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The effect of fucoxanthin as coloring agent on the quality of Shrimp Paste

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Abstract. Colour is one of quality attributes of shrimp paste where some industries add non-food grade colouring agent, which may possibly generate adverse effects for customer. The development of colouring agent for shrimp paste is a crucial field of study in the food technology. Fucoxanthin, a yellow pigment extracted from *Sargassum* sp. which was proven capable of possessing antioxidant activity was used in this study to colorize the produced shrimp paste. The fucoxanthin concentration of 0%, 3%, 6%, 9%, and 12% was designed by using a completely randomized design with four replications of each level. This research revealed that the addition of fucoxanthin significantly affected the colour (L*, a*, b*, and °hue), but did not significantly affect the preference of odour and texture. This study investigated that the fucoxanthin concentration of 12% was the best concentration.

1. Introduction

Shrimp paste is fermented shrimp or rebon shrimp that only processed salting, then left for some time the fermentation process. Shrimp paste is a fermented shrimp product which has a rough texture, a savory taste, and a sharp odour. The use of dyes in the food industry is a major factor, one of which is the shrimp paste food industry. Color is one of the important aspects in consumers' acceptance of a food product. Virtually, the color factor determines consumer choices for a product. Consumers will be attracted to the appearance of shrimp paste that looks bright and striking. Shrimp paste is less likeable by consumers because of its unattractive color appearance. Nowadays, textile dyes are often used for food. Synthetic dyes are often used in the food industry because they can produce stronger colors [1].

Fucoxanthin is the main pigment which is owned by brown seaweed *Sargassum* sp. and belongs to the carotenoid type [2]. Fucoxanthin can be used as a natural color in the fields of food, cosmetics, and pharmacology [3]. Fucoxanthin has biological activities that play a role in overcoming several health problems such as antiobesity, anticancer, antioxidants, and anti-inflammatory [4]. In addition, Fucoxanthin is not toxic to normal cells [5]. Utilization of natural pigment Fucoxanthin pigment from brown seaweed *Sargassum* sp. is an alternative to synthetic dyes.

2. Materials and method

The materials used to make shrimp paste include analytical scales *OHAUS CP214*, Electronic Kitchen Scale digital scales *CH-302*, Food Processor *PHILIPS HR 7627*, baking sheets, and cutting boards.



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Color analysis using the Color Reader KONICA MINOLTA Europe CECF-9. And the analysis of pH using a pH meter Accuracy 0.1 pH TDS3 & TDS EC Digital Meter. The main ingredient used in this study was fucoxanthin *Sargassum* sp. from Xi'an Quanao Biotech Co., Ltd, Ghuangzou, China. The materials used for pH test are aquadest, and supporting materials for shrimp paste production is rebon shrimp from Surabaya Pabean Market, KAPAL brand commercial salt from Surabaya, and ABC brand commercial shrimp paste from Jakarta as a control.

The research method used was an experimental method with a complete randomized design consisting of five treatments and 4 replications. The parameters observed were color values (L*, a*, b*, Hue), pH values, and sensory characteristics. Analysis of the data used is ANOVA followed by Duncan's multiple range test. Shrimp formulations are in Table 1.

Table 1. Formulation of shrimp paste

No.	Materials	P I (gr)	P II (gr)	P III (gr)	P IV (gr)	P V (gr)
1.	Rebon shrimp	40 gr	40 gr	40 gr	40 gr	40 gr
2.	Salt	6 gr	6 gr	6 gr	6 gr	6 gr
3.	Fucoxanthin pigment	0 gr	1,38 gr	2,76 gr	4,14 gr	5,52 gr

2.1 Shrimp Paste Production Process

The stages of making shrimp paste is the preparation of raw materials. Raw material for dried rebon shrimp. Next, rebon shrimp is added with 6 grams of salt. The added salt is mixed evenly with rebon shrimp. The next process is grinding. The milling process I aims to refine the shrimp meat rebon. The dough is then carried out in a closed fermentation process.

The next day, continued with grinding II, and after being milled thereafter it will be stored again for 24 hours. In the morning it is dried in the sun until it is completely dry (there is no dough attached to the hands). The drying process II is the same as drying I, but the rebon is dried in the sun with a tarpaulin. Rebon is turned upside down when it is dried so that the sun is distributed evenly. Brabon that has been dried and then ground (milling III) twice. Milling III was added to the fucoxanthin according to the treatment (0%, 3%, 6%, 9%, and 12%) which has been homogeneous with water, which is then added to the mixture. The brabon grind is then stored again in 24 hours.

The next day, printing is done manually using a mold with a cutting board. Dough that has been printed is then dried in the sun to make it denser. After drying, the shrimp paste is packed in plastic into several pieces and adjusted to the number of tests needed. The finished shrimp paste is then tested for color, color stability, pH, and characteristic of sensory.

2.2 Color Test

Color test is one of the tests to determine the level of brightness (L*) and color formed from shrimp paste. The color of shrimp paste can be determined by measuring the values of a* (component (+) red to (-) green) and b* (component (+) yellow to (-) blue). Color test is done using the Color Reader. Samples are 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 = redness, b = percentage of yellowish. The hue on the sample can be determined by continuing in the calculation of hue degree (Hue), using the following formula:

$$\text{Hue} = \tan^{-1}(b^*/a^*) \dots \dots \dots (1)$$

2.3 Color Stability Test

Color stability test is carried out on color changes during storage at room temperature at weeks 1, 2, 3 and 4 using the Color Reader.

2.4 pH test

pH analysis was performed using a digital pH meter. The pH meter must be rinsed first using distilled water and dried with a tissue before use. Sample preparation of 5 grams was weighed then crushed until smooth and dissolved in 50 mL of distilled water and then homogenized. Then the electrode on the pH meter is dipped into the sample until a stable figure is obtained on the pH meter projector.

2.5 Sensory Test

Sensory test is a subjective test from several panelists to determine whether or not a fishery product is consumed by the community. The test was conducted by 30 panelists by comparing existing products with specifications on the score sheet, then assessed.

The highest and lowest values of each specification are 9 and 1. These values will be calculated by the standard deviation and the standard deviation so that an interval value is obtained which indicates that the shrimp paste is suitable or not suitable for consumption. Terasi that is suitable for consumption has a minimum organoleptic value of 7.

3. Results and discussion

3.1. Lightness Value (L^*)

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

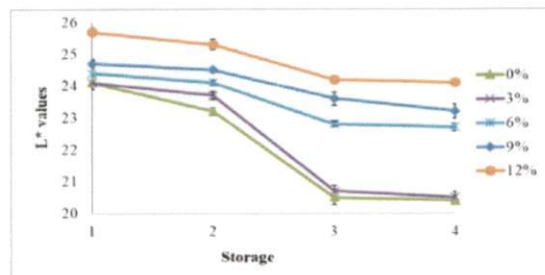


Figure 1. L^* values (Lightness) with Various Concentrations at Shrimp Paste during Storage

The results of the analysis of the L^* value shown in figure 1 show the L^* value is not stable during 4 weeks of storage. A value of L^* (brightness) indicates that the addition of fucoxanthin pigments gives a difference in brightness. The increasing concentration used, the higher the brightness value. This is because the increase in the concentration of the dye increases the color intensity of shrimp paste, so that the light reflected by the surface of the given shrimp paste is lower or the absorbance of the light on the surface of the shrimp paste is higher [6].

During 4 weeks of storage showed a decrease in the value of L^* . Decrease in L^* value every week due to pigment degradation [7], carotenoids have properties that are easily subjected to transient isomeration and eventually the original pigment color will fade [8]. Isomeration can result in changes in the structure of carotenoids from trans to cis shape. According to de Fretes *et al* [2] carotenoids in trans form have higher activity and color intensity compared to carotenoids in cis form.

The stability of fucoxanthin related to the presence of double bonds in the structure of fucoxanthin molecules, the double bonds in fucoxanthin easily damaged or termination reactions that cause decreased carotenoid activity and degradation [9]. Decrease in L^* value due to decreased intensity of fucoxanthin pigments due to fading accompanied by the emergence of the original color of shrimp paste. The natural color of the shrimp paste itself without the use of visual coloring tends to be dark brown. Brown absorbs more light than yellow, causing the L^* value to be smaller [6].

3.2. Redness value (a^*)

The redness value of shrimp paste during storage with the incorporation of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 2.

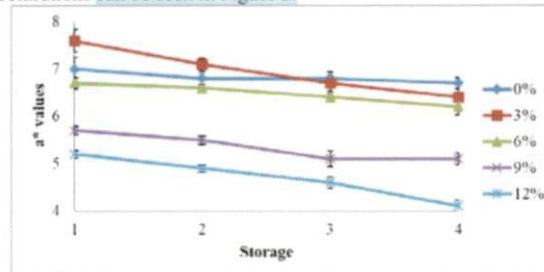


Figure 2. a^* values (Redness) with Various Concentrations at Shrimp Paste during Storage

The test results shown in figure 2 show the increasing concentration used, the value of a^* will be higher. However, at all concentrations (0%, 3%, 6%, 9%, and 12%) and tested for 4 weeks the value of a^* (redness) will decrease further. The red color in shrimp paste is due to the presence of astaxanthin pigment. Shrimp paste without the addition of coloring agent has a brownish red color. Astaxanthin is a pigment found in rebon shrimp. The results showed that the a^* value was not stable during 4 weeks storage, the decrease in a^* value was due to astaxanthin degradation. According to Li *et al.*, [10] astaxanthin is a red compound so that if a browning reaction occurs it can be seen that astaxanthin degradation has occurred. Astaxanthin can easily be degraded.

This degradation process is caused by an enzymatic process. Enzymatically, the polyphenol oxidase enzyme affects the stability of astaxanthin because it is destructive of astaxanthin. The activity of the polyphenol oxidase enzyme makes astaxanthin brownish. The activity of this enzyme can cause the brown pigment process, namely the change in color of astaxanthin to brown which indicates that astaxanthin degradation has occurred [10].

3.3. Yellowness value (b^*)

The yellowness value of shrimp paste during storage with the incorporation of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 3.

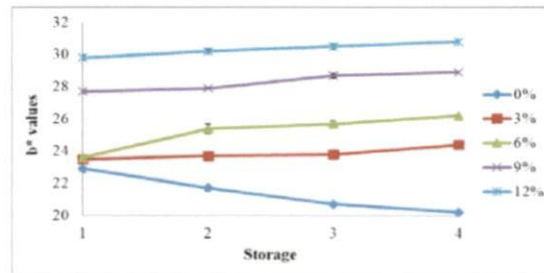


Figure 3. b^* values (Yellowness) with Various Concentrations at Shrimp Paste during Storage

The test results shown in figure 3 show the increasing concentration used, the b^* value will be higher. However, shrimp paste with the addition of coloring concentrations of 3%, 6%, 9%,

and 12% and tested for 4 weeks showed the value of b^* (yellowness) increased. Where as shrimp paste with a concentration of 0% or no coloring has decreased the value of b^* for 4 weeks. According to the results of the study Fitriyani *et al.*, [11] yellow or yellowness in shrimp paste showed a value of 20.75 - 21.50. The more additions of the dye will affect the yellow color of the shrimp paste.

The results show the value of b^* is unstable and has increased. This is because the red color in the shrimp paste is degraded and followed by the color of the fucoxanthin pigment, yellow is more visible in test the value of b^* . The a^* value corresponds to the b^* value in shrimp paste. A decrease in the value of a^* will be accompanied by an increase in the value of b^* , and vice versa. This is caused by the degradation of the astaxanthin pigment which is reversible and can be followed by the synthesis of fucoxanthin pigment [12].

Shrimp paste coloring using fucoxanthin pigment is in principle the same as other dyes that can dye an ingredient. Dyestuff molecules are formed as a result of a combination of unsaturated organic substances in materials with chromophores and auxochromes from coloring agents. The chromophore is the component responsible for the presence of color and auxochrome functions so that the color sticks to a material. In food, unsaturated organic substances such as hydrocarbons and their derivatives, phenol compounds and their derivatives and nitrogen-containing hydrocarbons react with chromophores such as nitro (-NN = OOH), nitroso (-N-OH) or carbonyl groups (-C = O-) forms color, in the presence of auxochromes such as -OH, -OCH₃ and -NH₂ then the color will be intermittently bound to food fibers and form irregular covalent bonds that give color [13].

The intensity of the yellow color in this case is caused by the fucoxanthin pigment having chromophores and auxochromes, that is = C = O as chromophore and -OH as auxochrome. Amino acids as organic material in shrimp paste will react with chromophore to form a coloring agent, in the presence of auxochrome, the coloring agent will stick to the product [13]. During the storage process the fermentation process occurs in shrimp paste, causing the protein to be hydrolyzed into amino acids. So that the amino acids that are formed increasingly react with chromophore to form dyes.

3.4. °Hue values

The °Hue value of shrimp paste during storage with the incorporation of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 4.

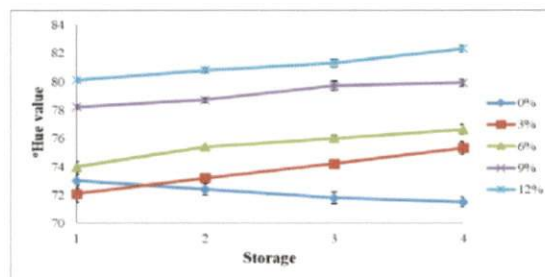


Figure 4. °Hue values with Various Concentrations at Shrimp Paste during Storage

The test results shown in figure 4 show that the higher the concentration of fucoxanthin dye used, the higher the °Hue value. According to Hutchings 1996 in Permatasari [6] °Hue is an attribute and visual sensation that refers to the area that looks the same as a proportion of the two parts to the reception of red, yellow, green, and blue. The °Hue value itself is a conversion of the value a^* and b^* . °Hue itself determines the color category of an object.

Analysis of shrimp shrimp °Hue value using fucoxanthin dye is included in the category of yellow red or red-yellow. This is in accordance with the statement of Permatasari [6] that the red-yellow color

category is at an interval of $54^\circ - 90^\circ$, ie at values below 54° it tends to be in the red category and above 90° is more likely to be in the yellow category.

The increase in $^\circ$ Hue value along with 4 weeks of storage showed a decrease in the intensity of shrimp paste color. The $^\circ$ Hue value category did not change and remained in the yellow red category until the last week but with a different redness value. Fucoxanthin pigment can give color due to the presence of chromophores and auxochromes that react with amino acids in shrimp paste, causing the formation of coloring agents [13].

3.5. pH values

The pH value of shrimp paste during storage with the incorporation of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 5.

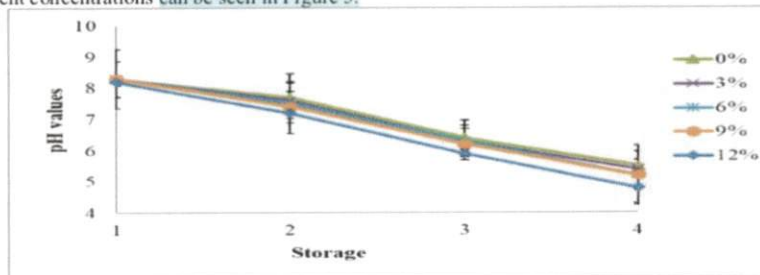


Figure 5. pH values with Various Concentrations at Shrimp Paste

The test results shown in figure 5 show a decrease in pH value from base to acid. This is caused by the nature of the fucoxanthin dye that is in the shrimp paste itself is acidic. According to Maeda [14] fucoxanthin can be applied to food with a pH ranging from 5-7.

The pH value is unstable and decreases in storage week after week. This can be caused by the effect of increasing storage temperature which causes respiration to take place quickly. The mechanism of pH reduction can be seen in the increase in organic acids so that the pH value will be more acidic [15]. According to Peralta *et al.*, [16] with increasing fermentation time the pH value of shrimp paste decreases until the end of fermentation.

The decrease in pH can be caused by the product interacting with CO_2 which causes the breakdown of compounds or the hydrolysis of compounds. According to Filho *et al.*, [17] a decrease in pH value can also be caused by the breakdown of fats into fatty acids and microbiological activity by bacteria because the oxidation reaction that occurs will produce compounds with low molecular weight, such as acids, fats, alcohols, and carbonyl.

According to Hii *et al.*, [18] fucoxanthin relatively stable under acidic conditions. This is due to the fact that fucoxanthin belonging to the carotenoid group contains hydroxyl groups, there is only a slight change in carotenoid reactivity, so the stability of carotenoids will be higher compared to other carotenoids that do not contain hydroxyl groups [18]. But according to Yip *et al.*, [19] that fucoxanthin is relatively unstable under conditions of high acid conditions, this is because high acids can cause pigment degradation.

3.6. Appearance Value

The appearance value of shrimp paste during storage with the incorporation of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 6.

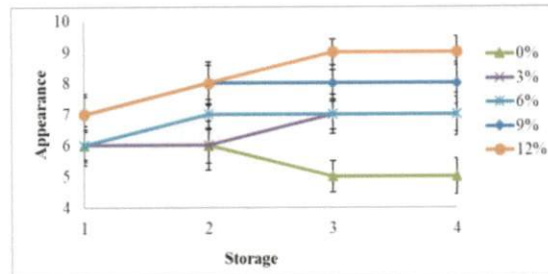


Figure 6. Appearance values with Various Concentrations at Shrimp Paste during Storage

The results of sensory analysis of shrimp paste appearance using scoresheet according to SNI 2716: 2016 are shown in figure 6 showing the change in the value of shrimp paste appearance at various concentrations. In shrimp paste with a concentration of 0% decreased appearance. This is due to the degradation of the dyes so that the color of the shrimp paste turns darker. This is supported by the results of the color test values L^* , a^* , and b^* .

Decrease in color test values is directly proportional to the sensory results at 0% concentration. Whereas the shrimp paste with the addition of coloring 3%, 6%, 9%, and 12% showed an increase in value. This is because the colored shrimp paste looks more displaying a brownish yellow color and clear. This is in line with the results of research by Fitriyani [20] showing that the addition of natural dyes to shrimp paste can improve color but does not affect the texture, taste, and odour of shrimp paste.

Shrimp paste storage at room temperature can be a factor that causes unstable fucoxanthin pigments. Fucoxanthin is stable at 4°C and 25°C during storage. Fucoxanthin is not stable at higher temperatures and cannot be stored at temperatures higher than room temperature. High temperatures can cause the breakdown of the double bonds in carotenoid molecules and cause pigment degradation [21].

3.7. Odour value

The odour value of shrimp paste during storage with the incorporation of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 7.

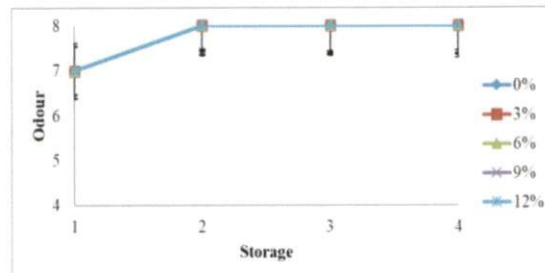


Figure 7. Odour values with Various Concentrations at Shrimp Paste during Storage

The results of the sensory analysis of shrimp paste odour are shown in figure 7 showing changes in the shrimp paste odour value at all concentrations but not significant, which only increases 1 value

above that is 8 which shows the specific odour of shrimp paste. This is because fucoxanthin coloring does not affect shrimp and shrimp odour is influenced by the fermentation process.

According to Aristyan [22] that the odour formed in shrimp paste is influenced by the presence of volatile compounds in shrimp paste due to the fermentation process. This is in accordance with the results of the sensory odour of shrimp paste that is in week 1 the odour produced is not specific to shrimp paste because there is no fermentation process in shrimp paste itself. While the increase in shrimp odour in the following weeks is due to the fermentation process involved in shrimp paste.

According to Rahman and Iffan [23] the odour of shrimp paste is produced by the fermentation process. The odour that appears on the shrimp paste comes from fatty acids that are volatile (acidic odors), ammonia and amines (rattan with ammonia) [11]. This is supported by the statement of Arita *et al.*, [24] saying that the odour of shrimp paste is produced from 16 kinds of hydrocarbon compounds, 7 kinds of alcohol, 46 carbonyl, 7 kinds of fat, 34 nitrogen compounds, 15 kinds of sulfur compounds, and other compounds. The different odour of shrimp paste that was received by the panelists explained that the addition of dyes to the shrimp paste did not inhibit the fermentation process of shrimp paste.

3.8. Flavour value

The flavour value of shrimp paste during storage with the incorporation of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 8.

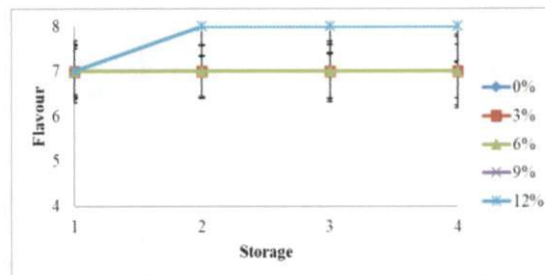


Figure 8. Flavour values with Various Concentrations at Shrimp Paste during Storage

The results of the sensory analysis of shrimp paste are shown in figure 8 showing changes in shrimp paste flavoured values at all concentrations but not significant, namely showing the specific taste of shrimp paste. This is because fucoxanthin coloring does not affect the flavour of shrimp paste at various concentrations having the same taste.

The flavour produced by shrimp paste comes from proteins that turn into amino acids, the typical flavour of shrimp paste comes from protein to amino acids that can cause good taste [16]. This is also supported by the statement of Hajeb and Jinap [25] that during the fermentation process the process of breaking down proteins into amino acids, one of which is glutamic acid which is a source of umami flavour from shrimp paste. The unique flavour of shrimp paste comes from amino acids that are formed during fermentation. The highest amino acid contained in shrimp paste is glutamic acid from non-essential while from the essential group is leucine [25].

3.9. Texture value

The texture value of shrimp paste during storage with the incorporation of fucoxanthin *Sargassum* sp. with different concentrations can be seen in Figure 9.

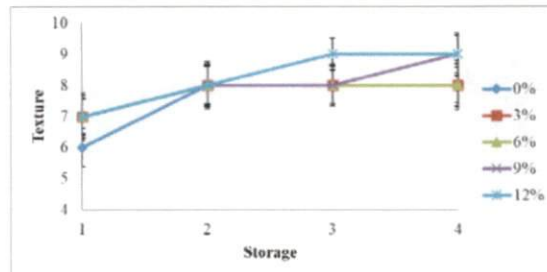


Figure 9. Texture values with Various Concentrations at Shrimp Paste during Storage

The results of sensory analysis of shrimp paste texture shown in figure 9 show changes in shrimp texture texture values at all concentrations but not significant. An increase in the value of shrimp paste texture for 4 weeks shows that the increase in shrimp paste has a solid texture and is not dry. The texture of shrimp paste can be influenced by the moisture content so the addition of water when adding coloring will affect the texture of the shrimp paste.

According to Fitriyani [11] that shrimp paste with water content is too low, the surface of the shrimp paste will be covered with salt crystals and the texture of the shrimp paste becomes not springy. If the water content is too high then the shrimp paste will become too soft. It is suspected that shrimp shrimp paste with the addition of fucoxanthin dye has a low water content. This is because after the process of adding powder coloring which is slightly mixed with water so that the process of mixing the dye with shrimp paste is more homogeneous.

After the color mixing is done drying again to the shrimp paste so that the water content becomes low. According to Fitriyani [11] the coloring is done after terasi dried in the sun is a modification to prevent the degradation of the dye used due to the influence of light and temperature, because high light intensity and high temperature can cause degradation of the dye.

4. Conclusion

The incorporating of fucoxanthin had a influence on color intensity, pH value, and characteristic of sensoric of shrimp paste during storage. Incorporation of fucoxanthin decrease L^* , a^* , pH value and increase the b^* , $^{\circ}$ Hue value, and characteristic of sensoric shrimp paste. This study investigated that the fucoxanthin concentration of 12% was the best concentration.

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