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The Addition Effect of Sea Cucumber Extract (*Stichopodidae*) on the Quality of Snakehead Fish oil (*C. striata*) during Storage

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors LP and Romadhon managed the analyses of the study. Author MHA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Snakehead fish (*Channa striata*) is one of the freshwater fish consumptions that has high economic value so that many people like it. Processing snakehead fish into fish oil can be an alternative to product diversification. Fish oil is an oil that contains high unsaturated fatty acids that are easily oxidized. Adding antioxidants to the oil can suppress oxidation so it is necessary to add antioxidant agents derived from natural ingredients such as sea cucumber extract (*Stichopodidae*). The purpose of this study was to examine the effectiveness of sea cucumber extract antioxidants with

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the best concentration on changes in the quality of snakehead fish oil obtained from previous research during storage. The material used in this study was sea cucumber extract and snakehead fish oil. The study was carried out using *experimental laboratories* method using *Split Plot in Time design* where the difference in adding sea cucumber extract as *the main plot* and storage duration as *a sub-plot* for Thiobarbituric acid (TBA), Peroxide Value (PV), and Free Fatty Acid (FFA) tests. The treatment of this study was the addition of sea cucumber extract (0% as a control; 0.1%; 0.2%; 0.3%) with 2 storage points (0 and 5 days) for 3 repeats. Making sea cucumber extract extraction method using 96% ethanol. Parametric data were analyzed with Analysis of Variance tests (ANOVA) and Honest Real Difference (HRD) while non-parametric data were analyzed with *Kruskal Wallis* and *Mann-Whitney*. The results of preliminary research obtained sea cucumber extract ration with 96% ethanol solvent of 16.1%, % inhibition of 80.30% and antioxidant activity of IC_{50} of 80.875 ppm (strong). The results of the main study obtained TBA values ranging from 0.07 to 0.27 mg. malonaldehyde/kg, PV range from 0.13 to 5.31 mEq/kg, and Free Fatty Acid (FFA) values range from 0.39 to 1.54%. Based on the results of the study showed that the interaction between the concentration of sea cucumber extract and the duration of storage had a real effect ($p < 0.05$). 0% sea cucumber extract produced the lowest TBA sapling, which was 0.07±0.05 mg. malonaldehyde/kg, 0.1% yielded the lowest peroxide number at 3.40±1.25 mEq/kg, and 0.2% yielded the lowest FFA number of 0.58±0.00% on day 5. The results suggest that ethanolic extract of sea cucumber does not act as an efficient antioxidant agent to prevent fish oil degradation.

Keywords: Fatty acid; fish oil; sea cucumber extract; snakehead fish.

1. INTRODUCTION

Indonesia is very rich in seafood production, and has the potential to be developed into a variety of processed seafood products. One potential seafood product is sea cucumber. According to [1], there are around 650 species of sea cucumbers in the world, and 60 species are found in Indonesia. This situation makes Indonesia the largest exporter of sea cucumbers, where many Indonesians use sea cucumbers for food needs and as export products. From the 2018 MMAF statistical data in 2015-2020, exports varied in the Sulawesi region obtained from the fish quarantine center. Sea cucumber product data for overseas shipments (export) Where in 2016 sea cucumber exports reached 251,784.4 kg and there was a decrease every year until 2019 reached 60,484.21 kg [2].

Teripang is another name for sea cucumber. Sea cucumber which has a Latin name (*Stichopodidae*) is an *invertebrate* or invertebrate type marine animal and usually lives on the bottom of waters such as substrates of sand, mud and coral reefs. Sea cucumbers play an important role as deposit *feeders* and suspension feeders. The potential of sea cucumber in the field of Fishery Product Technology is a material that can be utilized or processed into various preparations, one of the uses of sea cucumber extract. According to [3], sea cucumbers (*Stichopodidae*) contain high protein in wet conditions is 44-55% and in dry conditions is 82%, there are a number of essential and non-essential amino acids, especially arginine and glycine which reach 60.90 mg / kg and are high in unsaturated fatty acids. IC 50 value of sea cucumber extract *Stichopus hermanii* is 65.08 ppm, and *α-tocopherol* content is 2.75 ppm [4,5]. These results show that sea cucumber has potential as an antioxidant and protects cells from free radical damage.

Fish oil is very susceptible to damage due to fat oxidation. This can affect the stability of fish oil quality. The stability of fish oil is influenced by the high content of unsaturated fatty acids that contain many double bonds. According to [6], fat oxidation is a common phenomenon that often results in chemical changes that result in reduced nutritional value, reduced taste, and changes in product texture. These chemical changes cause the taste and aroma to become rancid. Prevention of oxidation that can maintain oil quality requires the addition of antioxidant compounds that can be obtained from sea cucumbers. According to [7], antioxidants are compounds that slow down and prevent the oxidation process. This compound is able to prevent the formation of free radical reactions (peroxides) during lipid oxidation.

Snakehead fish oil has the highest content of unsaturated fatty acids compared to other types of oil. The advantages of snakehead fish to be used as oil are high in albumin which can maintain the stability of fluid regulation in the

body. If the condition of body fluid levels decreases, then the protein that enters the body will break down so that it cannot function normally. Most types of oil are susceptible to oxidation due to long storage so it is necessary to add antioxidants from sea cucumbers. The oxidation process will increase in the presence of heat, light and oxygen. Fish oil oxidation damage is preceded by autooxidation of unsaturated fatty acids with the formation of free radicals caused by light, heat and fatty peroxides. These free radicals then react with oxygen to form active peroxide compounds that ultimately affect the physical and chemical properties of fish oil. Inhibition of oxidation can be done by adding antioxidants to fish oil [8].

Snakehead fish oil is produced from the extraction process, the use of fish oil to maintain a healthy body. Fish oil is a natural source of unsaturated fatty acids. Fish oil is widely used as a nutritional supplement, mainly because of the content of EPA and DHA in it which is beneficial to health, namely as an anti-inflammatory and anti-arithmetic substance that is beneficial for heart function [9]. According to [10], global demand for fish oil has increased over time for various purposes, including human or food consumption (14%), industrial purposes (5%), and aquaculture (81%). Global fish oil consumption reached 1 million tons in 2011. From 2005 to 2011, global fish oil use was mainly due to aquaculture use and human consumption.

2. MATERIALS AND METHODS

2.1 Materials

The material used in this study was sea cucumber (*Stichopodidae*) from Gegunung Village, Rembang Regency, Central Java. Fish oil is obtained from Klaten Regency, Central Java. Other ingredients used include 96% ethanol, DPPH. The main equipment used in this study includes *Rotary evaporator*, Spectrophotometer.

2.2 Preliminary Research

Preliminary research was conducted for the manufacture of sea cucumber extract in the manufacture of fish oil based on organoleptic test results with turbidity, odor and color parameters. The procedure for making sea cucumber extract refers to the research [8] with modifications. dried sea cucumbers (*Stichopodidae*) as much as 100 g cut into small pieces with scissors and put in a glass bottle. Simplicial is macerated with 500 ml of 96% ethanol solvent (polar) until all simplicial is submerged in a glass bottle that has been closed using aluminum foil. Maceration for 7x 24 hours at room temperature, then filtered with filter paper to separate the filtrate from the residue. The macerated filtrate is then evaporated with a rotary evaporator at a temperature of 40^{o}C $^{(\pm 1\ hour)}$ until an extract is formed that is no longer smelled solvent.

2.3 Primary Research

The main research was carried out the addition of evaporated extracts to add antioxidants to fish oil. After that, tests were carried out on Antioxidant Activity, Thiobarbituric Acid (TBA), Peroxide Number (PV), Free Fatty Acid (FFA) to determine changes in quality in fish oil. The next stage of research is the extraction of sea cucumber and the addition of fish oil as an antioxidant with a concentration of 0%; 0,1; 0.2% and 0.3%. TBA, FFA and peroxide number (PV) tests were carried out on days 0 and 5 to determine changes in quality in fish oil. After storage and testing, it will be known the effect of adding sea cucumber extract in inhibiting damage to fish oil.

2.3.1 DPPH method antioxidant activity test [8]

Extract samples with various concentrations were taken as much as 3 ml, then inserted 1 ml of 0.1 mM DPPH solution. The mouth of the tube is covered with aluminum foil and homogenized. The sample solution was incubated for 30 minutes at 37 ^{o}C. Then the absorption was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer.

$$\% \text{ Free radical capture} = \frac{Ao-A}{Ao} \times 100\%$$

Information:

Ao = DPPH initial absorbance
A = Sample absorbance + DPPH after 30 min incubation

From the value of percent inhibition as abscissa (x) and extract concentration as ordinate (y), then with the LR (Linear Regression) method a line equation is obtained and the concentration is determined when the percent inhibition is 50% (IC_{50})

2.3.2 Organoleptic analysis [11]

Organoleptic testing was conducted based on SNI 01-2346-2006_Rev. 2011. In the sensory test assessment, 25 semi-trained panelists were asked to give an impression of turbidity, color, and smell. Organoleptic assessment scores on the parameters used as follows; Turbidity (9 clear and transparent, 7 clear, slightly transparent, 5 slightly cloudy, 3 cloudy 1 very cloudy), Color (9 Light yellow, 7 Golden yellow, 5 Reddish yellow, 3 Brown, 1 Blackish brown), Odor (9 Specific fish oil, 7 Slightly sour odor, 5 Sour odor, 3 Rancid, 1 Rancid and foul odor). The assessment results are analyzed to calculate the standard deviation and confidence interval by the formula:

$$S2 = \frac{1}{n}\sum(Xi - X)^2$$

$$X - \frac{S}{\sqrt{n}}\ x\ 1{,}96 < \mu < X + \frac{S}{\sqrt{n}}\ x\ 1{,}96$$

2.3.3 Thiobarbituric acid [12]

Samples were weighed as much as 3 ml at each concentration (0%, 0.1%, 0.2%, 0.3%). Sample dissolved using 50 ml aquades the sample was inserted in a 1000 ml distillation flask while washed with 48.5 ml aquades, and added 1.5 ml 4 N HCL. Add 3 boiling stones and vaseline on the surface of the distilled flask lid. Pair a set of distillation tools, as well as turn on the ON button. Wait for the warm-up process for 10 minutes until the results come out. The distillate results were transferred into a test tube and 5 ml of TBA reagent was added (0.02 M *thiobarbituric-acid* solution in 90% glacial acetic acid). The next step, cool the test tube with running water and measure its absorbance at a wavelength of 528 nm with aquades as the zero point. TBA analysis is calculated in ppm units. TBA is determined by the formula:

$$\text{Number of TBA} = \frac{Abs\ x\ \text{Dilution Factor}\ x\ 7{,}8}{\text{Sample weight}}$$

Information:

Abs = Absorbance at 528 nm
7,8 = Number of TBA mg malonaldehyde/Kg sample

2.3.4 Peroxide value (PV) [13]

Weigh the sample about 5 ± 0.05 g of each concentration (0%, 0.1%, 0.2%, 0.3%) into a closed conical flask, then add 30 ml of a mixed solution of acetic acid and chloroform and stir until the sample dissolves completely. Add 0.5 ml of saturated potassium iodide solution using a pipette. Beat the Erlenmeyer pumpkin for 1 minute and then add 30 ml of distilled water. Continuous titration with 0.01N sodium thiosulfate solution and shake until the yellow color is almost gone (light yellow). Add the starch solution and continue to repeat while stirring until the end of the titration to release all the iodine present in the chloroform layer, adding the sodium solution drop by drop. thiosulfate until the blue color disappears. The peroxide number (PV) is determined by the formula:

$$\text{POV} = \frac{V\ x\ N\ x\ 1000}{W}$$

Information:

V = Sample Titration Volume
N = Normality of solution Na2S2O3.5H2O
In = Sample Weight

2.3.5 Free fatty acid (FFA) [13]

Oil samples were taken at 5 grams + 0.1 grams with different concentrations (0%, 0.1%, 0.2%, and 0.3%). The addition of IPA (*Iso PropyNeutral Alcohol* 50 ml) Plus using the indicator Phenolphthalein 3-5 drops. Then titrated using 0.1N NaOh until it turns pink. FFA is determined by the formula:

$$\%\ \text{FFA} = \frac{\text{V x N x Mr(BM)}}{\text{Sample weight}}$$

Information:

FFA = *Free Fat Acid* (Free Fatty Acids)
V = NaOH Titration Volume
N = Concentration (normality) Solvent (ethanol)
Mr/BM = Relative Molecular Time/Molecular Weight

3. RESULTS AND DISCUSSION

3.1 Preliminary Research

The yield obtained from the extraction of 100 grams of dried sea cucumber was 16.1 grams with a 16.1% extract in the form of a solid paste. This extract should be stored frozen, so its storage should be placed in the *freezer*. According to [14], the extraction process of *lerek* simplicial leaves uses the soaking method using 96% ethanol solvent. The soaking process is carried out in a cool place, away from light. Maceration filtration is carried out for 3x24 hours.

Maceration and maceration were first combined into one. The extract is evaporated using a rotary *evaporator* under vacuum conditions at 60°C until all ethanol evaporates until only the extract remains. The moisture content is removed by heating in a water bath, the temperature is maintained below 60°C until a viscous extract with a constant weight is obtained. The extract is stored in the refrigerator until use.

3.2 Primary Research

The main research was carried out the addition of evaporated extracts to add antioxidants to fish oil. After that, the Antioxidant Activity test was carried out, Thiobarbituric Acid (TBA), Peroxide Value (PV), Free Fatty Acid (FFA) to find out changes in the quality of fish oil. The next stage of research is the extraction of sea cucumber and the addition of fish oil as an antioxidant with a concentration of 0%; 0,1; 0.2% and 0.3%.

3.2.1 Antioxidant activity DPPH method

Based on the results of testing the antioxidant activity of sea cucumber extract. with the DPPH method, $IC_{50\ result}$ of 80.875 ppm were obtained. These results show that the antioxidant activity of sea cucumber extract is relatively strong. The content of antioxidants in sea cucumbers. The IC value of 50 was determined by calculating the regression analysis of % inhibition to the concentration of crude sea cucumber extract. The results of the % inhibition test on sea cucumber extract samples showed a % inhibition of 80.30%. The high antioxidant content of sea cucumber is said to inhibit the harmful effects of snakehead fish oil. According to [15], this shows that sea cucumber extract has a strong antioxidant capacity (80,875 ppm. A compound is said to have a very strong antioxidant capacity when the $IC_{50\ value}$ is less than 50 ppm (type 1), strong when the IC_{50} value is between 50 and 100 ppm (class 2), while the IC_{50} value is between 100-150 ppm (class 3), low when the IC_{50} value is between 150-200 ppm (class 4) and very low when the IC_{50} value is greater than 200 ppm.

The low value of IC_{50} extract is influenced by the solvent used during the extraction process. In this study, the solution used was an organic solvent. Organic solvents have a strong ability to penetrate animal cells. In addition, the bioactive ingredients contained in simplicial found outside the cell are easily soluble with organic solvents. Thus, the compound components in ethanol extracts are very strong compared to extracts in inorganic solvents. According to [16], ethanol is a polar and volatile compound so it is used as a solvent. In addition, ethanol is also used as an organic solvent that is widely used to dissolve organic compounds. Solvent extraction is carried out by exposing the material to be extracted to the solvent for a certain period of time, then separating the filtrate from the rest of the extracted material.

3.2.2 Organoleptic

Organoleptic evaluation is a science that uses human senses to measure the color, turbidity, aroma and taste of a food product. In this study the parameters used are turbidity, odor and color, these parameters are adjusted to INSA standards. Consumer acceptance of a product begins with an evaluation of turbidity, odor and color. Since its main objective is consumer acceptance, the sensory testing carried out by the panelists (25 panelists) is considered the most sensitive and therefore it is usually used to evaluate the quality of different foods to measure their shelf life or in other words to determine their shelf life. According to [17], the rating scale used includes turbidity, color, and odor. This has been adjusted in Indonesian National Standardization Agency.

Table 1. Snakehead fish oil sensory test value with sea cucumber extract concentration

Concentration Treatment	Length of Storage (Days)	
	Day 0	Day 5
0%	$8,76±0,65^{Bd}$	$7.80±0.89^{Ad}$
0,1%	$8.60±0.80^{Bc}$	$8.28±0.97^{Ac}$
0,2%	$8.49±0.86^{Bb}$	$7.93±1.00^{Ab}$
0,3%	$8.25±0.97^{Ba}$	$6.71±1.30^{Aa}$

Information:

- *The data is the average result of three repetition of the Sensory test ± standard deviation*
- *Superscripts with different capital letters on the same row show markedly different ($p<0.05$) on the storage duration factor*
- *Superscript with different non-capital letters in the same column showed a real difference ($p<0.05$) in the treatment factor of sea cucumber extract concentration in the sample*

Table 2. Turbidity value of snakehead fish oil with sea cucumber extract concentration

Concentration Treatment	Length of Storage (Days)	
	Day 0	Day 5
0%	8,84±0,55^{Bd}	7.56±0.91^{Ad}
0,1%	8,68±0,74^{Bc}	8.12±1.01^{Ac}
0,2%	8,60±0,81^{Bb}	7.56±0.91^{Ab}
0,3%	8.20±1.00^{Ba}	6.28±0.97^{Aa}

Information:
- *The data is the average result of three repetition of the Turbidity test ± standard deviation*
- *Superscripts with different capital letters on the same row show markedly different (p<0.05) on the sample factor of storage duration*
- *Superscript with different non-capital letters in the same column showed a real difference (p<0.05) in the treatment factor of sea cucumber extract concentration in the sample*

Table 3. Color value of snakehead fish oil with sea cucumber extract concentration

Concentration Treatment	Length of Storage (Days)	
	Day 0	Day 5
0%	8,76±0,66Bd	7.72±0.97Ad
0,1%	8,60±0,81Bc	8.12±1.01Ac
0,2%	8,44±0,91Bb	7.88±1.01Ab
0,3%	8,28±0,97Ba	5.96±1.01Aa

Information:
- *The data is the average result of three repetition of the Color test ± standard deviation*
- *Superscripts with different capital letters on the same line show markedly different (p<0.05) on the sample factor of storage duration*
- *Superscript with different non-capital letters in the same column showed a real difference (p<0.05) in the treatment factor of sea cucumber extract concentration in the sample*

Table 4. Value of fish oil odor with sea cucumber extract concentration

Concentration Treatment	Length of Storage (Days)	
	Day 0	Day 5
0%	8,68±0,74^{Bd}	8.12±1.01^{Ad}
0,1%	8,52±0,87^{Bc}	8.60±0.81^{Ac}
0,2%	8,44±0,86^{Bb}	8.36±0.95^{Ab}
0,3%	8,28±0,97^{Ba}	7.88±1.01^{Aa}

Information:
- *The data is the average result of three repetition of the Sensory test ± standard deviation*
- *Superscripts with different capital letters on the same row show markedly different (p<0.05) on the sample factor of storage duration*
- *Superscript with different non-capital letters in the same column showed a real difference (p<0.05) in the treatment factor of sea cucumber extract concentration in the sample*

Based on sensory testing conducted by 25 panelists. The following is a discussion of each sensory test specification performed:

a. Turbidity

Turbidity is a measure that uses the effect of light as a basis for measuring the state of raw water with a certain scale. Turbidity is an important parameter that is assessed before panelists assess other parameters. A product will look attractive if the appearance is in accordance with what consumers expect. The turbidity of a product is influenced by the characteristics of its constituent materials, color, and smell. The turbidity produced by the addition of extract on day 0 was the highest, namely at a concentration of 0%, which was 8.84% clear and transparent, while on day 5 it was also the same, namely clear and transparent. On the 5th day, the highest addition of extract was at a concentration of 0.1%, which was 8.12%. The turbidity rated best by the panelists was fish oil with the addition of 0.1% extract on day 5 compared to controls. According to [18], the degree of clarity of oil from this study ranges from 6.71 – 8.76% and the highest value at concentrations of 0% and 0.1%. This indicates that the small amount of active

compound components in the extract absorb the particles that cause turbidity in the oil even if only a small part, but produce oil with a higher degree of clarity than oil with a higher extract concentration.

The results of the *Kruskal-Wallis test* showed that the treatment had an effect on turbidity. The *Mann-Whitney* test showed that all treatments had significantly different effects. The results of the turbidity sensory test showed that the turbidity value on day 0 increased compared to snakehead fish oil with day 5 storage. These results showed that the turbidity value of snakehead fish oil with the addition of extracts (0%, 0.1%, 0.2%, 0.3%) decreased on day 5. Panelists disliked the cloudiness on day 5 with a concentration of 0.3% because it was slightly cloudy compared to snakehead fish oil with other concentrations. This turbidity is caused due to microbial activity. Longer storage leads to physical changes in oil and contamination during the storage process. According to [19], this turbidity is caused by mixed objects or colloidal objects in the oil. Foreign objects in the oil may be carried by outside air that enters the oil.

b. Color

Color is a physical characteristic of the oil, greatly influencing the appearance of the oil and the taste of the consumer. This color is influenced by natural dyes and the color is the result of degradation of natural dyes. The color produced by adding extract on the 0th day is the highest, namely at a concentration of 0%, which is 8.76% with a golden yellow color, while on the 5th day storage is also the same, namely golden yellow. On the 5th day, the highest addition of extract was at a concentration of 0.1%, which is 8.12% golden yolk. The color that was considered the best by the panelists was fish oil with the addition of 0.1% concentration extract on day 5 compared to other concentrations, according to [17], the color produced by oil in this study is the same, namely golden yellow. This color is a common color in fish oil and is caused by the presence of carotenoids. Carotenoids are organic pigments found in chloroplasts and chromoplasts that can be found in an organism.

The results of the *Kruskal-Wallis test* showed that the treatment had an effect on turbidity. The *Mann-Whitney* test shows all the different treatments have a real effect. Dyestuffs consist of 2 groups, the first group is natural dyes, which are naturally found in materials that contain oil and are extracted with oil in the extraction process. These dyes include α and β carotene (yellow), xanthophylls (brownish-yellow), chlorophyll (greenish) and anthocyanins (reddish). The second group is dyes from the degradation of natural dyes, namely dark color caused by the oxidation process to tocopherol (vitamin E), brown color caused by ingredients to make oil that has rotted or damaged, yellow color generally occurs in unsaturated oils. The results showed that the color value of snakehead fish oil plus extract (0%, 0.1%, 0.2%, 0.3%) decreased from day 0 to day 5. Panelists did not like the color on day 5 with a concentration of 0.3% because it was reddish-yellow compared to snakehead fish oil with other concentrations with the same storage period. This color is caused by oxidation reactions and contamination during the storage process. According to [20], color reduction can also be influenced by the influence of light on the storage process and other handling processes. The purification process has succeeded in reducing the oxidation value of fish oil and all parameters have met International Fish Oil Standards [21] (IFOS) standards. *Bleaching* is an adsorption process that will make the color of fish oil clearer by using Magnesol XL adsorbent which is used to remove free fatty acids, pigments, and other impurities.

c. Construction

The smell of a product greatly affects the taste of consumers. This is related to the sense of smell that causes the desire to consume a product. A good smell will be appetizing, while an unpleasant smell will reduce consumers' appetite to consume the product. Based on the test results, the odor value of snakehead fish oil with the addition of extract stored on day 0 is better than oil with the addition of extract on day 5 because of the smell of sour odor that masks the smell of oil, while oil on day 0 is dominated by specific oil. Storage for 5 days made the panelists' assessment of snakehead fish oil decrease due to the sour odor. This is because the oil is damaged by oxidation during storage. The odor caused by the presence of oxygen and microorganisms that break down protein compounds into simple compounds such as polypeptides, indoles and scatol. According to [22], the aroma of oil is influenced by Free Fatty Acid compounds oxidized in oil. Some of the factors causing oxidation are light and temperature. Unsaturated fatty acid molecules undergo oxidation, causing cooking oil to become rancid.

Table 5. TBA test results on snakehead fish oil (*Channa striata*)

TBA (mg. malonaldehyde/kg)	Length of Storage (Days)	
	Day 0	Day 5
0%	0.16±0.00^{Ba}	0.07±0.02^{Aa}
0,1%	0.14±0.02^{Aa}	0.11±0.05^{Aa}
0,2%	0.13±0.03^{Aa}	0.14±0.03^{Aab}
0,3%	0.12±0.03^{Aa}	0,27±0,02^{Bb}

Information:
- *The data is the average result of three repeats of the TBA test (mg, malonaldehyde / kg) ± standard deviation*
- *Superscripts with different capital letters on the same row show markedly different (p<0.05) on the sample factor of storage duration*
- *Superscript with different non-capital letters in the same column showed a real difference (p<0.05) in the treatment factor of sea cucumber extract concentration in the sample*

Table 6. Testing results of peroxide value on snakehead fish oil (*Channa striped*)

Peroxide Value (mEq/kg)	Length of Storage (Days)	
	Day 0	Day 5
0%	1.91±1.31^{Aa}	3,42±0,00^{Ba}
0,1%	1.13±0.03^{Aa}	3,40±1,25^{Ba}
0,2%	1.14±0.00^{Aa}	5.31±0.66^{Bb}
0,3%	1.15±0.01^{Aa}	4.57±1.13^{Bb}

Information:
- *The data is the average result of three repeats of the Peroxide Number (mEq/kg) test ± standard deviation*
- *Superscripts with different capital letters on the same line show markedly different (p<0.05) on the storage duration factor*
- *Superscript with different non-capital letters in the same column showed a real difference (p<0.05) in the treatment factor of sea cucumber extract concentration in the sample*

Table 7. Free fat acid *test results* on snakehead fish oil (*Channa striata*)

Free Fatty Acid (%)	Length of Storage (Days)	
	Day 0	Day 5
0%	0.97±0.67^{Ba}	0.77±0.33^{Aa}
0,1%	1.54±0.66^{Ba}	0.77±0.33^{Aa}
0,2%	0.39±0.33^{Aa}	0.58±0.00^{Ba}
0,3%	1.35±0.33^{Ba}	0.97±0.67^{Aa}

Information:
- *The data is the average result of three repetitions of the Free Fatty Acid(%) test ± standard deviation*
- *Superscripts with different capital letters on the same row show markedly different (p<0.05) on the storage duration factor*
- *Superscript with different non-capital letters in the same column showed a real difference (p<0.05) in the treatment factor of sea cucumber extract concentration in the sample*

3.2.3 Thiobarbituric acid (TBA)

The normality test results showed that the data of all treatments were spread normally. Results of TBA variant analysis at a concentration of 0%; 0,1%; 0,2%; and 0.3% showed a noticeable difference The results of the analysis of variance with ANOVA (Analysis of Variance) from the treatment of storage duration showed a noticeable difference, as well as the interaction between different concentrations and storage duration. The results of the ANOVA test showed that there was an interaction between the concentration of the given extract (Factor A) and the duration of storage (Factor B). The results of Analysis of Variance test (ANOVA) show a value (p <5%) against the resulting value. The results of the analysis were continued with the Honest Real Difference test to see the real different data between treatments. Based on the results of the study, the value of snakehead fish oil levels is good in influencing quality changes that have decreased due to the addition of sea cucumber extracts. The increase in levels is thought to be due to factors from oil storage caused by an increase in the rate of damage to snakehead fish

oil during storage. Sea cucumber extract provides additional antioxidants in snakehead fish oil so that levels decrease. The numbers on fish oil showed the highest value at all concentrations on day 5, especially in fish oil with the addition of extract concentration of 0.3% with the highest value. This is in accordance with the research of [8], that TBA (Thiobarbituric acid) levels in fish oil with the addition of lettuce extract on the same day i.e. day 5 had the highest value in all concentrations. However, at the same concentration factor, the highest value was also found in fish oil with the addition of a 0.3% concentration of sea lettuce extract but on different days. [23] added that the longer the repetition and storage of coconut oil, the results obtained are increasing and have experienced rancidity.

The results of TBA levels from snakehead fish oil with the addition of sea cucumber extract 0%; 0.1%; 0.2%; and 0.3% during storage day 0, day 5 are still within the safe limits allowed for food products. TBA content of oil with the addition of 0% extract; 0,1%; 0,2%; and 0.3% sea cucumber during storage day 0, day 5 had the lowest value at 0% concentration day 5 and the highest value at concentration 0.3% on day 5. The results showed that snakehead fish oil TBA levels were in accordance with Indonesian National Standardization Agency. This is in accordance with the provisions of International Fish Oil Standards [21] that the safe limit of TBA levels of fish oil products is a maximum of 3 mg. malonaldehyde / kg. According to [24], the TBA number is in accordance with INSA 01-2352-1991 [25], which is a maximum of 3 mg.malonaldehyde/kg. The results of the analysis of snakehead fish oil TBA ANOVA showed that the difference in sea cucumber extract concentration was significantly different from the TBA value.

The addition of sea cucumber extract, an antioxidant, added to fish oil, antioxidant properties can inhibit the formation of malonaldehyde compounds, which have the ability to destroy or inhibit free radicals. The lower the absorption value of malonaldehyde, the more effective the antioxidant is in inhibiting the formation of malonaldehyde compounds. The TBA value of fish oil given sea cucumber extract was lower than fish oil without extract (control), this showed that the formation of malonaldehyde compounds was inhibited in the presence of antioxidants. According to [26], antioxidant activity can be measured by measuring malonaldehyde compounds in the system. Antioxidant compounds can inhibit the formation of malonaldehyde compounds. The amount of malonaldehyde compounds in the fish oil mixture is indicated by the absorption value of absorbance. The lower the absorbance value of malonaldehyde, the more effective the extract is in inhibiting the formation of malonaldehyde compounds.

Peroxide compounds are unstable and can break down into simpler compounds such as malonaldehyde. The higher the TBA value (malonaldehyde content), the higher the oxidation number of the oil. The oxidation reaction begins with the formation of peroxides formed in the oil as the initial product of malonaldehyde formation. This is in accordance with the statement. According to [27], increased TBA levels are associated with increased peroxide as an initial product of malonaldehyde formation. Oxidation reactions usually begin with the formation of peroxides and hydroperoxides, which are essentially tasteless and odorless, but these components are very unstable and continue to oxidize rapidly to form various compounds such as aldehydes, ketones, acids, and other components that can cause unpleasant odors.

During storage, changes in fish oil TBA values are unstable and fluctuating. Malonaldehyde is a secondary product of the oxidation of unsaturated fatty acids. Malonaldehyde is highly reactive to proteins and amino acids. Malonaldehyde is formed by hydroperoxide, which is the initial product formed from the reaction of long-chain unsaturated fatty acids with oxygen. Changes in TBA numbers during storage show varying results. Because malonaldehyde is the result of hydroperoxide breakdown, it is considered very unstable and highly reactive to proteins and amino acids. According to [28], factors that affect the value of TBA or fat oxidation during storage include water activity, NaCl, and the type of fat used. The value of TBA increased, but the increase was relatively small. This shows the role of antioxidants and antibacterials in sea cucumber extract. Sea cucumber is a natural antioxidant found in extracts that can inhibit the oxidation of unsaturated fatty acids. Oil that has a lower TBA content will produce better oil. If TBA levels rise then the quality will decrease which results in oil that has a rancid odor. According to [23], to determine the rancidity of the Indonesian National Standardization Agency set a maximum

limit of oil rancidity of 3 mg malonaldehyde / kg sample. The quality of the oil will decrease, because rancid oils contain aldehydes and especially malonaldehyde.

3.2.4 Peroxide value (PV)

The normality test results showed that the data of all treatments were spread normally. Results of PV variant analysis at a concentration of 0%; 0,1%; 0,2%; and 0.3% showed a noticeable difference The results of the analysis of variance with ANOVA (Analysis of Variance) from the treatment of storage duration showed a noticeable difference, as well as the interaction between different concentrations and storage duration. The results of the ANOVA test showed an interaction between the concentration of the given extract (Factor A) and the duration of storage (Factor B) on the PV (Peroxide Value). The results of Analysis of Variance test (ANOVA) show a value (p<5%) of the PV produced. The results of the analysis were continued with the Honest Real Difference test to see the real different data between treatments. Based on the results of the analysis, it is continued with the Honest Real Difference test to see real different data between treatments. Based on the results of the study, the value of snakehead fish oil peroxide levels as a determinant of quality changes that decreased due to the addition of sea cucumber extract. Increased peroxide levels are thought to be due to factors from oil storage caused by an increase in the rate of damage to snakehead fish oil during storage. Sea cucumber extract provides additional antioxidants in snakehead fish oil so that peroxide levels decrease. The peroxide number in fish oil showed the highest value at all concentrations on day 5, especially in fish oil with the addition of 0.2% concentration extract with the highest value. This is in accordance with the research of [8], that peroxide levels in fish oil with the addition of sea lettuce extract at the same concentration are 0%; 0.1%; 0.2%; and 0.3% decreased in value and the storage factor on the same day also experienced an increase in peroxide numbers. The decrease in PV is caused by the primary oxidation compound formed (hydroperoxide) gradually decomposing into secondary oxidation products (malonaldehyde). After reaching the maximum value, the peroxide value decreases and decomposes into malonaldehyde. Peroxides become less stable and brittle when further modified to produce secondary oxidation products such as aldehydes, cotton, hydrocarbons, and other polymers. [29], adds that a significant decrease in peroxide value after reaching the maximum value indicates that peroxide is a less stable component and is very susceptible to undergo further changes that produce secondary oxidation products, such as aldehydes, ketones, hydrocarbons, and other polymers.

Based on the results of the PV test, the highest results in this study were achieved by samples with an extract concentration of 0.2% on day 5 namely (5.31) while the lowest results were found in samples with extract concentrations of 0.1% and 0.2% on day 0 namely (1.14). According to INSA standards, the maximum peroxide number in pure fish oil is < 5 mEq / kg, so that the number of fish oil peroxide in all samples on day 0 is in accordance with Indonesian National Standardization Agency and in the 5th day sample of 0% and 0.1% treatment according to Indonesian National Standardization Agency but in the treatment of 0.2% and 0.3% there are results that do not match Indonesian National Standardization Agency because it does not < 5 mEq / kg. The addition of sea cucumber extract treatment in snakehead fish oil contains antioxidants so that it can inhibit oxidation. The addition of the concentration of sea cucumber extract will increase the peroxide level of the oil. The addition of sea cucumber extract concentration can affect oil peroxide levels because there are antioxidants added so that it can inhibit the increase in peroxide levels in oil. According to Cikita et al., [30], the value of peroxide can increase with increasing storage time (exposure time), temperature and air. The longer the oxidation time, the peroxide number of the oil increases. This increase in peroxide index indicates that the oil is damaged in the oxidation stage due to the formation of peroxide compounds in the oil. The use of antioxidants can be said to inhibit the oxidation process, therefore although oxidation still occurs with increasing storage time, the amount of peroxide formed is less than without the addition of antioxidants to the oil. An increase in peroxide levels greatly affects the quality of snakehead fish oil, because rancidity can occur in the oil. The degree of rancidity of an oil can be determined based on its peroxide number. Oil with a peroxide content exceeding the specified limit can damage and endanger the health of the body. According to [31], damage contained in oil will affect the quality and nutritional value of oil and can affect health. Consuming oil containing peroxide will form free radicals in the body.

The results of peroxide levels in this study showed that the higher the sea cucumber extract, the higher the oil peroxide levels. This is because higher extract interactions are not effective. The addition of sea cucumber extract that has a low peroxide level is in the treatment of 0.1%. The decrease in peroxide levels produced is due to the addition of antioxidants that match the concentration in snakehead fish oil. This is supported by the research of [32], the process of oil damage is influenced by the presence of prooxidants and antioxidants. Prooxidants accelerate the oxidation process, while antioxidants inhibit it. The presence of antioxidants in fat will lower the rate of oxidation. Antioxidants are found naturally in vegetable and animal fats that are sometimes added intentionally. To inhibit the formation of peroxide and maintain the double bond in the oil, antioxidants are added, one of which is a natural antioxidant found in sea cucumber extract which has the ability to inhibit oxidation. According to [33], also mentioned that the addition of antioxidants can block oxidation reactions at the initiation and propagation stages.

The peroxide levels of snakehead fish oil on the table may vary. The difference can be caused by several factors, one of which is the type of sea cucumber used, differences in the concentration of sea cucumber extract, storage duration and the type of oil used. The higher the addition of sea cucumber extract concentration in snakehead fish oil will produce different PV values. Another influential factor is the presence of free radicals that can cause rancidity in oil. According to [30], peroxide is formed in the early stages of oxidation, when hydrogen produces free radicals. The formed free radicals react with oxygen to form peroxy radicals, then hydrogen atoms from other unsaturated molecules produce peroxides and new free radicals. The addition of antioxidants can prevent an increase in peroxide levels. The most effective antioxidants are those that resist oxidation, which is indicated by a slight increase in peroxide number. The higher the concentration or level of antioxidants used, the lower the peroxide index. Suboptimal concentrations or levels of antioxidants also lead to increased inhibition of oxidation reactions.

3.2.5 Free fatty acid (FFA)

The normality test results showed that the data of all treatments were spread normally. Results of Free Fatty Acid (FFA) variant analysis at a concentration of 0%; 0,1%; 0,2%; and 0.3% showed a noticeable difference The results of the analysis of variance with Analysis of Variance (ANOVA) from the treatment of storage duration showed a noticeable difference, as well as the interaction between different concentrations and storage duration. The results of the ANOVA test showed an interaction between the concentration of the given extract (Factor A) and the duration of storage (Factor B) on the FFA value. The results of the Analysis of Variance test (ANOVA) show a value ($p<5\%$) against the resulting FFA value. The results of the analysis were continued with the Honest Real Difference test to see the real different data between treatments. Based on the results of the study, the FFA content value of snakehead fish oil is good in influencing quality changes that have decreased due to the addition of sea cucumber extract. The increase in FFA levels is thought to be due to factors from oil storage caused by an increase in the rate of damage to snakehead fish oil during storage. Sea cucumber extract provides additional antioxidants in snakehead fish oil so that FFA levels decrease. FFA numbers in fish oil showed the highest value at all concentrations on day 5, especially in fish oil with the addition of 0.3% concentration extract with the highest value. This is in accordance with the research of [34], the longer the storage of Free Fatty Acid levels will increase. This proves that there has been a hydrolysis reaction in the oil. [35] added that the higher the concentration of antioxidants added, the lower the FFA levels produced.

Based on the results of the FFA test, the highest results in this study were achieved by samples with an extract concentration of 0.1% on day 0 namely (1.54) while the lowest results were found in samples with an extract concentration of 0.3% on day 0 namely (0.35). According to International Fish Oil Standards (IFOS) [21], the maximum FFA value in pure fish oil is 1.5%, so the FFA value of snakehead fish oil in all samples on day 0 and day 5 is in accordance with IFOS standards. IFOS is a third-party testing and certification program for fish oil. The results of the lowest value test (0.35) showed that fish oil with a concentration of 0.2% was better than fish oil with the highest result of 0.3% concentration (1.54). Oil that has high FFA levels can harm the body because there are carcinogenic properties in the human body. According to [36], if the Free Fatty Acid index determined is higher, the Free Fatty Acid content contained in it is higher, so that the quality of oil will be lower. Fish oil is damaged by hydraulic processes because the oil contains some water forming free fatty acids and

little glycerol. The higher the Free Fatty Acid content, the lower the quality of the oil. According to [37], unsaturated fatty acids will decompose due to the hot oil surface from direct contact with air, so that free fatty acids will increase. The presence of free fatty acids in raw fish oil is caused by heating during the extraction process. The carbon chain of double bonds in unsaturated fatty acids will react with heat to form free fatty acids that can affect the quality of fish oil.

Oil damage can be in the form of increased levels of free fatty acids (FFA), increased peroxide, dark discoloration, and rancid odor. In oil, there are two main types of damage, namely hydrolysis and rancidity. Rancidity occurs due to the oxidation process of unsaturated fatty acids exposed to oxygen and high temperatures which then form peroxide. From the oxidation process will cause a rancid odor in the oil and destruction of several kinds of vitamins in foodstuffs. Other damages such as FFA are formed due to the oxidation process and hydrolysis of enzymes during processing and storage. Antioxidants added in oil that function to inhibit hydrolysis and oxidative due to the presence of compounds that prevent the process. The most effective antioxidants are those that are able to resist oxidation, which is indicated by a slight increase in FFA levels. In the study, the lower the concentration or level of antioxidants used, the lower the FFA index. Excess concentrations or levels of antioxidants can also lead to increased inhibition of oxidation reactions. According to [38], oxidation and hydrolysis cause damage to oil. However, oxidation has a greater impact on oil damage than hydrolysis, because hydrolysis is a reaction that promotes oil oxidation. Free fatty oil resulting from the hydrolysis reaction will accelerate the oxidation of cooking oil by lowering the surface tension of the oil thereby increasing the rate of diffusion of oxygen in the oil.

4. CONCLUSION

The addition of sea cucumber extracts (0%, 0,1%, 0,2%, 0,3%) did not significantly maintain better quality of fish oils that had been stored for 5 days in terms of TBA, PV, FFA, and sensory values. The results implies that the addition of 0.1% of sea cucumber extracts obtained as the best treatment to prevent oil degradation. The results suggest that sea cucumber extract should be extracted using a non-polar or semi-polar solvent to obtained a more soluble extract in the fish oil. This method will help the antioxidant properties of sea cucumber to act more efficiently in preventing fish oil degradation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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