

ENCAPSULATION OF CANDLENUT OIL
BY FREEZE-DRYING METHOD

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ABSTRACT

Candlenut oil is a potential source of omega fatty acids that can be used as a food supplement or nutrient for food fortification. It contains high amount of omega fatty acids and also available in high quantity, especially in Indonesia. However, due to its off-odor and its thermal-sensitivity which makes candlenut oil prone to oxidation, the application into food products still needs more improvement. Encapsulation is one of the techniques that is used to protect the candlenut oil from oxidation. This research aimed to find the best encapsulating agent to protect the omega content from candlenut oil against oxidation through freeze-drying method. Factors such as encapsulating agent (whey protein isolate, sodium caseinate, β -cyclodextrin, gum Arabic) and ratios of encapsulating agent to oil (3:2, 1:1 and 2:3) were investigated to find out the most appropriate microcapsule and conditions to ensure there will be no change of the candlenut oil characteristics. Moisture content, microencapsulation efficiency (ME), and peroxide value (PV) were analyzed as the product parameter. The highest encapsulation efficiency was obtained by using sodium caseinate ($43.22 \pm 0.9\%$) with the ratio of encapsulating agent-oil was 3:2. The second stage of candlenut oil encapsulation was carried out to improve the efficiency of microcapsule, and the result showed that the efficiency of encapsulated oil with sodium caseinate as encapsulating agent was increased to 64.86%.

Keywords: *Candlenut oil, encapsulation, freeze-drying, omega fatty acid, oxidative stability*

ABSTRAK

Minyak kemiri merupakan sumber asam lemak omega yang potensial untuk digunakan sebagai suplemen atau nutrisi tambahan pada fortifikasi makanan. Minyak kemiri memiliki kandungan asam lemak omega yang tinggi serta kelimpahan yang cukup besar di Indonesia. Namun, minyak kemiri yang memiliki sifat sensitif terhadap panas menjadi mudah untuk teroksidasi. Hal ini menyebabkan diperlukannya pengembangan produk sebelum mengaplikasikan minyak kemiri tersebut ke dalam produk makanan. Enkapsulasi merupakan salah satu teknik yang digunakan untuk melindungi minyak kemiri dari reaksi oksidasi. Penelitian ini bertujuan untuk menentukan bahan penyalut (*encapsulating agent*) terbaik untuk melindungi kandungan asam lemak omega dari minyak kemiri dengan menggunakan metode *freeze drying*. Variasi bahan penyalut (*whey protein isolate, sodium caseinate, β -cyclodextrin, gum Arabic*) dan rasio bahan penyalut terhadap minyak (3:2, 1:1 dan 2:3) dilakukan untuk mendapatkan mikrokapsul minyak kemiri dengan kualitas terbaik. Kadar air, efisiensi mikroenkapsulasi dan bilangan peroksida dianalisis sebagai parameter produk hasil enkapsulasi. Efisiensi enkapsulasi terbaik didapatkan dengan menggunakan *sodium caseinate* ($43,22 \pm 0,9\%$) dan rasio bahan penyalut terhadap minyak 3:2. Optimasi enkapsulasi minyak kemiri pada tahap ke dua menunjukkan peningkatan efisiensi mikroenkapsulasi minyak kemiri dengan *sodium caseinate* sebagai bahan penyalut hingga 64,86%.

Kata kunci: *Asam lemak omega, enkapsulasi, freeze-drying, minyak kemiri, stabilitas oksidasi*

Article Information

Article Type: Research Article
Journal Type: Open Access
Volume: 2 Issue 1

Manuscript ID
v2n1351-1

Received Date
29 May 2020

Accepted Date
27 August 2020

Published Date
31 August 2020

DOI:
10.33555/jffn.v2i1.35

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Citation:

Yusri, S., Meidiana, C., Marpaung, A.M., Sutanto, H. 2020. Encapsulation of candlenut oil by freeze-drying method. *J. Functional Food & Nutraceutical*, 2(1), pp.53-61

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INTRODUCTION

Candlenut (*Aleurites moluccanus*) is widely known as culinary spices and herbs which can be easily found in Indonesia and South East Asia. Currently, candlenut is only being utilized as a spice, an ingredient for cooking, and for hair growth. In fact, the oil content from cold-press candlenut is relatively high, and it contains mostly polyunsaturated fatty acids (PUFAs) (Wijaya, 2008). Ander, Dupasquier, Prociuk, & Pierce (2003) stated that polyunsaturated fatty acids have more than one double bond in their backbone, hence they can provide health benefits. Moreover, omega-3 fatty acid can help to regulate the lipid circulation in the body, by increasing the high-density lipoprotein (HDL) and lowering the low-density lipoprotein (LDL) cholesterol and reducing atherosclerotic development (Ander, Dupasquier, Prociuk, & Pierce, 2003). PUFAs are essential oil, means that they are not produced in the human body.

Due to the fact that omega fatty acids can cause numerous health issues, there is an increasing interest from Indonesian consumers in foods which have been enriched or fortified with omega fatty acid. Omega fatty acid sources in Indonesia are still limited and usually they are relatively expensive. In this case, candlenut oil can be an alternative source since it contains high PUFAs and available abundantly, especially in Indonesia. However, due to its off-odor and its thermal-sensitivity which makes candlenut oil prone to oxidation, the application into food product still needs more improvement. Recent studies about food fortification with omega-3 from candlenut oil showed that, when the candlenut oil is added to the product, the omega fatty acid content is decreased (Kamil, 2016). The omega fatty acid in candlenut oil is also susceptible to promote oxidative degradation in oil when the product is exposed to oxygen and heat (Tjhin, 2014).

Microencapsulation is one of the techniques that is used to protect candlenut oil from oxidation (Kaushik, Dowling, Barrow, & Adhikari, 2015). In microencapsulation process, droplets or tiny particles are protected or surrounded by wall

matrix or encapsulating agent, thus forming very small capsules (Wang, Tian, & Chen, 2011; Raybaudi-Massilia & Mosqueda-Melgar, 2012). Selecting the encapsulating agent is important in microencapsulation process. The encapsulating agent must fulfill certain criteria. They must have high stability, high water solubility, emulsifying properties, a tendency to form a network during drying, and low viscosity for spray-drying (Calvo, Castaño, Lozano, & González-Gómez, 2012). According to Calvo et al. (2012), protein-based encapsulating agent was more effective in preserving the quality of microencapsulated extra-virgin olive oil. In the previous study about the encapsulation of candlenut oil with hydroxypropyl methylcellulose (HPMC), gum Arabic (GA), β -cyclodextrin (β -CD), and alginate by using spray-drying method, the best performance in microencapsulation efficiency was showed by β -cyclodextrin. However, gum arabic showed the best performance in terms of peroxide value (Verianto, 2018). It still cannot be concluded what is the best encapsulating agent for encapsulating candlenut oil.

In this research, sodium-caseinate (SC) and whey protein isolate (WPI) will be used as the alternative encapsulating agents with freeze-drying method. The result of this alternative encapsulating agent will be compared to the previous encapsulating agent used for candlenut oil, which are β -cyclodextrin and gum arabic, to know which type of encapsulating agent and formulation that could optimize the encapsulation of candlenut oil.

MATERIALS AND METHOD

Materials and equipments

Candlenut kernels were obtained from a local market in Tangerang. β - cyclodextrin, gum Arabic, sodium caseinate and whey protein isolate, maltodextrin, and lecithin were obtained from Richest Group Ltd., China. For analysis of microencapsulation, n-hexane for analysis, chloroform for analysis, acetic acid glacial 100%, saturated potassium iodide for analysis, starch, and sodium thiosulfate for analysis were obtained from Merck.

The equipment used for candlenut oil extraction was customized cold press expeller. For the candlenut oil microencapsulation, homogenizer (IKA Labortechnik RW 20N) was used for homogenizing the emulsion. Freeze dryer (Operon FDB – 5502) was used to dry the samples. Moisture content analyzer (Satorius MA 35) was used to determine the moisture content. Rotary evaporator (IKA HB 10) was used to evaporate n-hexane in microencapsulation efficiency analysis. The omega fatty acid content was analyzed at PT Saraswanti Indo Genetech by using gas chromatography with a flame ionization detector (GC-FID). The GC analysis was carried out by using a polyethylene glycol (PEG) capillary column (30m x 0.25mm ID x 0.25µm film thickness). Nitrogen was used as the carrier gas, with a flow rate of 1 mL/min. The injector and detector temperatures were set at 260 °C. The oven temperature was programmed from 120 °C to 240 °C at a rate of 4 °C/min.

RESEARCH METHODOLOGY

Extraction of candlenut oil.

Candlenut kernels were extracted by using cold-pressed expeller and then centrifuged at 6000 rpm and 15 °C for 20 minutes to obtain pure candlenut oil. The omega content of the centrifuged candlenut oil was analyzed by using GC-MS. Emulsification of the candlenut oil using four different types of encapsulating agents (Sodium caseinate, Whey Protein Isolate, β-cyclodextrin and Gum Arabic) with maltodextrin as the secondary encapsulating agent were prepared with the ratio 3:2, 1:1 and 2:3 of encapsulating agent to candlenut oil. The limited amount of water was added into the mixture. Homogenization process was conducted at room temperature for 5 minutes. Emulsion was frozen prior drying process. The drying process was done for 48 hours at –60 °C. Microencapsulation efficiency, moisture content analysis, and peroxide value analysis were conducted to examine the most appropriate material for encapsulating candlenut oil.

Microencapsulation efficiency analysis.

Method for microencapsulation analysis is adapted

from Calvo et al. (2012) with modification. Five grams of powder was mixed with 20 ml of hexane and then vortexed for 1 minute to extract free oil. The mixture was then filtered with a Whatman No. 41 filter paper. The washed powder on the filter paper was rinsed using 50 ml of distilled water. The hexane was evaporated using rotary evaporator at 60 °C and the internal oil was scaled. The oil was weighed and then the efficiency was measured. ME was calculated by using the following formula:

$$ME = \frac{\text{Total Oil} - \text{Surface Oil}}{\text{Total Oil}} \times 100\%$$

Peroxide value analysis.

Peroxide value was conducted by following the method from Toure (2007) with modification. Chloroform (6 mL) and glacial acetic acid (9 mL) were mixed together. Then, 1 gram of powder sample was dissolved in the solution. One mL of saturated solution of potassium iodide (KI) was added into the sample solution. The solution mixture was manually shaken for 30 seconds and stored in the dark for 30 minutes. Starch indicator solution (1%, 0.5 mL) was added into the mixture until the color of the solution changed from yellow to dark blue. Then 0.01 N of standardized sodium thiosulphate solution was used for titration against the sample solution until the the blue color almost disappeared. Blank titration was conducted under the same condition. To determine the POV value, the following equation was used:

$$POV \left(\frac{meq}{kg} \right) = \frac{Cx(V - V_0) \times 1000}{m}$$

Where,

C = concentration of sodium thiosulphate (mol/L)

V = volume of sodium thiosulphate for the sample titration (mL)

V₀ = volume of sodium thiosulphate for the blank titration (mL)

m = mass of candlenut oil (g)

Moisture content analysis.

The moisture content was measured by using moisture content analyzer Satorius MA35. Around 1 gram of powder sample was put in the analyzer for one hour at 105 °C. As a result of continuous heating, the moisture content would evaporate. The experiment was terminated once the sample mass had reached the constant value. For the moisture content after drying process, to minimize the time used, the moisture content was measured manually by using Memmert oven. The samples were weighed for about 1 gram and then heated at 105 °C for 24 hours, and then the weight was measured. The analysis stopped when constant weight was achieved.

RESULTS AND DISCUSSION

Candlenut oil extraction

The extraction of the oil from the candlenut kernels was carried out by using cold press expeller machine due to the sensitivity of the oil to heat. The friction between candlenut kernels and the expeller machine induced the increment of the temperature which leads to the degradation of omega content of the oil. Thus, the usage of cold press expeller becomes the critical factor to protect the oil from degradation. As much as 16 % of yield of candlenut oil was obtained from this extraction process.

The unsaturated fatty acids and omega content of the extracted candlenut oil were analyzed by using GC. The result of the analysis is shown in Table 1.

According to the GC data in Table 1, it is shown that oil obtained from candlenut is high in unsaturated fatty acid, which was 90.89%. The unsaturated fatty acid content obtained by cold-press extraction is higher than the previous study from Wijaya (2008), which was only 79.65% of unsaturated fatty acids. The difference in omega content might be caused by the different source of candlenut kernels. Extraction method also affects the omega content of the oil. Oil which was extracted using common extraction has lower omega content compared with oil which was extracted by using cold-press expeller. This is because heat degrade the omega content of the oil

through thermal oxidation. Wijaya (2008) stated that the unsaturated fatty acid content of candlenut oil extracted by cold expeller was higher (79,65%) than normal expeller (71.12 %). Comparing with another unsaturated fatty acid oil, unsaturated content of candlenut oil is still higher than olive oil (about 77%) which was studied by Susilo (2012) and fish oil (about 75%) which was studied by Aditia *et al.*, (2014). Based on the data, the highest omega content contained in the candlenut oil is omega-6 fatty acids, followed by omega-3 and omega-9. In this research, it is proved that extraction by using cold-press expeller can maintain the high content of omega fatty acids in the oil.

Table 1. Omega fatty acid content of pure candlenut oil extract

Parameter	Result (%)
Unsaturated fats	90.89 ± 0.06
Omega 3 fatty acids	26.31 ± 0.06
Omega 6 fatty acids	40.10 ± 0.00
Omega 9 fatty acids	24.30 ± 0.00

Encapsulation of candlenut oil

The efficiency of microencapsulation was analyzed to determine the amount of surface oil presents on the particles' surface and degree in which the encapsulating agent can prevent external or internal extraction of the oil. According to the result in Table 2, the highest efficiency was obtained by using sodium caseinate as the encapsulating agent with the ratio 3:2 of encapsulating agent to oil. In this case, sodium caseinate has an emulsification property and amphiphilic character, where they possess both hydrophilic and lipophilic properties. Because of the emulsifying properties, the stability of the emulsion by using sodium caseinate was higher than the other encapsulating agent hence the result showed that the efficiency of encapsulated oil with sodium caseinate was the highest.

However, there was no significant difference between each encapsulating agent ($P = 0.22$) but there was a significant difference between each ratio ($P = 0.0032$). This indicates that the efficiency

was influenced by the formulation's ratio. When the amount of oil load was higher than the encapsulating agent, the solid particle was

insufficient to give a full protection to the droplets of oil.

Table 2. Microencapsulation efficiency (me), peroxide value, and moisture content of the encapsulated candlenut oil

Encapsulating agent	Me (%)	Pov (meq/kg)	Moisture content (%)
B1	40.94 ± 2.0	1.99 ± 0.00	5.6 ± 0.35
B2	37.70 ± 1.6	1.49 ± 0.70	4.55 ± 0.82
B3	30.79 ± 4.8	2.73 ± 0.34	6.57 ± 0.37
W1	42.24 ± 0.5	0.99 ± 0.002	3.09 ± 0.54
W2	35.58 ± 1.8	1.24 ± 0.34	1.61 ± 0.09
W3	27.35 ± 6.1	2.97 ± 2.8	2.80 ± 0.55
S1	43.22 ± 0.9*	1.24 ± 0.35	2.07 ± 0.66
S2	36.22 ± 3.7	1.73 ± 0.35	2.42 ± 0.02
S3	28.40 ± 10.7	2.73 ± 0.34	2.45 ± 1.01
G1	38.37 ± 4.3	0.99 ± 0.0003	2.86 ± 0.62
G2	26.76 ± 13.0	1.24 ± 0.34	2.88 ± 0.38
G3	22.56 ± 8.8	1.74 ± 1.05	3.47 ± 2.40

B=β-cyclodextrin; W=whey protein isolate; S=sodium caseinate; G=gum Arabic. 1,2,3=formulation ratio, 1= 3:2 encapsulating agent to oil; 2= 1:1 encapsulating agent to oil; 3= 2:3 encapsulating agent to oil, * = highest microencapsulation efficiency.

The peroxide value of encapsulated candlenut oil was conducted to measure the oxidation occurred in the candlenut oil. Unsaturated fatty acids are prone to oxidation because of the double-bond presence in their structure. The presence of heat and oxygen promotes the oxidation. When unsaturated fatty acids are in contact with oxygen or exposed to heat, hydroperoxide is formed. This analysis measures hydroperoxide formed during oxidation. It is expected that by using freeze-dry method, could minimize the peroxide number of the product. The result of encapsulated candlenut oil in peroxide value is showed in Table 2.

The lowest peroxide number was found by using whey protein isolate and gum Arabic as the encapsulating agent. This is probably because gum Arabic has an antioxidant activity to prevent the formation of hydroperoxide during oxidation of the oil due to the presence of hydroxyl group which can scavenge the free radicals (Chew, Tan, & Nyam, 2018). However, there was no significant difference between each encapsulating agent ($P = 0.55$), the ANOVA result showed that there was a significant difference between each ratio ($P = 0.037$).

The higher oil loading showed higher peroxide value. This result corresponded with the efficiency of the microencapsulation process. Since the

surface oil was higher as the oil loading increase, the peroxide value was also increased. It is probably due to the oxidation of the un-encapsulated oil surface, thus it was not completely protected.

As can be seen in Table 2, the lowest moisture content after drying process through freeze-drying method was obtained by using whey protein isolate with ratio 1:1, where the highest moisture content was obtained by using β-cyclodextrin with the ratio 2:3. According to the data, the moisture content is affected by the ratio of the encapsulating agent to the oil. There was a significant difference between each encapsulating agent ($P = 0.001$). The post-hoc analysis showed that β-cyclodextrin was significantly different with whey protein isolate ($P = 0.0017$), sodium caseinate ($P = 0.0013$) and gum Arabic ($P = 0.0049$). The greater amount of solid particle in a system increases the volatile retention depending on the type of the solid and the type and amount of the volatile. It was suspected because the volatile compound was entrapped in the solid matrix system after the formation of the droplets. Higher solid content can reduce the circulatory system on the emulsion. This theory answers the result of β-cyclodextrin, as the higher amount of solid content has higher moisture content.

Screening of encapsulating agents for candlenut oil

Re-formulation of the total solid content and water used was conducted in order to achieve higher efficiency. At first, the utilization of water was limited in order to minimize the drying time, however, the limitation of water was responsible for the low efficiency of microencapsulation, besides the homogenization process. The screening of encapsulating agents for candlenut oil encapsulation was carried out by increasing the water content up to 50% and ratio encapsulating agent to oil 3:2. As a result, the efficiency of microencapsulated candlenut oil was increasing, as can be seen in Table 3.

Table 3. Microencapsulation efficiency (me), peroxide value, and moisture content of the encapsulated candlenut oil

Encapsulating agent	Me (%)	Pov (meq/kg)
B1	49.64	2.49 ± 0.50
W1	57.48	0.99 ± 0.00
S1	64.86	0.49 ± 0.76
G1	47.38	0.99 ± 1.40

By increasing the amount of water, the stability of the emulsion was increased and it was possibly because the size of oil droplets was also decreasing, so the encapsulating agent were more effectively in protecting the oil droplets. Based on the data, the highest microencapsulation efficiency was obtained by using sodium caseinate. Sodium caseinate is one of the best encapsulating agent for lipophilic compound because of its intrinsic stability to form protein-ligand complexes and casein micelles. In addition, the amphiphilic structure of caseins promote a rapid adhesion to the surface of oil droplets to form a thick layer that protects the newly formed droplets against flocculation and coalescence (Mujica-Álvarez, Gil-Castell, Barra, Ribes-Greus, Bustos, Faccini, & Matiacevich, 2020). The microencapsulation of the candlenut oil with sodium caseinate increased significantly from 43.22 to 64.86%. The lowest peroxide value of encapsulated candlenut oil also obtained by using sodium caseinate as

encapsulating agent. The peroxide value decreased from 1.24 to 0.49 meq/kg.

To analyze the stability of the microcapsules, heating process at 170 °C was conducted for 1.5 hours for the new formulation of encapsulated oil and also the un-encapsulated candlenut oil under the same treatment. During the exposure to the heat, it is noticeably that oxidation will occur. However, to evaluate the fluctuation of the peroxide value, the stability test was conducted. The result was explained in Table 4.

Table 4. Peroxide value of heated encapsulated candlenut oil with new formulation

Encapsulating agent	Pov (meq/kg) Day 0	Pov (meq/kg) day 5
Oil	23.75	24.60
B1	21.90	22.38
W1	20.36	22.43
S1	19.88	20.37
G1	24	>24

Un-encapsulated candlenut oil has the highest peroxide value after the heating process was done (at day 0). Sodium caseinate exhibited the lowest peroxide value at day-0, followed by whey protein and β -cyclodextrin. After five days of storage, peroxide value of all encapsulated oil and un-encapsulated oil were increasing. The auto-oxidation of the encapsulated and un-encapsulated oil was occurred during the heating process. However, both on day-0 and day-5, all the encapsulated oil showed lower peroxide value compared with the un-encapsulated candlenut oil. It was suspected that the peroxide value was increased due to the oxidation from the surface oil, suggesting the inside oil was well protected inside the matrix of encapsulating agent (Wang *et al.*, 2011). However, for gum Arabic, the peroxide value after being heated was increased significantly. Carneiro *et al.* (2013) stated that the peroxide oxidative stability was affected by the type of encapsulating agent used. A study about encapsulation of flaxseed oil by using spray drying and a combination of encapsulating agent between maltodextrin and gum Arabic, also showed the

poorest oxidative stability compared with whey protein concentrate. It can be concluded that gum Arabic exhibit inadequate protection against oxidation after heat was applied.

After the encapsulation process was carried out and the best encapsulating agent (in terms of

efficiency) was obtained, the omega content of the product was analyzed to check the decrement of the omega content. However, the omega fatty acid content of encapsulated oil by using sodium caseinate showed a decrement in all omega content as seen in Table 5.

Table 5. Omega content comparison between pure candlenut oil, encapsulated candlenut oil and commercial product

Parameter	Result (%)		
	Pure candlenut oil	Encapsulated candlenut oil	Commercial product
Unsaturated fats	90.89	50.75	36
Omega 3 fatty acids	26.31	14.31	5
Omega 6 fatty acids	40.10	22.01	21
Omega 9 fatty acids	24.30	14.21	10

This decrement corresponded with the low microencapsulation efficiency of the freeze-dried product. The purpose of this study was to retain the omega content through encapsulation process. However, the result of the first stage formulation showed that the surface oil was too high, which means that the oil was not completely encapsulated. Therefore, the un-encapsulated oil or surface oil was not well protected inside the encapsulating agent and hence the omega oil content was decreasing. Heinzelmann et al. (2000) explained that during emulsification and homogenization process, oil may degraded and oxidized with the presence of oxygen. Also, after the encapsulation process, during storage, the un-encapsulated oil or surface oil may be oxidized as well since there was no encapsulating agent that coated the droplets of oil (Kaushik et al. 2015). Thus, the omega content was decreasing significantly. However, the omega fatty acid content of encapsulated candlenut oil through freeze-drying method was higher compared to the commercial fat powder from MCT Lipids Life which was encapsulated with sodium caseinate through spray-drying method.

CONCLUSION

Four different encapsulating agents from carbohydrate and protein groups, i.e whey protein

isolate, sodium caseinate, β -cyclodextrin, and gum Arabic, were used for candlenut oil encapsulation. Both carbohydrate and protein based encapsulating agents were potentially protecting the oil against oxidation process from the aspects of microencapsulation efficiency, peroxide value (PoV), and moisture content. In terms of efficiency, the highest efficiency was obtained by using sodium caseinate with ratio 3:2. The screening of encapsulating agents for candlenut oil was conducted by using new formulation with higher moisture content and ratio encapsulating agent to oil 3:2. The result showed that sodium caseinate was the most suitable encapsulating agent to protect the candlenut oil from oxidation. The efficiency of encapsulated oil with sodium caseinate as encapsulating agent was increased from 43.22 to 64.86% and the peroxide value was decreased from 1.24 to 0.49 meq/kg.

ACKNOWLEDGMENT

This research was funded by the Ministry of Research, Technology, and Higher Education, Republic of Indonesia, through Program Kreativitas Mahasiswa (PKM) Research Grant 2018.

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