et al*., 2005). Differences in species, habitats, pre-treatment and the extraction method are the factors that can cause differences in the results of rendement.

4. CONCLUSION

Based on the results of the study, it can be 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 produces the highest yield compared to other combinations. The rendement yield is 6.18%.

Reference

[1] Aberoumand A. 2012. Comparative Study Between Different Extraction Methods of Collagen from Fish and its Properties. *World Applied Sciences Journal* 16 (3): 316-319.

[2] Bama,P., Vijayalakshimi, M., Jayasimman, R., Kalaichelvan PT, Deccaraman, M., and Sankaranarayanan, S. 2010. Extraction of collagen from cat fish (Tachysurus

maculatus) by pepsin digestion and preparation and characterization of collagen chitosan sheet. *International Journal of Pharmacy and Pharmaceutical Sciences* Vol.2: 133-137.

[3] Bhagwat PK and Dandge PB. 2016. Isolation, characterization and applications of fi sh valorizable scale collagen in food and agriculture industries. *Biocatalysis and Agricultural Biotechnology* 7: 234-240.

[4] Hema GS, Shyni K, Mathew S, Anandan R, Ninan G, and Lakshmanan PT 2013. A simple method for isolation of fish skin collagen-biochemical characterization of skin collgagen extracted from Albacore Tuna *(Thunnus Alalunga),* Dog Shark *(Scoliodon Sorrakowah),* and Rohu *(Labeo Rohita). Annals of Biological Research* 4 (1): 271-278.

[5] Junianto. 2015. The strategic role of fisheries product processing in national development. *Oration Scientific,* Faculty of Fisheries and Marine Sciences - Padjadjaran University. Jatinangor, Indonesia

[6] Junianto, Afrianto, E., Rohimma, R., Rostika. R., Andriani. Y., Handaka, AA and Nurhayati, A. 2015. Commercialization of products made from raw fish nilem as leverage local fish production in West Java.Research Report ALG, Unpad. Bandung

[7] Kittiphattanabawon, P., Benjakul, S., Visessaguan, W., Nagai, T., and Tanaka, M. 2005. Characterization of acid-soluble collagen from skin and bone of bigeye snapper *(Priacanthus tayenus). Food Chemistry* 221: 363-372

[8] Liu D, Wei G, Li T, Hu J, Lu N, Regenstein, JM and Zhou P. 2015. Effects of alkaline pretreatments and acid extraction conditions on the acid-soluble collagen from grass carp *(Ctenopharyngodon idella)* skin. *Food Chemistry* Vol. 172: 836-843.

[9] Sahubawa L and Putra ABN 2011. Effect of acetic acid concentration and extraction time on collagen quality of skin waste of black tilapia fish. *Journal of Science Technology,* No.1: 16-25

[10] Siddiqui, YD, Arief, EM, Yusoff, A., Suzina, AH and Abdullah, SY, 2013. Isolation of pepsin-Solublized (PSC) from the crude collagen extracted from the body wall of sea cucumber *(Bohadschia* spp). *International Journal of Pharmacy and Pharmaceutical Sciences* Vol. 5: 555-559.

[11] Suseno SH, Suptijah P and Wahyuni D S. 2004. Influence of addition of Nilem fish flesh (Ostheochilus hasselti) on Making Simping as Snack Food. *Technology Bulletin of Fishery Products.* Vol. VII no. 1. Pages: 44-55.

[12] Veeruraj A., Arumugam M., Ajithkumar T and Balasubramanian T. 2015. Isolation and characterization of collagen from the outer skin of squid *(Doryteuthis singhalensis). Food Hydrocolloids* 43: 708 - 716.

[13] Zhang, J., Duan, R., Ye, C., & Konno, K. (2010). Isolation and characterization of collagens from scale of silver carp (Hypophthalmichthys molitrix). *Journal of Food Biochemistry,* 34 (6), 1343-1354.

Appendix 1

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	B 4.38	C 5.16
0.7	A 5.17	B 6.18	C 5.91
0.9	A 5.62	B 5.74	B 5,71

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.