Estimation of the Shelf Life of Cod Liver Oil Supplemented with Arthrospira platensis Carotenoid Antioxidants Using the Accelerated Shelf-Life Test (ASLT) Method

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Abstract.

This study was aimed to determine the shelf life of cod liver oil added with the carotenoid antioxidant Arthrospira platensis using the Accelerated Shelf-Life Test (ASLT) method. The experimental design used was a completely randomized design with three factors, namely temperature, storage time and treatment with three replicates. The storage temperatures used were 30 ± 2oC, 40 ± 2oC and 50 ± 2oC while the treatments used were control cod liver oil and cod liver oil added with the carotenoid antioxidant Arthrospira platensis at a concentration of 600 ppm. Storage was carried out for 28 days with test points on days 0, 7, 14, 21, and 28 with parameters tested included peroxide value, anisidin number, total oxidation, carotenoid content, antioxidant activity and water content. Data were analyzed using ANOVA and Tukey HSD follow-up test. The test showed that the peroxide value is a critical parameter that can be used as a parameter for estimating shelf life. Based on ASLT calculations using the Arrhenius approach, the shelf life of cod liver oil added with the carotenoid antioxidant Arthrospira platensis at a storage temperature of 30 ± 2oC was 14,38 days.

Keywords: Accelerated Shelf-Life Test; Arrhenius; fish oil; Arthrospira platensis; shelf life

1. INTRODUCTION

Fish is a food product with high nutritional value, but it is also highly perishable and prone to quality deterioration. One fishery product with high nutritional content, including omega-3, omega-6, vitamins A and D, is fish oil. One type of fish oil with an annual production of nearly 10,000 tons that has attracted growing interest in the fats and oils industry over the past few decades is cod liver oil (CLO). Cod is one of the sources of fish oil because its processing by-products, such as extracted liver, can produce oil rich in unsaturated fatty acids, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Rohman, 2017).

Cod liver oil is currently in high demand because of its various health benefits, including lowering cholesterol and triglyceride levels, improving fat metabolism, and

increasing omega-3 levels in the body. However, fish oil is more susceptible to oxidation than other types of oil. Its stability is affected by its high content of unsaturated fats, which contain five to six double bonds, such as EPA and DHA. Fish oil stability can also be influenced by storage temperature. According to Pak (2005), fish oil without the addition of antioxidants remains suitable for consumption or can last for a maximum of 36 days.

The deterioration of oils or fats caused by oxidation reactions can be prevented by adding antioxidants. Antioxidants can stop oxidation reactions by preventing the formation of free radicals (Adawiah et al., 2015). Antioxidants can be classified into two types: natural and synthetic antioxidants. Commonly used synthetic antioxidants include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ). However, the use of synthetic antioxidants is limited because excessive consumption may cause toxic and carcinogenic effects. Therefore, safer alternative antioxidants are needed. Natural antioxidants derived from plants are a potential source that can be used to inhibit oxidation (Wulansari, 2018).

Arthrospira platensis is a microalga that has been widely used in the food, feed, pharmaceutical, and cosmetic industries (Suryaningtyas, 2019). Its active components have antitumor, antimicrobial, and antioxidant activities. Bioactive compounds in A. platensis that can act as natural antioxidants include phycocyanin, chlorophyll, flavonoids, beta-carotene, and tocopherol (Setyaningsih et al., 2013). One non-enzymatic antioxidant that can act as a precursor of vitamin A is carotenoid (Han et al., 2021). A. platensis contains active carotenoid compounds, particularly beta-carotene, which have the potential to act as natural antioxidants. According to Adadi et al. (2018), the carotenoid content in A. platensis is 6 mg/g, which is 30–480 times higher than that found in other vegetables and fruits. Beta-carotene is the most abundant carotenoid in A. platensis. One gram of A. platensis contains 2,300 IU of beta-carotene (Henrikson, 2009). Antioxidants obtained from A. platensis carotenoids can donate hydrogen atoms to free radicals, thereby preventing oxidation. This provides a basis for considering the addition of A. platensis carotenoids as antioxidants to inhibit the quality deterioration of fish oil. However, the shelf life of fish oil containing A. platensis carotenoids has not yet been determined.

Shelf life refers to the period during which a product undergoes a certain degree of quality degradation until it is no longer suitable for consumption or fails to meet the required standards. The Accelerated Shelf-Life Test (ASLT) is a method used to determine product shelf life by accelerating quality changes in critical parameters. In this method, storage conditions are set beyond normal conditions so that the product deteriorates more quickly, allowing its shelf life to be estimated. The advantages of the ASLT method include a relatively short testing period and a high level of accuracy (Mulyono et al., 2018).

Research on the estimation of fish oil shelf life was conducted by Fitriyani (2022). The shelf life of an emulsion product containing tuna fish oil and ikan kayu protein hydrolysate was estimated using the Arrhenius approach with a zero-order reaction, resulting in a shelf life of 144 days at 29°C. Research on the shelf life of fish oil supplemented with carotenoids has not yet been conducted. Therefore, it is necessary to estimate the shelf life by examining the quality of fish oil containing A. platensis carotenoids at different storage temperatures. Shelf life is an important factor

because it is closely related to the suitability and safety of a product (Harris and Fadli, 2014)

II. METHODS

Materials

The materials used in this study included commercial Arthrospira platensis powder (PT. Trans Pangan Spirulindo), cod liver oil (PT. Multi Kimia Raya Nusantara), n-hexane pro analysis, ethanol pro analysis, methanol, glacial acetic acid, chloroform, ammonium thiocyanate, isooctane, acetone, HCl, $BaCl_2$, $FeSO_4$ (Merck KGaA, Germany), p-anisidine reagent, $FeCl_2$, DPPH, beta-carotene (Sigma-Aldrich), distilled water, and nitrogen gas.

Equipment

The equipment used in this study included a three-neck flask, spiral reflux condenser, rubber stopper, thermometer, hot plate magnetic stirrer (AREC VELP Scientifica), water pump (Luckiness 1200, China), tubing, oven, analytical balance (Ohaus PX523), refrigerator (Sharp, Midea), vortex mixer, rotary vacuum evaporator (SCILOGEX RE100-PRO), moisture analyzer (Ohaus MB120), spectrophotometer (Genesys 10S UV-Vis), glass bottles, micropipettes, microtips, and cuvettes.

Research Procedures

In this study, A. platensis powder was extracted using solvents, and the resulting A. platensis carotenoid extract was then added to cod liver oil. The treatments consisted of a control cod liver oil sample and cod liver oil supplemented with Arthrospira platensis carotenoid antioxidants at a concentration of 600 ppm. The oil samples were then stored in vials wrapped in aluminum foil at room temperature (30 ± 2°C), in an oven at 40 ± 2°C, and in an oven at 50 ± 2°C. The samples were subsequently tested using several parameters, including peroxide value, anisidine value, total oxidation value, carotenoid content, antioxidant activity, and moisture content. Storage was carried out for 28 days, with testing conducted on days 0, 7, 14, 21, and 28.

Extraction of Arthrospira platensis Carotenoids

The extraction of A. platensis carotenoids was carried out according to Pahlevi et al. (2008). The extraction process began by weighing 9 grams of Arthrospira platensis powder and placing it into a three-neck flask wrapped in aluminum foil and connected to a spiral condenser and hot plate stirrer. Then, 27 mL of ethanol and 270 mL of hexane solvent were added. The extraction was performed for 3 hours and 24 minutes at a temperature of 51.9°C and a stirring speed of 1,500 rpm. The carotenoid extract solution was separated from the pellet and collected in a container whose outer surface had been wrapped in aluminum foil. The carotenoid extract solution was then evaporated using a rotary vacuum evaporator at 45°C for 20 minutes. Subsequently,

the evaporated extract was exposed to nitrogen gas until all remaining solvent had evaporated, resulting in a concentrated carotenoid extract. The carotenoid extract was transferred into an aluminum foil-wrapped container and stored in a freezer at −18°C.

Carotenoid Content (Desorby et al., 1997)

a. Preparation of the Carotenoid Standard Curve

A total of 5.5 mg of pro analysis-grade carotenoid was placed in a volumetric flask and dissolved in 10 mL of hexane up to the calibration mark. Then, 1 mL of the solution was transferred into a 10 mL volumetric flask and diluted with hexane up to the calibration mark. A series of dilutions was then prepared and measured at a wavelength of 450 nm.

b. Total Carotenoid Analysis

A total of 0.5 grams of the oil sample was weighed and placed in a 50 mL Erlenmeyer flask. Then, 250 µL of distilled water and 2.5 mL of n-hexane pro analysis were added. The solution was homogenized using a hot plate stirrer at 500 rpm for 30 minutes at room temperature. The absorbance was then measured using a UV-Vis spectrophotometer at a wavelength of 450 nm.

Antioxidant Activity (Guleria et al., 2013)

c. Preparation of DPPH Reagent

A total of 3.54 mg of DPPH powder was dissolved in 100 mL of methanol and stored in a container in a dark room.

d. Antioxidant Activity Test

A total of 1 mL of the fish oil sample was taken, followed by the addition of 1 mL of DPPH solution and 2 mL of methanol. The solution was then shaken and incubated in a dark bottle at room temperature for 30 minutes. After incubation, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.

Moisture Content (Ginting, 2008)

The moisture content of the fish oil samples was analyzed using an Ohaus MB120 moisture analyzer. The test was performed by turning on the instrument using the power button and setting the temperature and testing time. Then, 0.5 grams of the sample was placed on the aluminum pan, and the moisture content was displayed on the instrument's screen.

Shelf-Life Estimation (Arif, 2016)

The shelf life at a specific temperature can be determined by relating the k value to the known temperature value.

$\ln k = \ln k_0 - Ea/RT$

$k = k_0 e^{-Ea/RT}$

Description:
Ea = Activation energy
R = 1.986 cal/mol K

After determining the reaction order, the shelf life is calculated using the following equations:
For first-order reaction:
ts = [ln (No-Nt)]/k
ts = [ln (No-Nt)]/k0-e-Ea/RT,
For zero order reaction:
ts = (No-Nt)/ k0-e-Ea/RT
Description:
ts = Shelf life
No = Quality parameter value at the beginning of storage
Nt = Final quality parameter value (critical limit)
k = k value at storage temperature T

Data Analysis

The research data were analyzed using analysis of variance (ANOVA) at a 95% confidence level. If the analysis of variance showed a significant difference, the analysis was continued using the Tukey HSD test with SPSS 20 software. Data from the critical parameters were calculated using the Arrhenius approach.

III. RESULT AND DISCUSSION

Peroxide Value

Fish oil is a fishery product that is highly susceptible to oxidation due to its high content of unsaturated fats and relatively large number of double bonds. In this study, peroxide value was used as a parameter to indicate the stability of oil against oxidation reactions, with hydroperoxides serving as the primary lipid oxidation products (Pak et al., 2005). Peroxide value is a measurement used to determine the amount of peroxides present in fats or oils and is expressed in milliequivalents per kilogram (meq/kg). It is also used to measure the extent to which rancidity has occurred during storage, which subsequently determines product quality. An increase in peroxide value may occur during storage depending on the storage duration, temperature, and exposure to light or air (Khoirunnisa et al., 2020). The higher the peroxide value, the greater the extent of the oxidation reaction (Kusnandar, 2019).

Table 1. Peroxide Value

Treatment	Temperature (^{0}C)	Peroxide Value (mEq/kg)				
		Day-0	Day-1	Day-2	Day-3	Day-4
Control Oil	30	1,81 ± 0,00 CaE	4,09 ± 0,10 CaD	6,405 ± 0,09 CaC	11,02 ± 0,22 CaB	17,00 ± 0,07 CaA
	40	1,82 ±	4,098 ± 0,12	6,477 ± 0,05	11,42 ±	17,45 ± 0,09

		0,01 BaE	BaD	BaC	0,12 BaB	BaA
Oil + *A. platensis*	50	1,80 ± 0,00 AaE	4,233 ± 0,02 AaD	6,534 ± 0,07 AaC	12,01 ± 0,12 AaB	19,00 ± 0,29 AaA
	30	1,62 ± 0,01 CbE	3,898 ± 0,01 CbD	5,891 ± 0,06 CbC	9,983 ± 0,19 CbB	15,91 ± 0,25 CbA
	40	1,67 ± 0,02 BbE	3,943 ± 0,03 BbD	5,943 ± 0,08 BbC	10,45 ± 0,07 BbB	16,28 ± 0,17 BbA
	50	1,58 ± 0,08 AbE	3,949 ± 0,08 AbD	6,236 ± 0,13 AbC	10,94 ± 0,12 AbB	16,93 ± 0,16 AbA

Note: The first letter in different columns indicates a significant difference in temperature ($p<0.05$). The second letter in different columns indicates a significant difference between treatments ($p<0.05$). The third letter in different rows indicates a significant difference in storage duration ($p<0.05$).

Based on Table 1, the peroxide values of both the control cod liver oil and the cod liver oil supplemented with A. platensis carotenoid antioxidants increased as the treatment temperature increased from day 0 to day 28 of storage. Both the control cod liver oil and the oil supplemented with A. platensis carotenoid antioxidants showed the highest peroxide values on day 28 at 50°C. The control oil had a peroxide value of 19.00±0.29 mEq/kg, while the oil supplemented with A. platensis carotenoid antioxidants had a value of 16.93±0.16 mEq/kg. This finding is consistent with the study by Widodo et al. (2019), which reported that palm oil stored at 30°C, 40°C, 50°C, and 60°C for 28 days experienced an increase in peroxide values with increasing temperature and storage duration. An increase in peroxide values with rising temperature was also reported by Husain and Ismail (2021), who found that the peroxide value of palm cooking oil increased with every 20°C rise in storage temperature. The increase in peroxide values during storage is caused by chemical reactions between oil and oxygen, which lead to the formation of peroxide compounds and consequently promote oxidation (Montesqrit and Ovianti, 2013).

Based on the ANOVA results, temperature, treatment, and storage duration had significant effects on the peroxide values of fish oil ($p\<0.05$). Fish oil deterioration can be caused by exposure to light, heat, lipid peroxides, heavy metals, hemoglobin, chlorophyll, and lipoxygenase enzymes (Ketaren, 2005). Fish oil contains a relatively high number of double bonds, making it more susceptible to oxidation reactions (Andhikawati, 2020). The control fish oil showed a greater increase in peroxide values than the fish oil supplemented with A. platensis carotenoid antioxidants. This indicates that the addition of A. platensis, which contains carotenoids with antioxidant properties, can inhibit fish oil deterioration during storage. Antioxidants can donate hydrogen atoms to stabilize free radical compounds (Adawiah et al., 2015). Higher peroxide values indicate lower oil quality (Rozi et al., 2016). According to CODEX WHO, the acceptable peroxide value for fish oil is ≤5 meq/kg, while the IFOS standard is ≤3.5 meq/kg. Based on the observations, on day 0, the peroxide values of both the control fish oil and the fish oil supplemented with A.

platensis were still within the established standards. However, from day 7 to day 28, the peroxide values of both the control fish oil and the oil supplemented with A. platensis exceeded the limits set by CODEX WHO and IFOS.

Anisidine Value

The anisidine value is a measure of secondary oxidation products formed through the decomposition of hydroperoxides, resulting in compounds such as aldehydes, ketones, acids, alcohols, hydroxyl compounds, hydrocarbons, and other polymeric compounds classified as secondary oxidation products (Panagan et al., 2011). The reaction of these secondary compounds with p-anisidine produces a yellow-colored complex, whose absorbance is measured at a wavelength of 350 nm (Kusharto et al., 2015). Factors that can promote oxidation include temperature, storage duration, oxygen exposure, repeated heating, light, metal catalysts such as iron and copper, and the degree of unsaturation of fatty acids in the oil (Estiasih, 2009).

Table 2. Anisidine Value

Treatement	Temperature (^{0}C)	Anisidine Value(mEq/kg)				
		Day-0	Day-1	Day-2	Day-3	Day-4
Control Oil	30	8,843 ± 0,75 BaD	9,89 ± 0,62 BaD	14,13 ± 0,55 BaC	24,44 ± 0,49 BaB	33,80 ± 1,05 AaA
	40	8,77 ± 0,98 BaD	9,77 ± 1,89 BaD	14,79 ± 1,04 BaC	25,17 ± 1,58 BaB	34,14 ± 1,28 AaA
	50	9,061 ± 0,39 AaD	10,4 ± 0,44 AaD	17,24 ± 0,50 AaC	29,05 ± 1,28 AaB	37,82 ± 0,47 AaA
Oil+*A. platensis*	30	7,13 ± 0,34 BbD	8,50 ± 0,48 BbD	13,33 ± 0,29 BbC	18,77 ± 1,22 BbB	28,25 ± 1,81 BbA
	40	7,28 ± 0,19 BbD	8,75 ± 0,72 BbD	12,49 ± 2,37 BbC	19,68 ± 0,96 BbB	29,52 ± 2,21 BbA
	50	6,87 ± 1,32 AbD	9,00 ± 1,47 AbD	13,51 ± 0,23 AbC	21,01 ± 0,82 AbB	31,27 ± 1,40 AbA

Note: The first letter in different columns indicates a significant difference in temperature ($p<0.05$). The second letter in different columns indicates a significant difference between treatments ($p<0.05$). The third letter in different rows indicates a significant difference in storage duration ($p<0.05$).

Based on Table 2, the anisidine values of both the control cod liver oil and the cod liver oil supplemented with A. platensis carotenoid antioxidants increased as the

treatment temperature increased from day 0 to day 28 of storage. Both the control fish oil and the oil supplemented with A. platensis carotenoid antioxidants showed the highest anisidine values on day 28 at 50°C. The control cod liver oil had an anisidine value of 37.82±0.47 mEq/kg, while the cod liver oil supplemented with A. platensis carotenoid antioxidants had a value of 31.27±1.40 mEq/kg. This finding is consistent with the study by Wiyani et al. (2022), which reported that Ulva lactuca fatty acids stored at 30°C, 40°C, and 50°C showed an increase in anisidine values during storage as the temperature increased, with the highest anisidine value observed at 50°C on day 15. An increase in anisidine values with storage temperature was also reported by Yulianto et al. (2022), who found that tuna oil treated with 1.5% NaOH showed an increase in anisidine values at temperatures of 60°C, 70°C, and 80°C, with the highest value obtained at 80°C. The increase in the anisidine values of fish oil in this study indicates that oxidation occurred, as evidenced by the formation of secondary oxidation products during storage. Storage duration and temperature can affect the levels of secondary oxidation products (Estiasih, 2009).

Based on the ANOVA results, temperature, treatment, and storage duration had significant effects on the anisidine values of fish oil ($p<0.05$). The control fish oil showed a greater increase in anisidine values than the fish oil supplemented with A. platensis carotenoid antioxidants. This indicates that the addition of A. platensis, which contains carotenoids with antioxidant properties, can inhibit fish oil deterioration during storage. Antioxidants can protect cells from free radical damage by donating an electron to free radicals, thereby stabilizing them and preventing lipid damage (Andarina and Djauhari, 2017). According to CODEX WHO, the acceptable anisidine value for fish oil is ≤20 meq/kg, while the IFOS standard is ≤15 meq/kg. Based on the observations, from day 0 to day 14, the anisidine values of both the control fish oil and the fish oil supplemented with A. platensis remained within the established standards. However, from day 21 to day 28, the anisidine values of both the control fish oil and the fish oil supplemented with A. platensis at 50°C exceeded the CODEX WHO limit and IFOS

Total Oxidation

The peroxide value and anisidine value obtained from an analysis can be used to determine the total oxidation value of a product. The total oxidation value (TOTOX) is calculated as the sum of twice the peroxide value and the anisidine value (Pokorny et al., 2001). The oxidation of oil occurs in two stages: primary oxidation and secondary oxidation. The TOTOX value provides an overall indication of the oxidation process occurring in the oil. Measuring

Table 3. Total Oxidation

Treatment	Temperature (^{0}C)	Total Oksidasi (mEq/kg)				
		Day-0	Day-1	Day-2	Day-3	Day-4
Control Oil	30	12,47 ± 0,74 AaE	18,07 ± 0,48 AaD	26,94 ± 0,68 AaC	46,50 ± 0,60 AaB	67,81 ± 1,16 AaA
	40	12,41 ±	17,96 ± 1,75	27,74 ±	48,01 ±	69,05 ±

		0,94 BaE	BaD	1,15 BaC	1,57 BaB	1,12 BaA
Oil + *A. platensis*	50	12,66 ± 0,39 CaE	18,91 ± 0,46 CaD	30,31 ± 0,34 CaC	53,07 ± 1,05 CaB	75,83 ± 0,96 CaA
	30	10,38 ± 0,35 AbE	16,29 ± 0,46 AbD	25,11 ± 0,28 AbC	38,74 ± 1,30 AbB	60,08 ± 1,42 AbA
	40	10,63 ± 0,16 BbE	16,63 ± 0,79 BbD	24,37 ± 2,20 BbC	40,59 ± 1,08 AbB	62,08 ± 2,26 BbA
	50	10,03 ± 1,29 CbE	16,90 ± 1,56 BbD	25,98 ± 0,15 CbC	42,89 ± 0,94 AbB	65,13 ± 1,71 CbA

Note: The first letter in different columns indicates a significant difference in temperature ($p<0.05$). The second letter in different columns indicates a significant difference in treatment ($p<0.05$). The third letter in different rows indicates a significant difference in storage duration ($p<0.05$).

Based on Table 3, it can be seen that the total oxidation values of both control cod liver oil and cod liver oil supplemented with *A. platensis* carotenoid antioxidants increased as the temperature increased from day 0 to day 28 of storage. Both control cod liver oil and cod liver oil supplemented with *A. platensis* carotenoid antioxidants showed the highest total oxidation values on day 28 at 50°C. The control cod liver oil had a value of 75.83±0.96 mEq/kg, while the cod liver oil supplemented with *A. platensis* carotenoid antioxidants had a value of 65.14±1.71 mEq/kg. This is in line with the study by Montesqrit and Ovianti (2013), which stated that storage duration and temperature affect the total oxidation value of fish oil microcapsules. The lowest total oxidation value was obtained at 80°C, while storage for 15–45 days resulted in an increase in the total oxidation value. The increase in the total oxidation value of fish oil is influenced by peroxide and anisidine values. The TOTOKS value obtained can be used to estimate oxidative damage to lipids (Moigradean *et al*., 2012).

Based on the ANOVA results, temperature, treatment, and storage duration showed significant differences in the total oxidation values of fish oil ($p<0.05$). The control fish oil showed a greater increase in total oxidation than the fish oil supplemented with *A. platensis* carotenoid antioxidants. This indicates that the addition of *A. platensis*, which contains carotenoids with antioxidant properties, can inhibit fish oil deterioration during storage in terms of peroxide and anisidine values. Antioxidants can donate hydrogen atoms, thereby stabilizing free radical compounds (Adawiah *et al*., 2015). According to CODEX WHO, the acceptable total oxidation value for fish oil is ≤ 26 meq/kg, while the IFOS standard is ≤ 20 meq/kg. Based on the observations, from day 0 to day 7, the total oxidation values of both control fish oil and fish oil supplemented with *A. platensis* remained within the established standards. However, from day 14 to day 28, the total oxidation values of both control fish oil and fish oil supplemented with *A. platensis* exceeded the limits set by CODEX WHO and IFOS.

Carotenoid Content

Carotenoids are pigments ranging from yellow and orange to red, which can be obtained from natural sources such as higher plants and algae. In general, carotenoids

function as natural antioxidants that can counteract free radicals and prevent oxidation (Maleta *et al*., 2018). Carotenoids are easily degraded by factors such as light, heat, and oxygen (Mertz *et al*., 2010). In this study, carotenoids were extracted from *Arthrospira platensis* and applied to fish oil. Carotenoid content analysis was conducted to determine the stability of the carotenoids added to the fish oil.

Table 4. Carotenoid Content

Treatment	Temperature (^{0}C)	. Carotenoid Content (µg/g)				
		Day-0	Day-1	Day-2	Day-3	Day-4
Control Oil	30	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA
	40	0 ± 0 AaA	0 ± 0 AaA	0 ± A AaA	0 ± 0 AaA	0 ± 0 AaA
	50	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA
Oil + *A. platensis*	30	23,16 ± 0,73 AbA	21,60 ± 0,38 AbB	18,56 ± 0,49 AbC	13,96 ± 0,25 AbD	8,93 ± 0,81 AbE
	40	22,75 ± 0,62 BbA	20,94 ± 0,52 BbB	18,21 ± 1,17 BbC	12,72 ± 0,27 BbD	8,36 ± 0,65 BbE
	50	22,95 ± 0,47 CbA	19,74 ± 0,53 CbB	16,14 ± 0,56 CbC	10,97 ± 0,56 CbD	7,21 ± 0,64 CbE

Note: The first letter in different columns indicates a significant difference in temperature ($p<0.05$). The second letter in different columns indicates a significant difference in treatment ($p<0.05$). The third letter in different rows indicates a significant difference in storage duration ($p<0.05$).

Based on Table 4, the control cod liver oil showed no carotenoid content, whereas the cod liver oil supplemented with *A. platensis* carotenoid antioxidants showed a decrease in carotenoid content as the temperature increased from day 0 to day 28 of storage. The lowest carotenoid content was observed on day 28 of storage at 50°C, with a value of 7.21±0.64 µg/g. This is in line with the study by Susanti and Siregar (2016), which reported that red fruit oil stored for 98 days at temperatures of 35°C, 45°C, and 55°C experienced a decrease in carotenoid content as temperature and storage duration increased. This condition indicates that storage duration and temperature have a significant effect on the carotenoid content of a material. The rate of carotenoid degradation at high temperatures increases more rapidly due to the

isomerization of trans-carotene into its cis form. In addition, oxidation also increases as temperature rises (Aryayustama *et al*., 2018). The decrease in carotenoid content occurs because carotenoids are easily degraded. The degradation of carotenoids during storage is influenced by temperature and exposure to sunlight. Carotenoid stability is affected by the double bonds in the molecular structure of carotenoids, which make structural changes more likely to occur (Heriyanto and Limantara, 2010). Based on the ANOVA results, temperature, treatment, and storage duration showed significant differences in the carotenoid content of fish oil ($p<0.05$).

Antioxidant Activity

Antioxidants are compounds that can stop oxidation reactions by preventing the formation of free radicals. Antioxidants can be divided into two types: natural and synthetic antioxidants. Natural antioxidants can be obtained from fruits, plants, or algae containing compounds such as polyphenols, flavonoids, vitamin C, vitamin E, and carotenoids (Hani and Milanda, 2021). Antioxidant activity can be influenced by several factors, including pH, oxygen, light, and temperature. Temperature and storage duration can affect the degradation of compounds with antioxidant activity (Khotimah *et al*., 2018). Antioxidant activity testing was conducted to determine the ability of carotenoids to act as antioxidants in inhibiting oxidation during storage.

Table 5. Antioxidant Activity

Treatment	Temperature (^{0}C)	Antioxidant (% Inhibition)				
		Day-0	Day-1	Day-2	Day-3	Day-4
Control Oil	30	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA
	40	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA
	50	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA	0 ± 0 AaA
Minyak + *A. platensis*	30	76,37 ± 1,72 BbA	66,66 ± 3,33 AbB	55,38 ± 3,24 AbC	41,63 ± 2,05 AbD	22,99 ± 1,84 AbE
	40	76,11 ± 1,59 AbbA	66,05 ± 1,81 ABbB	54,13 ± 3,21 ABbC	41,27 ± 4,27 AbbD	20,44 ± 7,19 ABbE
	50	73,43 ±	64,58 ±	50,50 ±	39,35 ±	17,07 ± 1,84

0,79 AbA	2,21 AbB	2,44 AbC	1,30 AbD	AbE

Note: The first letter in different columns indicates a significant difference in temperature ($p<0.05$). The second letter in different columns indicates a significant difference in treatment ($p<0.05$). The third letter in different rows indicates a significant difference in storage duration ($p<0.05$).

Based on Table 5, the control cod liver oil showed no antioxidant activity, whereas the cod liver oil supplemented with *A. platensis* carotenoid antioxidants showed a decrease in antioxidant activity as the temperature increased from day 0 to day 28 of storage. The lowest antioxidant activity was observed on day 28 of storage at 50°C, with a value of 17.07±1.84%. This is in line with the study by Perdisen *et al*. (2021), which stated that the antioxidant activity of mandarin peel extract containing carotenoid compounds decreased from week 0 to week 4. The same results were also reported by Prabowo *et al*. (2013), who found that the antioxidant activity of ethanolic extract of *Sargassum* sp. in fish oil emulsions decreased from day 0 to day 38 of storage at an incubator temperature of 30 ± 4°C. This condition occurs because antioxidants are compounds that are easily oxidized. The decrease in antioxidant activity can be caused by light, heat, peroxide metals, and direct contact with oxygen. Antioxidants are particularly unstable when exposed to heat. The higher the temperature, the lower the antioxidant activity. The decrease in antioxidant activity in a material due to temperature and storage duration affects the ability of a compound to donate hydrogen atoms to inhibit free radicals (Farikha *et al*., 2013).

Based on the ANOVA results, temperature, treatment, and storage duration showed significant differences in the antioxidant activity of fish oil ($p<0.05$). The greatest decrease in antioxidant activity occurred at 50°C on day 28. The carotenoid content obtained from *A. platensis* influenced antioxidant activity. The decrease in antioxidant activity was also accompanied by a decrease in carotenoid content due to the effect of high temperatures. Although antioxidant activity decreased, the fish oil supplemented with *A. platensis* was able to maintain its antioxidant activity compared to the control fish oil. This indicates that carotenoids, as antioxidants, can inhibit oxidation reactions during storage by donating hydrogen atoms to free radicals (Utami, 2013).

Moisture Content

Water is one of the main components in food products that can affect color, texture, and flavor. Moisture content is an important quality parameter of a product because water in a material can trigger reactions and reduce its quality, thereby affecting its shelf life (Prasetyo *et al*., 2019). In general, moisture content is used to determine the quality of a product. The lower the moisture content of a product, the longer its shelf life (Winarno, 1984).

Table 6. Moisture Content

Treatment	Temperature (^{0}C)	Moisture Content (%)				
		Day-0	Day-1	Day-2	Day-3	Day-4
Control	30	0,38 ±	0,51 ± 0,05	0,66 ±	0,70 ± 0,05	0,84 ±

Oil		0,00 AaD	AaC	0,05 AaB	AaB	0,11 AaA
	40	0,38 ± 0,00 AaD	0,55 ± 0,06 AaC	0,61 ± 0,04 AaB	0,63 ± 0,06 AaB	0,77 ± 0,02 AaA
	50	0,38 ± 0,00 AaD	0,44 ± 0,04 AaC	0,58 ± 0,01 AaB	0,61 ± 0,04 AaB	0,72 ± 0,04 AaA
	30	0,59 ± 0,00 AbD	0,70 ± 0,11 AbC	0,82 ± 0,06 AbB	0,85 ± 0,09 AbB	0,99 ± 0,11 AbA
Oil +*A. platensis*	40	0,61 ± 0,04 AbD	0,67 ± 0,06 AbC	0,81 ± 0,10 AbB	0,88 ± 0,24 AbB	1,16 ± 0,03 AbA
	50	0,58 ± 0,01 AbD	0,62 ± 0,04 AbC	0,68 ± 0,08 AbB	0,76 ± 0,04 AbB	1,24 ± 0,30 AbA

Note: The first letter in different columns indicates a significant difference in temperature ($p<0.05$). The second letter in different columns indicates a significant difference in treatment ($p<0.05$). The third letter in different rows indicates a significant difference in storage duration ($p<0.05$).

Based on Table 6, it can be observed that the moisture content of the control cod liver oil increased from day 0 to day 28 of storage. However, as the treatment temperature increased, the moisture content of the control oil tended to decrease. Cod liver oil supplemented with carotenoid antioxidants from *A. platensis* also showed an increase in moisture content as the storage temperature increased from day 0 to day 21. However, on day 28, the moisture content decreased with increasing temperature.

This finding is consistent with the study by Hikmawan *et al.* (2019), which reported that the moisture content of palm oil increased with longer storage periods in storage tanks, from 0.17% on the first day to 0.27% on the sixth day. A decrease in fish oil moisture content with increasing temperature was also reported by Nurfiqih and Hakim (2021), who found that higher reaction temperatures resulted in lower moisture content in the produced palm oil. The lowest moisture content, 0.38%, was obtained at a temperature of 60°C.

An increase in product moisture content during storage may be caused by environmental humidity or oxidation reactions. Oxidation in fish oil produces CO_2 gas, volatile compounds, and a certain amount of water molecules. High moisture content can affect oil quality. The higher the moisture content, the lower the oil quality, as it may promote hydrolysis, which can eventually lead to rancidity (Hikmawan *et al.*, 2019). Furthermore, an increase in moisture content during storage may be caused by the relative humidity (RH) of the surrounding environment. When the moisture content of a material is low while the surrounding RH is high, the material may absorb water vapor from the air, resulting in an increase in its moisture content (Suastuti, 2009).

Based on the ANOVA results, treatment and storage duration showed significant differences in the moisture content of fish oil ($p<0.05$). Meanwhile,

temperature did not show a significant effect on fish oil moisture content. The increase in moisture content in oil supplemented with *A. platensis* was greater than that in the control fish oil. This finding is similar to the study by Mayasari (2019), which reported that jelly candy supplemented with Spirulina had a higher moisture content than the control jelly candy. Similarly, Negara *et al.* (2014) reported that chocolate bars supplemented with 5% *A. platensis* had a moisture content of 1.63%, which was higher than that of the control chocolate bars. This condition is presumably due to the ability of *A. platensis* to readily absorb water from its surrounding environment (Negara *et al.*, 2014).

The moisture content standard for fish oil established by IFOMA is 0.5–1%. Based on the observations, from day 0 to day 28, the moisture content of both the control fish oil and the fish oil supplemented with *A. platensis* remained within the standard range. However, the fish oil supplemented with *A. platensis* at temperatures of 40°C and 50°C exceeded the IFOMA threshold, with moisture contents of 1.16 ± 0.03% and 1.24 ± 0.31%, respectively.

Shelf-Life Estimation

Shelf life is the period from production until the product reaches consumers during which its quality remains consistent with the specified standards or intended use, in terms of organoleptic properties, morphology, and physical and chemical characteristics. Shelf-life estimation is necessary to determine the storage period of a product, which is ultimately related to product safety.

In this study, the shelf life of fish oil supplemented with carotenoid antioxidants from *A. platensis* was estimated using the Accelerated Shelf-Life Test (ASLT) method with the Arrhenius approach, involving three temperature points and a storage period of four weeks. The Arrhenius approach is suitable for products whose quality deterioration is caused by oxidation reactions, Maillard reactions, and protein denaturation. Shelf-life estimation using the Arrhenius approach can be conducted through the following steps:

a. Regression Plotting and Determination of Reaction Order

The test data for each parameter are plotted, with the measurement results on the y-axis and storage time on the x-axis, followed by the calculation of the linear regression equation. Determining the reaction order is necessary to establish the rate of quality deterioration in fish oil.

If the rate of quality change occurs constantly or linearly, the reaction follows zero-order kinetics. However, if the rate of quality change is not constant and follows a logarithmic or exponential pattern, the reaction follows first-order kinetics. Zero-order reactions can be represented by a graph showing the relationship between k and storage time, whereas first-order reactions can be represented by a graph showing the relationship between $\ln k$ and storage time. The reaction order selected for determining the fish oil shelf-life model is the one with the highest R^2 value or the value closest to 1 (Arif, 2016).

Table 7. Regression Equations of the Testing Parameters for Control Cod Liver Oil

		Kontrol			
		Ordo 0		Ordo 1	
Parameter	Suhu	Persamaan regresi	R^2	Persamaan regresi	R^2
	30°	y = 0,5331x + 0,6067	0,95	y = 0,0781x + 0,7264	0,98
	40°	y = 0,5514x + 0,5347	0,95	y = 0,0792x + 0,7251	0,98
Angka peroksida	50°	y = 0,6027x + 0,2793	0,93	y = 0,0822x + 0,7163	0,98
	30°	y = 0,9211x + 5,332	0,91	y = 0,0512x + 2,0504	0,96
	40°	y = 0,9451x + 5,3027	0,92	y = 0,0524x + 2,0476	0,97
Angka anisidin	50°	y = 1,0876x + 5,504	0,94	y = 0,0554x + 2,1043	0,97
	30°	y = 1,9871x + 6,5433	0,94	y = 0,0619x + 2,4875	0,99
	40°	y = 2,0477x + 6,372	0,94	y = 0,0631x + 2,4842	0,99
Total oksidasi	50°	y = 2,2928x + 6,0613	0,95	y = 0,0659x + 2,5159	0,99
	30°	0	0	0	0
	40°	0	0	0	0
Aktivitas antioksidan	50°	0	0	0	0
	30°	0	0	0	0
	40°	0	0	0	0
Kadar karotenoid	50°	0	0	0	0
	30°	y = 0,0158x + 0,4008	0,98	y = 0,0268x - 0,8859	0,96
	40°	y = 0,0122x + 0,4202	0,93	y = 0,0218x - 0,8551	0,9
Kadar Air	50°	y = 0,0121x + 0,3798	0,97	y = 0,0228x - 0,9432	0,96

Table 8. Regression Equations of the Testing Parameters for Cod Liver Oil Supplemented with A. platensis Carotenoid Antioxidants

Minyak + *A. platensis*					
Parameter	Suhu	Ordo 0		Ordo 1	
		Persamaan regresi	R^2	Persamaan regresi	R^2
Angka peroksida	30°	y = 0,4953x + 0,528	0,95	y = 0,0787x + 0,636	0,98
	40°	y = 0,5104x + 0,514	0,95	y = 0,0789x + 0,6572	0,98
	50°	y = 0,5385x + 0,390	0,95	y = 0,0823x + 0,6254	0,97
Angka anisidin	30°	y = 0,7503x + 4,698	0,93	y = 0,0506x + 1,8851	0,98
	40°	y = 0,7916x + 4,467	0,91	y = 0,0516x + 1,8874	0,98
	50°	y = 0,8688x + 4,174	0,93	y = 0,0554x + 1,8679	0,99
Total oksidasi	30°	y = 1,7407x + 5,756	0,94	y = 0,0625x + 2,3463	1
	40°	y = 1,8124x + 5,492	0,93	y = 0,0632x + 2,356	0,99
	50°	y = 1,9458x + 4,951	0,94	y = 0,0667x + 2,3309	0,99
Aktivitas antioksidan	30°	y = -1,8825x + 78,9	0,98	y = -0,041x + 4,457	0,91
	40°	y = -1,9446x + 78,8	0,98	y = -0,0443x + 4,4703	0,89
	50°	y = -1,9709x + 76,5	0,97	y = -0,0488x + 4,4619	0,87
Kadar karotenoid	30°	y = -0,5157x + 24,4	0,96	y = -0,0334x + 3,261	0,9
	40°	y = -0,5288x + 24,0	0,96	y = -0,0357x + 3,2475	0,91
	50°	y = -0,5752x + 23,4	0,99	y = -0,0415x + 3,2346	0,96
Kadar Air	30°	y = 0,0137x + 0,603	0,97	y = 0,0177x - 0,4919	0,97
	40°	y = 0,0186x + 0,568	0,92	y = 0,0221x - 0,5221	0,96
	50°	y = 0,0207x + 0,489	0,74	y = 0,0243x - 0,6287	0,81

Based on Table 7, the control cod liver oil showed that the peroxide value, anisidine value, total Oxidation, and moisture content parameters followed first-order kinetics, as their R^2 values were higher than those of zero-order kinetics. Meanwhile, the antioxidant activity and carotenoid content parameters also followed first-order kinetics. Based on Table 8, cod liver oil supplemented with *A. platensis* carotenoid antioxidants showed that the peroxide value, anisidine value, and TOTOKS parameters followed first-order kinetics because their R^2 values were higher than those of zero-order kinetics, whereas antioxidant activity, carotenoid content, and moisture content followed zero-order kinetics.

These results indicate that the reaction rates of parameters following zero-order kinetics occur at a constant rate. Meanwhile, parameters following first-order kinetics are often referred to as unimolecular reactions because their reaction rates are directly proportional to the concentration of only one component (Hariyadi, 2019). Quality deterioration processes that follow zero-order kinetics include enzymatic degradation, enzymatic browning, and oxidation reactions. Meanwhile, quality deterioration processes that follow first-order kinetics include rancidity, microbial growth, off-flavour development, vitamin degradation, and the decline in vitamin quality (Wulandari, 2014).

b. Arrhenius Plotting

The Arrhenius equation is commonly used to determine quality deterioration caused by physical and chemical changes in food products during processing and storage (Hariyadi, 2019). In the previous stage, the reaction order for each parameter was determined as the basis for constructing the Arrhenius plot.

To obtain the Arrhenius equation, a graph is constructed by plotting 1/T against ln k for each testing parameter. The Arrhenius equation obtained for each parameter is expressed as follows:

Table 9. Arrhenius Regression Equations

No	Parameter	Control		Oil + *A. platensis*	
		Regression Equation	R^2	Regression Equation	R^2
1	Peroxide Value	y = -249,08x - 1,7317	0,92	Y = -216,73x – 1,8334	0,78
2	Anisidine Value	y = -383,98x - 1,7104	0,94	Y = -440,65x – 1,5383	0,90
3	Total Oxidation	y = -305,05x - 1,7797	0,94	Y = 315,93x + 0,5654	0,86
4	Antioxidant Activity	0	0	Y = -225,47x + 1,3796	0,95
5	Carotenoid Content	0	0	Y = -531,07x + 1,0804	0,90
6	Moisture Content	y= 1318,2x - 8,5372	0,79	Y = -1556,9x + 1,1229	0,96

Based on Table 9, the Arrhenius regression equations and coefficients of determination (R^2) were obtained for each parameter in both the control cod liver oil and cod liver oil supplemented with *A. platensis* carotenoid antioxidants. The resulting regression equations were then adjusted to the Arrhenius equation in the form of $\ln k = \ln k_0 - Ea/RT$, where $\ln k_0$ represents the intercept and Ea/R represents the slope. From this equation, the activation energy (Ea) for each parameter and the quality deterioration rate (k) at each temperature for the critical parameter can be determined. The parameter with the lowest activation energy is selected as the critical parameter and subsequently used to calculate the shelf life of the product.

c. Determination of the Critical Parameter

A critical parameter is a parameter considered susceptible to or easily affected by quality deterioration. The determination of critical parameters is based on the decline in product quality during storage. The selection of the critical parameter for estimating the shelf life of fish oil supplemented with *A. platensis* carotenoid antioxidants is based on the parameter with the lowest activation energy.

Activation energy (Ea) is the minimum energy required by molecules for a reaction to occur. A high activation energy indicates strong interaction energy, meaning that a greater amount of energy is required to initiate a reaction. Conversely, low activation energy indicates that only a small amount of energy is required to initiate a reaction, resulting in faster quality deterioration of a product (Oceanic *et al.*, 2017).

Table 10. Activation Energy of the Testing Parameters

No	Parameter	Control		Oil + *A. platensis*	
		Ea (kJ/mol)	k0	Ea (kJ/mol)	k0
1	Peroxide Value	494,67	$1,76 \times 10^{-1}$	430,42	$1,59 \times 10^{-1}$
2	Anisidine Value	762,58	$1,80 \times 10^{-1}$	875,13	$2,14 \times 10^{-1}$

3	Total Oxidation	605,82	$1,68 \times 10^{-1}$	627,43	1,76
4	Antioxidant Activity	0	0	447,78	3,97
5	Carotenoid Content	0	0	1054,7	2,94
6	Moisture Content	2617,94	$1,96 \times 10^{-1}$	3092	3,07

Based on Table 10, the activation energy and k_0 values were obtained for each parameter in both the control cod liver oil and cod liver oil supplemented with *A. platensis* carotenoid antioxidants. The activation energy was calculated from the slope of the Arrhenius equation, which was equated to −Ea/R and then multiplied by the ideal gas constant (R = 1.986 cal K^{-1} mol^{-1}).

Activation energy is used to determine the minimum energy required to activate a reaction as a result of molecular collisions or vibrations, while k_0 represents the frequency factor, which is related to the frequency of molecular collisions during a reaction. The k_0 value is obtained from the intercept of the Arrhenius equation, which is equated to ln k_0.

Based on the calculated activation energy values, moisture content had the highest activation energy, whereas peroxide value had the lowest activation energy in both the control cod liver oil and the cod liver oil supplemented with *A. platensis* carotenoid antioxidants. This indicates that peroxide value is the parameter most susceptible to quality deterioration and can therefore be identified as the critical parameter. This condition occurs because storing fish

d. Calculation of the Quality Deterioration Rate of the Critical Parameter

The rate of quality deterioration (k) describes the extent to which a parameter undergoes quality deterioration due to the influence of temperature. The reaction rate constant (k) is affected by environmental factors, one of which is reaction temperature (Hariyadi, 2019).

In this study, the k values for cod liver oil supplemented with *A. platensis* carotenoid antioxidants were calculated using three temperature points for the parameter most susceptible to quality deterioration, namely peroxide value, which was identified as the critical parameter.

The k value can be calculated using the following equation:

$$k = k0 \; x \; e^{-\frac{Ea}{RT}}$$

Where:

k = Rate of quality deterioration of the critical parameter

k_0 = Frequency factor constant

Ea/R = Value obtained from the slope of the critical parameter in the Arrhenius plot

T = Storage temperature in Kelvin

Table 11. Regression Equations and Quality Deterioration Rates of the Critical Parameter

Suhu	Kontrol				Minyak + *A. platensis*			
	Persamaan regresi	Ea (kJ/mol)	k0	k	Persamaan regresi	Ea (kJ/mol)	k0	k
30°				$7,78 \times 10^{-2}$				$7,82 \times 10^{-2}$
40°	y = -249,08x - 1,7317	494,67	$1,76 \times 10^{-1}$	$7,99 \times 10^{-2}$	y = -216,73x - 1,8334	430,42	$1,59 \times 10^{-1}$	$7,99 \times 10^{-2}$
50°				$8,19 \times 10^{-2}$				$8,17 \times 10^{-2}$

Based on Table 11, the quality deterioration rates of the control cod liver oil and cod liver oil supplemented with *A. platensis* carotenoid antioxidants were obtained using the critical parameter, namely peroxide value, at three storage temperature points. The slope calculated from the Arrhenius regression equation, which relates ln k to the storage temperature at three different temperature levels, provides the k value or the quality deterioration rate constant of the product (Arif, 2016). The k value is subsequently used in the shelf-life calculation formula for fish oil supplemented with *A. platensis* carotenoid antioxidants.

e. Shelf-Life Calculation

The shelf life of cod liver oil supplemented with *A. platensis* carotenoid antioxidants was calculated using the Arrhenius approach at three temperature points. Shelf life was calculated using first-order kinetics, with the critical parameter determined based on the lowest activation energy value, namely peroxide value.

Shelf-life calculation using the Arrhenius approach can be determined from the quality deterioration rate and the difference between the initial storage value and the critical limit. The shelf life of fish oil supplemented with *A. platensis* carotenoid antioxidants can be calculated using the following equation:

Where:

No = Initial storage value

Nt = Critical limit value

k = Quality deterioration rate constant

Table 12. Shelf Life of Control Cod Liver Oil

Temperature	Control						
	K	No	Nt	Ln No	Ln Nt	Ln Nt – Ln No	Shelf Life (Days)
30°	$7,78 \times 10^{-2}$	1,82	5	0,6	1,61	1,01	13,01
40°	$7,99 \times 10^{-2}$	1,82	5	0,6	1,61	1,01	12,67
50°	$8,19 \times 10^{-2}$	1,82	5	0,6	1,61	1,01	12,36

Temperature	Oil + *A. platensis*

	k	No	Nt	Ln No	Ln Nt	Ln Nt – Ln No	Shelf Life (Days)
30°	7,82 x 10^{-2}	1,62	5	0,48	1,61	1,12	14,38
40°	7,99 x 10^{-2}	1,62	5	0,48	1,61	1,12	14,06
50°	8,17 x 10^{-2}	1,62	5	0,48	1,61	1,12	13,76

Table 13. Shelf Life of Cod Liver Oil Supplemented with A. platensis Carotenoid Antioxidants

Based on Table 12, the shelf-life estimation of cod liver oil using the Arrhenius approach showed that control cod liver oil stored at temperatures of 30 ± 2°C, 40 ± 2°C, and 50 ± 2°C had shelf lives of 13.01 days, 12.67 days, and 12.36 days, respectively. Meanwhile, Table 13 shows that cod liver oil supplemented with *A. platensis* carotenoid antioxidants, stored at temperatures of 30 ± 2°C, 40 ± 2°C, and 50 ± 2°C, had shelf lives of 14.38 days, 14.06 days, and 13.76 days, respectively. In this study, cod liver oil supplemented with *A. platensis* carotenoid antioxidants had a longer shelf life than the control cod liver oil.

f. Q_{10}

In addition to the Arrhenius approach, another method that can be used to estimate the shelf life of a product is the Q10 method. The Q10 value indicates that the rate of a chemical reaction increases by a certain factor when the ambient temperature rises by 10°C. Shelf-life estimation using the Q10 method is carried out by using the activation energy (Ea) value. The Q10 method can be used to estimate shelf life at various storage temperatures (Syarief and Halid, 1993).

Shelf life using the Q10 method can be calculated using the following formula:

$$Q_{10} = e^{Ea} \frac{(T2 - T1)}{R\ x\ T1\ x\ T2}$$

$$Q_{10} = 2{,}718^{494{,}16} \frac{(313 - 303)}{1{,}986\ x\ 303\ x\ 313}$$

$$Q_{10} = 2{,}718^{0{,}026}$$

$$Q_{10} = 1{,}02$$

This value indicates that for every 10°C increase in temperature, the reaction rate increases by a factor of 1.02. Therefore, the shelf-life estimation based on the Q10 calculation is presented in Table 14.

Table 14. Shelf Life Based on the Arrhenius and Q_{10} Approaches

Control	Oil + *A. platensis*

Temeperature	Shelf Life (Days) (Arrhenius)	Shelf Life (Days) (Q_{10})	Temperature	Shelf Life (Days) (Arrhenius)	Shelf Life (Days) (Q_{10})
30°	13,01	13,01	30°	14,38	14,38
40°	12,67	11,65	40°	14,06	13,04
50°	12,36	11,34	50°	13,76	12,74

Based on Table 14, it can be seen that control cod liver oil estimated using the Q10 method at temperatures of 30 ± 2°C, 40 ± 2°C, and 50 ± 2°C had shelf lives of 13.01 days, 11.65 days, and 11.34 days, respectively. Meanwhile, the table shows that cod liver oil supplemented with *A. platensis* carotenoid antioxidants at temperatures of 30 ± 2°C, 40 ± 2°C, and 50 ± 2°C had shelf lives of 14.38 days, 13.04 days, and 12.74 days, respectively. In general, the shelf-life estimation results obtained using the Arrhenius and Q10 approaches did not differ considerably. Cod liver oil supplemented with *A. platensis* carotenoid antioxidants had a longer shelf life than the control cod liver oil. This condition occurred because the addition of *A. platensis* carotenoids, which contain antioxidants, to the fish oil was able to inhibit oxidation reactions caused by storage temperature compared with the control fish oil without antioxidant supplementation. Antioxidants can donate hydrogen atoms, thereby stabilizing free radical compounds (Adawiah *et al*., 2015).

The shelf-life calculation of cod liver oil supplemented with *A. platensis* carotenoid antioxidants was carried out using peroxide value as the critical parameter. The selection of peroxide value as the critical parameter was based on its having the lowest activation energy (Ea). A similar finding was reported by Fitriyani (2022), who stated that the shelf-life estimation of an emulsion product containing fish oil and fish protein hydrolysate, using peroxide value as the critical parameter, resulted in a shelf life of 141 days at 29°C. In a study by Wiyani *et al.* (2022), the estimated shelf life of *Ulva lactuca* fatty acids, using peroxide value as the critical parameter, was 8.93 days, 7.90 days, and 6.86 days at temperatures of 30 ± 2°C, 40 ± 2°C, and 50 ± 2°C, respectively. In the present study, the Arrhenius approach showed that control cod liver oil at temperatures of 30 ± 2°C, 40 ± 2°C, and 50 ± 2°C had shelf lives of 13.01 days, 12.67 days, and 12.36 days, respectively, whereas cod liver oil supplemented with *A. platensis* carotenoid antioxidants at the same temperatures had shelf lives of 14.38 days, 14.06 days, and 13.76 days, respectively. A similar study on the shelf life of fish oil and its stability during storage was conducted by Hanifah (2023). The study reported that pure tuna oil supplemented with 0.6% tocopherol, whose stability was evaluated using peroxide value, anisidine value, and total oxidation (TOTOKS), remained stable for up to 9 days of storage. A study on fish oil stability using peroxide value as a parameter was conducted by Pak (2005), who reported that fish oil supplemented with 0.05% tocopherol had a shelf life of 41 days, while fish oil supplemented with 0.05% Ronoxan had a shelf life of 44 days. In this study, the shelf lives of control cod liver oil and cod liver oil supplemented with *A. platensis* carotenoids were relatively close. This was presumably because the addition of *A. platensis* carotenoids at a concentration of 600 ppm did not significantly affect the inhibition of oxidation in cod liver oil. In addition, carotenoids are unstable compounds

that can be easily degraded by temperature or heat exposure. One approach that can be used to improve carotenoid stability is encapsulation. Encapsulation is the process of coating easily degradable core materials with a specific coating material that can protect the core substances from factors that cause deterioration during processing and storage (Boer *et al*., 2019).

A study on the shelf life of a similar oil product was conducted by Shen *et al.* (2019), who reported that DHA oil containing algae-derived antioxidant composites had a shelf life of 7.11 days. A study on the shelf life of palm oil, which has been widely commercialized and exported to various countries, was conducted by Widodo *et al.* (2019). The study reported that the estimated shelf life of palm oil was 6.1 months at room temperature, 1.6 months at 40°C, 0.7 months at 50°C, and 0.6 months at 60°C. Meanwhile, research on the shelf life of fruit-derived oils, such as red fruit oil, was conducted by Susanti and Siregar (2016), who reported that the shelf life of red fruit oil estimated using the Arrhenius method was 8 months.

IV. CONCLUSION

A. platensis carotenoids demonstrated a significant effect on inhibiting cod liver oil oxidation, as indicated by the longer shelf life of cod liver oil supplemented with *A. platensis* carotenoids compared with the control cod liver oil. The shelf life of cod liver oil supplemented with *A. platensis* carotenoid antioxidants at a storage temperature of 30 ± 2°C was 14.38 days.

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