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Characteristics of virgin coconut oil (VCO) microencapsulation using O/W emulsification and complex coacervation methods

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Abstract

Virgin coconut oil (VCO) is produced by processing the flesh of coconut (*Cocos nucifera* L.). It has beneficial health effects due to the medium-chain fatty acids (MCFA), vitamins, and antioxidant contents. Since liquid VCO is not widely accepted by consumers for direct consumption, further processing into powder VCO can be achieved using microencapsulation methods, such as single-layer emulsification (SL), layer-by-layer emulsification (LBL), and complex coacervation (CC). Therefore, the present work aimed to determine the characteristics of microencapsulation of VCO powder by emulsification and coacervation complex methods over 30 days of storage period. Results showed that SL VCO emulsion remained stable for three days of storage, while LBL VCO emulsion experienced syneresis. The particle size of the reconstituted VCO powder was also larger than the fresh emulsion. LBL microcapsule had the highest yield at 74.17%, and there was an increase in moisture content (MC) during 30 days of storage in CC microcapsule. Meanwhile, MC of SL microcapsule was constant, and the equilibrium MC of LBL was retained during 15 days of storage. A microencapsulation efficiency of up to 78.65% was obtained for CC, and the value decreased for all microcapsules during the 30 days of storage. Different oxidative characteristics were also reported, resulting in increased peroxide value of VCO powder. Furthermore, all microcapsules had amorphous and glassy surface morphology, as well as different characteristics and stability during storage.

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Introduction

Coconut oil is one of the main products derived from the processing of coconut (*Cocos nucifera* L.) flesh or copra. Virgin coconut oil (VCO) is different from crude coconut oil (CCO) due to the fundamental differences in the production methods. VCO is obtained through dry extraction process of copra without heat to prevent the alteration of the intrinsic characteristics and nutritional composition (Hee *et al*., 2017). The oil is not subjected to a process of refining, bleaching, or deodorising (RBD) to maintain the nutritional value, and distinctive aroma and taste. The high content of medium-chain fatty acids in VCO increases the metabolic rate in the liver, and reduces the risk of obesity (Assunção *et al*., 2009).

There is a consumer preference challenge associated with the direct consumption of VCO despite its considerable health benefits. This is because the oil contains solid saturated fatty acids at room temperature, which impacts the textural characteristics (Hee *et al*., 2017). Therefore, further processing is required by converting liquid VCO into powdered form using the microencapsulation method. Microencapsulation is a food processing method where VCO droplets are encapsulated within a delicate layer of coating material (Timilsena *et al*., 2017). According to Meirowitz (2018), this process serves to protect VCO from direct interaction with its surrounding environment. Consequently, the encapsulated content within microcapsule can be released either at once or gradually, depending on the specific target within the organs of the body.

The most suitable microencapsulation method for a particular type of sample depends on the core, application of microcapsule, required particle size, physical and chemical properties of the core and walls, release mechanism, scale, and cost of production. The types of microencapsulation methods currently used in the food industry are emulsification and complex coacervation (Meirowitz, 2018). The emulsification method includes making a food

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emulsion, which is a system with at least two immiscible liquid phases. However, this system is unstable, and must be controlled with an agent, commonly referred to as an emulsifier (Norton *et al*., 2013). Furthermore, the complex coacervation method is considered the simplest, cheapest, scalable, reproducible, and most effective method for microencapsulation of hydrophobic materials. This results in very high microencapsulation efficiency with low oil surface, and provides high oxidative stability of the core material (Timilsena *et al*., 2017).

Therefore, the aim of the present work was to evaluate the influence of microencapsulation methods, which were single-layer, layer-by-layer emulsification, and coacervation complex. This was conducted on the properties of VCO emulsion and the stability of powdered oil microencapsulated by oven vacuum drying for 30 days of storage. In this experiment, the emulsifiers used were non-ionic surfactants (Tween 80), a combination of protein and polysaccharide (whey protein isolate and gum Arabic), transglutaminase enzyme as the cross-linking agent for the coacervation complex method, and maltodextrin DE 18-20 for the glass former material with the highest VCO concentration.

Materials and methods

Materials

VCO, Tween 80, and whey protein isolate (WPI) were purchased from Bali Coconut (Indonesia), Croda (UK), and Milk Specialties Global (USA), respectively. Instant gum Arabic (GA) was purchased from El-Nasr for Food Industries (Sudan), maltodextrin DE 18-20 (MD) was purchased from Qin Huang Dao Lihua Starch Co. Ltd. (China), and distilled water was obtained from the Department of Chemistry, Universitas Padjadjaran (Indonesia). Transglutamynase-enzyme (TG) with a nominal activity of 100 U/g powder was purchased from CV. Nura Jaya (Indonesia).

Emulsion preparation

Oil-in-water (O/W) single-layer emulsion (SL)

Tween-80 was mixed with a concentration of 2% (w/w) (of the total oil) into a glass beaker containing 70% (w/w) distilled water, and homogenised using a magnetic stirrer at a speed of 3,500 rpm until complete dissolution to prepare the emulsion. A total of 33% (w/w) (from total solid) was continuously added to the glass beaker during homogenisation using ultrasonic homogeniser at frequency of 20 kHz, amplitude of 70%, and pulse of 3 s off 3 s. Furthermore, the elapsed time for emulsion formation was 20 min, and an ice bath was used to prevent a sudden increase in temperature.

Oil-in-water (O/W) layer-by-layer emulsion (LBL)

Emulsifiers were made from a combination of biopolymers, WPI, and GA. WPI and GA at a ratio of 3:1 [36% (w/w) of the total VCO] were placed in a beaker glass containing 210 mL of distilled water [70% (w/w)], and homogenised using a magnetic stirrer at room temperature with a speed of 3,500 rpm for 3 - 5 min. Subsequently, the pH solution was adjusted to 3.5 using citric acid 10% (w/w), and the biopolymer solution was prepared at 25°C for 24 h to ensure the formation of an equilibrium solution. A total of 9.9% (w/w) VCO was added continuously to a glass beaker containing a biopolymer solution while being homogenised using ultrasonic homogeniser at frequency of 20 kHz, amplitude of 70%, and pulse of 3 s off for 3 s. The elapsed time for emulsion formation was 20 min, and an ice bath was used to prevent a sudden increase in the emulsion temperature.

Oil-in-water (O/W) complex coacervation (CC)

Approximately 9.9% (w/w) of VCO was added to a glass beaker containing WPI solution [2.7% (w/w) in distilled water] continuously during homogenisation using ultrasonic homogeniser at 20 kHz frequency, amplitude 70%, and pulse 3 s on 3 s off for 20 min. After O/W emulsion was formed, GA solution [0.9% (w/w) in distilled water] was added to the mixture of WPI and VCO, and the solution was adjusted to pH 4 by adding 10% (w/w) citric acid to induce electrostatic interactions between WPI and GA. The solution was homogenised using a magnetic stirrer at 800 rpm for 3 h, and the temperature was maintained at 10°C. A cross-linking agent was added in the next step with 2.7% (w/w) transglutaminase enzyme 100 U/g solution at pH 6. In addition, total ratio of solvent and total solid was 70:30. This process was carried out with constant stirring using a magnetic stirrer at 400 rpm for 3 - 6 h, and complex coacervation was monitored by precipitate formation.

Emulsion characterisation

Stability of emulsion

Aliquots of each SL and LBL emulsion were transferred into test tubes, sealed, and stored at 25°C

after the emulsification process. Oil phase separation was monitored daily during the interval between day 0 and day 3 after the preparation of emulsion. The stability (non-phase separation) was expressed quantitatively through the creaming index (CI) after three days of storage, as described in Eq. (1):

$$\%CI = \left(\frac{Hi}{H_0}\right) \times 100 \quad \text{(Eq. 1)}$$

where, H_0 and H_i = initial height of the emulsion, and upper phase after day 1, respectively.

Droplet size of emulsion

The size distribution and mean droplet diameter of emulsion were determined using laser diffraction particle size analyser (Beckman Coulter LS-13 320; Central Laboratory of Universitas Padjadjaran, Indonesia). The analysis was conducted immediately after the preparation of emulsion, in duplicate and three cycles, using distilled water as a dispersant. Furthermore, the mean diameter was obtained and the polydispersity index (PDI) was calculated using Eq. (2):

$$PDI = \left(\frac{\sigma}{d}\right)^2 \quad \text{(Eq. 2)}$$

Microencapsulation by oven vacuum drying

The emulsion produced in the three treatments was added to the glass former material, maltodextrin DE 18-20, at different concentrations [19.9% (w/w) for SL, 16.5% (w/w) for LBL, and 13.8% (w/w) for CC], and homogenised with a magnetic stirrer for 30 min to ensure complete dissolution. For CC, the coacervate phase at the bottom was separated from the supernatant by taking the upper phase using a pipette. In addition, emulsion and coacervate were dried using oven-vacuum drying (Pilot Plant Laboratory, Universitas Padjadjaran, Indonesia). The temperature was adjusted to 60°C, and the drying time ranged from 3 - 5 h, depending on the crust-formed wall material. The microencapsulated VCO was subjected to size reduction using a mortar and pestle, and sieved (40 mesh) to obtain microencapsulated powder with uniform size.

Characterisation of microencapsulated VCO powder
Droplet size of microcapsule

The size distribution and mean droplet diameter of microcapsule were determined using laser diffraction particle size analyser (Beckman Coulter LS-13 320; Central Laboratory of Universitas Padjadjaran, Indonesia). The analysis was conducted immediately after the preparation of microcapsule, in duplicate and three cycles, using distilled water as a dispersant. The mean diameter and the polydispersity index (PDI) were also calculated using Eq. (2).

Yield process (%) of microencapsulation

The VCO powder microcapsule yield process was the ratio of the weights to microcapsule-forming material before being processed, multiplied by 100%. Determination of the yield based on Eq. (3) was calculated according to Hasrini *et al*. (2017), as follows:

$$Yield\ Process\ (\%) = \frac{weight\ of\ microcapsuled\ (g)}{weight\ of\ the\ microcapsules\ forming\ material} \times 100 \quad \text{(Eq. 3)}$$

Colour

The colour of microcapsule was described by the digital colorimetry method using a chromameter (Spektrofotometer CM-5 Konica Minolta), and the instrument was first calibrated using a white reference tile. The results were expressed as L* [(lightness); black (0) to white (100)], a* [green (-) to red (+)], and b* [blue (-) to yellow (+)]. Subsequently, the browning index (BI), chroma, and colour angle were calculated according to Caliskan and Dirim (2016):

$$\text{Browning Index (BI)} = \frac{[100(x-0.31)]}{0.17} \quad \text{(Eq. 4)}$$

where, $x = \frac{(a*+1.75L*)}{(5.645L*+a-3.012b*)}$

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2} \quad \text{(Eq. 5)}$$

$$\text{Hue Angle (°)} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad \text{(Eq. 6)}$$

Moisture content of microcapsule

The moisture content of microcapsule was determined gravimetrically in an oven drying for 3 h at 105°C (BSN, 1992).

Surface oil of microcapsule

The surface oil of microcapsule was determined according to Lim and Roos (2017) (Eq. 7) with slight modifications, and surface oil was measured using washing method. Filter paper containing 2 g of powdered microcapsule was washed

four times with 10 mL of *n*-hexane at each step, and the filtrate was collected in a glass beaker known for its constant weight. It was then stored in a fume hood overnight or until the solvent evaporated completely. The filtrate was dried in an oven at 105°C for 30 min to remove the solvent residue until a constant weight was achieved.

$$\text{Surface Oil (\%)} = \frac{surface\ oil\ (gram)}{weight\ of\ powdered\ microcapsuled\ (gram)} \times 100 \quad \text{(Eq. 7)}$$

Total oil content of microcapsule

The total oil content (Eq. 8) was quantified according to Badan Standardisasi Nasional (BSN, 1992) with slight modification; microcapsule samples being weighed 1 - 2 g into hull (filter paper). VCO powder sample was hydrolysed (the Weibull method) using 20 mL of distilled water and 30 mL of 25% HCl in the fume hood. The boiling stone was added and covered with a cover glass while boiling in a hotplate on a scale of 3 for 15 min. The sample was filtered with two-layer filter paper, and the residue was washed with warm distilled water until the filtrate had a neutral pH. Subsequently, the residue was dried in the oven at a temperature of 105°C overnight to evaporate the remaining water on filter paper. Dried filter paper containing residue was put in a hull, and the hydrolysed sample was extracted using 200 mL of *n*-hexane for 3 h in the Soxhlet instrument to ensure complete oil extraction process. The sample was dried using the oven at a temperature of 105°C for 30 min. The sample drying process was carried out until a constant weight was achieved.

$$\text{Total Oil (\%)} = \frac{Total\ oil\ (gram)}{weight\ of\ powdered\ microcapsuled\ (gram)} \times 100 \quad \text{(Eq. 8)}$$

Microencapsulation efficiency

The microencapsulation efficiency (Eq. 9) was determined by the fraction of encapsulated oil (total oil - surface oil) over the total oil content:

$$\text{Encapsulation Efficiency (\%)} = \frac{(\%\text{total oil} - \%\text{surface oil})}{total\ oil\ (\%)} \times 100 \quad \text{(Eq. 9)}$$

Peroxide value

The peroxide value and the extraction of the oil from microcapsule were determined following the method proposed by the International Dairy Federation (ISO, 2006) and Alcântara *et al*. (2019) with modification. Briefly, 0.5 g sample of VCO powder was weighed into a test tube, and suspended in 5 mL of distilled water. The test tube was shaken for 30 s using a vortex mixer to ensure complete dissolution of powder. Subsequently, 400 µL portion was removed, and 1.5 mL of isooctane/isopropanol (2:1) mixture was added to extract the oil from the powder. The mixture was separated by centrifugation (5,000 rpm for 5 min, 25°C), and the upper phase was collected with micropipette for further analysis. The extractions were performed in triplicate, and 100 µL aliquot of the extraction yield was added to 9.8 mL chloroform/methanol (7:3) mixture solvent. For the complex colour formation, 50 µL of Fe^{2+} solution (clear solution of mixture of 0.4 g of $BaCl_2.2H_2O$ in 50 mL of distilled water, and 0.5 g of $FeSO_4.7H_2O$ in 50 mL of distilled water) and 50 µL of NH_4CNS (7.5 g dissolved in 25 mL of distilled water) were added to the mixture of sample and solvent. The sample was shaken with a vortex mixture, and allowed to stand in the dark for 10 min. Absorbance was then measured at 500 nm, and the peroxide value was determined using a standard curve of Fe^{3+} (1 - 5 µg). The obtained absorbance was corrected with test sample and reagent blanks to obtain the absorbance of red iron (III) complex.

Morphology of particle

The microstructure of microcapsule was observed in scanning electron microscope (SEM) (HITACHI model TM 3000, Japan). The analysed sample was placed on metallic supports (stubs) by adhesion in double-sided metallic tape, and observed in SEM for image acquisition.

Dissolution time of microcapsule in water

The determination of dissolution time of microcapsule in water was performed according to El-Tinay and Ismail (1985). Briefly, 1.0 g sample of VCO powder was weighed into a glass beaker, dissolved in 50 mL of distilled water, and homogenised using magnetic stirrer (892 rpm, 25°C). Subsequently, the dissolution time was calculated for VCO powder until the sample has completely dissolved.

Results and discussion

Characteristics and stability of emulsion

Emulsion is thermodynamically unstable, and tends to emulsify over time resulting in the separation of the oil phase (Zhao *et al*., 2018). The destabilisation process in O/W or W/A emulsion is caused by gravitational separation from the difference in density between the dispersed and continuous phase. This leads to the movement of the dispersed phase droplets to the surface of the sample, and forms a concentrated region known as cream (Hutapea *et al*., 2018; Zhao *et al*., 2018). The formation of the cream increases creaming index value, while emulsion has low stability (Hutapea *et al*., 2018).

Based on the experimental results, SL VCO emulsion using Tween 80 emulsifier [2% (w/w) of the total oil] had good stability during storage. The incorporation of the emulsion particles into larger and unstable molecules can be prevented when the equilibrium of attractive and repulsive forces is maintained or controlled (Mandei, 2019).

LBL VCO emulsion exhibited limited stability during storage, as evidenced by the separation into distinct phases. These include the unseparated emulsion solution and clear serum at the bottom, observed on day 3, a phenomenon commonly referred to as syneresis. According to Laplante *et al*. (2006), syneresis occurs due to a decrease in the net surface charge on the oil droplets, thus resulting in a neutralisation flocculation mechanism that depends on the pH conditions of the system. According to Ozturk *et al*. (2015), WPI forms a thin interfacial layer that can stabilise emulsion by electrostatic force. Destabilisation is caused when there is a decrease in the droplet charge, and the pH approaches the isoelectric point of the adsorbed protein. Therefore, the Van der Walls force dominates the electrostatic force at this pH, thus resulting in flocculation of the droplets.

The instability of LBL VCO emulsion could have been caused by the insufficient amount of surfactant used. The emulsifier WPI and GA used had a concentration of 3.6% (w/w) of the total solution. The lack of concentration could cause the non-optimal coating of the oil droplets, thus affecting the process of forming the multi-molecular layer (Husni *et al*., 2019). Furthermore, Laplante *et al*. (2006) stated that the weak hydrophobicity caused the low efficiency of absorption of polysaccharides such as GA on the oil surface, thus reducing the steric stabilisation mechanism against the increase in the strength of the emulsion which caused flocculation, neutralisation, and syneresis.

In the present work, SL emulsion had an average particle diameter size of 1.4805 ± 0.013, 1.1357 ± 0.023 for LBL emulsion, and 1.4607 ± 0.071 for CC particles (Table 1). According to Roldan-Cruz *et al*. (2016), the minimum threshold concentration of the surfactant Tween 80 required for SL emulsion is 2%. At this concentration, further reduction in the particle diameter of emulsion through the addition of additional surfactants becomes exceedingly challenging. This limitation arises from Tween-80 constrained capacity to effectively stabilise the curvature of small droplets.

Table 1. Stability and particle size of emulsions and coacervates.

Sample	% CI after 3rd day	Mean particle size (μm)	PDI
SL emulsion	0	1.48 ± 0.01	0.13 ± 0.01
LBL emulsion	0*	1.14 ± 0.02	0.22 ± 0.03
CC coacervates	-	1.46 ± 0.07	0.17 ± 0.03

Values are mean ± standard deviation. Asterisk (*) indicates that syneresis has occurred in LBL emulsion.

The mean particle diameter of LBL emulsion was the lowest as compared to the other treatments. This can be caused by the use of an emulsifier mixture of WPI with higher concentration than GA. The mean size of the emulsion particle diameter in CC was slightly larger than LBL, caused by the length of the agitation process during the formation of the coacervate. This phenomenon could be attributed to the stirring process, and when the oil droplets are formed in a continuous phase, the oil droplets will move continuously, collide, and make the particle size larger (Carpenter *et al*., 2020).

Polydispersity index (PDI) is a measure of the uniformity of particle size in a sample. According to ISO 22412:2017 (ISO, 2017), PDI value of < 0.05 indicates that the sample belongs to the monodispersed standard (uniform), while > 0.7 is classified as polydispersed, or has a very wide particle distribution. There is no established standard for the minimum acceptable PDI value for specific products. Based on experimental results, PDI value for the three treatment groups exceeded the monodispersed standard.

Characteristics of microcapsule

Microencapsulation yield

A high yield value indicates that the running drying process is efficient (Hasrini *et al*., 2017). Based on the experimental results shown in Table 2, the treatments with the highest and lowest yield percentages were LBL and CC, and the last was SL VCO powder. According to Karrar *et al*. (2020), GA has a very good ability in film-forming. The combination of WPI-GA-MD is considered an efficient combination of emulsifier and film-forming where GA functions as a matrix-forming and filler. Emulsion made from a mixture of WPI and GA biopolymers tends to have a higher viscosity than using Tween-80 as an emulsifier. This is because WPI has the ability to bind and retain water as well as increase the viscosity of the emulsion. However, the SL VCO powder with the lowest yield value among other treatments can be caused by the large number of liquid emulsion samples that collapse and stick to the walls of the vacuum oven dryer, and are difficult to recover, hence less VCO powder can be dried.

Colour

The SL VCO powder had lower L* value than the other two treatments as shown in Table 2. SL and CC VCO powders had negative a* value, where the samples were green. Meanwhile, LBL VCO powder was red as evidenced by positive a* value. The b* values of the three treatments were positive; yellowish colour.

According to Di Giorgio *et al*. (2019), the difference in the emulsification process does not have a significant effect on the colour of the powder sample, but is influenced by the formulation or use of coating materials in the microencapsulation process. The addition of protein fraction causes the microencapsulated results to have higher brightness level (L*), and increases the b* value. Therefore, the powder turns yellowish, and reduces the change in colour value (ΔE*). Based on the experimental results, the colour angle in the three samples showed reddish colour; value < 10° (Mahdavee Khazaei *et al*., 2014).

According to Şahin Nadeem *et al*. (2011), the addition of larger amount of maltodextrin in the microencapsulated formulation increases L* value in the powder. This contrasted with the experimental results where VCO powder with single layer emulsification method had the largest maltodextrin content in its formulation as compared to other treatments. The drying process of microcapsule causes a non-enzymatic browning reaction seen from the value of the browning index (BI). According to Caliskan and Dirim (2016), the BI value can be reduced by increasing the amount of maltodextrin in the microencapsulated formulation.

The browning reaction in the present work was non-enzymatic, and produced in thermally-treated food (Rufián-Henares *et al*., 2009), namely caramelisation and Maillard reaction. The caramelisation reaction occurred because maltodextrin used as one of the coating materials is a saccharide polymer $(C_6H_{10}O_5)n.H_2O$ which is composed of monomers in the form of glucose bound. Meanwhile, Maillard reaction occurred due to the presence of reducing sugars from the addition of maltodextrin as well as amino acids from WPI and GA.

Moisture content

Factors indicating the quality of powder and the stability during storage include moisture content, glass transition temperature (T_g), and water activity (a_w) (Karrar *et al*., 2020). Based on the experimental results shown in Table 3, the moisture content in the three treatments ranged from 1.89 - 5.13% in dry basis. In SL VCO powder, the moisture content of microcapsule was constant for 30 days of storage. This could have been due to the change in weight being relatively small. Equilibrium moisture content can be affected by relative humidity (RH) and ambient temperature, varieties of materials, and methods of measurement. Even though the moisture content was constant, microcapsule showed tendency to caking or hardening of the powder. This possibility could have been caused by the storage of microcapsule in conditions with a higher water activity (a_w) than CSC (critical storage condition). Microcapsule stored at a_w above CSC causes the coating material to melt, thus resulting in an extensive coalescence as well as triggering a collapse and caking (Escalona-García *et al*., 2016).

The moisture content of LBL VCO powder fluctuated, and the increase on day 15 occurred because the a_w of the material was lower than the RH of the environment. Therefore, the material absorbs water from the surrounding environment, and the opposite occurred in moisture content on day 30. The high value of moisture content in LBL VCO powder could have been caused by the addition of GA, where GA has high molecular weight of 0.25×10^6 - 1.0 ×

Table 2. Characterisation of VCO microcapsules and reconstituted powder.

Sample	Yield (%)	L*	a*	b*	Chroma	Hue angle (°)	Browning index	Dissolution time (s)	Particle size (µm)	PDI
SL	58.87 ± 0.03	79.82 ± 0.46	-0.84 ± 0.44	8.34 ± 1.75	8.38	-1.98	9.98	76.80 ± 11.46	1.58 ± 0.00	0.12 ± 0.00
LBL	74.17 ± 2.75	91.87 ± 0.17	0.33 ± 0.26	12.35 ± 0.28	12.35	3.22	14.36	158.10 ± 10.61	1.53 ± 0.01	0.13 ± 0.00
CC	65.00 ± 7.07	94.14 ± 0.24	-0.23 ± 0.01	8.68 ± 0.29	8.68	2.45	9.25	108.15 ± 22.70	1.38 ± 0.00	0.20 ± 0.00

Values are mean ± standard deviation. Dissolution time, particle size, and polydispersity index (PDI) were obtained from the reconstituted microcapsules of VCO powder.

Table 3. Stability of microcapsules of VCO powder during storage.

Sample	Day(s)	Moisture content (% db)	Encapsulation efficiency (%)	Peroxide value (mEq/kg)
SL	0	1.97 ± 0.55	13.73 ± 2.28	0.4290 ± 0.06
	15	1.89 ± 0.22	8.56 ± 2.93	0.4984 ± 0.05
	30	1.93 ± 0.20	10.28 ± 3.25	0.4655 ± 0.02
LBL	0	4.28 ± 0.86	70.14 ± 1.52	0.4638 ± 0.00
	15	5.13 ± 0.67	71.48 ± 2.00	0.5245 ± 0.00
	30	4.39 ± 0.69	69.17 ± 4.04	0.3874 ± 0.00
CC	0	2.63 ± 0.13	78.65 ± 0.80	0.4375 ± 0.01
	15	3.22 ± 0.37	75.97 ± 2.63	0.4506 ± 0.09
	30	3.68 ± 0.86	75.13 ± 0.52	0.4751 ± 0.01

Values are mean ± standard deviation.

10^6 Da with a multi-branched structure and an anionic group in the outer section. The bond between GA and water molecule is stronger, thus leading to difficulty in evaporation.

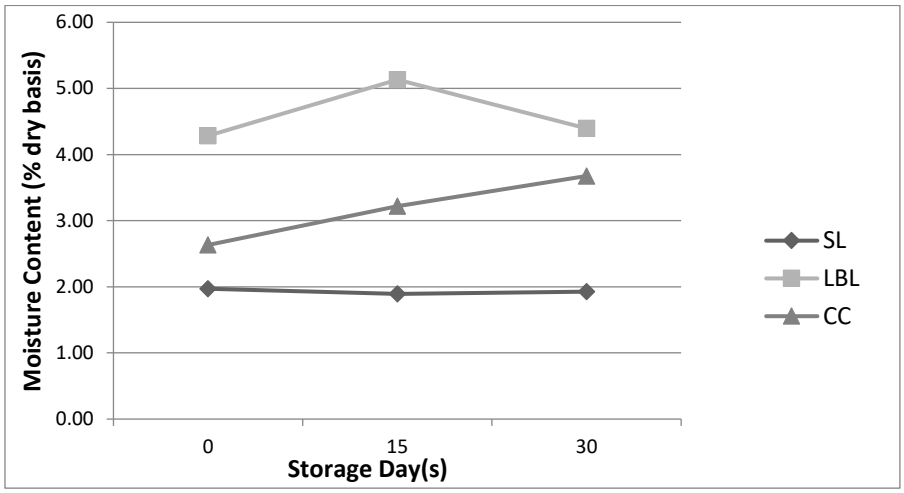
CC VCO powder had increased moisture content value during 30 days of storage. This increase occurred because WPI had fairly large amount of protein, where the water holding capacity is better when the protein is in an amorphous state (Karrar *et al*., 2020). However, the microcapsule experienced an increase in the value where equilibrium was not achieved (Figure 1a).

According to Gallardo *et al*. (2013), the minimum moisture content criteria for powder products often used in industry are 3 - 4%. The moisture content of a powder and the a_w in the material are directly proportional, thus preventing the fat oxidation process in the sample. Based on the experiment, SL and CC VCO powder samples met the moisture content requirements in the industry, unlike LBL VCO powder with value > 4%.

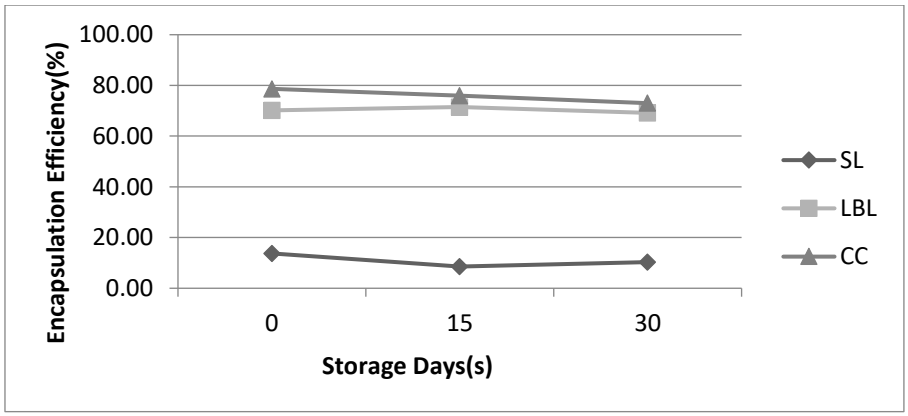
Microencapsulation efficiency

Based on the experimental results, SL VCO powder had very low microencapsulation efficiency of 13.73%, and decreased throughout storage (Table 3). The low value microencapsulation efficiency could have been caused by the high concentration of VCO used in the formulation [33% (w/w) per total solid]. According to Alcântara *et al*. (2019), the higher the concentration of the oil phase used, the lower the microencapsulation efficiency due to the reduced availability of sufficient coating material to form a matrix structure for the encapsulated oil droplets. This can be clarified from the experimental results where the surface oil in VCO powder sample had very large percentage, ranging from 25 - 30%. The low value of microencapsulation efficiency could have been due to the availability of sufficient coating materials used which were only Tween 80 and maltodextrin. Even though the use of maltodextrin can increase the resistance of the encapsulated oil to oxidation, the compound does not have lipophilic properties, and provides lower protection than GA (Nhu Quynh *et al*., 2016). According to Supriyadi and Rujita (2013), the factor that affects the value of the encapsulated oil is the viscosity of the emulsion. The low viscosity during the drying process of the microcapsules will result in a weak crust, and encapsulated VCO becomes less protected from damage. In addition, the low viscosity of the emulsion can cause a longer drying process, thus lowering the amount of encapsulated VCO.

According to Barbosa *et al*. (2005), microencapsulation efficiency is influenced by emulsion form. Stable emulsion decreases the amount of unencapsulated material, and increases the level of efficiency. However, this statement does not apply to the experiments carried out, where LBL VCO emulsion produced powder with high microencapsulation efficiency of 69 - 71%. Before drying microcapsule, emulsion can be homogenised to minimise the phase separation. According to Di Giorgio *et al*. (2019), the addition of WPI in sufficient concentrations can reduce the time required to create a semi-permeable surface layer of droplets and air during the drying process. This creates oil droplets that are more difficult to diffuse to the particle surface during the drying process, and also causing an increase in oil retention ability. Furthermore, the addition of GA and maltodextrin as coating materials provided a synergistic effect where the combination



(a)



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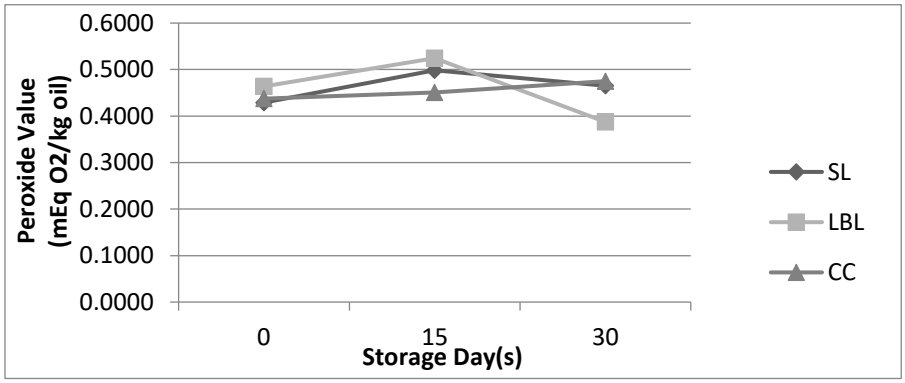


(c)

Figure 1. (a) Moisture content, **(b)** encapsulation efficiency, and **(c)** peroxide value of VCO powder microcapsules during storage.

could increase encapsulation efficiency; it could increase the viscosity of the LBL emulsion to accelerate the drying rate of microcapsules and maintain the amount of encapsulated core material (Premi and Sharma, 2017) (Figure 1b).

The 73 - 78% of microencapsulation efficiency in CC VCO powder was slightly higher as compared to 69 - 71% LBL VCO powder. The occurrence of this phenomenon could have been influenced by a common factor, namely the use of the same type of coating material (WPI-GA-MD). The high efficiency of microencapsulation in complex coacervation is influenced by the combination of proper coating and achieving the optimum pH in the process (Dong *et al.*, 2011). The use of cross-linking agents is needed because the coating materials is not certain to form

microcapsule with a dense structure. Addition of cross-linking agent such as TG enzyme in the fabrication of complex coacervation tends to increase microencapsulation efficiency. This is consistent with the research conducted by Dong *et al*. (2011), where the use of TG enzyme provides rigidity to microcapsule wall by forming intermolecular cross-linking bonds with protein molecules [ε-(λ-glutamyl)-lysyl)].

Peroxide value

Peroxide value is a parameter that shows the level of fat oxidation calculated as the number of primary products of fat and oil oxidation (hydroperoxide). Based on the experimental results shown in Table 3, the SL and LBL VCO powders had slight increase and decrease in the peroxide number on days 15 and 30. The CC VCO powder sample had increase in peroxide value on day 30. The factors that affect the rate and degree of fat oxidation include the composition of its constituent fatty acids, available oxygen concentration, temperature, surface area, water activity, and the presence of antioxidant and pro-oxidant compounds. A material containing fat with a concentration of polyunsaturated fatty acids (PUFA) will have the potential to undergo fat oxidation (Sikorski *et al*., 2020).

Based on the experimental results, VCO powder from the three treatments stored for 30 days at room temperature had a peroxide value of < 2.0 mEq O_2/kg oil, in compliance with SNI 7381:2008 (BSN, 2008). As a comparison, fresh VCO samples were also tested for the peroxide value (0.0524 mEq O_2/kg of oil). There was an increase in peroxides value during microencapsulation process in all treatments at an average of ten times. Even though VCO is classified as saturated oil, it still contains unsaturated fatty acids in the form of oleic acid (C18:1) with a concentration of 5 - 10%, linoleic acid (C18:2) of 1 - 2.5%, and long-chain unsaturated fatty acids (C18:3 - C24:1) with a concentration of < 0.5% of the total fatty acids that can be oxidised (APCC, 2009).

CC VCO powder showed an increase in peroxide value where the oxidation process was still ongoing. Meanwhile, the peroxide value in SL and LBL VCO samples decreased due to the achievement of the maximum concentration of hydroperoxides. Under these conditions, hydroperoxides decompose into secondary oxidation products and alkoxyl radicals (RO·), which are the starting point for cleaving the aliphatic fatty acid chains known as β-scission reactions. These reactions produce different products such as aldehydes, hydrocarbons, hydroperoxides, ketones, and epoxides (Frankel, 2005) (Figure 1c).

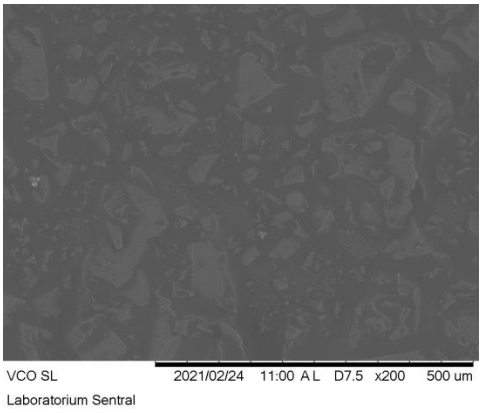
Morphology and particle size of microcapsule

The three samples of VCO powder had surface morphology with amorphous and glossy shapes formed during the drying, grinding, and sieving processes using a vacuum oven dryer (Figure 2). Microcapsule resulting from vacuum drying has a surface morphology similar to freeze-drying, such as the research conducted by Mahdavee Khazaei *et al*. (2014) with grinding and sieving processes. Mahdavee Khazaei *et al*. (2014) also stated the glassy structure protected the encapsulated active ingredients from exposure to heat and oxygen. Microcapsule resulting from drying using a vacuum oven has a more asymmetrical structure than spray drying method, which are spherical with various sizes (Alcântara *et al*., 2019).

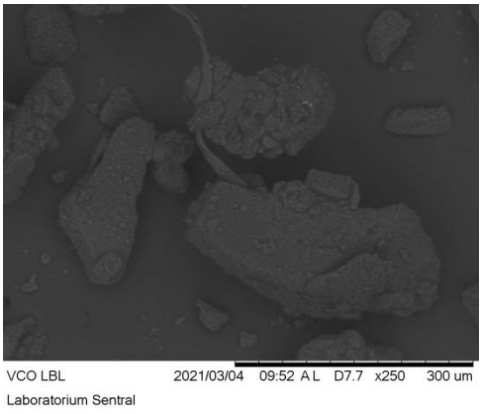
The surface morphology of LBL and CC VCO powders was dense. The use of coating materials in the form of WPI-GA-MD can produce microcapsule with better stability and retention of encapsulated active ingredients (Karrar *et al*., 2020). This is supported by the high-efficiency value of LBL and CC VCO powders and the use of TG enzyme that can provide rigidity to microcapsule wall (Dong *et al*., 2011). However, the opposite occurred in SL VCO powder, where the morphology cracked and formed flakes. The shape can be caused by the grinding and sieving process of microcapsule, resulting in the release of encapsulated material and increasing the amount of surface oil. This was seen in the value of VCO powder microencapsulation efficiency of the single-layer emulsification method which was very low. According to Premi and Sharma (2017), the presence of cracks and dents increases gas permeability to reduce the protective effect around the material (Figures 2a, 2b, and 2c).

Characterisation of reconstituted microcapsule

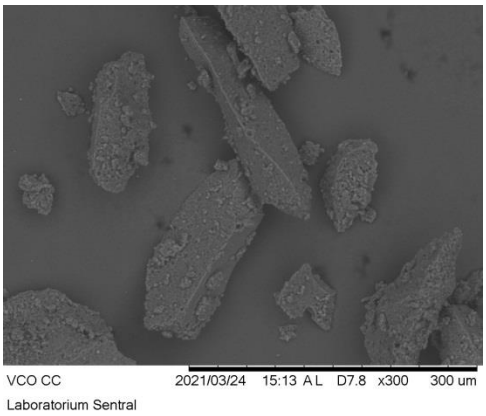
Powder with good dispersion and solubility properties is considered critical in food industry. The quality of the reconstituted product must be the same in terms of chemical composition and nutritional content as compared to the original material. SL VCO



(a)



(b)



(c)

Figure 2. Micrographs from scanning electron microscopy of VCO powder microencapsulated by **(a)** SL, **(b)** LBL, and **(c)** CC.

powder had shorter time to dissolve in water than the other two samples (Table 2). This could have been caused by the morphology which was in the form of flakes, and there were cracks in the microcapsule wall. The increased permeability of microcapsule wall reduces the protective effect around the encapsulated material (Premi and Sharma, 2017). SL VCO powder had higher maltodextrin concentration which could increase the wetting characteristics of the formulation due to the smaller contact angle with water (Korma *et al*., 2019).

The longer time required to dissolve LBL and CC VCO powders and the presence of insoluble components could have been caused by WPI protein denaturation in microcapsule drying process. This is because the drying process lasted quite a long time using a vacuum oven dryer with a duration of 3 - 5 h at 60 - 65°C. The denatured protein powder thus exhibited poor solubility upon reconstitution, thus leading to potential loss of therapeutic efficacy and a reduction in certain functional properties, such as the ability to gel and emulsify (Amdadul Haque *et al*., 2013).

The reconstituted SL and LBL VCO powders had larger particle size and smaller PDI value than the fresh emulsion particle size. Meanwhile, a different result was observed in CC VCO powder, characterised by smaller particle size and higher PDI value as compared to the fresh coacervate. According to Turchiuli *et al*. (2017), the increase in reconstituted powder particle size and PDI value was caused by the occurrence of coalescence in small oil droplets (< 0.2 m). The depletion of the carbohydrate polymer layer can also be caused by the presence of larger oil droplets originating from unencapsulated oil in powder microcapsule.

Conclusion

In conclusion, SL VCO emulsion was stable for three days of storage, while LBL VCO emulsion was destabilised with syneresis. The average particle size in all treatments had similar values ranging from 1.13 to 1.48 m. The treatments showed polydispersity index values in the range of 0.13 - 0.22, and were considered as monodispersed. Furthermore, the use of different types of microencapsulation methods and coating materials affected several characteristics of the VCO powder. The microcapsules produced by using a combination of several wall materials such WPI-GA-MD in LBL and CC VCO powder provided the highest yield and microencapsulation efficiency. All treatments of VCO microencapsulation showed a slight increase in peroxide value as compared to the fresh VCO, and tended to decrease in peroxide value during the storage. Therefore, the microencapsulation process could be used to develop stable VCO in powder form.

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