Effect Collagen Concentration Tilapia Scales on The Quality of Burn Ointment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript

ABSTRACT

Collagen is one of the main connective tissue animal proteins and has been widely used as a biomedical material. Collagen is divided into XIX types. Type I collagen, among others, is obtained from bone, scales and skin. Collagen derived from type I can repair tissue or accelerate tissue regeneration to heal burns. The purpose of this research was to determine the addition of fish scale collagen extract to the characteristics of the burn ointment preparation in accordance with the Indonesian National Standard (SNI) and the best quality. The method used in this research is an experimental method of Completely Randomized Design (CRD) consisting of 4 collagen addition treatments: 0%, 2%, 4% and 6% repeated 5 times. Parameters in this method include physical-chemical parameters (pH, spreadability, shelf life and homogeneity) and organoleptic parameters (appearance, aroma, texture and color). Bayes test results, the concentration of the addition of tilapia scale collagen in the ointment preparation of 4% resulted in a value close to the control treatment. The addition of 4% collagen was the best treatment compared to 2% and 6% with a pH value of 6.12, dispersion of 3.22 cm, safe ointment preparation did not change at all during 28 days of storage. Based on the results of the organoleptic test parameters, the ointment at this concentration had a homogeneous appearance, slightly yellowish white color, a distinctive smell of collagen and a semi-solid texture, this was in accordance with the quality standard of the ointment and had the best quality characteristics.

Keywords: Fish waste, Fish scales, Burn ointment, collagen concentration, SNI

1. INTRODUCTION

Tilapia (Oreochromis niloticus) is a type of fish that has high economic value and is an important commodity in the world's freshwater fish business [1]. Based on data, tilapia production per year has increased by around 13.5%. In 2016 the production of tilapia was 1,114,156 tons while in 2017 it was 1,265,201 tons [2]. Tilapia production continues to increase, causing the processing of tilapia-based fishery products to also increase. According to [3] the fish processing industry produces an average of 75% of the total weight of waste. Fish scales are only 3% of the total fish waste, the waste is estimated to have a proportion of about 30-40% of the total fish weight, consisting of 12.0% head, 11.7% bones, 3.4% fins, 4 skin, 0%, spines 2.0%, and entrails 4.8%.

Processing waste into collagen is one way to increase added value and to reduce environmental pollution [4]. Collagen is one of the main connective tissues of animal protein and has been widely used as a biomedical material. Collagen is divided into 19 types, namely types I to XIX. Collagen types I, II, III and V are fibrous collagen. Type I collagen, among others, is obtained from bone, scales and skin. Collagen derived from type I can repair tissue or accelerate tissue regeneration to heal burns [5].

Collagen is applied in the pharmaceutical field, one of which is burn ointment. Ointment is a semi-solid preparation that is easy to apply and is used as an external medicine [6]. According to [7] collagen as the active substance in the ointment (vaselin flavum) is able to accelerate the growth of new cells on the skin. Vaseline flavum is widely used as a base for hydrocarbon ointments (fatty base) used primarily for its emollient effect (protects the skin). The speed of wound healing can be influenced by the substances contained in the drug given, if the drug has the ability to increase healing by stimulating faster growth of new cells in the skin [8]. Hydrocarbon ointment base lasts on the skin for a long time, does not easily evaporate into the air and is difficult to wash off, thus prolonging drug contact with the skin, its action is only as a cover. According to [9] addition of collagen can affect the characteristics and quality of ointment products. Characteristics of hydrocarbon ointments can be known from several factors including pH, homogeneity, dispersibility, organoleptic and shelf life.

Based on this, it is necessary to further investigate the effect of using the concentration of collagen as an active substance on the characteristics of burn ointment.

2. MATERIALS AND METHODS

2.1 Time and Place

The research was carried out for three months starting on March 29, 2021 until June 24, 2021, Fishery Products Processing Laboratory Building 2, Faculty of Fisheries and Marine Sciences, and Sentran Laboratory, Padjadjaran University, Sumedang, West Java, Indonesia.

2.2 Materials and Tools

The tools used are beaker glass, analytical scale, measuring cup, 1 l volumetric flask, thermometer, pH meter, refrigator or cooling tank, measuring cup, tray, funnel, spatula, filter paper, freeze dryer, glass or petri dish, weight of iron weighing 100 g , scoring sheet, hot plate stirrer, ruler, ointment pot, mortar, aluminum foil. Materials used in the isolation process of tilapia scale collagen. Dry tilapia scales waste, 0.5-1cm in size as much as 50g as raw material for collagen, 1M NaOH, 1.5M CH3COOH, aquades, 0.9M NaCl, pH indicator, buffer solution pH 4 and 7. manufacture of ointment with the addition of tilapia fish scale collagen. Cera alba, Vaseline album, tilapia scale collagen extract (0%, 2%, 4% and 6%), as observation variables.

2.3 Research Procedure

This research consists of several stages, namely the isolation of collagen [10]. Second prisoner of making ointment [11]. The last stage of observation with parameters pH, homogeneity, dispersion, shelf life and oraganoleptic [9].

2.4 Research Methods

The method used in this research is experimental [9]. The experimental design used for the parameters of the ointment pH, spreadability, shelf life and homogeneity of the ointment using a Completely Randomized Design (CRD) consisting of four treatments: (A) Addition of fish scale collagen 0%, (B) Addition of fish scale collagen 2%, (C) Addition of fish scale collagen 4%, (D) Addition of fish scale collagen 6% and five replications. The organoleptic parameters of appearance, color, aroma and texture using four treatments and 15 semi-trained panelists as replicates were then analyzed by non-parametric statistics Friedman test. the results of the description test of each panelist on the research sheet are compiled and analyzed into a conclusion that states the specifications for appearance, aroma, texture and color.

2.5 Data Analysis

The data from the homogeneity test of the ointment were analyzed descriptively comparatively. The data from the ointment pH test, the spreadability of the ointment, were analyzed by parametric statistics using analysis of variance (ANOVA) at a 95% confidence level. Further tests were carried out if there were differences in the treatment used with Duncan's real difference test. The data obtained from the organalleptic test of the ointment were analyzed by non-parametric statistical Friedman test. Multiple comparison test (multiple comparison) was carried out, there were significant differences. The hedonic test usually produces many of the same numbers so it is necessary to carry out an analysis that can provide differences between treatments. Determination of the best treatment using the Bayes method by considering the weight of the criteria and the median value.

3. RESULT AND DISCUSSION

3.1 Ointment Homogeneity

Homogeneity is one of the evaluations of the physical properties of the ointment that can determine the effectiveness of the ointment preparation. Ointment preparations can be said to be homogeneous when there are no lumps or coarse granules in the ointment preparation. The results of the homogeneity test of the ointment are presented in (Table 1).

Table 1. Results of Homogeneity of Tilapia Fish Scales Collagen Extract Ointment

Treatment	Homogeneity
A (Addition of fish scale collagen 0%)	Homogeneous
B (Addition of fish scale collagen 2%)	Homogeneous
C (Addition of fish scale collagen 4%)	Homogeneous
D (Addition of fish scale collagen 6%)	Homogeneous

Based on Table 1. shows that all treatments have homogeneous characteristics. Homogeneous results show that the distribution of the particles in the ointment preparation looks even. According to [9] homogeneous ointment preparations indicated that the mixture of the ointment ingredients and the extract of the active substance used was good so that no lumps or coarse

granules were found in the preparation. An ointment preparation must be homogeneous and even so that it does not cause irritation and is evenly distributed when used. The requirements for a good preparation are homogeneous [12].

3.2 pH Ointment

Test pH is a form of evaluating the physical properties of ointment preparations which aims to detect the safety of the ointment before being applied to the skin. According to [9] a pH value that is too acidic can irritate the skin while a pH value that is too alkaline can make the skin scaly. The test results for the pH of the ointment are presented in (Table 2).

Table 2. Average pH Value of Tilapia Scale Collagen Ointment

Treatment	pH value
A (Addition of fish scale collagen 0%)	$6,48 \pm 0.08^{d}$
B (Addition of fish scale collagen 2%)	$6,30 \pm 0.07^{c}$
C (Addition of fish scale collagen 4%)	$6,12 \pm 0,08^{b}$
D (Addition of fish scale collagen 6%)	$5,72 \pm 0,08^{a}$

Note: Numbers followed by the same letter notation mean that there is no significant difference with 95% confidence level

Based on Table 2. the average pH values in the four ointment formulations ranged from 5.72 to 6.48. The results of testing the pH value in all treatments showed that the fish scale collagen ointment was in accordance with the SNI 16-4399-1996 standard as a quality requirement, which ranged from 4.5-8 [12]. All treatments with addition of fish scale collagen extract were in accordance with the normal pH value of the skin, namely 4.5-6.5 [6]. This indicates that the test results of the pH value of the ointment have met the SNI standard and are in accordance with the normal pH of the skin. The results of analysis of variance (ANOVA) showed that Fcount>Ftable, meaning that the addition of fish scale collagen extract had a significant effect on the pH value. This corresponds to [13] showed that the addition of collagen with different concentrations had a significant effect on the pH value of water-based ointments. The decrease in the pH value of the ointment is thought to be due to the process of making collagen using an acid solution (CH3COOH) so that it produces collagen which tends to be acidic [14]. The higher the concentration of collagen addition, the lower the pH level of the ointment..

3.3 Spreadability Ointment

The spreadability test was carried out to see the ability of the preparation to spread on the skin, where an ointment base should have good spreadability to ensure satisfactory administration of medicinal ingredients. The results of testing the spreadability of the ointment are presented in (Table 3).

Table 3. The Average Value of Dispersion of Tilapia Scale Collagen Ointment

Treatment	Dispersion
A (Addition of fish scale collagen 0%)	$4,04 \pm 0.11^{c}$
B (Addition of fish scale collagen 2%)	$3,64 \pm 0.11^{b}$
C (Addition of fish scale collagen 4%)	$3,22 \pm 0.10^{a}$

D (Addition of fish scale collagen 6%)	3,18 ± 0.08[a]

Note: Numbers followed by the same letter notation mean that there is no significant difference with 95% confidence level

Based on Table 3. the average value of dispersion in the four formulations ranged from 3.18 to 4.04 cm. The fish scale collagen ointment in this study can be categorized as easily spreadable because the diameter of the spread ranges from 3-4 cm [15]. This shows that the test results of the spreadability of the ointment have met the standard of being easy to spread on the skin. Based on analysis of variance (ANOVA) shows that Fhit>Ftab means that the addition of fish scale collagen extract has a significant effect on the dispersion value. This is in accordance with the statement of [16] collagen has thickening properties. Collagen mixed into ointment preparations as an active substance can affect the ointment base itself, so that the spreadability is lower. According to [17] difference in dispersion greatly affects the rate of diffusion of the active substance across the membrane. The wider the membrane where the preparation is spread, the greater the diffusion coefficient which results in increased drug diffusion, so the greater the spreadability of a preparation, the better.

3.4 Organoleptic

Testing the quality of the ointment made begins with an organoleptic test. Observations made in this test are the appearance of the preparation, aroma, texture and color. Parameters of good quality ointment are semi-solid dosage form, the ointment has a characteristic smell to the extract used and the color is like the extract.

Appearance

Appearance has an important role in consumer acceptance because it becomes the initial assessment of a product produced. The panelists' preference value given to the appearance of the ointment is shown in (Table 4).

Table 4. The Average Value of Panelists' Preference for the Appearance of the Ointment

Treatment	Median	Average
A (Addition of fish scale collagen 0%)	9	8.6 [a]
B (Addition of fish scale collagen 2%)	9	8.2 [a]
C (Addition of fish scale collagen 4%)	9	8.3 [a]
D (Addition of fish scale collagen 6%)	9	8.0 [a]

Note: Numbers followed by the same letter notation mean that there is no significant difference with 95% confidence level.

Based on the results of hedonic testing, the appearance of the ointment showed that the panelists' preference value ranged from 8.07 to 8.60, which means that the fish collagen ointment was still acceptable to the panelists. The results of the Friedman test at the 95% confidence level showed that the addition of collagen was not significantly different so that it did not affect the panelists' preference for the appearance of the ointment. The requirements for a good ointment preparation are homogeneous.

Aroma

Aroma is an important component to determine consumer acceptance and preference for a product that describes the characteristics of the product. The value of the panelists' preference for the aroma of the ointment is shown in (Table 5).

Table 5. The Average Value of Panelists' Preference for the Aroma of Ointment

Treatment	Median	Average
A (Addition of fish scale collagen 0%)	9	8.6 [a]
B (Addition of fish scale collagen 2%)	9	8.0 [a]
C (Addition of fish scale collagen 4%)	7	7.6 [ab]
D (Addition of fish scale collagen 6%)	7	6.4 [b]

Note: Numbers followed by the same letter notation mean that there is no significant difference with 95% confidence level.

Based on the results of the hedonic ointment test, the panelists' preference value ranged from 6.47 to 8.60, which means that the fish collagen ointment was still acceptable to the panelists. Friedman test results at the 95% confidence level showed that the addition of collagen to the ointment had an effect on the level of preference for the aroma of the ointment. This is in accordance with [18] collagen tends to have a less pleasant aroma so that it can affect the aroma produced in the product. The preparations of ointment without the addition of collagen, 2% 4%, and 6% addition of collagen were still acceptable to the panelists with a neutral and distinctive smell of collagen.

Texture

Texture of the ointment shows the level of smoothness and uniformity of the resulting ointment. Texture is a tactile value character that can be felt physically and imaginatively. The panelists' preference values given to the texture of the ointment are shown in (Table 6).

Table 6. The Average Value of Panelists' Preference for the Texture of the Ointment

Treatment	Median	Average
A (Addition of fish scale collagen 0%)	7	7.4 [a]
B (Addition of fish scale collagen 2%)	7	7.4 [a]
C (Addition of fish scale collagen 4%)	9	8.3 [a]
D (Addition of fish scale collagen 6%)	7	7.1 [a]

Note: Numbers followed by the same letter notation mean that there is no significant difference with 95% confidence level.

Based on the results of hedonic testing, the texture of the ointment shows that the panelists' preference value ranges from 7.13 to 8.33, which means that the fish collagen ointment is still acceptable to the panelists. Friedman test results at the 95% confidence level showed that the addition of collagen to the ointment had no effect on the level of preference for the texture of the ointment. This is in accordance with [9] addition of collagen to the ointment preparation with a good mixing process will not affect the texture of the resulting ointment.

Color

The color of the ointment is influenced by the color component of the ointment base and the nature of the ointment base used. Color characteristics determine the selection and acceptance of a

product by consumers which can be observed through the sense of sight. The panelists' preference values given to the color of the ointment are shown in (Table 7).

Table 7. The Average Value of Panelists' Preference for the Color of the Ointment

Treatment	Median	Average
A (Addition of fish scale collagen 0%)	9	8.3 [a]
B (Addition of fish scale collagen 2%)	9	8.3 [ab]
C (Addition of fish scale collagen 4%)	7	7.4 [ab]
D (Addition of fish scale collagen 6%)	7	6.3 [b]

Note: Numbers followed by the same letter notation mean that there is no significant difference with 95% confidence level.

Based on the results of hedonic testing, the color of the ointment shows that the panelists' preference value ranges from 6.33 to 8.33, which means that the fish collagen ointment is still acceptable to the panelists. Friedman test results at the 95% confidence level showed that the addition of collagen to the ointment had an effect on the level of preference for the color of the ointment. Based on research [19] in the preparation of skin moisturizing gel, the higher the addition of collagen concentration which is formulated to give a yellowish color. This is in accordance with the opinion of [14] collagen from tilapia scales has a low brightness. Tilapia fish scale collagen has a yellowish white color and low brightness.

3.5 Shelf life

Shelf life is a test to determine changes in physical and chemical properties of ointment preparations during storage. Changes in the physical properties of the ointment during storage were observed, including appearance, aroma, texture, and color, while the chemical properties observed were the pH of the ointment.

Physical Ointment

The purpose of this examination is to determine the physical changes of the ointment preparation during organoleptic storage time. The results of the observation of the physical shelf life of the ointment carried out every week for 28 days of storage at room temperature 25°C are shown in (Table 8).

Table 8. Results of Observation of Physical Shelf Life of Ointment

Treatment	Appearance			
	1st Week	2nd Week	3rd Week	4th Week
A (Addition of fish scale collagen 0%)	-	-	-	-
B (Addition of fish scale collagen 2%)	-	-	-	-
C (Addition of fish scale collagen 4%)	-	-	-	-
D (Addition of fish scale collagen 6%)	-	-	-	-
Treatment	**Aroma**			
	1st Week	2nd Week	3rd Week	4th Week
A (Addition of fish scale collagen 0%)	-	-	-	-
B (Addition of fish scale collagen 2%)	-	-	-	-
C (Addition of fish scale collagen 4%)	-	-	-	-
D (Addition of fish scale collagen 6%)	-	-	-	-
Treatment	**Texture**			
	1st Week	2nd Week	3rd Week	4th Week
A (Addition of fish scale collagen 0%)	-	-	-	-
B (Addition of fish scale collagen 2%)	-	-	-	-
C (Addition of fish scale collagen 4%)	-	-	-	-
D (Addition of fish scale collagen 6%)	-	-	-	-
Treatment	**Color**			
	1st Week	2nd Week	3rd Week	4th Week
A (Addition of fish scale collagen 0%)	-	-	-	-
B (Addition of fish scale collagen 2%)	-	-	-	-
C (Addition of fish scale collagen 4%)	-	-	-	-
D (Addition of fish scale collagen 6%)	-	-	-	-

Note : + : There are changes
- : No changes
Each formula is replicated 3 times

Based on Table 8. it can be seen that the four ointment formulas did not change in appearance, aroma, texture, and color during the 4-week storage period. This means that the difference in the addition of fish scale collagen used in the manufacture of fish collagen extract ointment can be said to have a fairly good physical stability against a storage period of 4 weeks.

Chemistry Ointment

The purpose of this examination is to determine the chemical changes of the ointment preparation during storage. The results of the observation of the chemical shelf life of the ointment carried out every week for 28 days of storage at room temperature 25°C are shown in (Table 9).

Table 9. Results of Observation of Chemical Shelf Life of Ointment

Treatment	Nilai pH			
	1st Week	2nd Week	3rd Week	4th Week
A (Addition of fish scale collagen 0%)	6	6	6	6
B (Addition of fish scale collagen 2%)	6	6	6	6
C (Addition of fish scale collagen 4%)	6	6	6	6
D (Addition of fish scale collagen 6%)	5	5	5	5

Note : Each formula is replicated 3 times

Based on Table 9. it can be seen that the four ointment formulas did not change the average pH value during 4 weeks of storage. The pH value of hydrocarbon-based ointments tends to maintain the pH value. This means that the difference in the addition of fish scale collagen used in the manufacture of fish collagen extract ointment can be said to have a fairly good chemical stability against a storage period of 4 weeks.

3.5 Determination of the Best Ointment Bayes Method

Bayes method is a technique used to perform analysis in making the best decision from a number of alternatives [20]. Parameters in this method include physical-chemical parameters (pH, spreadability, shelf life and homogeneity) and organoleptic parameters (appearance, aroma, texture and color). The importance value for the objective parameter is given the same value, namely 3 because it is considered the most important parameter. The assessment of the importance of each parameter is presented in (Table 10).

Table 10. Characteristics and Values of Importance of Physical-Chemical and Organoleptic Parameters

No	Parameter	Interest Selection Basis	Value of Interest
	Physical-Chemistry:		
1	pH	The pH value is an important parameter because it is directly related to the skin	3
2	Spreadability	Spreadability is related to the ability of the ointment to disperse the active ingredients	3
3	Shelf life	Shelf life is related to the safety level of the ointment product	3
4	Homogeneity	Homogeneity relates to the effectiveness of the active ingredients and ingredients of the ointment.	3
	Organoleptic :		
5	Appearance	Appearance relates to the overall appearance of the ointment.	3
6	Color	The color of the ointment is related to the first impression of the appearance of the product	1
7	Aroma	Aroma relates to consumer acceptance and preference for a product	2
8	Texture	Texture is related to physical perceived characteristics	1

The ranking scale used ranged from 1 to 4 according to the concentration of tilapia fish scale collagen addition treatment. The total value of the multiplication between the ranking value and the weight value is used to determine the best concentration of collagen addition treatment in the ointment preparation. The highest total value obtained indicates the best ointment. The calculation results of the Bayes method can be seen in (Table 11).

Table 11. Bayes Method Calculation Results

Parameter	Alternatif (Treatment)				
	0%	2%	4%	6%	Weight
pH	1	2	3	4	0,155
Spreadability	4	3	2	1	0,155
Homogeneity	4	4	4	4	0,155
Shelf life	4	4	4	4	0,155
Appearance	4	2	3	1	0,136
Color	4	3	2	1	0,071
Aroma	4	3	2	1	0,102
Texture	1	2	4	3	0,068
Total value	3,32	2,95	3,00	2,53	
Priority	0,280	0,249	0,257	0,214	
Rating	1	3	2	4	

Based on table 11. the results of calculations using the Bayes method show that the best ointment with the highest concentration of 0% tilapia scale collagen addition produces the highest total value, which is 3.32. Ointment with a concentration of collagen addition of 4% has a total value that is close to the control preparation, which is 3.00. These results concluded that the ointment with a concentration of 4% added collagen was the best treatment compared to the ointment with a concentration of 2% and 6%.

4. CONCLUSION

The results showed that based on the results of the Bayes test, the concentration of the addition of tilapia scale collagen in the ointment preparation of 4% resulted in a value close to the control treatment. The addition of 4% collagen was the best treatment compared to 2% and 6%. Based on the physical-chemical evaluation of the ointment with a concentration of 4% tilapia scale collagen addition, it had a pH value of 6.12, spreadability of 3.22 cm, and the safe ointment preparation did not change at all during 28 days of storage according to the quality standard of the ointment. Based on the results of the organoleptic test, the ointment at this concentration had a homogeneous appearance, slightly yellowish white color, a distinctive smell of collagen and a semi-solid texture.

CONSENT

As per international standard or university standard, respondents' written consent has been collected and preserved by the author(s)

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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