OCL 2023, 30, 4

https://doi.org/10.1051/ocl/2023002

OCL Oilseeds & fats Crops and Lipids

Available online at:
www.ocl-journal.org

RESEARCH ARTICLE OPEN ACCESS

Extraction of astaxanthin from fermented *Acetes* using virgin coconut oil with the glass beads vortex method★

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Received 12 June 2022 – Accepted 24 January 2023

Abstract – Astaxanthin is an antioxidant that can be extracted from crustaceans such as shrimp, lobster, crawfish, and crabs. Fermented *Acetes* shrimp (called cincalok) is a traditional side dish from Indonesia rich in astaxanthin. This study extracted astaxanthin from cincalok using the glass beads vortex method with virgin coconut oil (VCO) as a green solvent. Several parameters that affect the amount of astaxanthin extracted, such as the ratio between sample and solvent, contact time, and particle size of glass beads, have been tested. The physicochemical characteristics of VCO before and after extraction were also analyzed. The UV-Vis spectrophotometer analysis results showed that the optimum ratio between sample and solvent was 1:10 g/mL, the optimum contact time was 15 minutes, and the optimum particle size of the glass beads was 60–80 mesh. Under these optimum conditions, the amount of astaxanthin extracted was 27.97 μg/g dry weight of cincalok or 2.79 μg/mL VCO. After extraction, the viscosity and density of VCO still meet the Indonesian National Standard with values of 17.98 mm^2/s and 917.2 kg/m^3, respectively. Before and after extraction, the GC-MS chromatogram shows the main component of fatty acids in VCO is lauric acid, with a percentage of 27.28 and 26.72%, respectively. VCO can also extract omega-3 fatty acids DHA and EPA from cincalok with the same rate of 0.04%.

Keywords: astaxanthin / extraction / fermented *Acetes* / glass beads vortex / virgin coconut oil

Résumé – Extraction de l'astaxanthine de crevettes *Acetes* fermentées à l'aide d'huile de noix de coco vierge par la méthode du vortex avec des billes de verre. L'astaxanthine est un antioxydant qui peut être extrait de crustacés tels que les crevettes, les homards, les écrevisses et les crabes. La crevette *Acetes* fermentée (appelée cincalok) est un plat d'accompagnement traditionnel d'Indonésie riche en astaxanthine. Cette étude a permis d'extraire l'astaxanthine du cincalok en utilisant la méthode du vortex de billes de verre avec, comme solvant vert, de l'huile de noix de coco vierge (VCO). Plusieurs paramètres affectant la quantité d'astaxanthine extraite, tels que le rapport entre l'échantillon et le solvant, le temps de contact et la taille des particules des billes de verre, ont été testés. Les caractéristiques physicochimiques de VCO avant et après extraction ont également été analysées. Les résultats de l'analyse au spectrophotomètre UV-Vis ont montré que le rapport optimal entre l'échantillon et le solvant était de 1:10 g/mL, le temps de contact optimal était de 15 minutes, et la taille optimale des particules des billes de verre était de 60–80 mesh. Dans ces conditions optimales, la quantité d'astaxanthine extraite était de 27,97 μg/g de poids sec de cincalok ou 2,79 μg/mL de VCO. Après extraction, la viscosité et la densité du VCO sont toujours conformes à la norme nationale indonésienne avec des valeurs de 17,98 mm^2/s et 917,2 kg/m^3, respectivement. Avant et après extraction, le chromatogramme GC-MS montre que le principal composant des acides gras dans le VCO est l'acide laurique, avec un pourcentage de 27,28 et 26,72 %, respectivement. Le VCO peut également extraire les acides gras oméga-3 DHA et EPA du cincalok avec le même taux de 0,04 %.

Mots clés : astaxanthine / extraction / acétates fermentés / vortex de billes de verre / huile de coco vierge

★ Contribution to the Topical Issue "Lipids from aquatic environments / Lipides issus des milieux aquatiques"

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Highlight

- Cincalok, a traditional side dish in Indonesia, is rich in astaxanthin.
- Astaxanthin compounds are soluble in oil solvents such as virgin coconut oil.
- The glass bead vortex method by using VCO as a solvent is a potential method that can be used for extracting astaxanthin.
- This method can also extract omega-3 fatty acids DHA and EPA from cincalok.

1 Introduction

Rebon (*Acetes* sp.) is one of the marine products of the type of crustacean with a tiny size compared to other types of shrimp. The active compounds in *Acetes* shrimp include chitosan, minerals, lipids, and carotenoids; astaxanthin is the highest carotenoid (Trung and Phoung, 2012). *Acetes* shrimp are widely used to make fermented products such as shrimp paste and cincalok. Cincalok is a traditional side dish in West Kalimantan Province, Indonesia, made by spontaneous fermentation of *Acetes* shrimp for 7–15 days. Fermentation becomes a crucial process because it involves lactic acid, which can dissolve calcium salts in shrimp that contain pigment. This process can release astaxanthin from protein and increase astaxanthin levels when extracted (Nofiani and Ardiningsih, 2018; Rahmalia *et al.*, 2022).

Among almost all extraction methods, one of crucial points in their process is the solvent used; it has ability to separate analytes of interests from a mixture of compounds due to affinity with the solvent. In case of astaxanthin, it is hydrophobic with limited solubility in water. Efforts have, therefore, recently been made to identify the most appropriate solvent for this molecule, to increase extraction efficiency. Mauludia *et al.* (2021) reported that astaxanthin extracted from cincalok using acetone was 14.7 μg/g wet weight, higher than unfermented *Acetes* shrimp (2.55 μg/g). In the other study, Prayitno *et al.* (2022) reported the maximum astaxanthin extraction yield from cincalok when used virgin coconut oil as a solvent is 100.62 μg/g dry weight. Rahmalia *et al.* (2022) have reported that cincalok oil extracted using n-hexane by the soxhletation method contained 0.38 mg/L astaxanthin.

Astaxanthin (3,3'-dihydroxy-β'-carotene 4,4'-dione) is a carotenoid compound composed of 40 carbon atoms connected by single and double bonds that form a phytoene chain. Astaxanthin contains both a hydroxyl and a keto group, and this unique structure plays important roles in neutralizing reactive oxygen singlet (ROS) (Brotosudarmo *et al.*, 2020). Astaxanthin application has been well documented for over two decades and is currently the primary market driver for the pigment. Synthetic astaxanthin dominates the world market, but recent interest in natural pigment sources has increased substantially. Astaxanthin can be obtained from aquatic organisms such as shrimp, salmon, and lobster and is produced by several microorganisms such as algae, yeast, and bacteria by giving these species a characteristic pink color (Higuera-Ciapara *et al.*, 2006).

Several methods have been developed to extract astaxanthin, including maceration, supercritical fluid, and enzymatic. However, each of these methods has drawbacks. Karnila *et al.* (2020) was extracted astaxanthin from the carapace of *L. vannamei* using the maceration method with toxic solvents such as chloroform. This process also takes quite a long time, which was 12 hours. Whereas Wang *et al.* (2012) extracted astaxanthin from the *Haematococcus pluvialis* using the supercritical fluid method requiring a cosolvent such as ethanol and taking place at high pressure (43.5 MPa). The enzymatic method requires a long time for the incubation process (Karnila *et al.*, 2020; Lindahl *et al.*, 2013). Recently, Prayitno *et al.* (2022) extracted astaxanthin in cincalok using the ultrasonication method and various vegetable oils as solvent. They reported that the best astaxanthin extraction yield was 100.62 mg/g with virgin coconut oil (VCO) as solvent. In this study, the other extraction method used is the glass beads vortex, which uses glass beads (broken glass). This method functions in the process of cell lysis due to the collision of glass beads with cells. The collision can cause damage to the cell wall, so this process is sure also to remove the astaxanthin content that is still in the cell (Hermana *et al.*, 2005). Solvent selection is also vital in the exploration of potentially active components.

This study also refers to the principle of green chemistry. The solvent used in this study is the vegetable oil for extracting astaxanthin in cincalok, as Prayitno *et al.* (2022) reported, namely VCO, which is pure coconut oil made from fresh coconut meat. As an extraction solvent, VCO has advantages such as being easy to obtain because the availability of coconut oil is easily found in tropical country such as Indonesia, the price is low and it is safe for consumption (non-toxic) (Handayani *et al.*, 2008). In addition, because of the high content of medium-chain fatty acids such as lauric acid, VCO is claimed as a healthy food (Cassiday, 2016).

Astaxanthin in shrimp is usually found in esterified form with fatty acids and a little in free form, so astaxanthin in shrimp will be more soluble in oil solvents (Clark *et al.*, 2000). Sindhu and Sherief (2011) have extracted astaxanthin from red shrimp shell waste using various vegetable oils and reported that coconut oil could attract the most astaxanthin compounds (3.32 ± 0.23 μg/g dry weight) compared to soybean and sunflower oil (1.36 ± 0.15 μg/g and 1.48 ± 0.20 μg/g, respectively). Coconut oil or VCO contains more medium-chain fatty acids (C6–C12) than long-chain fatty acids (C12–C18) (Cassiday, 2016). Meanwhile, soybean and sunflower oil are the opposite. Based on the fatty acid content, the polarity of VCO is higher than that of soybean or sunflower oil. Astaxanthin is a carotenoid with several ionone rings reveals more polar than the other carotenoids. Hence, astaxanthin is more soluble in VCO than soybean or sunflower oil.

Hence, this study was conducted to extracted astaxanthin from cincalok using the glass bead vortex method with VCO as a solvent. In this process, it is not necessary to separate or isolate astaxanthin from the solvent. The extracted product is VCO enriched with astaxanthin, which can be used in various applications such as healthy food and cosmetics.

2 Materials and methods

2.1 Materials

Acetes sp. were obtained from Ketapang Waters, West Kalimantan, Indonesia. Standard astaxanthin with 98% purity was purchased from Sigma Aldrich (SML0982 SIGMA) and stored in the dark at −4 °C. Other ingredients include acetone (Merck), sugar, salt, glass beads, and VCO (VICO).

2.2 Methods

2.2.1 Cincalok preparation

The cincalok was made by adopting the method of Prayitno *et al.* (2022). Fresh *Acetes* shrimp were washed with seawater to remove impurities such as sand, stones, and small fish. A total of 10 kg of clean *Acetes* were added with 1 kg of sugar and 1 kg of salt and mixed until blended. The mixture was stored in a plastic container that had been covered with aluminum foil and stored at room temperature for 8 days. Cincalok, produced after eight days of fermentation, was then filtered. The residue was dried using vacuum drying at a temperature of 50 °C for three hours. Dried cincalok was ground and ready for further treatment.

2.2.2 Astaxanthin extraction

2.2.2.1 Effect of sample and solvent ratio

The cincalok sample was added with VCO. The various samples and solvents ratios (1:2.5, 1:5, 1:10, and 1:15 w/v) was applied. The extraction process was carried out with 60–80 mesh particle size of glass beads using vortex for 6 × 5 minutes with a break of 5 minutes each (total vortex time was 30 minutes). After that, the mixture was centrifuged to separate the filtrate and residue. The filtrate was analyzed using a UV-Vis spectrophotometer to determine the extracted astaxanthin content. The experiment was carried out three times.

2.2.2.2 Effect of extraction time

The cincalok sample was added with VCO with the optimal ratio obtained from the previous stage. The extraction process was carried out with 60–80 mesh particle size of glass beads using vortex by the variating total time of 15, 30, 45, and 60 minutes. After that, the mixture was centrifuged to separate the filtrate and residue. The filtrate was analyzed using a UV-Vis spectrophotometer to determine the extracted astaxanthin content. The experiment was carried out three times.

2.2.2.3 Effect of glass beads size

The dried cincalok was added with VCO with the optimal ratio obtained from the previous stage. The extraction process was carried out with various particle sizes of glass beads (40–60, 60–80, and 80–100 mesh) using vortex for an optimum time obtained from the second stage. After that, the mixture was centrifuged to separate the filtrate and residue. The filtrate was analyzed using a UV-Vis spectrophotometer to determine the extracted astaxanthin content. The experiment was carried out three times.

2.2.2.4 Extracted astaxanthin content analysis

Astaxanthin levels were calculated as total carotenoids in the sample by adopting the method of Prayitno *et al.* (2022). In the first stage, standard astaxanthin solution was prepared in acetone with various concentrations of 0.4375, 0.875, 1.75, 3.5, and 7 ppm. The absorbance of each solution was measured at the maximum wavelength in the range 300–700 nm, then a linear curve of the relationship between absorbance and concentration was made, which will be used to calculate astaxanthin levels in the sample.

As much as 1 mL of each extracted filtrate was put into 5 mL of measuring flask, then added with acetone until the mark. The mixture was shaken until homogeneous; the absorption was read at the maximum wavelength obtained previously from the standard astaxanthin. The treatment was repeated 3 times. Pure VCO before extraction was also given the same treatment to see the absorption of VCO in acetone in the wavelength range of 300–700 nm. The absorbances obtained at the maximum wavelength were converted into concentration units through the resulting equation on the obtained calibration curve.

2.2.2.5 Data analysis

The relationship between the extracted astaxanthin content and test parameters such as sample and solvent ratio, extraction time, and glass beads size was analyzed using One-Way ANOVA with a 95% confidence level. A One-Way ANOVA test was conducted to determine whether there was a significant difference between astaxanthin levels in each extraction parameter effect.

3 Results and discussion

3.1 Cincalok manufacturing and processing

Cincalok was made by a fermentation process. The ingredients used in this process are shrimp, salt, and sugar. Salt added to the fermentation process aims to inhibit the growth of spoilage bacteria. While the addition of sugar aims to make the product tastes sweet and stimulate the growth of lactic acid bacteria (LAB) (Destrosier, 1988). Before the extraction process, the cincalok sample obtained was then filtered. Cincalok that has been filtered is then dried using vacuum drying for 3–6 hours at a temperature of 50 °C, which aims to dry or reduce the water content contained in cincalok so that it is not quickly overgrown with fungi which can cause the product to rot (Jha *et al.*, 2015). Drying was carried out at a temperature of 50 °C to keep astaxanthin stable. It is due to the stable temperature of astaxanthin being below 70 °C (Ambati *et al.*, 2014). The resulting dried cincalok was then ground. This process aims to reduce the size and increase the surface area of the sample so that in the astaxanthin extraction process, the contact of the sample with the solvent will increase, which can optimize the extraction of the target compound in cincalok. Cincalok and dry cincalok produced in this work can be seen in Figure 1.

3.2 Astaxanthin extraction

Astaxanthin was extracted in cincalok using the glass beads vortex method with VCO as a solvent. The study results

Fig. 1. Cincalok (left), and dried cincalok (right).

Fig. 2. VCO and cincalok residues before (a) and after (b) extraction.

on the centrifugation process showed that the cincalok oil extract was at the top while the residue was at the bottom (Wong and Hartina, 2014). This is because the specific gravity of the cincalok residue is heavier than the specific gravity of the cincalok oil extract. The results of the extraction are presented in Figure 2.

In this study, VCO after extraction was analyzed using thin-layer chromatography (TLC), and it was compared with standard astaxanthin. The stationary phase used in the form of 60 F_{254} silica gel, while the mobile or eluent phase is acetone and hexane with a ratio (1:4 v/v). The results of TLC of VCO after extraction, a mixture of VCO after extraction and standard astaxanthin, and standard astaxanthin are presented in Figure 3. The results of the TLC analysis prove the extraction results contain astaxanthin with Rf of 0.19.

A UV-Vis spectrophotometer analysis was conducted to determine the astaxanthin concentration in VCO after extraction. In this case, astaxanthin is calculated as a total carotenoid, as has also been done by Prayitno *et al.* (2022). Based on Figure 4, standard astaxanthin dissolved in acetone shows an absorption spectrum in the range of 300–600 nm. Conjugated double bonds in carotenoid structures can cause an absorption spectrum at visible light wavelengths (Rahmalia *et al.*, 2014). In the standard astaxanthin spectrum, there is only one peak whose maximum wavelength is 477 nm in accordance with previous studies (Rahmalia *et al.*, 2022; Prayitno *et al.*, 2022). Therefore, the calculation of astaxanthin extracted in VCO was carried out at 477 nm. The standard astaxanthin standard curve has a linear regression value ($R = 0.9996$), indicating a linear relationship between absorbance and astaxanthin concentration according to Lamber-Beer's law where $y = 0.2443 \times +0.0141$. The gradient and intercept values obtained are also similar to previous studies (Rahmalia *et al.*, 2022; Prayitno *et al.*, 2022).

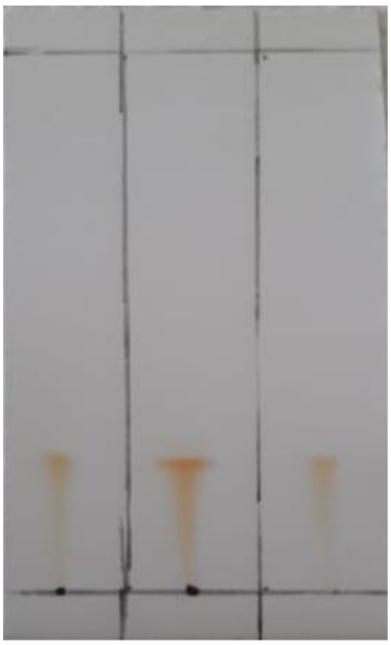

Fig. 3. Results of TLC of VCO after extraction (a), a mixture of VCO after extraction and standard astaxanthin (b), and standard astaxanthin (c).

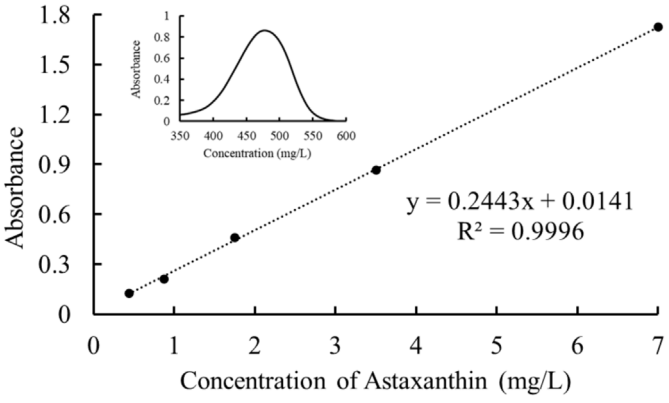

Fig. 4. Spectrum and calibration curve of standard astaxanthin.

3.2.1 Effect of sample and solvent ratio on extracted astaxanthin content

Table 1 shows that the effect of the sample and solvent ratio of the ratio 1:2.5; 1:5; 1:10; and 1:15 w/v resulted in extracted astaxanthin levels of 10.00; 19.24; 27.97, and 26.78 g/g. The results of SPSS analysis with ANOVA test showed a significant difference, namely 0.000 ($P < 0.05$), between the ratios of 1:2.5 w/v, which were significantly different from the ratios of 1:5, 1:10, and 1:15 w/v. The 1:5 w/v ratio is also significantly different from the 1:2.5 ratio; 1:10 and 1:15 w/v, while the ratio of 1:10 w/v is significantly different from 1:2.5 and 1:5 w/v, but not significantly different from 1:15 w/v and vice versa. 1:15 w/v was significantly different with the ratio 1:2.5 and 1:5 w/v but not significantly different with 1:10 w/v.

Table 1. Measurement results of sample and solvent ratio on extracted astaxanthin content.

Sample and solvent ratio (g/mL)	Average (μg/g) ± SD*	%RSD
1:2.5	10.00 ± 0.57^{bcd}	5.70
1:5.0	19.23 ± 0.66^{acd}	3.34
1:10	27.92 ± 0.35^{ab}	1.25
1:15	26.70 ± 1.75^{ab}	6.53

* Lowercase letters indicate a significant difference between the levels of astaxanthin in each ratio.

Table 2. Measurement results of time effect on extracted astaxanthin levels.

Extraction time (minute)	Average (μg/g) ± SD*	%RSD
5	15.56 ± 3.32^{cd}	21.3
10	19.23 ± 1.16^{c}	6.03
15	27.92 ± 0.35^{abdef}	1.25
20	20.11 ± 1.50^{ac}	7.54
25	18.89 ± 1.02^{c}	5.39
30	18.82 ± 1.33^{c}	7.07

* Lowercase letters indicate a significant difference between the levels of astaxanthin at each time.

Based on the analysis, the ratio of the sample and the solvent used affects the yield of the extraction of astaxanthin. The ratio of sample and solvent until a ratio of 1:10 w/v increased astaxanthin levels and resulted in the most considerable astaxanthin levels that were not significantly different from the ratio of 1:15 w/v. However, the levels of astaxanthin produced slightly decreased. In this condition, VCO was already at the saturation point, and the intensity of the cavitation process was reduced. Therefore, there will be no increase in extraction yield with the addition of solvent (Brennan, 2006).

3.2.2 Effect of time on extracted astaxanthin levels

Table 2 shows that the contact time or extraction time affected the amount of astaxanthin extracted. At the extraction time of 5, 10, 15, 20, 25, and 30 minutes the extracted astaxanthin levels were 12.88; 19.28; 27.97; 20.14; 18.87 and 18.79 g/g. The results of the SPSS analysis with the ANOVA test showed a significant difference, namely 0.000 ($P < 0.05$) between 5 minutes, significantly different from 15 and 20 minutes, but not significantly different from 10, 25, and 30 minutes. The time of 10, 25, and 30 minutes was significantly different from 15 minutes. The time of 15 minutes was significantly different from the time of 5.10, 20, 25, and 30 minutes. Meanwhile, 20 minutes was significantly different from 5 and 15 minutes but not significantly different from 10, 25, and 30 minutes.

Based on the analysis, the extraction time used influences the astaxanthin extraction level. The effect of extraction time

Table 3. Measurement results of glass beads size effect on extracted astaxanthin content.

Glass beads size (mesh)	Average (μg/g) ± SD*	%RSD
40–60	19.09 ± 0.38^{bc}	1.99
60–80	27.92 ± 0.35^{ac}	1.25
80–100	13.65 ± 1.27^{ab}	9.30

* Lowercase letters indicate a significant difference between the levels of astaxanthin at each size of glass beads.

Table 4. VCO quality requirements according to Indonesian National Standard (SNI).

No.	Test parameter	Unit	Requirements
1	Density	kg/m^3	915.0–920.0
2	Viscosity	–	–
3	%FFA (calculated as lauric acid)	%	Maximum 0.2

up to 15 minutes increased astaxanthin levels and resulted in the most considerable astaxanthin levels, which were significantly different at 20, 25, and 30 minutes resulting in decreased astaxanthin levels. During the first 15 minutes, the extraction was fast, and the longer the extraction process, the higher the level of astaxanthin extracted. This is because of the length of the contact time between the sample and the solvent increases. During the extraction process, the cell membrane breaks due to the pressure difference between inside and outside the cell, which causes the astaxanthin content to increase. However, after 15 minutes, the amount of astaxanthin extracted decreased for a long time during the extraction process. This happens because extraction using too long a time can also make the solvent quickly saturated and unable to extract optimally to reduce the value of astaxanthin levels.

3.2.3 Effect of glass beads size on extracted astaxanthin content

Table 3 shows that the extraction using glass beads measuring 40–60, 60–80, and 80–100 mesh resulted in astaxanthin levels of 19.12, 27.97, and 13.65 g/g, respectively. The results of SPSS analysis with ANOVA test showed a significant difference, namely 0.000 ($P < 0.05$) between sizes 40–60, 60–80, and 80–100 mesh. Based on the analysis, the size of the glass beads used influences the extraction of astaxanthin.

The effect of glass beads size up to 60–80 mesh size increases astaxanthin content and produces the most considerable astaxanthin content, which is significantly different with 80–100 mesh size produces the most negligible astaxanthin content. These results indicate that the most optimal use of glass beads is found in the glass beads size of 60–80 mesh, which produces the most considerable astaxanthin content. This is because the smaller the size of the glass beads used, it can cause the breakdown of cell walls and membranes in the material, resulting in many damaged cell walls which can then make it easier for compounds in the material to rise to the

Table 5. GC-MS interpretation results.

Fatty Acid	Formula	Percentage (%)	
		VCO before extraction	VCO after extraction
Caproic acid	C6:0	1.19	1.14
Caprylic acid	C8:0	9.21	9.09
Pelargonic acid	C9:0	0.02	0.02
Capric acid	C10:0	8.97	8.80
Hendecanoic acid	C11:0	0.08	0.08
Lauric acid	C12:0	27.28	26.72
Tridecanoic acid	C13:0	0.11	0.11
Myristic acid	C14:0	17.46	17.42
Pentadecanoic acid	C15:0	0.04	0.04
Palmitic acid	C16:0	13.11	13.32
Palmitoleic acid	C16:1	–	0.08
Margaric acid	C17:0	0.08	0.05
Oleic acid	C18:1	11.21	11.52
Linoleic acid	C18:2 (n-6)	3.17	3.40
Arachidic acid	C20:0	0.12	0.15
Stearic acid	C18:0	7.47	7.29
Eicosapentaenoic acid (EPA)	C20:5	–	0.04
Arachidonic acid	C20:4	–	0.01
Nonadecanoic acid	C21	0.24	0.31
Docosahexaenoic acid (DHA)	C22:6 (n-3)	–	0.04
Docosanoic acid	C22:0	0.03	0.09
Lignoceric acid	C24:0	0.05	0.07

surface and make it easier for solvents to attract compounds from the material (Nwabanne, 2012). These results follow Sembiring *et al.* (2006) research, which showed that the particle size of 60 mesh was the best treatment for ginger extraction using glass beads. While the extraction process using glass beads with a size of 80–100 mesh (about 0.18–0.15 mm) produces the most negligible astaxanthin content. This is in accordance with research conducted by Grima *et al.* (2004), that the size of the glass beads of 0.1 mm is effective for breaking down the cell walls of bacteria, while the size of 0.5 mm is more effectively used for breaking down the cell walls of the microalgae species. In addition, the smaller the size of the glass beads used, the longer the extraction time to obtain the maximum extract because the smaller the size of the glass beads, the lower the ability to break down the cell walls.

3.3 Physico-chemical properties of VCO before and after extraction

The VCO quality requirements set according to the Indonesian National Standard (SNI) 7381:2008 are shown in Table 4. VCO solvents were tested for their characteristic properties, then compared with these standards to determine the quality of the oil produced. The characteristics tested were viscosity, density, fatty acid (%FFA) and fatty acid profile based on GC-MS analysis.

The density test results of VCO before and after extraction in this study were 916.7 and 917.2 kg/m^3, respectively, following the VCO quality requirements based on SNI 7381:2008. These results indicate that the VCO before and after extraction is in accordance with the established standards. The VCO density after extraction was slightly higher than the VCO before extraction because the VCO after extraction contained several carotenoid pigment compounds, which caused the VCO density after extraction to be higher. The viscosity test results of VCO before and after extraction in this study were 16.71 and 17.98 mm^2/s, respectively, while the VCO viscosity, according to the SNI, was not explicitly determined. The viscosity of VCO increased after the extraction process was also caused by astaxanthin compounds contained in this oil, which caused higher VCO viscosity after extraction.

Free fatty acids (%FFA) are fatty acids that have been separated from the triglycerides contained in the oil. Free fatty acids produced by hydrolysis and oxidation processes are usually combined with neutral fats (Nurhasnawati *et al.*, 2015). Fatty acids in the oil are undesirable because they increase free fatty acids produce an unpleasant taste and odor. The amount of free fatty acids in the oil can indicate the quality of the oil, where the higher the value of free fatty acids, the lower the quality. In this study, %FFA VCO before and after extraction was obtained with 0.17 and 0.24% results due to the contribution of free fatty acids in cincalok.

The fatty acid profile of VCO before and after extraction was determined using GC-MS analysis, where the results of data interpretation are presented in Table 5. This table shows that the VCO before and after extraction had 25 and 32 detectable fatty acids, respectively. The fatty acids contained in VCO after extraction were more than VCO before extraction. This is because VCO after extraction also contains fatty acid components from cincalok.

The analysis showed that the highest fatty acid component contained in VCO both before and after extraction was lauric acid, with a percentage of 27.28 and 26.72%, respectively. These results follow Bergsson *et al.* (2001) research, which was reported that the most abundant fatty acid in VCO before extraction was lauric acid, which had the highest percentage of 32.73%. Caprylic and capric acids are also present in high percentages of about 8–9% in VCO (both before and after extraction). This is in accordance with Cassiday (2016) who wrote that coconut oil contains medium chain fatty acids such as lauric, caprylic and capric acid. These fatty acids are beneficial for health.

In addition, there are other fatty acids in VCO after extraction, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of 0.04% each arachidonic acid, behenic acid, and palmitoleic acid each of 0.01; 0.01; 0.08% which came from cincalok. Omega-3 fatty acids that have an essential role for health, namely DHA and EPA, are 0.04% each. DHA and EPA are only found in VCO after extraction, and this is in accordance with the research of Balange *et al.* (2017), who reported that dried shrimp contained 8.19 and 13.11% DHA and EPA, respectively.

4 Conclusion

Cincalok is a potential source of astaxanthin. The green extraction method using glass beads vortex with VCO solvent can enrich VCO with astaxanthin and omega-3. The results showed that the optimal condition for astaxanthin extraction from cincalok using the glass beads vortex method with VCO as solvent was using a sample:solvent ratio of 1:10 w/v and a glass beads size of 60–80 mesh for 15 minutes. Under these conditions, VCO was produced containing 2.79 μg/mL VCO, 0.4% DHA, and 0.4% EPA. In this process, the separation or isolation of astaxanthin from the VCO is not necessary. The VCO enriched with astaxanthin and omega-3 can be used directly in various applications such as healthy food and cosmetics. The use of Hansen's theory also becomes a future perspective to determine the best type of green solvent besides VCO for extracting astaxanthin.

Acknowledgments. This study received financial support from Tanjungpura University through the DIPA Mathematics and Natural Sciences Faculty.

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Cite this article as: Fitriani D, Rahmalia W, Ardiningsih P, Usman T, Nurbaeti SN, Prayitno DI. 2023. Extraction of astaxanthin from fermented *Acetes* using virgin coconut oil with the glass beads vortex method. *OCL* 30: 4.