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5 The effect of fucoxanthin as coloring agent on the quality of catfish sausage

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Abstract. Fucoxanthin is the main carotenoid produced from brown seaweed *Sargassum* sp. Fucoxanthin has an orange color and antioxidant activity. Fucoxanthin as coloring agent and antioxidants can be used to maintain the quality of catfish sausage during storage. The purpose of this study was to determine the effect of incorporating fucoxanthin from *Sargassum* sp. to color intensity, pH value and peroxide value of catfish sausages during storage. The method was implemented as the experimental method with Completely Randomized Design (CRD). Five different catfish sausages formulations were produced containing 0%, 1%, 2%, 3%, and 4% fucoxanthin. The results showed that the incorporating of fucoxanthin had a significantly different ($p < 0.05$) on the value of color intensity, pH value and peroxide value of catfish sausages during storage. Incorporation of fucoxanthin decreases the * L value, pH value, peroxide value and increases the * a and * b value of catfish sausage. Hedonic test of catfish sausages shows that panelist score on appearance, odor, flavor, texture, color and overall quality were significantly different ($p < 0.05$). Catfish sausage with 1% fucoxanthin is the most preferable by the panelist.

1. Introduction

Catfish sausage is a value-added product in the form of catfish meat mixed with additional ingredients that are put into the casing and processed by heating [2]. Catfish meat contains unsaturated fatty acids which can cause catfish sausage susceptible to oxidation during storage [5]. Zakaria and Sarbon [21], reported that the oxidation process can affect taste, color, odor, texture, and decrease in nutritional value. This can cause a rancid odor in fish sausages during cold storage.

The food industry generally uses synthetic antioxidants such as hydroxyanisole butylated (BHA) and butylated hydroxytoluene (BHT) to control the oxidation process. However, the use of this synthetic compound can have a carcinogenic effect if consumed in the long run [18]. The utilization of natural ingredients is an alternative to synthetic antioxidants that are added to prevent oxidation [1]. One source of natural antioxidants is fucoxanthin carotenoid pigments found in seaweed *Sargassum* sp. [10]. Fucoxanthin as a coloring agent and antioxidant can be used to maintain product quality during storage [17].

Sellimi *et al.* [18] stated that the use of fucoxanthin pigment as a natural coloring agent and antioxidant is one of the utilization efforts that can be studied further. Therefore, this study aims to show the effect of adding fucoxanthin from *Sargassum* sp. value of color intensity, pH and peroxide value in catfish sausages during storage.

2. Materials and Method

The material used in this study was fucoxanthin *Sargassum* sp. obtained from Xi'an Quanao Biotech Co., Ltd., Guangzhou, China. The ingredients used for making fish sausages are catfish meat, cassava starch (*rosebrand*), water, salt (*kapal*), ice, sugar (*sugar*), cooking oil (*sania*) and spices such as pepper (*ladaku*), and garlic. The ingredients for the test are potassium iodide (KI), 0.01N sodium thiosulfate (Na_2SO_3), 1% starch indicator solution, chloroform (*Merck*), acetic acid (*Merck*), and aquades.

Equipment used for making fish sausages are food processors (*Philips HR 7627*), balance sheets (*CH-302*), and casings. Equipment for analyzing catfish sausages are color reader (*KONICA MINOLTA Europe CECF-9*), 210 gr analytical balance (*Ohaus pioneer*), Accuracy pH meter 0.1 pH TDS3 & TDS ¹⁴ Digital Meter.

This study is experimentally using a completely randomized design (CRD) with 5 treatments and 4 replications. Parameters in this study include color intensity, pH and peroxide number during storage. Data from the test results were analyzed using ANOVA and further tests of multiple distances (Duncan) with a degree of confidence of 5%. Non-parametric data were analyzed quantitatively using the Kruskal-Wallis, which is a hedonic or likeness test. Formulation to make catfish sausages are in Table 1, as follows:

¹⁶ **Table 1.** Formulation of Catfish Sausage

| No | Ingredients | Formulation I | Formulation II | Formulation III | Formulation IV | Formulation V |
|----|----------------|---------------|----------------|-----------------|----------------|---------------|
| 1 | Fish | 100 g | 100 g | 100 g | 100 g | 100 g |
| 2 | Fucoxanthin | 0 g | 1 % | 2% | 3% | 4% |
| 3 | Ice Water | 27 ml | 20 ml | 20 ml | 20 ml | 20 ml |
| 4 | Sugar | 2 g | 2 g | 2 g | 2 g | 2 g |
| 5 | Salt | 2 g | 2 g | 2 g | 2 g | 2 g |
| 6 | Garlic | 3 g | 3 g | 3 g | 3 g | 3 g |
| 7 | Pepper | 9 g | 1 g | 1 g | 1 g | 1 g |
| 8 | Cooking Oil | 5 ml | 5 ml | 5 ml | 5 ml | 5 ml |
| 9 | Cassava Starch | 10 g | 10 g | 10 g | 10 g | 10 g |

2.1 Catfish Sausage Making

The initial stage of making fish sausage begins with weeding local catfish, in the *fillets* and discarded skin, cleaned of skin that is still attached. The meat is mashed using a food processor so that it is obtained mashed meat and weighed as much as 100 grams. The finished meat is given (2g) of salt and stirred evenly. Furthermore, finely added meat, tapioca flour (10 g), cooking oil (5 g), granulated sugar (2 g), pepper (1 g), garlic (3 g), and added fucoxanthin with 5 levels of treatment (0%, 1%, 2%, 3%, and 4%) , stirred and added water (20 mL) until homogeneous. In the next stage, the dough is put into the charger and then stuffed into the casing by way of pressing slowly until solid so that there are no air cavities inside the casing. The length of the sausage is determined at 10 cm, then the two ends are tied together. After that the sausage is boiled at 45 ° C for 15 minutes then the temperature is raised 90 ° C for 30 minutes. The next stage after becoming a fish sausage is a hedonic test and color test, pH test, and peroxide value test for 21 days (days 0, 7,14,21) at chilling temperature storage.

2.2 Hedonic Test

The hedonic test of catfish sausage uses parameters of appearance, color, aroma, taste, texture, and overall. The panelists used were 30 panelists who knew the ways of assessing hedonic tests. The hedonic quality testing aims to determine the response of panelists and sensory quality responses from the samples produced [4]. Each panelist of the hedonic test was asked to observe the appearance, texture, smell, taste, color, and overall of catfish sausage made with various concentrations of seaweed

pigment *Sargassum* sp. The panelists filled in the questionnaire column that had been given and gave grades 1-5.

2.3 Color Intensity Test

Color analysis is done using a *color reader*, the sample is placed at the end of the tool and obtained data. The value that is read on the tool is the value L = percentage of brightness, a = percentage of redness, b = percentage of yellowish. The Hue value is calculated by the formula = $\tan^{-1}(b^* / a^*)$ The color intensity test is carried out for 21 days at chilling temperature storage [21].

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2.4 pH Test

The pH value was measured according to Erkkila *et al.* [5], as many as 10 grams of the sample were destroyed using a *homogenizer*, then homogenized with 90 ml of distilled water. The homogeneous solution was measured by a *pen* pH that had been calibrated using a standard buffer solution. The stability of the value shown by the pH *pen* is the result of measuring the degree of acidity of the sample pH.

2.5 Peroxide Test

The method of determining peroxide numbers based on Zakaria and Sarbon [21] uses the principle of iodine titration released from potassium iodide compounds by peroxide using standard thiosulfate solution as titrant and starch solution as indicators. This method detects all substances that oxidize potassium iodide under acidic conditions. A total of 5 g of sample was put into a 250 ml erlenmeyer flask, then 30 ml of acetic acid and chloroform was added in a ratio of 3: 2, then 0.5 ml of potassium iodide (KI) was added, the solution was then carefully shaken to mix, then add 30 ml of distilled water. Then the solution is titrated with 0.01 N sodium thiosulfate (Na_2SO_3), until the solution turns yellow, after that 0.5 ml of 1% indicator starch solution is added which will change the color of the solution to blue, then continued with the titration along with continuing shaking the solution until it turns light blue which indicates the release of iodine from the chloroform layer, continue titrating carefully until the blue color in the solution disappears. Peroxide value calculation is done by the following equation:

$$\text{Peroxide Value (meq/kg)} = \frac{S \times M \times 1000}{\text{sample weight (gr)}}$$

No. 10

S= Volume of sodium thiosulfate (ml)

M= Concentration of sodium thiosulfate (0.01)

3. Result and Discussion

3.1 Lightness Intensity L*

The lightness value of catfish sausage during storage with the incorporation of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 1.

The highest L* color value is that of P1 sausage (Fucoxanthin 0%) and the lowest L* value is that of sausage P5 (Fucoxanthin 4%). The addition of fucoxanthin causes the catfish sausage to be darker in color, so the L* value decreases. According to Susanto *et al.* [19], fucoxanthin causes a darker color because it has an L* brightness value of 27, 70 which indicates a low brightness intensity. Carotenoid pigments provide color because they are chromophore groups or color-bearing groups.

Fucoxanthin is a carotenoid in brown seaweed [15]. Carotenoids have chromophores and auxochrome groups which are = C = O as a chromophore and -OH as auxochrome [13]. The chromophore group is the component responsible for the presence of color and the auxochrome functions so that the color sticks to a material [3].

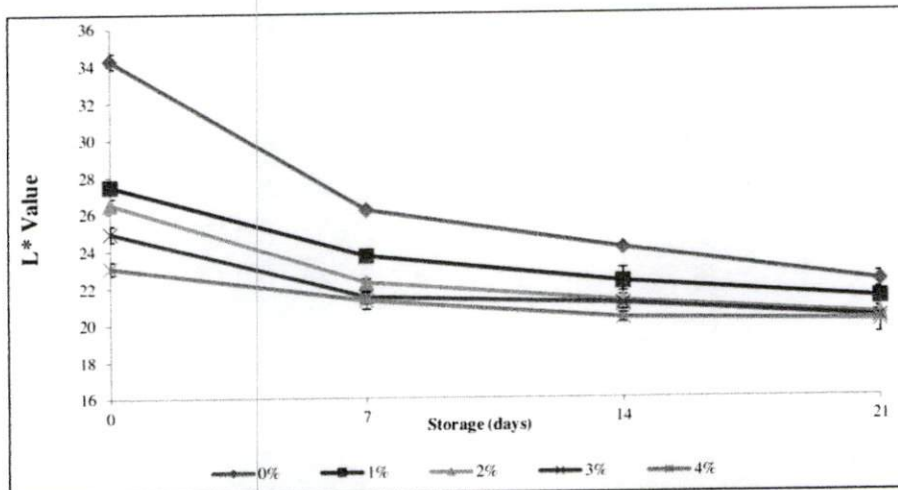


Figure 1. Lightness Value of Catfish Sausage during Storage

Amino compounds as the organic material in the meat will react with dye chromophores form, with the auxochrome, the dye will be attached to the product, in which case this is a sausage [3].

3.2 Redness Value a^*

The results of testing the redness intensity of catfish sausages during storage with the addition of fucoxanthin *Sargassum* sp with different concentrations can be seen in Figure 2

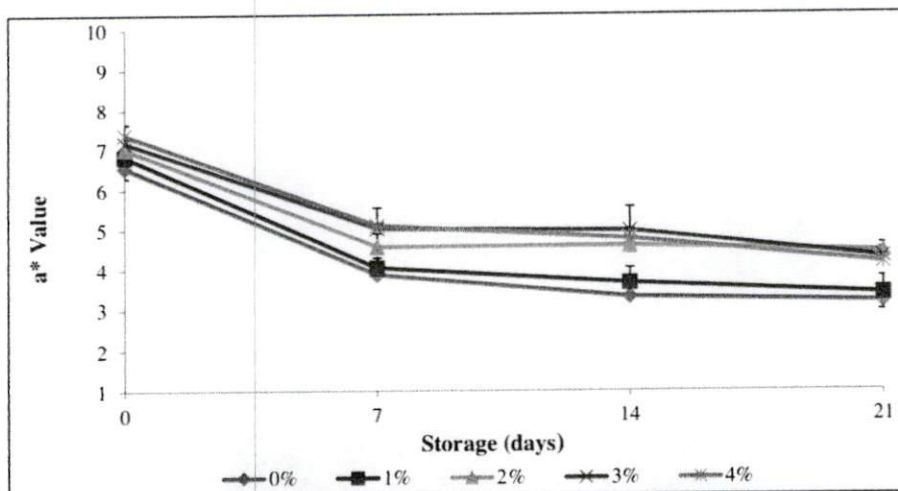


Figure 2. a^* Value of Catfish Sausage during Storage

The highest a^* value is in sausage P5 (Fucoxanthin 4%) and the lowest a^* value is in the P1 treatment (Fucoxanthin 0%). Based on research, it is known that the addition of fucoxanthin can increase the value of a^* (redness) in catfish sausages. Fucoxanthin has a positive a^* which means

towards red with a* value of 0,73 [11]. Fucoxanthin is a red or yellow pigment that has an absorbance of 350-600 nm [17].

3.3 Yellowness Intensity (b^*)

The results of testing the yellowness intensity during storage with the addition of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 3.

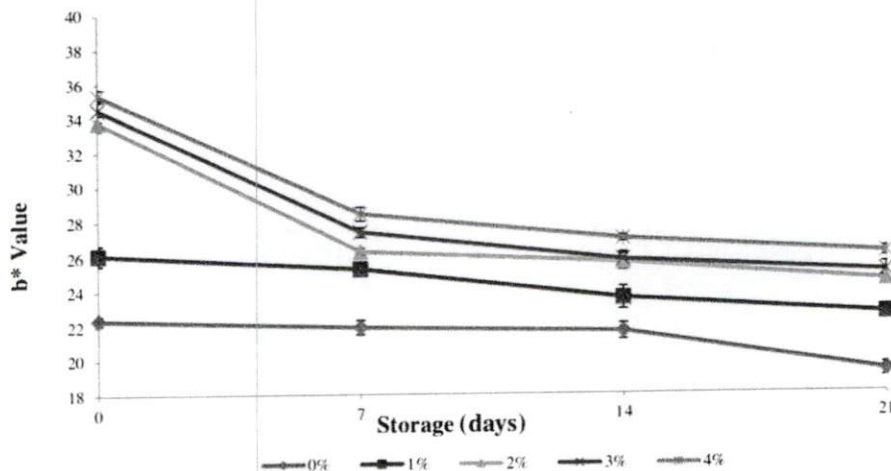


Figure 3. b^* Value Catfish Sausage during Storage

The highest b^* value is sausage P5 (Fucoxanthin 4%) and the lowest b^* value is P1 treatment (Fucoxanthin 0%). The results show that the addition of fucoxanthin can increase the b^* value of catfish sausage. This is because fucoxanthin shows b^* positive value which means the pigment is yellow with value of 5, 21 [11]. Fucoxanthin is an orange pigment that belongs to the carotenoid group which produces yellow, orange and red color absorption [12].

The results of L^* , a^* , and b^* sausage color during storage for 21 days decreased. The highest changes in L^* , a^* and b^* values were found in the treatment P1 (0% fucoxanthin). This shows that the addition of fucoxanthin can inhibit the decrease in the value of L^* , a^* , and b^* catfish sausages during storage [18]. Fucoxanthin as a natural pigment can donate hydrogen atoms to free radicals, so it can inhibit discoloration [16].

According to Mercadante *et al.* [16], the discoloration of sausages with the addition of carotene during storage occurs due to the loss of carotenoid color. Natural pigments have lower color stability than artificial pigments because they are very susceptible to oxidation which causes color fading [9]. This is because when pigments donate hydrogen atoms to free radicals, changes in absorbance occur that cause discoloration and color bleaching [16].

3.4 °HUE value

The results of the HUE value of catfish sausages during storage with the addition of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 4

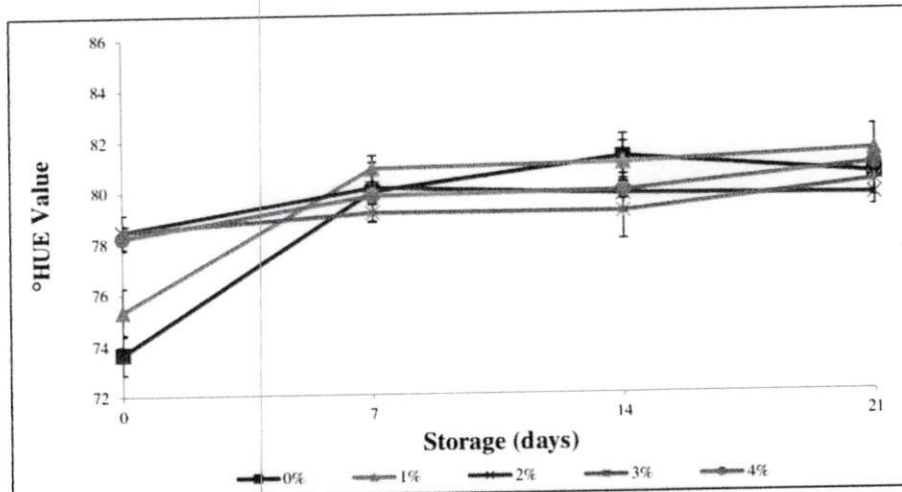


Figure 4. °HUE Value of Catfish Sausage during Storage

The addition of fucoxanthin can increase the value of HUE in catfish sausage. This is because fucoxanthin has a °HUE value of 40, 60 -41.90 [11] Based on the results that the color of catfish sausage is red-yellow [8]. Fucoxanthin gives color because carotenoid has chromophore and auxochrome groups [13]. In food, organic substances react with chromophores to form colors, and in the presence of auxochromes, these colors will be intermittently bound alternately to foodstuffs and form irregular covalent bonds that give color [3].

3.5 Peroxide Value

The peroxide value of catfish sausage during storage with the addition of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 5.

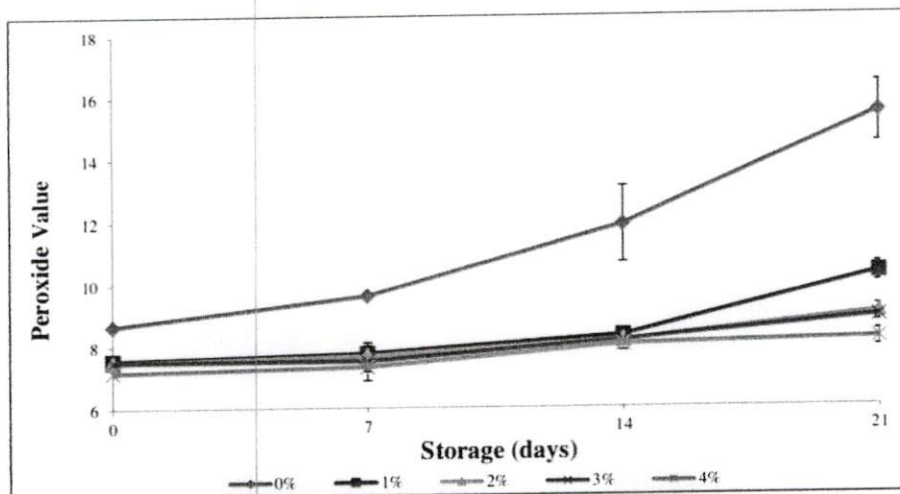


Figure 5. Peroxide Value of Catfish Sausage during Storage

Based on the results of the peroxide test on catfish sausages, it was found that the addition of fucoxanthin significantly ($p < 0.05$) on the peroxide value. Catfish sausage peroxide value during 21 days of storage has increased. This is because oxidation of catfish sausage. In sausages, oxidation can occur in phospholipid membranes or adipose fat emulsified in all parts of the product [14]. Emulsion of oil in water in food there are three parts, namely oil on the inside, oil on the surface and the water phase. In meat emulsions, most of the oxidation occurs on the surface of oil droplets or fat membranes [16].

During the storage periode, the smallest change in the value of peroxide was shown in sausages with 4% fucoxanthin and the highest change in peroxide value occurred in sausages without the addition of fucoxanthin. These results indicate that the addition of fucoxanthin to sausages can inhibit oxidation during storage [18]. This is because fucoxanthin is a polar carotenoid [11]. Polar compounds such as carotenoids tend to be present in the phospholipid membrane, precisely where oxidation usually occurs in sausages so it can inhibit the oxidation process. Also, antioxidant activity depends on the groups contained in carotenoid molecules, where the substitution of hydrogen with polar groups will increase the ability to capture free radicals because the side that contains many electrons will more easily react with free radicals [14].

3.6 pH value

The pH value of catfish sausages during storage with the addition of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 6.

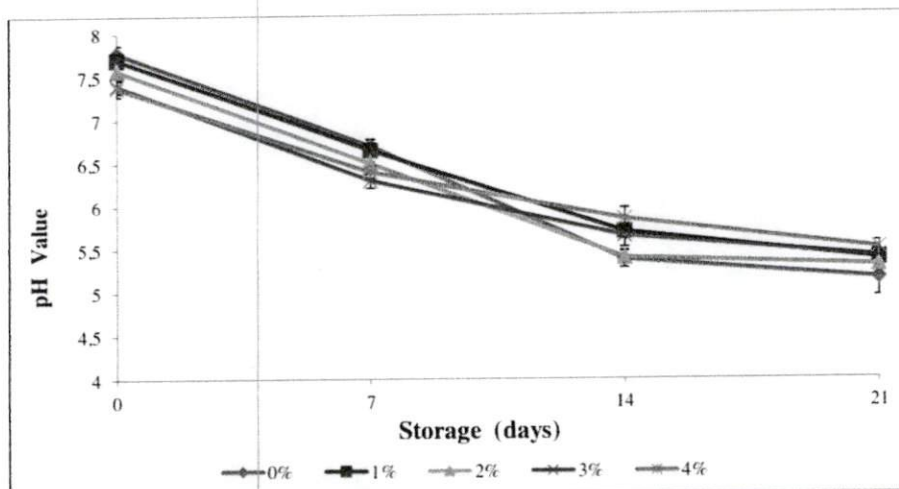


Figure 6. pH Value of Catfish Sausage during Storage

Based on the results, it has been known that the addition of fucoxanthin significantly influences ($p < 0.05$) on the pH value of catfish sausages during storage. The addition of fucoxanthin concentration resulted in a decreased pH value. According to Yip *et al.* [20], fucoxanthin has a pH of 6.1 so that when added to food can reduce the pH of the product.

During storage for 21 days, the pH value of catfish sausage in each treatment decreased. The decrease in pH value is caused by the breakdown of fat into fatty acids due to oxidation reactions that occur will produce compounds with low molecular weight, such as acids, fats, alcohol, and carbonyl [7].

3.7 Hedonic Test

The results of testing the pH value of catfish sausages during storage with the addition of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 7.

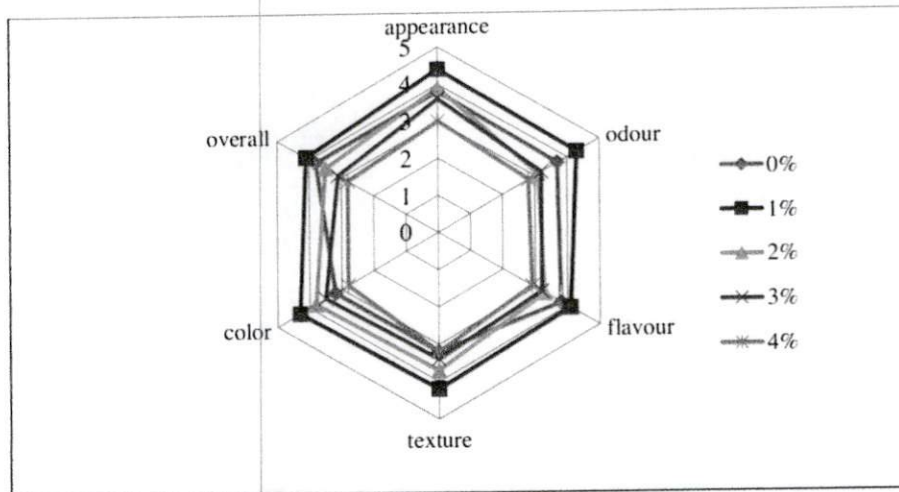


Figure 7. Hedonic Test of Catfish Sausages

The hedonic test of the study was carried out on storage day 0. The results of the hedonic test of catfish sausage showed that catfish sausage with a concentration of 1% fucoxanthin was most preferred compared to other treatments. The higher the concentration of fucoxanthin added, the panelist preference level decreases. The decrease in panel preference level is related to color, pH and peroxide value.

4. Conclusion

The results of this study concluded that the incorporation of fucoxanthin *Sargassum* sp. can increase the color intensity value (a^* , b^* value), and decrease L^* value, the peroxide value, and the pH value in catfish sausages during storage.

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