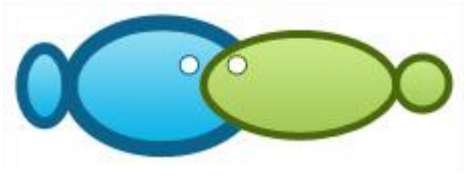

Advantages of virgin coconut oil enriched with astaxanthin (VCOA) in protection against light- and heat-induced oxidation

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Abstract. This study examines the antioxidant activity and quality of virgin coconut oil containing astaxanthin (VCOA) from fermented *Acetes* sp. (cincalok) when exposed to light and heat. The concentration of astaxanthin, the content of free fatty acids, and the peroxide value were determined using spectrophotometric based methods. The results showed that VCOA has a high antioxidant activity with an inhibitory concentration 50 (IC_{50}) of 31.67 mg L^{-1}. VCOA consistently maintains good quality up to a temperature of 90ºC, with minimal changes in peroxide value and free fatty acid concentration. This allows it to withstand high temperatures without significant changes in quality. The concentration of astaxanthin in VCOA remains stable up to a temperature of 60ºC. The main advantage of VCOA is its strong antioxidant activity, which helps protect the body from damage caused by free radicals. In conclusion, enrichment of virgin coconut oil (VCO) with astaxanthin gives it a strong antioxidant activity, allowing it to help protecting biological cells to light and heat induced degradation.
Key Words: antioxidant, free fatty acids, peroxide value, spectrophotometer.

Introduction. Virgin coconut oil enriched with astaxanthin (VCOA) represents an innovative green technology that utilizes virgin coconut oil (VCO) as an eco-friendly solvent to extract astaxanthin from *Acetes* sp., a species used in *cincalok*, a traditional Indonesian fermented shrimp product. This green extraction method leverages the oil-solubility of astaxanthin, thereby eliminating the need for petroleum-based solvents.

Virgin coconut oil is an ideal solvent for applications in food and health fields due to its high solvency, high flash point, low toxicity, and biodegradability. It is also derived from renewable resources, is reasonably priced, and can be recycled easily without adverse effects. The resultant VCOA retains the antioxidant activity of astaxanthin, making it beneficial for health and nutritional applications (Prayitno et al 2022).

Astaxanthin is a natural pigment found in some types of algae, fish, and shrimp. It is known to have strong antioxidant properties, thereby defending cells from damage caused by free radicals and oxidative stress (Sztretye et al 2019; Yaqoob et al 2021; Zheng et al 2023).

Virgin coconut oil (VCO) is mainly composed of saturated fatty acids (around 90%) and the addition of astaxanthin will bring a highly unsaturated carotenoid structure, which is easily oxidizable (Prayitno et al 2022; Sabahannur & Alimuddin 2022) giving then an antioxidant protection to the lipids. Lipid oxidation can lead to undesirable taste and aroma, deteriorate nutritional quality, and cause the production of toxic

compounds (Loganathan et al 2022; Ansorena et al 2023). Lipid oxidation can be influenced by various factors such as degree of unsaturation, heat, light, oil processing, antioxidants, and transition metals (Asnaashari et al 2017). When VCOA is exposed to light and heat during storage or processing, there is a possibility of degradation of astaxanthin and therefore oxidation of lipids contained in VCOA. The degradation of astaxanthin can reduce the ability of VCOA to protect cells from damage, while lipid oxidation can produce compounds that have the potential to damage health (Anarjan & Tan 2013; Takeungwongtrakul & Benjakul 2016).

Currently, monitoring and controlling lipid oxidation in a product is becoming increasingly important due to the potential link between the intake of oxidized fats and the development of degenerative diseases such as Alzheimer's and Parkinson's, heart disease, and cancer (Petrovic et al 2020; Ali et al 2022). Therefore, there is a need for information on the important contribution of VCOA in the protection against light- and heat-induced oxidation, as well as providing a strong knowledge base for the development of better products in the future. This information is crucial as it brings significant benefits to VCOA producers and consumers. For producers, this research can provide information about the influence of light and heat exposure on the quality of VCOA, so that appropriate measures can be taken in the storage, processing, and packaging of VCOA. As for consumers, the information found can assure that the VCOA being used still maintains its quality and effective astaxanthin content in defending health. Based on this, the research objective is to determine the characteristics of astaxanthin degradation and lipid oxidation when VCOA is exposed to light and heat. By knowing these characteristics, it can be determined how far VCOA can withstand and maintain its quality under light and heat exposure. This is important to ensure that VCOA remains effective in defending cells and does not cause negative effects on consumer health.

Material and Method

Research materials. The research materials used include fresh *Acetes* sp. purchased from fishermen in Teluk Majantu, Sedau Village, Singkawang Selatan District, West Kalimantan, Indonesia; sugar (Kaisar), salt (Subur Kimia Jaya), and virgin coconut oil (VCO) (MD Natural) purchased from a supermarket in Pontianak City. In addition, trans-astaxanthin (Mr: 596.84 g mol^{-1}, CAS: 472-61-7) produced by Sigma Aldrich and 2,2-diphenyl-1-picrylhydrazyl (DPPH, CAS: 1898-66-4) produced by Himedia were also used. Other analytical grade materials were used such as glacial acetic acid (CH_3COOH, CAS: 64-19-7), acetone ($(CH_3)_2CO$, CAS: 67-64-1), ethanol (C_2H_5OH, CAS: 64-17-5), ethyl acetate ($CH_3COOC_2H_5$, CAS: 141-78-6), phenolphthalein indicator (CAS: 77-09-8), starch indicator (CAS: 9005-84-9).

Preparation of fermented Acetes sp. Fermented *Acetes* sp. was made from fresh small shrimp (about 5 kg) taken directly from fishermen. The making of fermented *Acetes* sp. adopts a method passed down through generations by the community for making cincalok, which involves washing the shrimp repeatedly with seawater. A cloth filter separates impurities such as stones and small fish from the miniature shrimp. Next, sugar and salt are added to the cleaned shrimp in a ratio of shrimp:sugar:salt of 10:1:1 w/w, and mixed well. Furthermore the mixture is incubated in a covered container at room temperature and kept in the dark for 10 days.

Production of VCOA from fermented Acetes sp. Fermented *Acetes* sp., which is the result of the preparation process, was filtered to separate the water from the shrimp. The shrimp residue was dried at a temperature of 50°C for 3 hours using vacuum drying. The dried fermented *Acetes* sp. was ground using a screw press. Production of VCOA was carried out through the extraction process of astaxanthin from fermented *Acetes* sp. using ultrasonication and VCO as a solvent, as described in our previous study (Prayitno et al 2022).

A total of 5 g of dried fermented *Acetes* sp. was dispersed in 50 mL of VCO and placed in a 100 mL plastic container. A probe (Vibra Cell-750 HV, 20 kHz, 13 mm) was immersed to a depth of 5 cm. The total ultrasonication time was 3 minutes at 40% amplitude, with an ultrasonication time of 5 seconds and a rest time of 5 seconds to prevent the sample from getting too hot. Separation was done by filtration using a syringe filter (0.45 µm), and the supernatant was collected in a dark bottle. This extraction method was repeated until 1000 mL of supernatant (VCOA) were obtained.

Determination of antioxidant activity of VCOA from fermented Acetes sp. The antioxidant activity test was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method using a UV-Vis spectrophotometer. The DPPH solution was prepared at a concentration of 40 mg L^{-1} by weighing 4 mg of DPPH solid and dissolving it in ethyl acetate to a total volume of 100 mL. The maximum wavelength of the DPPH standard solution was determined by realizing a spectrum in the range of 400-800 nm.

Samples of VCO and VCOA were prepared with a concentration of 1000 mg L^{-1} by weighing 50 mg of the sample and dissolving it in ethyl acetate to a total volume of 50 mL. Then, they were pipetted at a specific volume for dilution to obtain varying concentrations of 50, 100, 150, 200, and 250 mg L^{-1} in a 10 mL volume. Each sample solution with varying concentrations was pipetted at 2 mL and mixed with a 40 mg L^{-1} DPPH solution, followed by incubation for 30 minutes until a colour change from purple to yellow occurs. The absorbance was then measured at the maximum wavelength of DPPH. As a control, the absorbance of a 40 mg L^{-1} DPPH solution pipetted at 2 mL and mixed with 2 mL of ethyl acetate solvent (without the sample) was also measured. The percentage of inhibition can be calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Furthermore, the concentration of astaxanthin required to achieve 50% inhibition (IC_{50}) was determined as an indicator of the antioxidant activity of the VCO and VCOA samples. The IC_{50} value was calculated using the linear regression equation ($y = ax + b$), where y represents the percentage of inhibition, x denotes the concentration of the measured substance, and a and b are the slope and intercept of the regression curve, respectively. The IC_{50} corresponds to the x-value at which y equals 50%.

Determination of VCOA advantages exposed to light and heat. The concentration of astaxanthin and free fatty acids (FFA), as the peroxide value (PV) of VCOA after exposition to light and heat were determined by modifying the method of Takeungwongtrakul & Benjakul (2016). Each 25 mL of oil was put into 2 measuring flasks and closed. One measuring flask containing oil was placed in a black box in which a halogen lamp with an intensity of 300 lux has been installed. Meanwhile, another bottle was stored at room temperature in the dark (without light). Astaxanthin-enriched vegetable oil samples from cincalok were taken randomly at times 0, 1, 2, 3, 4, 5, and 6 hours and analyzed for astaxanthin concentration, FFA, and PV. All treatments were repeated 3 times (triplicate).

Determination of the advantages of VCOA when exposed to light. A volume of 25 mL of VCOA were poured into a measuring flask and tightly sealed, then placed in a black box with a halogen lamp installed inside with an intensity of 300 lux. The sample was illuminated continuously for 6 hours. Every hour, 5 mL of the sample were taken and analyzed for astaxanthin concentration, FFA content, and PV. The same treatment was done on VCOA placed in a dark condition (without light). All treatments were repeated 3 times (triplicate).

Determination of VCOA degradation indicators when exposed to heat. Amber bottles containing 15 g of VCOA were placed in a water bath at different temperatures (30, 45, 60, and 90°C) for 6 hours. Every hour, a 5 mL sample was taken and analyzed

for astaxanthin concentration, FFA content, and PV. All treatments were repeated 3 times (triplicate).

Quantitative analysis of astaxanthin using UV-Vis spectrophotometer. As the total concentration of carotenoids extracted in VCO, astaxanthin is measured by spectrophotometry by modifying the procedures described by Goula et al (2017) and Corbu et al (2019). Standard astaxanthin solutions were prepared in acetone with varying concentrations of 0.44, 0.88, 1.75, 3.50, and 7.0 mg L^{-1}. The absorbance of each standard solution was measured at a wavelength of 477 nm using a UV-Vis Spectrophotometer U-1800 SHIMADZU. Subsequently, a graph of concentration versus absorbance was plotted to obtain a linear equation with a coefficient of determination (R^2) close to 1 (the marking value).

A volume of 1 mL of VCOA was transferred to a 5 mL volumetric flask, then acetone was added to the mark. The mixture was shaken until homogeneitiy, and the absorbance was read at a wavelength of 477 nm. The pure VCO before extraction was also treated in the same manner to observe its absorption spectrum in acetone in the wavelength range of 300-700 nm. The absorbance of VCOA at a wavelength of 477 nm obtained was then subtracted by the value of VCO absorption at the same wavelength (if absorption occurred) as a correction factor and then converted into concentration units through the equation obtained from the standard curve.

Determination of free fatty acid (FFA) content. A total of 0.5 mL of VCOA was placed into a 25 mL Erlenmeyer flask, and then 5 mL of ethanol were added. Two (2) drops of phenolphthalein indicator were added to the mixture. Next, the mixture was titrated with a standardized 0.05 N KOH solution until it changed color to pink. Then, the amount of KOH used was calculated to determine the FFA content using the following formula (Sudarmadji et al 1996):

$$\text{FFA (\%)} = \frac{\text{M x A x N}}{\text{1000 x G}} \text{ x 100}$$

where: FFA = free fatty acids content;
M = the molecular weight of fatty acid (200);
A = the volume of KOH for titration (mL);
N = the normality of the KOH solution;
G = the mass of the sample (g).

Determination of peroxide value (PV). A total of 0.3 g of VCOA was dissolved with 10 mL of chloroform in a 250 mL Erlenmeyer flask. Next, 15 mL of glacial acetic acid and 1 mL of saturated potassium iodide solution were added. The flask was then closed and incubated for 30 minutes in a dark place. After 30 minutes, 50 mL of distilled water were added and the flask was vigorously shaken. The mixture was then titrated with a standardized sodium thiosulfate solution. To clarify the titration endpoint, 3 drops of starch solution were added (after adding this indicator, the solution turned blue). The titration was continued until the blue color disappeared. The peroxide value was calculated and the measurements were done in triplicate (according to SNI 7381:2008).

Data analysis. The relationships between astaxanthin concentration, FFA levels, and PV with test parameters such as exposure duration, heating temperature, and heating time were analyzed using a One-Way ANOVA with a 95% confidence level (significance level of 0.05). If the p-value is smaller than the significance level, then Ho is not rejected, leading to the conclusion that there is no significant difference between the independent and dependent variables. Conversely, if the p-value is greater than the significance level, then Ho is rejected, indicating a significant difference between the independent and dependent variables (One-Way ANOVA).

Results

Antioxidant activity VCO and VCOA. The antioxidant activity of VCO and VCOA was determined using the DPPH method using a UV-Vis spectrophotometer. According to Purwanti et al (2019), the DPPH method is based on the oxidation-reduction reaction. DPPH is a free radical compound because it has one unpaired electron, making it highly reactive and able to damage cells in the body. Figure 1 shows the maximum wavelength of DPPH in ethyl acetate was 516 nm. This is consistent with the studies by Song et al (2016) and La et al (2021) who reported that the maximum wavelength of DPPH in ethyl acetate was 514 nm.

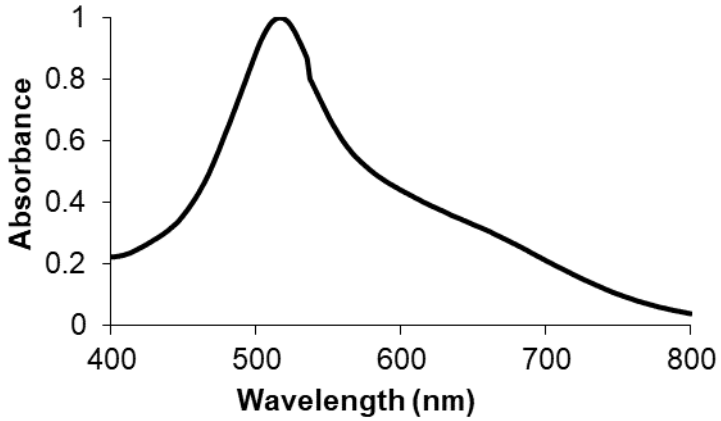

Figure 1. UV-Vis spectra of 40 mg L^{-1} DPPH solution in ethyl acetate.

Samples of VCO and VCOA were dissolved in ethyl acetate, then tested for antioxidant activity by adding a solution of DPPH 40 mg L^{-1}, and incubated for 30 minutes. When antioxidant compound is mixed with DPPH it will donate one or several electrons to the reactive DPPH molecule. After receiving the electron from the antioxidant compound in the sample, the initially deep purple DPPH solution will become pale purple or yellow (Shekhar & Anju 2014), indicating that the DPPH radical has been neutralized by the antioxidant compound in the sample. The antioxidant activities of VCO and VCOA can be determined by their IC_{50}, which is a parameter used to measure the effectiveness of a compound in inhibiting a specific chemical reaction. IC_{50} refers to the concentration of a compound required to inhibit the activity by 50%. The lower the IC_{50} value, the stronger the compound is in inhibiting the target activity, and the higher the IC50 value, the weaker the compound's ability to inhibit the target (Shahidi & Zhong 2015). If the IC_{50} value is below 50 mg L^{-1} the antioxidant activity is categorized as very strong. If the IC_{50} value is between 50-100 mg L^{-1} the antioxidant activity is categorized as strong. IC_{50} values between 100-150 mg L^{-1} indicate moderate activity. IC_{50} values above 150 mg L^{-1} indicate weak activity (Molyneux 2004). The IC50 results obtained from this study can be seen in Table 1.

Table 1

IC_{50} values for VCO and VCOA samples

Variant	*IC_{50} values (mg L^{-1})*
VCO	164.89
VCOA	31.67

Advantages of VCOA when exposed to light and heat. DPPH test shows that the presence of astaxanthin in VCO can increase its antioxidant activity. However, astaxanthin is a chemical compound that is not stable due to its sensitivity to various environmental factors such as light, oxygen, acidity, and temperature, which can cause a loss of its bioactivity (Aneesh et al 2022). The oxidative stability of oil is influenced by the fatty acids, which are the main composition of the oil. Apart from the FFA content, the PV

is a useful indicator to determine the level of oxidation experienced by the oil (Maszewska et al 2018). Therefore, in this study, the advantages of VCOA exposed to light and heat were determined based on the concentration of astaxanthin, FFA content, and PV.

Advantages of VCOA when exposed to light. Light is one of the factors that can degrade the quality of oil (de Souza et al 2020). Overexposure of vegetable oil/fat to light can cause lipid oxidation and rancidity, production of undesirable flavors, resulting in a decrease in quality (Ahmed et al 2011). To assess the degradation of VCOA when exposed to light, FFA content and PV were measured. VCOA was illuminated with a halogen lamp at an intensity of 300 lux for 6 hours. Halogen lamps have a range of polychromatic light wavelengths of 320-1100 nm, which is similar to the natural polychromatic light wavelength range of the sun, which is 360-760 nm (Sterhov & Loshkarev 2019).

The results of the astaxanthin concentration test, FFA level, and PV from VCOA can be seen in Table 2. Due to exposure to light, there is a decrease in astaxanthin concentration. The astaxanthin concentration for each hour interval for 6 consecutive hours is 9.20, 9.14, 9.01, 9.01, 8.77, 8.22, and 8.08 mg L^{-1} respectively. The conjugated double-bond system of astaxanthin causes this molecule to absorb excited singlet oxygen energy into its carotenoid chain, triggering the degradation of the carotenoid molecule even though this event prevents other molecules or tissues from damage caused by radicals (Ambati *et al*., 2014). Further astaxanthin protection results that it had no significant effect on the FFA value and there was a change in the peroxide value of VCOA, but it still remained in the SNI quality range. Apart from that, the fatty acids in VCO have the highest number of saturated bonds compared to other oils, so VCO is more resistant to light.

Table 2

Astaxanthin concentration, FFA levels, and PV when VCOA is exposed to light

Irradiation time (hours)	*Astaxanthin concentration (mg L^{-1})*	*Free fatty acid (%)*	*Peroxide value (meq kg^{-1})*
0	9.20±0.89	0.289±0.020	32.22±1.57
1	9.14±0.27	0.300±0.027	27.78±1.57
2	9.01±0.40	0.309±0.007	26.67±2.72
3	9.01±0.41	0.297±0.011	26.67±2.72
4	8.77±0.56	0.305±0.011	21.11±1.57
5	8.22±0.44	0.305±0.011	20.00±2.72
6	8.08±0.55	0.303±0.006	21.11±1.57

Advantages of VCOA when exposed to heat. The quality of oil is also influenced by temperature (Pramitha & Juliadi 2019). During heating, numerous complex reactions such as hydrolysis, isomerization, cyclization, oxidation, and polymerization occur, which alter the taste and degrade compounds in the oil, resulting in a change in oil quality. Oxidized products of fatty acids release a taste and odor (Nduka et al 2021). Therefore, this study also observed the quality of oil when heated at certain temperatures. The heating temperature was set at 30, 60, 90, and 120°C, while the test parameters included astaxanthin concentration, FFA content, and PV. This data is very useful for predicting the oxidative level of oil under various processing, storage, and heat distribution conditions.

The research results presented in Tables 3, 4, and 5 respectively indicate the advantages of VCOA when exposed to heat based on the concentration of astaxanthin, FFA levels and PV of VCOA.

Table 3

Concentration of astaxanthin (mg L^{-1}) when VCOA is exposed to heat

Heating time (hours)	*Temperature (°C)*			
	30	*60*	*90*	*120*
0	11.63±0.69	11.63±0.69	11.63±0.69	11.63±0.69
1	11.78±0.14	11.93±0.52	11.30±0.28	11.16±0.79
2	11.22±0.39	10.51±1.11	10.96±0.74	8.32±0.41
3	11.30±0.49	11.28±0.03	10.37±1.09	6.42±0.58
4	11.72±0.20	10.52±0.86	9.91±0.86	4.76±0.09
5	11.52±0.36	10.39±1.15	9.82±0.85	3.19±0.49
6	11.62±0.47	10.69±0.94	9.93±0.90	2.81±0.42

Table 3 presents the evaluation of the influence of temperature and heating time on the concentration of astaxanthin in VCOA. The astaxanthin concentration remained stable when VCOA was heated at 30°C and 60°C for 6 hours. Heating at 90°C led to a decrease in astaxanthin concentration in VCOA, although the level remained within a safe range. After 6 hours at this temperature, the concentration decreased from 11.63±0.69 to 9.93 ±0.90 mg L^{-1}, indicating that astaxanthin in VCOA is relatively stable up to 90°C. However, heating at 120°C caused a pronounced reduction in astaxanthin concentration, suggesting that degradation becomes more substantial at this temperature. The goal was to ensure that the astaxanthin content in VCOA remains unaffected during storage and application, as maintaining the stability and quality of astaxanthin-rich oil is essential.

Table 4

FFA levels (%) when VCOA is exposed to heat

Heating time (hours)	*Temperature (°C)*			
	30	*60*	*90*	*120*
0	0.313±0.007	0.337±0.040	0.368±0.015	0.374±0.006
1	0.318±0.004	0.352±0.011	0.415±0.053	0.420±0.026
2	0.311±0.016	0.350±0.013	0.424±0.019	0.557±0.023
3	0.316±0.006	0.369±0.020	0.409±0.016	0.584±0.028
4	0.317±0.001	0.359±0.021	0.396±0.002	0.656±0.010
5	0.306±0.002	0.368±0.021	0.392±0.006	0.656±0.010
6	0.294±0.002	0.455±0.017	0.386±0.032	0.655±0.009

Table 4 shows that the FFA value of VCOA heated for 6 hours at temperatures of 30, 60, and 90° C does not show a significant increase. However, when heated at a temperature of 120°C, there is a significant increase in the FFA value. This indicates that hydrolysis and oxidation processes have occurred in VCOA. High levels of FFA in oil can cause an unpleasant taste and shorten the shelf life of the oil. Damaged oil can cause adverse health effects on the human system due to the accumulation of toxic substances in the oil and products produced with oil as raw material (von Hanstein et al 2020).

Table 5

Peroxide value (meq kg^{-1}) when VCOA is exposed to heat

Heating time (hours)	*Temperature (°C)*			
	30	*60*	*90*	*120*
0	33.33±1.89	30.67±1.89	33.33±1.89	38.67±1.89
1	33.33±1.89	30.67±1.89	29.33±1.89	128.00±3.27
2	33.33±1.89	30.67±1.89	29.33±1.89	169.33±3.77
3	30.67±1.89	33.33±1.89	26.67±1.89	218.67±1.89
4	32.00±3.27	30.67±1.89	28.00±3.27	184.00±5.66
5	33.33±1.89	26.67±1.89	28.00±0.00	174.67±3.77
6	33.33±1.89	26.67±1.89	28.00±3.27	166.67±3.77

Table 5 shows that, in accordance with SNI (2008) standards, the PV of VCOA remained within the acceptable range when heated at 30°C, 60°C, and 90°C, with an average PV between 26.67 and 33.33 meq kg^{-1}. However, at 120°C, the PV of VCOA rose markedly over a 1- to 6-hour heating period, suggesting the initiation of oil oxidation and the onset of rancidity at this elevated temperature. Notably, fluctuations in PV measurements are likely due to concurrent decomposition reactions that occur as peroxide concentrations reach their peak.

Table 6 presents data on the effects of temperature on astaxanthin concentration, FFA levels, and PV in a specific context. As the temperature increases, the astaxanthin concentration shows a declining trend, with significant reduction noted at 120°C (6.99± 3.60 mg L^{-1}) compared to lower temperatures ($p < 0.05$). This suggests that higher temperatures may degrade astaxanthin, likely due to thermal instability.

The FFA levels exhibit an upward trend with increasing temperature, ranging from 0.31±0.01% at 30°C to 0.56±0.12% at 120°C. The significant increase at 120°C ($p < 0.05$) indicates that higher temperatures may promote lipid hydrolysis, contributing to elevated FFA levels.

For PV, an indicator of lipid oxidation, values decrease initially with temperature increases up to 90°C but show a substantial increase at 120°C (154.29±57.56 meq kg^{-1}, $p < 0.05$). This sharp rise at higher temperatures indicates accelerated oxidative degradation, a common effect of prolonged exposure to elevated heat.

Overall, Table 6 underscores the detrimental effects of high-temperature exposure on astaxanthin stability, FFA accumulation, and lipid oxidation, highlighting the importance of controlling temperature to maintain bioactive and lipid quality.

Table 6

Statistical analysis of astaxanthin, FFA, and PV with temperature

Temperature (°C)	*Concentration of astaxanthin (mg L^{-1})*	*FFA levels (%)*	*Peroxide value (meq kg^{-1})*
30	11.54±0.2[a]	0.31±0.01[a]	32.76±1.04[a]
60	10.99±0.62[a]	0.37±0.04[a]	29.91±2.42[a]
90	10.56±0.74[a]	0.40±0.02[ab]	28.95±2.14[a]
120	6.99±3.60[b]	0.56±0.12[b]	154.29±57.56[b]

Note: values with different superscripts within the same column are significantly different ($p < 0.05$).

Discussion. Based on the results of Table 1, the VCO sample had a weak ability to scavenge free radicals, with an IC_{50} value of 164.89 mg L^{-1}. Meanwhile, VCOA had a very strong ability to scavenge free radicals, with an IC_{50} value of 31.67 mg L^{-1} indicating a relatively high antioxidant activity. This is similar to the findings on *Tetraselmis suecica* microalgae, where the IC_{50} value was 37.32 mg L^{-1}, which is higher than the IC_{50} value of VCOA even though VCOA is classified as a very strong antioxidant (Sedjati et al 2021).

The strength of this VCOA antioxidant is due to the presence of astaxanthin, which has a stronger activity compared to vitamin E, vitamin C, and beta-carotene (Sztretye et al 2019; Singh et al 2021). Astaxanthin contains conjugated double bonds, hydroxyl groups, and a ketone group. The conjugated double bond also gives the red color to astaxanthin. The conjugated double bond acts as an electron donor and reacts with free radicals to neutralize and stop the chain reaction of free radicals (Jiang et al 2020). Astaxanthin also has more hydroxyl groups, making it a better antioxidant.

The results of photostability show that it does not have a significant effect on the FFB value of VCOA. The increase in FFB value in oil is generally caused by the oxidation process of the oil, as seen in Table 2. In this case, the presence of astaxanthin can inhibit the increase in the FFB value. Additionally, the fatty acids in VCO have the highest amount of saturated bonds compared to other oils, making VCO more resistant to light. Based on the Indonesian National Standard (2008), the FFB value of VCOA slightly exceeds the limit, as the maximum FFB limit for VCO is 0.2% (calculated as lauric acid). The high FFB value in VCOA is possibly influenced by the pH of fermented *Acetes* sp.,

which ranges from 4 to 5, causing acidity in VCOA. However, if referring to the SNI for animal oil, this value is still within the quality range, which is between 1 and 8.

PV from VCOA shows a decrease, but still remains in the SNI quality range. This shows that even though it was exposed to continuous light for 6 hours, no lipid oxidation occurred in VCOA. This is likely to happen because VCOA contains astaxanthin, an antioxidant compound that can slow down lipid oxidation (Ekpe et al 2018). Karppi et al (2007) and Choi et al (2011) reported that the ability of astaxanthin to maintain a lipid profile, including preventing lipid oxidation, is due to its strong antioxidant properties. Astaxanthin is a natural carotenoid with powerful antioxidant properties both *in vitro* and *in vivo*. Like other carotenoids, astaxanthin can absorb excited singlet oxygen energy into its carotenoid chain, leading to degradation of the carotenoid molecule but preventing damage to other molecules or tissues from radicals. The terminal astaxanthin ring is capable of trapping radicals both on the surface and inside the phospholipid membrane. Unsaturated polyene chains only trap radicals in the membrane. Additionally, the possibility of 6 hours is not enough to cause oxidation of lipids in VCOA.

Based on the data shown in Table 3, it can be seen that the concentration of astaxanthin does not show any changes when VCOA is heated at temperatures of 30 and 60^{o}C for 6 hours. Heating at a temperature of 90^{o}C causes a decrease in the concentration of astaxanthin in VCOA, but it is not significant. During the 6-hour heating, the concentration of astaxanthin in VCOA decreased from 11.63±0.69 to 9.93±0.90 mg L^{-1}. This indicates that astaxanthin in VCOA is relatively stable up to 90^{o}C. However, heating at 120^{o}C causes a significant decrease in the concentration of astaxanthin, indicating that more astaxanthin is being degraded. Astaxanthin works by neutralizing free radicals, preventing chain reactions that can damage cells.

Based on the data shown in Table 4, VCOA still shows stability when heated up to a temperature of 90^{o}C. The results obtained in this study are consistent with Zhuang et al (2022), who reported that certain vegetable oils still maintain their quality if the processing temperature is below 100^{o}C. However, during processing above 100^{o}C, unsaturated fatty acid oxidation leads to the formation of various radicals, including •CH_3, •CO, and •CHO, which can then result in the formation of malondialdehyde (MDA), α-dicarbonyl compounds (α-DCs) such as glyoxal (GO), methylglyoxal (MGO), and 2,3-butanedione (2,3-BD), α,β-unsaturated aldehydes such as 4-hydroxy-2-hexenal (4-HHE) and 4-hydroxy-2-nonenal (4-HNE), and easily volatile oxidation products such as 2-butene and pentanal (Jiang et al 2013; Ma et al 2019; Zhuang et al 2022).

Based on the data shown in Table 5, the peroxide number of stabilized VCOA remains stable up to a temperature of 90°C. In this case, there is no increase in the peroxide number of VCOA due to heating at temperatures of 30, 60, and 90°C, even when heated for 6 hours. However, a decrease in the peroxide number of VCOA occurs at 60 and 90°C with increasing heating time. This is likely due to the decomposition reaction when the peroxide reaches its maximum value, as seen in Table 5. These results are consistent with Ali et al (2022), who reported that adding antioxidants to oil can produce low peroxide values. Astaxanthin in VCOA can prevent rancidity and improve shelf life.

In addition to antioxidants, a decrease in peroxide value also indicates that VCOA contains low molecular weight fatty acids with low unsaturation due to hydrolytic rancidity. Fluctuations in peroxide value can be associated with the decomposition of peroxides formed during primary oxidation into secondary oxidation (Guillen & Cabo 2002). A decrease in peroxide value with increasing heating time has been observed by several researchers (Guillen & Cabo 2002; Nduka et al 2021; Ali et al 2022).

According to SNI 7381:2008, the peroxide number of VCOA due to heating at 30, 60, and 90°C is still classified as good, with an average peroxide number ranging from 24 to 34 meq kg^{-1}. However, at a temperature of 120°C, the peroxide number of VCOA increases significantly, starting from 1 to 6 hours of heating, resulting in rancidity. Based on the results obtained, it can be concluded that VCOA remains stable up to a heating temperature of 90°C.

Conclusions. This research has produced virgin coconut oil enriched with astaxanthin from fermented *Acetes* sp., called VCOA. The results of the study showed that VCOA has

a very strong antioxidant activity, with an IC_{50} of 31.67 mg L^{-1}, stronger than VCO (IC_{50} 164.89 mg L^{-1}). Exposure to light of 300 lux for 6 hours did not decrease the quality of VCOA in terms of astaxanthin concentration, FFAumin level, and peroxide value. Similarly, heating process only reduced the quality of VCOA when heated above 90°C. Thanks to its high antioxidant activity and good resistance to light and temperature, VCOA has the potential to be a valuable compound for use in the health field, in both food and cosmetics.

Conflict of interest. The authors declare that there is no conflict of interest.

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