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ORIGINAL ARTICLE

# Supercritical $CO_2$ extraction of candlenut oil: process optimization using Taguchi orthogonal array and physicochemical properties of the oil

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**Abstract** A series of experiments was conducted to determine optimum conditions for supercritical carbon dioxide extraction of candlenut oil. A Taguchi experimental design with $L_9$ orthogonal array (four factors in three levels) was employed to evaluate the effects of pressure of 25–35 MPa, temperature of 40–60 °C, $CO_2$ flow rate of 10–20 g/min and particle size of 0.3–0.8 mm on oil solubility. The obtained results showed that increase in particle size, pressure and temperature improved the oil solubility. The supercritical carbon dioxide extraction at optimized parameters resulted in oil yield extraction of 61.4% at solubility of 9.6 g oil/kg $CO_2$. The obtained candlenut oil from supercritical carbon dioxide extraction has better oil quality than oil which was extracted by Soxhlet extraction using n-hexane. The oil contains high unsaturated oil (linoleic acid and linolenic acid), which have many beneficial effects on human health.

**Keywords** Supercritical carbon dioxide extraction · Candlenut · *Aleurites moluccana* · Optimization · Taguchi

## Introduction

*Aleurites moluccana L.*, commonly known as candlenut (English) or Kukui nut (Hawaii) or kemiri (Bahasa Indonesia), is a flowering tree (normally up to 20 m height) belonging to the Euphorbiaceae family. The tree is native to the Indo-Malaysia region and become one of the world's great domesticated multipurpose trees (Krisnawati et al. 2011). The productivity of *A. moluccana* seed yields up to 16 ton/ha/year and the oil yield is approximately 3200 kg/ha annually. Oil derived from the seeds provides useful material for varnish, soap and pharmaceuticals. Candlenut oil is widely sold in the cosmetics industry and may currently be moluccana's primary commercial product. After removal of the oil, the remaining seed cake can be used for fertilizer or animal fodder (Elevitch and Manner 2006).

Different techniques have been used to obtain extracts of ground unshelled candlenut, the most notable being mechanical pressing, solvent extraction and more recently, supercritical fluid extraction (SCFE) (Rosiwati and Syarief 1996; Siddique et al. 2011). Our laboratories have investigated the different extraction techniques and biological assays on natural products (Trisina et al. 2011; Tandrasasmita et al. 2015; Karsono et al. 2014; Tjandrawinata et al. 1995). Supercritical fluid extraction (SCFE), which is a powerful technique in separation processes, has become a focus of interest in the field of extraction from natural materials (Salea et al. 2013, 2014). Higher solubility, mass transfer rates and selectivity are the advantages of SCFE method. The selectivity of the component to be extracted depends on the density of supercritical fluid, which can be altered by varying process conditions. The low latent heat of evaporation and high volatility of the solvent make the extract free from residual solvent. Supercritical carbon dioxide (SC-$CO_2$) is the most frequently used solvent for this extraction because of its practical advantages, including inexpensive, odorless, colorless, non-toxic, non-flammable, non-corrosive nature of the solvent and moderate critical temperature (Tc = 31.05 °C) as well as critical pressure (Pc = 7.38 MPa). The dielectric constant of SC-

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$CO_2$ extraction is in the range of 1.05–1.60, depending on the temperature and pressure (Zhang et al. 2005). This value is close to dielectric constant of hexane (1.88 at 25 °C) (Mopsik 1967). Thus, desired non-polar component such as triglycerides can be extracted by SC-$CO_2$.

Several studies have been done in recent years on the applications of SC-$CO_2$ on various oil-bearing materials such as soybean (Jokic et al. 2012), walnut (Salgın and Salgın 2006), rice bran (Tomita et al. 2014), peanut (Anggrianto et al. 2014), palm kernel (Zaidul et al. 2007), cottonseed (Bhattacharjee et al. 2007), rapeseed and sunflower (Boutin and Badens 2009).

Taguchi is one of the simple and systematic approaches for designing an experiment with less number of experimental runs. It could be employed to optimize the performance characteristics of process parameters, which is proved to be a powerful tool that differs from traditional full factorial investigation. This approach could economically satisfy the needs of problem solving and design optimization. Thus, it is possible to reduce the time and cost for the experimental investigations (Galtonde et al. 2009). Taguchi experimental design method is employed in some applications related to the supercritical carbon dioxide extractions. Ansari et al. (2012) employed Taguchi method to optimize supercritical extraction of oil from spearmint (*Mentha spicata* L.) leaves. Salea et al. (2013) compared Taguchi and full factorial design to optimize various parameters of the supercritical extraction of black cumin (*Nigella sativa*) seeds. Salea et al. (2014, 2016) also utilized Taguchi method to optimize supercritical extraction of oil from Javanese turmeric (*Curcuma xanthorrhiza* Roxb.) and emprit ginger (*Zingiber officinale* var. Amarum) rhizome.

The objective of this study is to investigate the effect of pressure, temperature, $CO_2$ flow rate and particle size on the solubility of candlenut according to Taguchi Method. The extraction was conducted at optimized parameters and the resulted yield and oil quality were compared with Soxhlet extraction method using n-hexane.

## Materials and methods

### Material

Dried-shelled candlenut was obtained from West Sumatra and supplied by CV Cipta Pratama (Indonesia). Moisture content in candlenut kernel was determined as 5 w% according to AOAC official method 925.40. Food-grade liquid carbon dioxide (purity 99.99%) was supplied in cylinder tube by PT Inter Gas Mandiri (Cikarang, Indonesia). Analytical grade of n-heptane (JT Baker, USA), n-hexane (J.T. Baker, USA), chloroform (Mallinckrodt Baker, USA), diethyl ether (Merck, Germany), hydrochloric acid (Merck, Germany), acetic acid (Merck, Germany), Hydranal® solvent (Merck, Germany) and Hydranal® titrant 5 (Merck, Germany), ethanol (96%, PT Brataco, Indonesia), phenolphthalein indicator (Merck, Germany), potassium hydroxide (pellets, 85%, Merck, Germany), potassium iodide (Merck, Germany), sodium thiosulphate pentahydrate (Merck, Germany), the FAME standard solution (mixtures of 34 FAME, 1000 ppm in DCM, Supelco, USA), glyceryl tridecanoate (5 mg/ml in $CHCl_3$, Sigma, USA) and $BF_3$ reagent (14% $BF_3$ in methanol, Aldrich, USA) were used.

### Material preparation

Prior to the extraction process, all dried plant materials were grounded in milling machine (Comil U5, Quadro Engineering, Canada) at impeller speed of 3500 rpm. Three stainless steel filters were used with screen size of 0.062, 0.040 and 0.024 inch, respectively. These ground samples were packaged in a polyethylene bag and stored in the refrigerator. The resulting particle size of samples which was grounded with Comil U5 are 0.8, 0.55 and 0.3 mm average particle size for screen size of 0.062, 0.040 and 0024 inch, respectively.

### Soxhlet extraction

Extraction of oil from candlenut was carried out by Soxhlet extraction method using n-hexane. About 50 g of powdered candlenut (0.3 mm) was put into a porous thimble and placed in a Soxhlet extractor. The extraction was performed using 500 ml of n-hexane (boiling point of 68.5 °C). The heating power was set to 5 cycles/h so that 30 cycles of extraction were achieved within 6 h of extraction time. The oil was obtained after the solvent was removed under reduced temperature and pressure. The oil was then stored in refrigerator at 7 °C for subsequent physicochemical analysis.

### Supercritical carbon dioxide extraction

The supercritical carbon dioxide (SC-$CO_2$) extraction was performed using a customized supercritical fluid apparatus which described in the previous study (Salea et al. 2013, 2014; Anggrianto et al. 2014). The SC-$CO_2$ extraction was carried out using supercritical extractor with $CO_2$ cycle system. The extractor vessel with 1000 ml capacity was loaded with 50 g powdered candlenut. Food-grade liquid $CO_2$ was delivered to the extraction vessel using high pressure pump (Thar, USA). The pressure in the extraction vessel was controlled by back pressure regulator (Tescom, USA). Heat exchangers (Lab. Companion, USA)

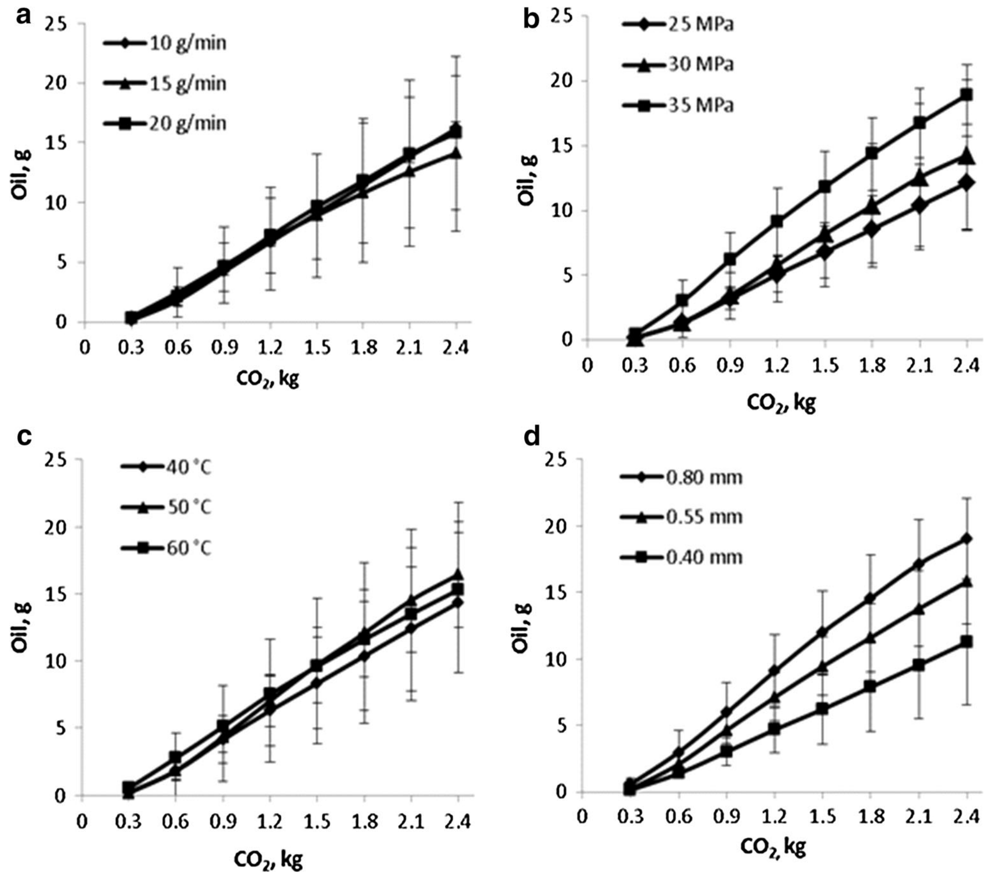


**Fig. 1** Extraction curve at average value of each parameter: **a** $CO_2$ flow rate, **b** pressure, **c** temperature and **d** particle size

were provided in system to maintain temperature in the extractor and separator vessel. Extraction was started with static extraction for 60 min (static extraction time was fixed for all extractions) followed by dynamic extraction. The dynamic extraction time was varied from 120 to 240 min, depending on $CO_2$ flow rate to give a final solvent-to-feed ratio (SFR) of 48. Mass of the extracted oil was collected every 15–30 min.

Experimental data was plotted as mass of the extracted oil versus total $CO_2$ mass used to dissolve the solute. Solubility values at each temperature and pressure were at equilibrium concentrations that are indicated by linear portion of the extraction curve. A linear regression was performed at constant extraction rate (CER) for each condition. The solubility at each condition was obtained from the slope of the fitted line on experimental data.

## Taguchi experimental design and statistical analysis

Taguchi experimental design was applied for determination of the influence of supercritical fluid carbondioxide extraction (SCFE-$CO_2$) parameters on oil solubility. Four parameters of extraction with SC-$CO_2$, including pressure, temperature, $CO_2$ flow rate and particle size were studied. Each of the four parameters was treated at three levels. Experiments were performed at pressure of 25, 30 and 35 MPa, temperature of 40, 60 and 80 °C, $CO_2$ flow rate of 10, 15, 20 g/min and particle size of 0.8, 0.55 and 0.3 mm. The influence of SCFE-$CO_2$ parameters on oil solubility was determined by Taguchi method.

The data obtained from SCFE-$CO_2$ was subjected to signal-to-noise (S/N) ratio calculation. The S/N ratio calculation is an evaluation of output performance stability which measures level of performance and effect of noise parameters on performance. In this study, target values of 'larger is better' was used since the purpose of this study was to obtain the highest oil solubility. The larger difference (Δ) value in parameters indicated that the parameters will affect the process since changes in signal causes a larger effect on the output factor being measured. The S/N ratio is calculated using the following Eq. (1):

$$S/_N = -10 * \mathrm{Log}_{10}\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{Y_i^2}\right) \tag{1}$$

where n is the number of trials in experiments, i is the experiment number and Y is the response.

## Analysis of candlenut oil

### *Physicochemical properties*

Candlenut oil which still contained some fine particles was centrifuged at 3000 rpm for 30 min. The clear oil was recovered and analyzed for their physical and chemical characteristics. The analysis for relative density, refractive index, acid value, peroxide value, saponification value, unsaponifiable matter and water content were carried out according to the standard test methods of AOAC 985.19, AOAC 921.08, AOAC 920.160, AOAC 940.28, AOAC 965.33, AOAC 972.28 and AOAC 984.20, respectively. Refractive index of the oil was determined using a refractometer (Atago, Atago Co. Ltd., Japan). Water content was analyzed using volumetric KF titrator (V30 Compact Volumetric KF Titrator, Mettler-Toledo AG, Switzerland). Viscosity was determined by viscometer (DV-E Viscometer, Brookfield Engineering Labs Inc., USA). All measurements were carried out in duplicate.

### *Fatty acid composition analysis*

Fatty acid was transformed to fatty acid methyl ester by direct transesterification using $BF_3$ catalyst (AOAC 969.33, Fatty acids in oils and fats—Preparation of methyl esters of fatty acids). Fatty acid composition was determined by gas chromatography (GC) (Clarus 680, Perkin Elmer), equipped with a flame ionization detector (FID) and a 100 m × 0.25 mm steel column, 0.2 µm film Agilent HP88 capillary column. The injector and detector (FID) temperature was 260 °C. The oven temperature program was at initial temperature of 40 °C with heating rate of 10 °C/min to 240 °C and hold for 60 min at split ratio of 1:5. Nitrogen was used as a carrier gas at flow rate of 1.2 ml/min. Either FAME standard solution or sample of 1 µl was injected to GC column. Fatty acid was identified by comparing the retention time (Rt) to those of standards (Supelco, 37 components FAME mixtures). All determinations were carried out in duplicates.

## Results and discussion

### Supercritical carbon dioxide extraction

The effects of four operating conditions of SC-$CO_2$, namely pressure, temperature, $CO_2$ flow rate and particle size on the extraction of candlenut oil were investigated using Taguchi method. The experimental responses in term of oil solubility are summarized in Table 1. Figure 1a shows extraction curves at various $CO_2$ flow rate. It is shown that $CO_2$ flow rate of 10, 15 and 20 g/min have similar extraction curves. Brunner (1994) explained that in extraction process, the extraction curves can be described by a three-step process. The first linear portion is denoted by constant extraction rate period which is characterized by the convective mass transfer between the solid material surface and the fluid phase. In this period, the amount of free oil in the cell of oil-bearing plant was sufficient to allow for solvent saturation. The second part of the extraction curve represents the falling rate period. In this step both convection and diffusion in the solid must be considered. For the third step or the diffusion-controlled rate period, the diffusion in the solid controls the rate of mass transfer. It can be concluded that by varying $CO_2$ flow rate from 10 to 20 g/min, solvent saturation was achieved and analysis based on solubility can be calculated from extraction curves.

The effect of pressure was studied at 25–35 MPa (Fig. 1b). In Fig. 1, the value of oil solubility was increased by an increase in pressure. Same phenomenon was reported by Soares et al. (2007) in the study of supercritical extraction of sunflower and corn oil at 20–35 MPa. The increase in solubility was due to the fact that higher pressure resulted in an increase in solvent

**Table 1** Solubility of shelled candlenut oil at various SCFE-$CO_2$ conditions

| No | P (MPa) | T (°C) | $CO_2$ (g/min) | Size (mm) | Time (min) | | Solubility (%) |
|---|---|---|---|---|---|---|---|
| | | | | | Static | Dynamic | |
| 1 | 25 | 40 | 10 | 0.8 | 60 | 240 | 5.49 |
| 2 | 25 | 50 | 15 | 0.55 | 60 | 160 | 4.58 |
| 3 | 25 | 60 | 20 | 0.3 | 60 | 120 | 3.02 |
| 4 | 30 | 40 | 15 | 0.3 | 60 | 160 | 3.17 |
| 5 | 30 | 50 | 20 | 0.8 | 60 | 120 | 7.95 |
| 6 | 30 | 60 | 10 | 0.55 | 60 | 240 | 6.09 |
| 7 | 35 | 40 | 20 | 0.55 | 60 | 120 | 7.46 |
| 8 | 35 | 50 | 10 | 0.3 | 60 | 240 | 5.88 |
| 9 | 35 | 60 | 15 | 0.8 | 60 | 160 | 9.60 |

density and subsequently the solvent power of solvent (dielectric constant of SC-$CO_2$ was increased as pressure increased). As the density increased, the distance between the molecules were decreased. Therefore, interaction between solutes and $CO_2$ was increased and it leads to greater solubility of the solutes in $CO_2$. The increase in pressure will also accelerate solute and solvent mass transfer in supercritical extractor vessel system and increase the solubility of oil (Reverchon and Marco 2006).

From Fig. 1c, it can be seen that as temperature increased from 40 to 60 °C, the solubility was also increased. This can be explained that vapor pressure of the oil was increased and its solubility and extraction was improved at higher temperature. Same phenomenon was also observed by Salgin and Salgin (2006) in the study of supercritical extraction of walnut oil.

In general, the oil solubility was increased with decreased particle size. In this study, the oil solubility was reduced by decreased particle size of candlenut from 0.8 to 0.3 mm (see Fig. 1d). The amount of broken cells was increased by reducing particle size by grinding. With an increase in broken cells, the amount of free oil on the surface of material was also increased. The free oil act as an adhesive between the candlenut particles resulted in lumpy-structured materials, as observed during the experiment. The particle was agglomerated with each other. As a result, there were a decrease in the specific surface area and an increase in the diffusion path and intraparticle resistance. These were caused by channeling during process and also an increase in mass transfer limitation, thus solvent saturation could not be achieved (Salgın and Korkmaz 2011).

S/N value of oil solubility is shown in Table 2 and Fig. 2. In terms of maximize response of candlenut oil solubility, particle size has the largest effect on SCFE-$CO_2$ process based on S/N ratio calculation followed by pressure, temperature and $CO_2$ flow rate.

## Comparison of SCFE-$CO_2$ with Soxhlet n-hexane extraction

The oil yield resulted from Soxhlet extraction using n-hexane was 62.8%, while the maximum yield obtained with SC-$CO_2$ extraction was 61.4% (using sample with particle size of 0.8 mm, extracted at 35 MPa with temperature of 60 °C and SFR 96). Relative density, kinematic viscosity, refractive index, acid value, peroxide value, saponification value and unsaponifiable matter of the candlenut oil were determined to characterize the oil. The physicochemical properties of the oil are shown in Table 3. The oil quality of candlenut obtained by SC-$CO_2$ extraction generally has better quality compared to Soxhlet extraction

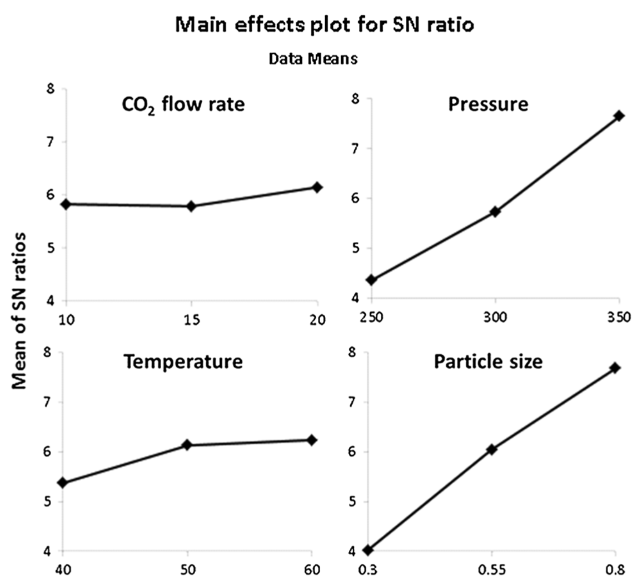


**Fig. 2** Main effects plot for oil solubility

**Table 2** S/N value of oil solubility calculated for each factor and level

| Level | Pressure | Temperature | $CO_2$ flow rate | Particle size |
|---|---|---|---|---|
| 1 | 4.36 | 5.37 | 5.82 | 7.68 |
| 2 | 5.74 | 6.14 | 5.78 | 6.04 |
| 3 | 7.65 | 6.24 | 6.14 | 4.02 |
| Δ (max–min) | 3.28 | 0.87 | 0.36 | 3.66 |
| Rank | 2 | 3 | 4 | 1 |

**Table 3** The physicochemical properties of candlenut oil

| Parameters | Test methods | Unit | Value | |
|---|---|---|---|---|
| | | | SCFE-$CO_2$ | Soxhlet |
| Relative density (20 °C) | AOAC 985.19 | | 0.904 | 0.904 |
| Kinematic viscosity (20 °C) | – | $m^2/s$ | 76.44 | 76.55 |
| Refractive index (20 °C) | AOAC 921.08 | | 1.478 | 1.477 |
| Saponification value | AOAC 920.160 | mg KOH/g oil | 187.6 | 184.9 |
| Acid value | AOAC 940.28 | mg KOH/g oil | 1.5 | 0.85 |
| Peroxide value | AOAC 965.33 | mEq $O_2$/1000 g oil | 4.1 | 7.3 |
| Unsaponifiable matter | AOAC 972.28 | % | 0.69 | 0.91 |
| Moisture content | AOAC 984.20 | % | 0.26 | 0.16 |

using n-hexane. The higher acid value of oil obtained by SCFE-$CO_2$ (1.5 mg KOH/g oil) than Soxhlet extraction (0.85 mg KOH/g oil) is due to the use of lower temperature in SCFE-$CO_2$. The temperatures used were 60 and 80 °C, respectively. Deactivation of lipase responsible for free fatty acid development is effective at higher temperature. However, the use of high temperature may have detrimental effect to oxidative stability of the oil. High temperature is a catalyst for autoxidation process. As shown in Table 3, oil obtained by Soxhlet extraction (7.3 mEq $O_2$/kg oil) has higher peroxide value than those obtained by SCFE-$CO_2$ (4.1 mEq $O_2$/kg oil). Both acid value and peroxide value are indicators of oil rancidity. According to Codex Alimentarius (FAO/WHO), a standard minimum acid value and peroxide value for virgin and cold press oil are 4 mg KOH/g oil and 15 mEq/kg oil, respectively. The oil obtained by SCFE-$CO_2$ has higher purity as it has lower unsaponifiable matter than oil obtained by Soxhlet extraction. The result of saponification value of candlenut oil was 184 (mg of KOH/g oil). The saponification value was within range of 175–250, which was normally found in other seed oils such as safflower, sunflower and corn (Yong and Salimon 2006).

The fatty acid analysis of candlenut oil as determined by GC analysis showed that candlenut oil has high content of oleic acid, linoleic acid and linolenic acid, as shown in Table 4. Oyen et al. (2007) reported that the dominant fatty acid present in candlenut oil were palmitic acid (5–9%), stearic acid (2–7%), oleic acid (11–35%), linoleic acid (34–49%) and linolenic acid (21–35%), which were similar to those found in this present study. This work showed that

**Table 4** Fatty acid composition of candlenut oil

| Fatty acid | | SCFE-$CO_2$ | Soxhlet | Literature (Oyen 2007) |
|---|---|---|---|---|
| Palmitic | C 16:0 | 6.41 | 6.23 | 5–9 |
| Stearic | C 18:0 | 2.53 | 2.46 | 2–7 |
| Oleic | C 18:1 | 26.35 | 26.60 | 11–35 |
| Linoleic | C 18:2 | 39.71 | 40.08 | 34–49 |
| Linolenic | C 18:3 | 25 | 24.63 | 21–35 |
| Σ saturated | | 8.69 | 8.94 | 7–16 |
| Σ mono-unsaturated | | 26.60 | 26.35 | 11–35 |
| Σ poly-unsaturated | | 64.71 | 64.71 | 55–74 |

candlenut oil is a rich source of linoleic acid (C18:2) and linolenic acid (C18:3), accounting for up to 40 and 25% of the total oil, respectively. According to a study by Alabdulkarim et al. (2012), alpha-linolenic acid (ALA) and gamma-linoleic acid (GLA) have many beneficial effects on human health. However, due to the high content of unsaturated oil of approximately 91% (see Table 4), the oil is highly sensitive to oxidation either by light, oxygen and/or temperature. Good storage condition and addition of antioxidant are important to maintain stability of the oil.

## Conclusion

The present study successfully applied Taguchi method to determine optimum supercritical fluid extraction conditions for high solubility of candlenut oil with particle size as the

main parameter affecting the supercritical extraction of candlenut oil. The optimum condition for candlenut extraction is at pressure of 35 MPa, temperature of 60 °C and particle size of 0.8 mm. The oil obtained by SCFE-$CO_2$ has better quality than Soxhlet extraction using n-hexane. The oil contains high unsaturated oil (linoleic acid and linolenic acid) which have many beneficial effects on human health.

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