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Authors' Affiliation:
1. Biology Department, Institut Teknologi Sepuluh Nopember - Indonesia
2. Chemistry Department, Institut Teknologi Sepuluh Nopember - Indonesia

***Corresponding Authors:**
Dewi Hidayati
Email: dewhidayati@gmail.com

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Acceptability Test of Modified Transglutaminase Gelatin from Striped Catfish (*Pangasius hypophthalmus*) Skin Based on Organoleptic and Toxicology

Nurul Fitria[1], Dewi Hidayati[1]*, Fredy Kurniawan[2], Arif Luqman[1], Noor Nailis Sa'adah[1], Edo Danilyan[1]

Abstract

Background: Skin is one of the abundance side products of the striped catfish (*Pangasius hypophthalmus*) fillet industry in tropical countries. The previous histological study revealed that the skin of striped catfish contains high collagens which has the potential to supply the increasing halal gelatin demands. However, the texture of striped catfish skin gelatin (SCSG) at room temperature is mushy. This can be enhanced by the transglutaminase (TG) modification. SCSG modified with TG (SCSG_TG) needs to be tested for safety and consumer acceptance before being marketed. The research aims to decide the level of acute toxicity of SCSG_TG in mice (*Mus musculus*) and discover the organoleptic quality of SCSG_TG.

Methods: Striped catfish skin gelatin modified with transglutaminase (SCSG_TG) was acquire by CH_3COOH (acid) and NaOH (base) extraction at a temperature of 58˚C. Toxicity tests using mice were worked by the Limit Test Procedure of the Organization for Economic Cooperation and Development (OECD) and then analyzed descriptively and qualitatively. While organoleptic tests were worked using hedonic assessment and the Friedman test.

Results: The result shows SCSG_TG did not induce any toxic effects or death in mice. SCSG_TG 10 mg film was the most favored treatment group by the panelists (4.9-6.5) compared with SCSG_TG 0 mg (3.8-6.0) and SCSG_TG 50 mg film (4.1-6.3).

Conclusion: Hence, the striped catfish skin gelatin film modified with transglutaminase (SCSG_TG) was declared safe, non-toxic, and accepted according to the organoleptic assessment that has met the standard ("dislike" criteria <25%).

Introduction

Striped catfish is a type of freshwater fish that is widely used as a product fillet in fresh or frozen [1,2]. Fillet fish products use 45% of the fish and the rest is waste, such as skin, head, bones and stomach contents. Thus, many studies have developed catfish skin waste from the production of catfish fillet becomes a product that has economic value [3]. One way to utilize catfish skin waste is to process it into gelatin [4].

Catfish skin has the potential to be a source of gelatin to meet consumer needs for halal gelatin. According to [5], catfish skin produced a total protein content of >85% in gelatin with a yield value of 50.83%. One of the uses of fish gelatin is as a raw material for films. However, the use of fish gelatin in films still has drawbacks, it is very easy to damage, and the texture is easy to soften at room temperature [6,7]. At present, many studies have been carried out to improve the functional properties of gelatin, one of which is the cross-linking method. Cross linking enzymatically with the addition of transglutaminase (TG), the enzyme plays a role in catalyzing the bond cross-linking between l-lysine and l-glutamine in gelatin so that it can improve the rheological properties of proteins [7,8].

Appearance, flavor, and consistency of the film are very important characteristics and must be considered before being marketed. Thus, it is necessary to test the safety and level of consumer acceptance [6,9]. Safety tests are needed to determine whether the product has a toxic effect or not, it can be done using a toxicity test [10]. As well as to determine the level of consumer acceptance can be done through organoleptic tests using an assessment of flavor, texture, appearance, and odor with the help of the five senses [11]. Research on the toxicity and organoleptic properties of modified TG gelatin from striped catfish has never been done before, so this study is expected to decide the level of acute toxicity of SCSG_TG in mice (*Mus musculus*) and discover the organoleptic quality of SCSG_TG.

Methods

Preparation of Striped Catfish Skin

The skin of a large striped catfish (total body length 48.6 ± 1.9 cm with an average weight of 1.194 ± 27.0 grams) was cleaned from the remaining meat and fat by manual scrapping. The skin is washed with distilled water and stored in the freezer (-20°C).

Gelatin Extraction

First, pretreatment was carried out. The skin of striped catfish was soaked in acid (CH_3COOH) and base (NaOH). Each striped catfish skin (30 grams) was wrapped in filter cloth and soaked in 0.12 mol CH_3COOH. The skin weight/solution ratio is 1/10 (w/ v) and stirred for 60 minutes at room temperature (18-24°C). Then, the skin was rinsed with running water until the pH is neutral (6.0-7.0). Furthermore, the skin was soaked using 0.2 mol of NaOH. The skin weight/solution ratio is 1/10 (w/ v) and stirred for 60 minutes at room temperature (18-24°C). Subsequently, the skin was rinsed with running water until the pH is neutral (6.0-7.0) [5]. After pretreatment, the skin was put in distilled water with a ratio of skin weight/distilled water 1/10 (w/v) and heated using a water bath (58˚C) for 60 minutes [5]. The drying process of gelatin was performed refer to [12] with modification. The extract of gelatin was filtered using filter cloth before transferring onto a petri dish for drying at a temperature of 65˚C.

Modified TG Gelatin Production

A piece of dried gelatin (1 gram) was dissolved in 20 ml of distilled water and homogenized by a magnetic stirrer at 50˚C. Three of 30 ml beakers were prepared, each filled with 10 ml of striped catfish skin gelatin (SCSG) extract. Transglutaminase (TG) was added into the solution with the formulation G0: SCSG_TG 0 mg; G1: SCSG_TG 10 mg; G2: SCSG_TG 50 mg. Then 0.1 ml of D-sorbitol was added to each beaker glass to soften and reduce the level of brittleness of the catfish skin gelatin film. The solution was homogenized with magnetic stirrer (50˚C) for 10 minutes and poured into a petri dish. Lastly, the solution was dried at a refrigerator temperature (4-6˚C) [13].

Preparation of Test Animals

The test animals used in this study were mice (*Mus musculus*) strain ddy, male, aged 2 months, with the average body weight of mice in each treatment group is 32 grams. 15 test animals were acclimatized in advance for 1 week. Then, the test animals were grouped into three treatment groups with five individual replications per group (G0: SCSG_TG 0 mg; G1: SCSG_TG 10 mg; G2: SCSG_TG 50 mg). Mice are housed in animal testing cages, using conventional open-top cages and subject to a light dark cycle. The room temperature for mice should be 20–24°C and humidity should be kept at 45-65%. Extraneous noise and in particular ultrasound should be kept to a minimum. Food and water were supplied ad libitum. The test animals were weighed before and after treatment.

Toxicity Test

The toxicity test refers to guidelines [14] using the limit test to identify chemicals that tend to have low toxicity. Five mice were used in each treatment group. The dose of SCSG to be injected into mice was calculated based on the dose limit test at 2000 mg/kg and then converted according to the weight of the mice so that the dose of gelatin to be fed was 640 mg/ml. Mice were not given food (fasting) for 1-2 hours before injection. The dose was administered to one mice in each treatment group at first 24 hours. If the test animal survives, then the dose is continued to the other four test animals and observed for the next 24 hours.

Organoleptic Test

Based on the procedure in [15], organoleptic tests were carried out on the texture, odor, flavor, and appearance using the five senses. A total of 30 panelists were selected for this sensory testing. During the organoleptic test, the panelists were placed in a condition room where interferences for the five senses were minimized, such as smell disturbances and groans after performing the flavor test.

Data Analysis

The results from toxicity test were analyzed statistically using ANOVA, while the results from organoleptic test was analyzed statistically using Friedman Test. R-Studio open-source version 4.2.1 were used to perform the data preparation and visualization process. The libraries and functions used in analysis were the following: for the data preparation the tidyverse library were used, for the colour palette usage ggsci library were used [16], and the ggplot2 were used for visualization.

Results

Toxicity Test Results of Transglutaminase Modified Catfish Skin Gelatin Film (SCSG_TG)

Acute toxicity test is one of the preclinical tests that aims to see the toxic effect by giving a dose within 24 hours [14]. The toxicity test used in this study was an acute toxicity test at a dose of limit test 2000 mg/kg using five mice as test animals [14]. All mice that were treated with acute dose (2000 mg/kg) orally in all groups of SCSG_TG (G0, G1, and G2) showed no mortality (O) either in the first or second 24 hours (Table 1). It infers that SCSG_TG is non-toxic.

Time	Sample ID	Mice Treatment		
		G0	G1	G2
Day 1	1	O	O	O
Day 2	2	O	O	O
	3	O	O	O
	4	O	O	O
	5	O	O	O

Note: G0 = SCSG_TG 0 mg; G1 = SCSG_TG 10 mg; G2 = SCSG_TG 50 mg; O = no mortality.

Table 1: Toxicity test and body weight measurement results after administration of a 2000 mg/kg acute dose (SCSG_TG/weight of mice).

In this study, toxic effects were also observed by measuring the body weight of mice before and after treatment (Table 2). There are did not show any significant difference (p >0.05) in the average weight of the test animals in each treatment group before and after treatment. This declares that the administration of SCSG_TG does not affect the weight of the mice.

No	Treatment	Average weight of mice (gr) ± SD	
		Before treatment	After treatment
1	G0	32.02 ± 2.89	32.26 ± 2.88
2	G1	32.27 ± 4.92	31.71 ± 4.49
3	G2	32.12 ± 4.36	32.31 ± 4.85

Table 2: Note: G0 = SCSG_TG 0 mg; G1 = SCSG_TG 10 mg; G2 = SCSG_TG 50 mg; SD = Standard Deviation.

The Results of SCSG_TG Film Organoleptic Quality Assessment

The organoleptic test in this study used a descriptive test and a hedonic test based on the parameters of appearance, texture, odor, and flavor with 30 panelists who assessed or responded to their likes or dislikes on the SCSG_TG film. After conducting the organoleptic test, each panelist made an assessment. The results of organoleptic tests are presented in Figure 1. Average and standard deviation data of preference level of SCSG_TG film organoleptic quality assessment is available in the supplementary material.

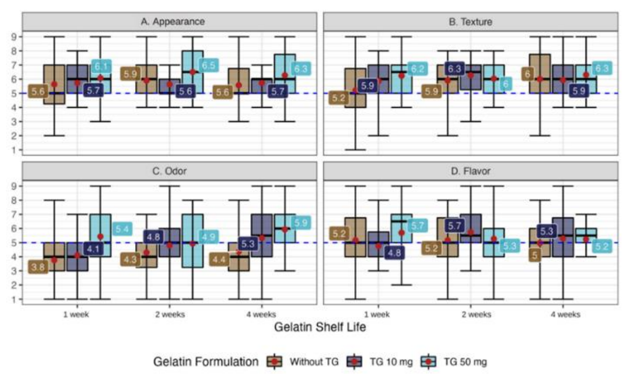

Figure 1: Graph of SCSG_TG film favorite level based on hedonic test.

The organoleptic test (Figure 1) exhibited that SCSG_TG 0 mg film (organoleptic value 3.8-6.0) and SCSG_TG 50 mg film (organoleptic value 4.1-6.3) were tended to be "disliked slightly" to "liked slightly". Meanwhile, for the SCSG TG 10 mg film, the panelists gave a better range value, from "neutral" to "like" (organoleptic value 4.9-6.5). Based on preference level by a semi qualitative assessment, it is known that the panelists gave a best assessment of "neutral" to "like" to the SCSG_TG 10 mg. This is also supported by the results of the Friedman test analysis, which show that the mean value of the SCSG_TG 10 mg film has the highest value of 2.22, then followed by SCSG_TG 50 mg (mean rank = 2.01), and finally SCSG_TG 0 mg (mean rank = 1.77) (p-value <0.05). So, the treatment of SCSG_TG concentration and shelf-life affect the organoleptic value of the panelist. The SCSG_TG 10 mg film is more preferred by panelists on all parameters.

Shelf life	Result											
	G0				G1				G2			
	Appearance	Texture	Odor	Flavor	Appearance	Texture	Odor	Flavor	Appearance	Texture	Odor	Flavor
1 Week	2.20	2.3	2.37	2.00	2.40	2.7	2.73	1.97	2.20	2.73	2.53	2.07
Qualitative	ST	SS	O	SS	ST	S	SO	SS	ST	S	SO	SS
2 Week	2.53	2.87	2.97	2.10	2.83	2.83	2.97	1.43	2.57	2.87	3.27	1.93
Qualitative	T	S	SO	SS	T	S	SO	SS	T	S	SO	SS
4 Week	2.43	2.57	2.57	1.93	2.37	3.00	2.97	1.90	2.47	2.73	3.13	2.13
Qualitative	ST	S	SO	SS	ST	S	SO	SS	ST	S	SO	SS

Note: Appearance parameters assessment: 1=Not transparent (NT); 2=Slightly Transparent (ST); 3=Transparent (T); 4= Very Transparent (ST). Texture parameters assessment: 1=Coarse (C); 2=Slightly Smooth (SS); 3=Smooth (S); 4= Very Smooth (VS). Odor parameters assessment: 1= Very Odor (VO); 2= Odor (O); 3= Slightly Odor (SO); 4= Odorless (O). Flavor parameters assessment: 1= Flavorless (Ts); 2= Slightly Sweet (SS); 3= Sweet (S); 4= Very sweet (VS).

Table 3: The average descriptive test value of the appearance, texture, odor, and flavor parameters.

For the appearance parameter, panelists most preferred SCSG_TG 10 mg film at 2-week shelf life (organoleptic value: 6.5) and rated "like". By semi-qualitative assessment (Table 3) the appearance of the SCSG_TG film at 2-week shelf life was considered "transparent". Based on the texture parameter, panelist most preferred SCSG_TG 10 mg film at the entire shelf life (organoleptic value: 6.0-6.3) and rated "like slightly". By semi-qualitative assessment the texture, SCSG_TG 10 mg film for the entire shelf life rated "smooth". The panelist gives the best assessment for SCSG_TG 10 mg film at 4 weeks shelf life (organoleptic value: 5.9) and rated 'like slightly' in the odor parameter. SCSG_TG 10 mg film at the entire shelf life was rated "slightly odor" or "slightly fishy". For the flavor parameter, panelist most preferred SCSG_TG 10 mg film at 1-week shelf life (organoleptic value: 5.7) and rated "like slightly". For the entire shelf life, SCSG_TG 10 mg film rated "slightly sweet". In addition, based on the significance test, it shows that SCSG_TG concentration treatment and shelf life affect the panelist's preference for appearance and odor parameter (p-value <0.05). Meanwhile, the SCSG_TG concentration treatment and shelf life do not affect the panelist's preference for the SCSG_TG texture and flavor (p-value > 0.05).

SCSG_TG Organoleptic Quality as the Basic Determination of Consumer Acceptance

Based on the Panelist's acceptance percentage (Figure 2), it is known that panelists most preferred in SCSG_TG 10 mg film at 4-weeks shelf life meanwhile the SCSG_TG 0 mg at 1-week shelf-life film was like slightly. This graphic shows that SCSG_TG is organoleptically acceptable to panelists because the acceptance percentage is more than 25%.

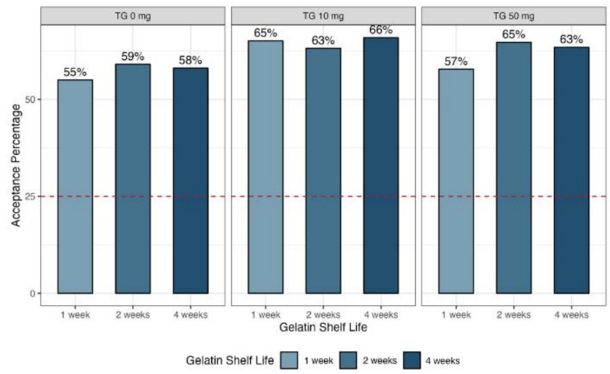

Figure 2: Panelist's Acceptance Percentage of SCSG_TG. The red dashed line represents the assessment percentage minimum standards of products that are organoleptically acceptable.

Discussion

According to Table 1, all mice that were treated with acute doses (2000 mg/kg) orally in all SCSG_TG groups (G0, G1, and G2) did not show mortality (O) either in the first or second 24 hours. Therefore, it can be said that SCSG_TG is not toxic and safe for consumption because it has a limit test dose of >2000 mg/kg. This is in accordance with the protocol [14] that states that if there are three or more live test animals after administration of a dose of 2000 mg/kg, the test material is said to be safe and non-toxic. The material tested in this study is fish gelatin, it is known that fish is a nutritious food ingredient and is rich in amino acids as a building block for protein [17]. Therefore, it can be said that SCSG_TG is a material that has safety for use and does not have a toxic effect. In addition to the absence of mortality, the toxicity test also did not find any behavioral signs associated with toxicity, such as vomiting, convulsions, bleeding, and increased respiration. The signs of toxicity can be seen from changes in the behavior of test animals, including increased respiration, tremors, seizures, and itching [18]. A substance is declared to have a toxic effect if it causes a weight loss in test animals of more than 10% of the initial body weight [19].

The results of the descriptive test of the appearance parameters are in accordance with the statement [20] in which the observed fish gelatin showed a transparent and al-most appearance less film. According to [21] stated that the concentration of gelatin can affect the appearance of the film. In his research, the higher the concentration of gelatin, the more transparent the resulting film is. The smooth texture of the SCSG_TG film is derived from the gelatin concentration. In this study, the concentration of gelatin used in each treatment is the same (10 ml of SCSG). According to [21] the higher the concentration of gelatin, the smoother the texture on the surface of the film. Furthermore, the addition of sorbitol to the SCSG_TG film was also used to reduce friability or increase elasticity of the fish gelatin film [13].

Referring to research [22], catfish gelatin has a characteristic fishy flavor. The fishy smell or unpleasant flavor in catfish which is a type of freshwater fish comes from geosmin and 2-methylisoborneol compounds [23]. These compounds are produced by several species of cyanobacteria and bacteria [24]. These compounds will be synthesized by algae or bacteria and then dissolve into water which will be absorbed by fish through the gills, skin, and digestive tract [23]. Flavor is one of the factors that can determine the delicacy of a food related to the sense of smell. Common problems with gelatin products derived from fish are fishy odor or a fishy smell and are less liked by consumers [22]. The odor flavor (fishy) and slightly odor (slightly fishy) from the SCSG_TG film are derived from the raw material for making the SCSG_TG film, which is striped catfish. According to [25] gelatin is a "flavorless" food ingredient (unflavored). The slightly sweet flavor in SCSG_TG is obtained from the film material, namely sorbitol which gives a sweet flavor [26]. Referring to Figure 2, it can be said that the SCSG_TG film is organoleptically acceptable to consumers (not rejected). According to [27] a product is organoleptically acceptable to consumers if the percentage of "dislike" (reject) criteria is <25%. So, it can be said that the SCSG_TG film can be accepted according to the organoleptic assessment that has met the standards.

This study shows that 10 mg SCSG_TG film has the best preference value, especially from appearance, odor, and flavor parameters. Transparency, odorless, and tasteless are the essential characteristics that most determine the commercial quality of gelatin [28]. It can be said that SCSG_TG 10 mg film can be made as commercial gelatin and as food packaging. Usually, transparent film packaging has a higher demand in the market be-cause customers can clearly see the food they will consume. Moreover, transparent film packaging can indicate that the packaging does not contain an additive ingredient because of its clean appearance. Fish gelatin is a good film material because it is transparent and almost colorless [29].

Conclusively, based on toxicity limit test orally, striped catfish skin gelatin film modified with TG (SCSG_TG) was non-toxic. From the average organoleptic value, it is known that SCSG_TG 10 mg film has the highest preference value of the panelists meanwhile the SCSG_TG 0 mg film has the lowest preference value of the panelists. In addition, based on the significance test, it shows that SCSG_TG concentration treatment and shelf life affect the panelist's preference for appearance and odor parameter (p-value <0.05). Meanwhile, the SCSG_TG concentration treatment and shelf life do not affect the panelist's preference for the SCSG_TG texture and flavor (p-value >0.05). This is also supported by the results of the Friedman test analysis, which show that the mean value of the SCSG_TG 10 mg film has the highest value of 2.22, then followed by SCSG_TG 50 mg (mean rank = 2.01), and finally SCSG_TG 0 mg (mean rank= 1.77) (p-value <0.05). Therefore, the treatment of SCSG_TG concentration and shelf life affect the organoleptic value of the panelist.

Author Contributions

Nurul Fitria contributed to conceptualization, collected the experimental data, interpreted and analyzed the result, and visualization, and wrote the original draft preparation. Dewi Hidayati contributes to the conceptualization, methodology, review, and editing of the manuscript, funding acquisition, and supervision. Fredy Kurniawan, reviewing and editing the manuscript, and supervision. Arif Luqman, review and edit the manuscript. Noor Nailis Sa'adah, as a project administrator, and Edo Danilyan, visualization of the data.

Conflict of Interest

The authors declare that there is no conflict of interest.

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