Engineering Journal
issn: 0125-8281

Article

Lipids from *Vitellaria paradoxa* Gaertn Seeds by Supercritical CO$_2$: Extraction and Optimization of Parameters by Response Surface Methodology

Naomasa Yamamoto[1], Kazuya Murakami[2], Chhouk Kimthet[3], Wahyudiono[1], Siaw Onwona–Agyeman[4], Hideki Kanda[1,a,*], and Motonobu Goto[1]

1 Department of Materials Process Engineering, Nagoya University, Furo–cho, Chikusa–ku, Nagoya 464–8603, Japan
2 Process Engineering, Process Engineering Department, Nippon Steel & Sumitomo Metal Corporation, Tokai-city, 476–8686, Japan
3 Department of Chemical Engineering, Nagoya University, Nagoya, 464–8603, Japan
4 Laboratory of Organic Geochemistry, Tokyo University of Agriculture and Technology, Tokyo, 183–8509, Japan
*E-mail: kanda.hideki@material.nagoya-u.ac.jp

Abstract. Supercritical carbon dioxide (CO$_2$) was employed to extract lipids from *Vitellaria paradoxa* Gaertn seeds in semi–batch process at various operating conditions to optimize extraction process. The extraction processes were carried out at 40 – 60 ^{o}C and 20 – 40 MPa with 2 – 4 ml min^{-1} CO$_2$ flow rates. Analysis of variance (ANOVA) with 33 factorial design combined with statistical was used to optimize variables in the process of *Vitellaria paradoxa* Gaertn seeds lipids extraction with supercritical CO$_2$. The FT–IR spectra indicated that the *Vitellaria paradoxa* Gaertn seeds components were extracted by supercritical CO$_2$. The SEM images also indicated that the physical changes in the surface of the *Vitellaria paradoxa* Gaertn seeds occurred after supercritical CO$_2$ extraction treatment. The maximum of extracted lipids was 0.47 g/g–sample when the extraction was conducted at a temperature of 80 ^{o}C and pressure of 40 MPa with CO$_2$ flow rate of 3 ml min^{-1}. The GC–MS analysis showed that the extracted lipids mainly composed of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2). The analysis of experimental design demonstrated that pressure and temperature were the influential variables on the lipids extraction.

Keywords: Supercritical CO$_2$, *Vitellaria paradoxa* Gaertn, shea butter, extraction, lipids.

ENGINEERING JOURNAL Volume 22 Issue 5
Received 12 March 2018
Accepted 26 June 2018
Published 30 September 2018
Online at http://www.engj.org/
DOI:10.4186/ej.2018.22.5.31

1. Introduction

Vitellaria paradoxa Gaertn has been recognized as a major plant of the woody flora of the Sudan and Guinea savannah vegetation zones of sub–Saharan Africa. This woody flora is generally known as the shea butter tree and has been known to be an iconic fruit tree in the dry savanna woodlands of Africa [1]. The shea butter tree was of African origin and may grow in zones with 400 – 1800 mm rainfall per year. This species has spread out from Senegal to Uganda (West–East Africa) and up to the Adamaoua Province in Cameroon.

The fruit of *Vitellaria paradoxa* Gaertn can be consumed by both humans and animals, however shea butter is the major product of *Vitellaria paradoxa* Gaertn which is marketed on local and international markets [2, 3]. The *Vitellaria paradoxa* Gaertn butter can be used as an alternate for cocoa butter in chocolate and confectionery industries. The extracted butter from the *Vitellaria paradoxa* Gaertn seed kernel also might be applied for manufacturing body care products while the oil product of this seed kernel can be applied as margarines to substitute olive oil in baking process. Moreover, different parts of this plant (leaves, roots, and stem bark) have been applied in the pharmaceutical industry. They are used for enteric infections treatment such as helminthes, dysentery, diarrhea, and other gastrointestinal tract infections, wound infections and skin diseases [4].

Here, supercritical fluid would be applied to extract value added compounds including phytochemicals which have potential nutrient and therapeutic properties from *Vitellaria paradoxa* Gaertn seeds. This extraction process can recover phytochemicals from *Vitellaria paradoxa* Gaertn seeds while leaving the *Vitellaria paradoxa* Gaertn seeds in a state in which they can be used as a renewable source of energy. Supercritical fluids may act as selectively dissolving liquid solvents when temperature and pressure are tuned, therefore they have been widely applied in many industries for extraction, purification, re-crystallization, and fractionation processes that are accepted to be more efficient extraction fluids than conventional liquid solvents [5]. Supercritical carbon dioxide (CO_2) is the most general employed media as low temperatures may be applied. This makes it appropriate for the thermolabile compounds extraction from plant biomass [5–11]. It also can be applied to extract the easily oxidized compounds from plant matrix [12–14]. Compared with other traditional extraction techniques, such as soxhlet extraction, supercritical CO_2 extraction is very gainful and environmentally friendly. Supercritical CO_2 became an interesting extraction media due to its high diffusivity, coupled with its high solvent strength that can be easily adjusted by shifting of temperature and/or pressure. In addition, CO_2 exists in the gaseous phase at room temperature thus it can be easily removed and re-used as the solutes dissolved in the supercritical CO_2 will separate after depressurization process. Consequently, supercritical CO_2 extraction may pave way for an efficient extraction and fractionation technique which may applied in the food and pharmaceutical industries.

2. Experimental Section

2.1. Materials

Dried seeds of *Vitellaria paradoxa* Gaertn were purchased from Sanoflore Co., France. Before extraction, the grinder device (IKA, MF–10–B–S1, USA) was applied to crush the *Vitellaria paradoxa* Gaertn seeds into certain particle sizes and sieved through fine–mesh sieves (MF–Sieb, Germany). The particle size of *Vitellaria paradoxa* Gaertn seeds was then 0.556 mm. The fatty acids methyl esters standard (FAME; Supelco 37 Component. FAME Mix.) was bought from Sigma–Aldrich St. Louis, MO, USA. Hexane (C_6H_{14}; 96.0%), sodium chloride (NaCl; 99.5%), potassium chloride (KCl; 99.5%), and hydrochloric acid (HCl; 35.0 – 37.0%) were obtained from Wako Pure Chemical Industries, Ltd., Japan. Carbon dioxide (CO_2: 99%) was bought from Sogo Kariya Sanso, Inc. Japan.

2.2. Experimental Setup and Procedure

Figure 1 illustrates the apparatus scheme of supercritical CO_2 extraction system. The extraction system consists of a high–pressure pump for CO_2 (PU–2080; Jasco, Japan), oven (WFO–400; EYELA, Tokyo, Japan), a 10 ml extractor (Thar Technologies, Inc., PA, USA) and back pressure regulator (AKICO, Tokyo, Japan). The supercritical CO_2 extraction experiments were performed at 40 – 80 ^{o}C and 20 – 40 MPa in a semi–batch process. The flow rates of CO_2 were 2 – 4 ml min^{-1}. 2.0 g of feed (*Vitellaria paradoxa* Gaertn seeds) was loaded into the Thar extraction cell, while the packed cotton wool was also placed at the sides of the Thar extraction cell (top and bottom) at each extraction process. This packed cotton wool may prevent the solid samples from being moved into the tubing and clogging the extraction system. The Thar extraction cell was located in the oven to maintain the extraction temperature. Briefly, the extraction step could be described as follow. First, the Thar extractor that has been filled with the dried *Vitellaria paradoxa* Gaertn seeds was put in the oven. Next, the oven power was turned on when the set up extraction apparatus was completed. After the oven temperature reached the desired temperature, CO_2 from a cylinder was introduced into the Thar extraction cell via high pressure pump. The extracts were catched and saved in vials every 30 min for 240 min. They were then weighed and immediately placed in the refrigerator at 5 °C. The vials containing extracts were covered with aluminum sheet. These steps were performed until next analysis.

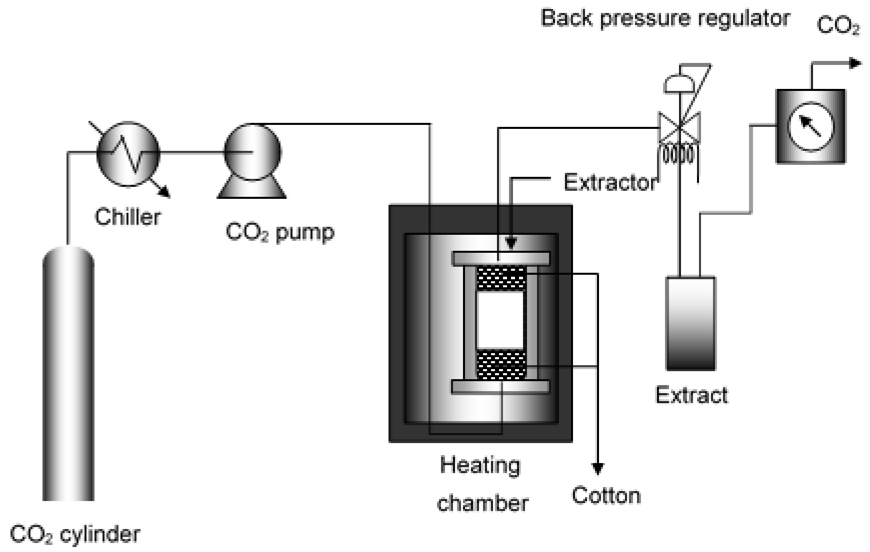

Fig. 1. Schematic diagram of supercritical CO_2 extraction apparatus.

2.3. Analytical Methods

The component in each extract was analyzed by GC–MS (gas chromatograph mass spectrometer; Agilent 7890A–GC) which was equipped with an Agilent 5975C mass spectrometer. The used column was a phenyl arylene capillary column (HP–5MS; 30 m x 0.25 mm with 0.25 μm Film Thickness). The program temperatures were ramped from 60 °C (5 min) to 320 °C with 4 °C min^{-1}. The temperatures of injector and detector were 250 °C. The helium gas flow rate as a carrier gas was 24 ml min^{-1} and the 1.0 μL sample was injected via autosampler device. The FAME standard was employed to quantify the amount of extracted FAME from *Vitellaria paradoxa* Gaertn seeds. Solid residues obtained at each extraction process were placed in the oven at 60 °C. Next, they were characterized by Fourier transform infrared spectrometer (FT–IR Spectrum Two; Perkin–Elmer Ltd., England). A SEM device (Scanning Electron Microscope; JEOL JSM–6390–LV) was also applied to characterize the morphologies of *Vitellaria paradoxa* Gaertn seeds before and after extraction process.

3. Results and Discussion

Extraction has been known as the process to remove soluble components from insoluble substances (liquid or solid) by introducing a solvent, especially liquid solvent. It might take place when the liquid solvent was passed in the plant matrix at high temperatures and/or high pressures. Thus, this phenomenon will also happen when the supercritical CO_2 was introduced into the plant matrix since the solvent power change that can drive the extraction process [5, 15, 16]. Figure 2 shows the *Vitellaria paradoxa* Gaertn seeds spectra by FT–IR before and after supercritical CO_2 extraction treatment when extraction experiment was carried out at 40 °C and 40 MPa with CO_2 flow rate 3 ml min^{-1}. It could be seen that the similar spectras of FT–IR were found on the *Vitellaria paradoxa* Gaertn seeds before and after supercritical CO_2 extraction treatment. Table 1 shows the wave number assignments of FT–IR spectra [17]. The spectra indicates that the supercritical

CO_2 extraction treatment on the *Vitellaria paradoxa* Gaertn seeds did not change its functional groups distribution. However, due to the removing or reducing *Vitellaria paradoxa* Gaertn seeds substances happened during extraction, the different peaks intensities of them could be obtained clearly. At these conditions, supercritical CO_2 can diffuse into the *Vitellaria paradoxa* Gaertn cells and then dissolve *Vitellaria paradoxa* Gaertn seed components through the generation of *Vitellaria paradoxa* Gaertn seeds components–CO_2 complexes via van der Waals associations [18]. Due to this phenomenon, when the supercritical CO_2 flows out from *Vitellaria paradoxa* Gaertn seeds matrix, the *Vitellaria paradoxa* Gaertn seed components might also go out simultaneously. Hence, it could be said that the applied supercritical CO_2 on the *Vitellaria paradoxa* Gaertn seeds matrix may remove the chemical compounds from them [5, 19–21]. The peak intensities of the band at 2922.65 – 2922.09 and 2852.71 – 2851.5 cm^{-1} were attributed to C–H stretching from a decline in methylene and methyl groups after supercritical CO_2 extraction treatment. A similar phenomenon was also obtained in other functional groups at regions of 1744.74 – 1740.6; 1367.14 – 1336.64; and 1207.15 – 1203.30 cm^{-1} corresponding to the vibration of C=O stretching of carboxylic acids, aldehydes, ketones or esters; the stretching of C–O, the deformation of C–H and N–H ; and amide III collagens groups. This revealed that they might be destroyed and removed during treatment of supercritical CO_2 extraction on *Vitellaria paradoxa* Gaertn matrix. Similar results were obtained by Ono *et al.* 2017 [22], they conducted experiments on phytochemicals extraction from grains of paradise seeds by using supercritical CO_2. They reported that the functional groups intensities which exist in the grains of paradise seeds declined after introducing supercritical CO_2.

Table 1. The typical functional groups and the infrared signal with the possible compounds.

Wave number [cm^{-1}]	Functional groups	Compounds
3600 – 3000	O–H stretching	Acid, methanol
2860 – 2970	C–H_n stretching	Alkyl, aliphatic, aromatic
1700–1730, 1510–1560	C = O stretching	Ketone and carbonyl
1632	C = C	Benzene stretching ring
1613, 1450	C = C stretching	Aromatic skeletal mode
1470–1430	O–CH_3	Methoxyl–O–CH_3
1440–1400	O–H bending	Acid
1402	C–H bending	
1232	C–O–C stretching	Aryl–alkyl ether linkage
1215	C–O stretching	Phenol
1170, 1082	C–O–C stretching vibration	Pyranose ring skeletal
1108	O–H association	C–OH
1060	C–O stretching and C–O deformation	C–OH (ethanol)
700–900	C–H	Aromatic hydrogen
700–650	C–C stretching	

Figure 3 shows the SEM images of *Vitellaria paradoxa* Gaertn seeds before and after supercritical CO_2 extraction treatment at 80 °C with 20 – 40 MPa extraction pressures and 3 ml min^{-1} CO_2 flow rate. As mentioned earlier, supercritical CO_2 has the ability to penetrate into *Vitellaria paradoxa* Gaertn seeds and to remove their components via van der Waals interactions. Consequently, the different surface structures of *Vitellaria paradoxa* Gaertn seeds before and after treatment by supercritical CO_2 extraction could be observed clearly. It shows that the surface morphology of *Vitellaria paradoxa* Gaertn seeds was wrapped by the membrane–like structure before introducing supercritical CO_2 treatment. There are no pores or any surface cracks, and appear smooth and tight with some boundary edges clearly. After supercritical CO_2 extraction treatment, it could be seen that the surface morphologies of the *Vitellaria paradoxa* Gaertn seeds were seemingly damaged. It indicates that supercritical CO_2 possibly breaks the physical structural barriers of the *Vitellaria paradoxa* Gaertn seeds and extract their substances. The disrupted physical structures or the removed membrane–like structures were obtained and observed clearly at a higher extraction pressure (40 MPa). At this condition (40 MPa), the increasing operating pressure may enhance CO_2 diffusivity to penetrate into the

Vitellaria paradoxa Gaertn seeds. As a result, supercritical CO_2 will result in a more powerful swelling action to disrupt and transfer the *Vitellaria paradoxa* Gaertn seeds components [23].

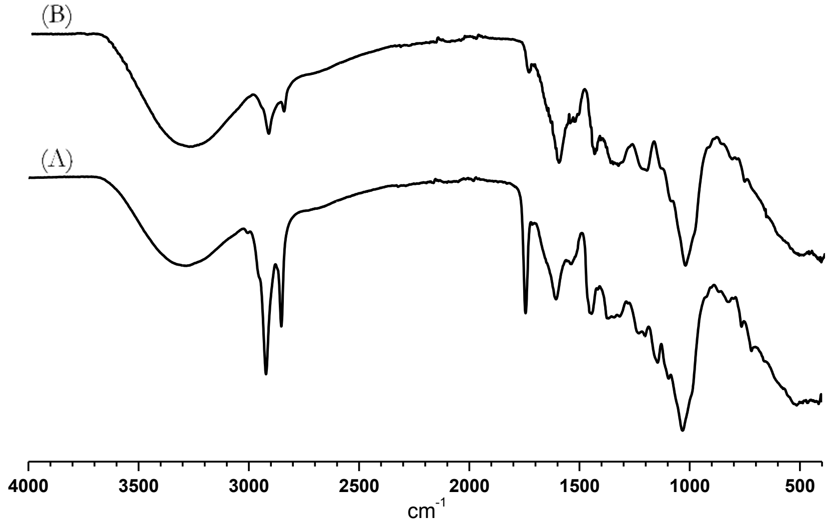

Fig. 2. FT–IR spectra of *Vitellaria paradoxa* Gaertn seeds and its solid residue.

Sovova [24] observed the vegetable oil extraction rate by supercritical CO_2. She reported that CO_2 under supercritical conditions can break vegetable cells or the outer surface of vegetable matrix to reach extractable components. She described that supercritical CO_2 extraction process consisted of a three–step procedure. First, is the constant extraction rate period. At this step, the convective mass transfer between the surface vegetable components and the fluid phase occurred. She explained that supercritical CO_2 as a solvent might carry easily removable vegetable components from rupture vegetable cells during this step followed by the falling extraction rate period and the diffusion controlled rate period. Consequently, as shown in the SEM images, the physical surface structures of vegetable matrix including *Vitellaria paradoxa* Gaertn seeds occurred after supercritical CO_2 extraction treatment.

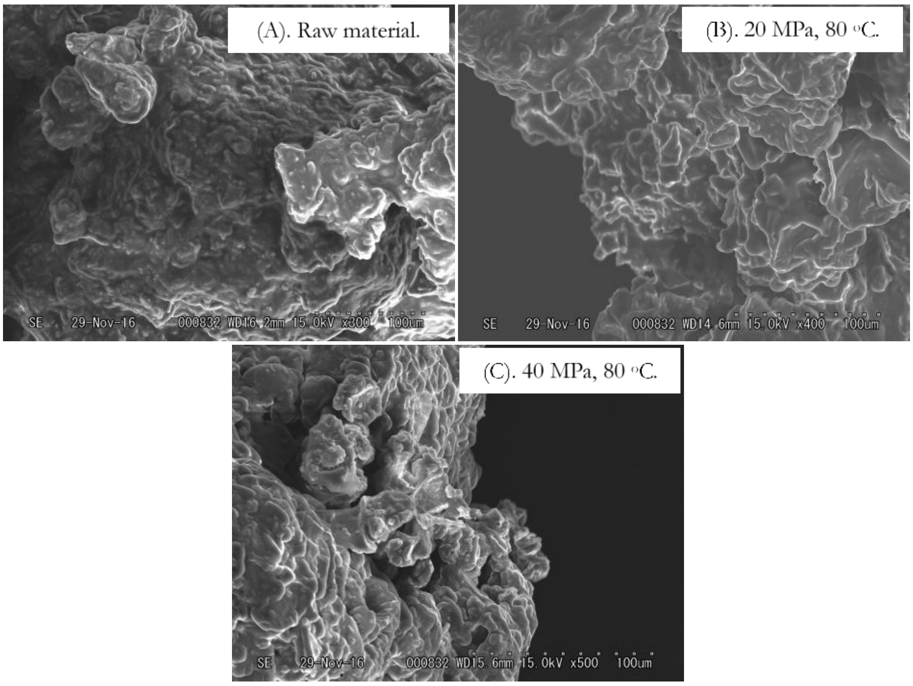

Fig. 3. SEM images of *Vitellaria paradoxa* Gaertn seeds and its solid residue.

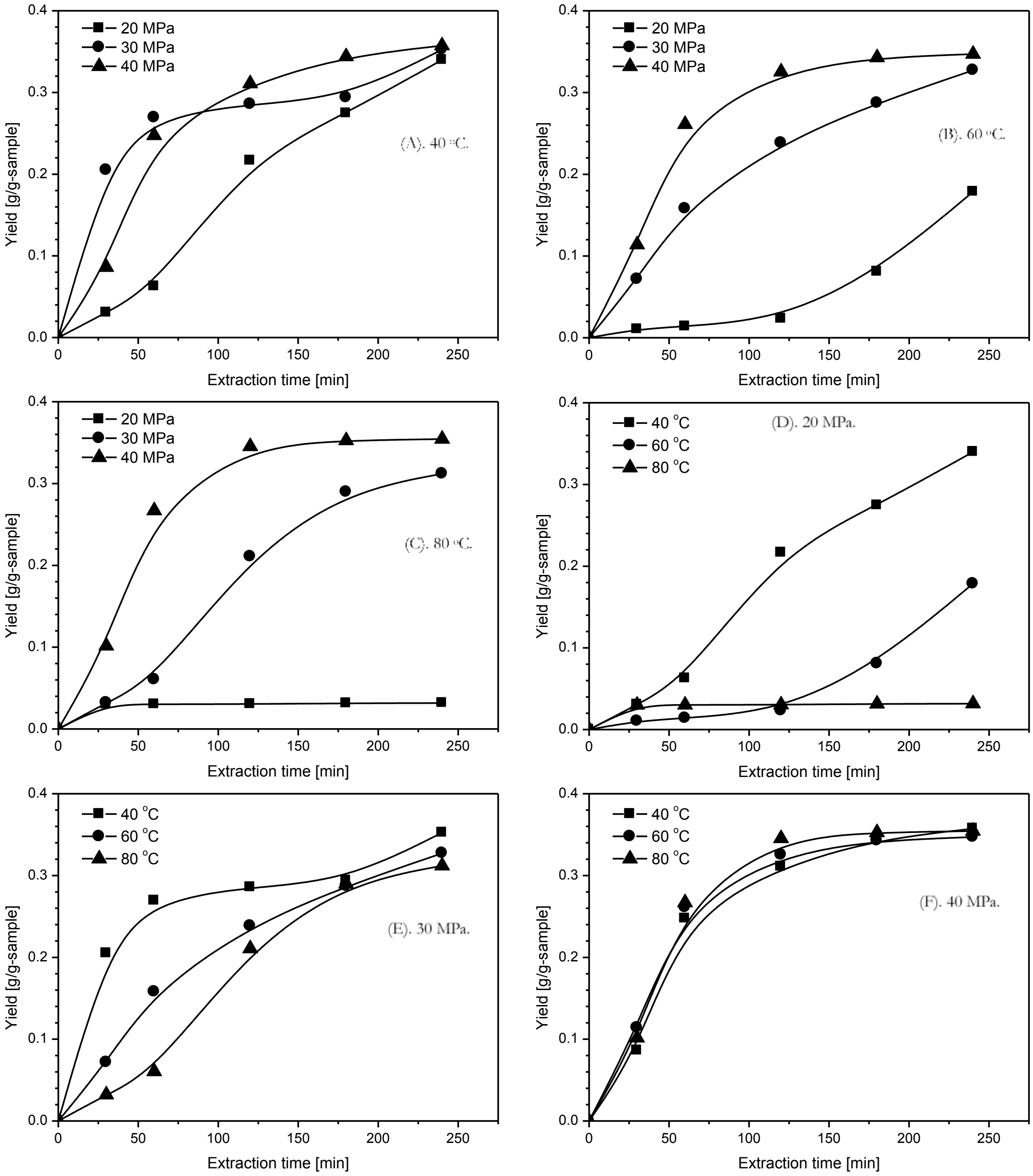

Fig. 4. Yields of *Vitellaria paradoxa* Gaertn seeds extraction.

Figure 4 (A–C) shows the yields of *Vitellaria paradoxa* Gaertn seeds extraction curves under varying operating pressures and temperatures with CO_2 flow rate of 3 ml min^{-1}. The color of extract which was obtained under each extraction condition was a clear oily liquid to pale yellow color with increasing extraction time. Clearly, the figure illustrated that the extraction yield increased at constant temperature with elevating pressure. The amount of extraction yields were about 0.03 and 0.06 g/g–sample when the extraction process was conducted at 20 and 30 MPa with 80 °C and 60 min extraction time, respectively. Significantly, it could approach 0.27 g/g–sample when the extraction pressure was raised to 40 MPa with the same extraction conditions (extraction time and extraction temperature). Similar results were obtained in other extraction conditions. This phenomenon is corresponded to a direct raising of supercritical CO_2 density and therefore the solvating power of supercritical CO_2 was also increased [25]. Conversely, Egydio *et al.* 2010 [26] informed that operating pressures did not result in a significant effect on the extraction yield when they applied supercritical CO_2 on tomato juice as a starting material at 40 – 80 °C and 20 – 35 MPa. Next, Machmudah *et al.* 2012 [25] informed that the increasing operating pressure of supercritical CO_2 extraction system beyond an optimum point may drive to improve in the yield of extraction. Next, they explained that a further rise in operating pressure led to a decline in the extraction yield at the same operating temperature. Similar to operating pressures, the change of operating temperatures in supercritical CO_2 extraction system will also influence the yields of *Vitellaria paradoxa* Gaertn seeds extraction. At these conditions, the increasing operating temperatures will lead to a decline in density of CO_2 at a constant pressure, consequently, the reduction of CO_2 solvent power will occur. Simultaneously, the operating temperature also affected the solute volatility. Therefore, the operating temperature effect is not easy to estimate due to its dependence on the raw material nature. However, it could be seen in Fig. 4 (D – F) that increasing operating temperature at a constant pressure with CO_2 flow rate 3 ml min^{-1} seemed to decrease the yields of *Vitellaria paradoxa* Gaertn seeds extract. It indicated that the CO_2 density might possess dominant effect during the extraction process.

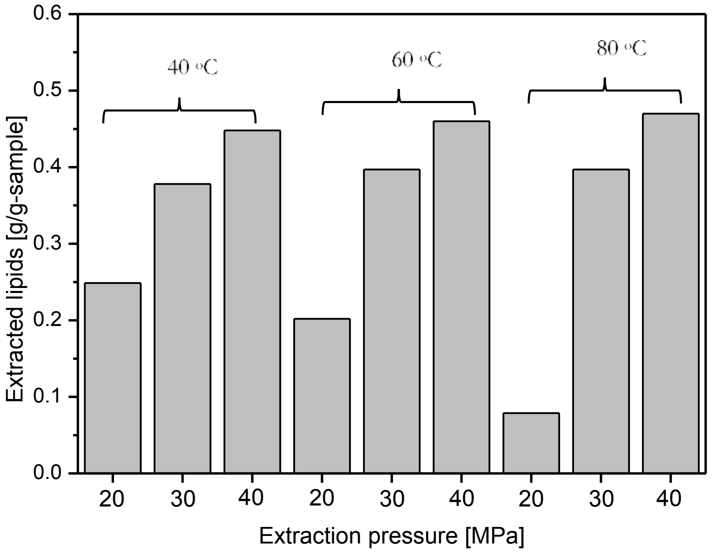

Fig. 5. Yields of lipids from *Vitellaria paradoxa* Gaertn seeds extraction.

Table 2. FAME composition of *Vitellaria paradoxa* Gaertn seeds.

Fatty acids	Components [Peak area %]		
	Supercritical CO_2	Hexane	Ethanol
C16:0	3.3	2.7	3.3
C18:2	10.1	7.6	4.7
C18:1	48.0	54.2	45.0
C18:0	37.2	35.5	46.0
Unidentified	1.4	0	1.0
Saturated fatty acid	40.5	38.2	49.3
Unsaturated fatty acid	58.1	61.8	49.7

To understand the amount of lipids in the extract of *Vitellaria paradoxa* Gaertn seeds, the hexane solvent was introduced to each extract sample of *Vitellaria paradoxa* Gaertn seeds. This solvent is the most commonly used to extract lipids from biomass matrix. Compared to other common solvents such as chloroform or petroleum ether, hexane is a nontoxic organic solvent. Figure 5 shows the lipids amount from the extract of *Vitellaria paradoxa* Gaertn seeds at various extraction conditions. As the major substances of biological membranes, lipids may provide many functions in plants and they are comprised of a hydrophobic barrier. This compound generally contains hydrocarbons with long chains, however, some elements such as oxygen, nitrogen, phosphorus or sulfur were also found. As illustrated in Fig. 5, the lipids amount improved clearly with raising extraction pressure at a constant extraction temperature. At these conditions, the increasing operating pressure will increase the density of CO_2, next, the raising in the supercritical CO_2 density in the extraction system promotes the solvating power of CO_2. Thus the extraction efficiency enhancement including lipids extraction might be happened. At a constant pressure, when the operating temperature was raised, the amount of extracted lipids from *Vitellaria paradoxa* Gaertn seeds seems confound at each condition.

This can be explained as follows. At a constant pressure, the CO_2 density decreases with raising temperature and promotes to the fluid solvent power reduction, however, the increasing temperature may increase vapor pressure which affects solubility. Due to this isotherm phenomena, the confound effects on the extraction yields in overall including lipids extraction may occur. In plant seeds including those of *Vitellaria paradoxa* Gaertn, lipids are comprised of a mixture of many types, but triacylglycerols are generally the main component. These triacylglycerols contain three fatty acids esterified to each hydroxyl position of a glycerol molecule. Hence, before GC–MS analysis, the esterification of extracted lipids were needed. Here, the acid–catalyzed esterification technique was applied in all extracted lipids. The main components in the GC–MS spectra (based on NIST mass spectral database) were matched with the FAME standard. Table 2 shows the major compositions of FAME in extracted lipids from *Vitellaria paradoxa* Gaertn seeds. It shows that irrespective of the extraction method introduced, the compositions of FAME in extracted lipids from *Vitellaria paradoxa* Gaertn seeds remained similar consisting C16 – C18 as major components. These fatty acids (C16 – C18) are generated from the transesterification of extracted lipids using acidic conditions and these compounds were also needed for human metabolism due to the higher portion of unsaturated fatty acids. These typical FAME components were similar to those of vegetable oils and suitable for generating biofuels, such as biodiesel [27].

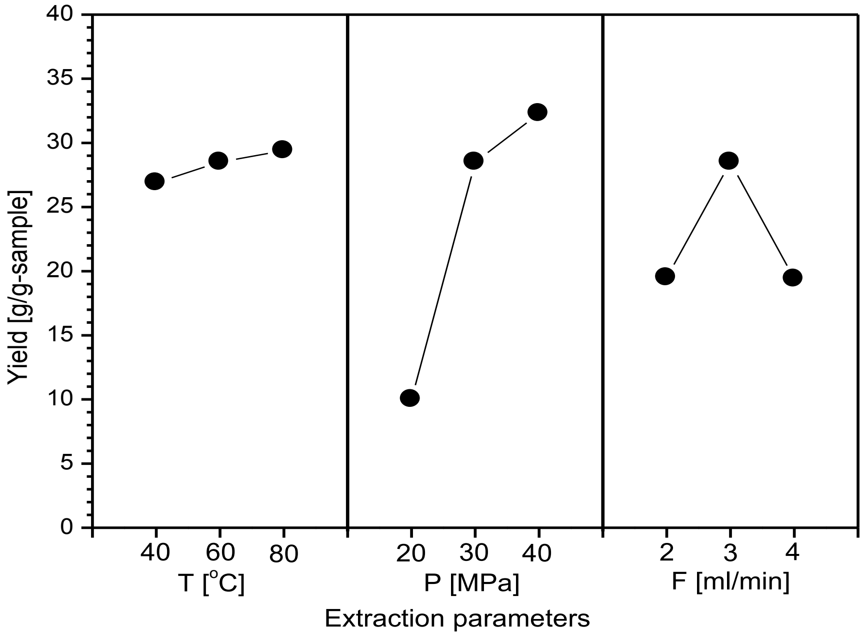

Fig. 6. Plot of main variables on the *Vitellaria paradoxa* Gaertn seeds extraction yield in average.

Table 3. Values of independent variables.

Factor		Units	Levels		
Variable	Key		Low (–)	Middle (0)	High (+)
Temperature	*T*	°C	40	60	80
Pressure	*P*	MPa	20	30	40
CO_2 flow rate	*F*	ml min^{-1}	2	3	4

In this work, response surface methodology was applied to optimize the extraction process of *Vitellaria paradoxa* Gaertn seeds by using supercritical CO_2. In this method, the statistical and the optimization methods were combined hence it can be applied to the model and optimize experimental designs. This method was applied to study the effects of pressure (*P*), temperature (*T*), and flow rate of CO_2 (*F*) on the extraction yield as independent variables. These selected factors were chosen due to the fact that they have been known as important parameters in the supercritical CO_2 extraction process. The effect of these variables on supercritical CO_2 extraction was simultaneously observed using a three–factor design, with three levels for each factor high (+1), medium (0), and low (–1); see Table 3. Table 4 lists experiments number that were performed to optimize extraction processes. Each experiment process was conducted in duplicated and/or triplicated. As informed in this Table, the maximum amount of yield from *Vitellaria paradoxa* Gaertn seeds extraction is about 0.418 g/g–sample when the experiment was conducted at run #25 (80 °C, 40 MPa, 2 ml min^{-1}). It was not as expected that the higher amount of extraction yield would be found at high extraction pressure with low extraction temperature owing to the high CO_2 density. This phenomenon might be caused by the competition between the density of solvent and vapor pressure of solute. At these conditions, Machmudah *et al.* 2007 [28] reported that the smaller change in density of solvent occurs and therefore the vapor pressure of solute alteration serves more effective to overcome the solvent density effect resulting to the amount of extraction yield. The higher extraction temperature may also contribute to the cell walls degradation. Consequently, the *Vitellaria paradoxa* Gaertn seeds extraction yield is increase.

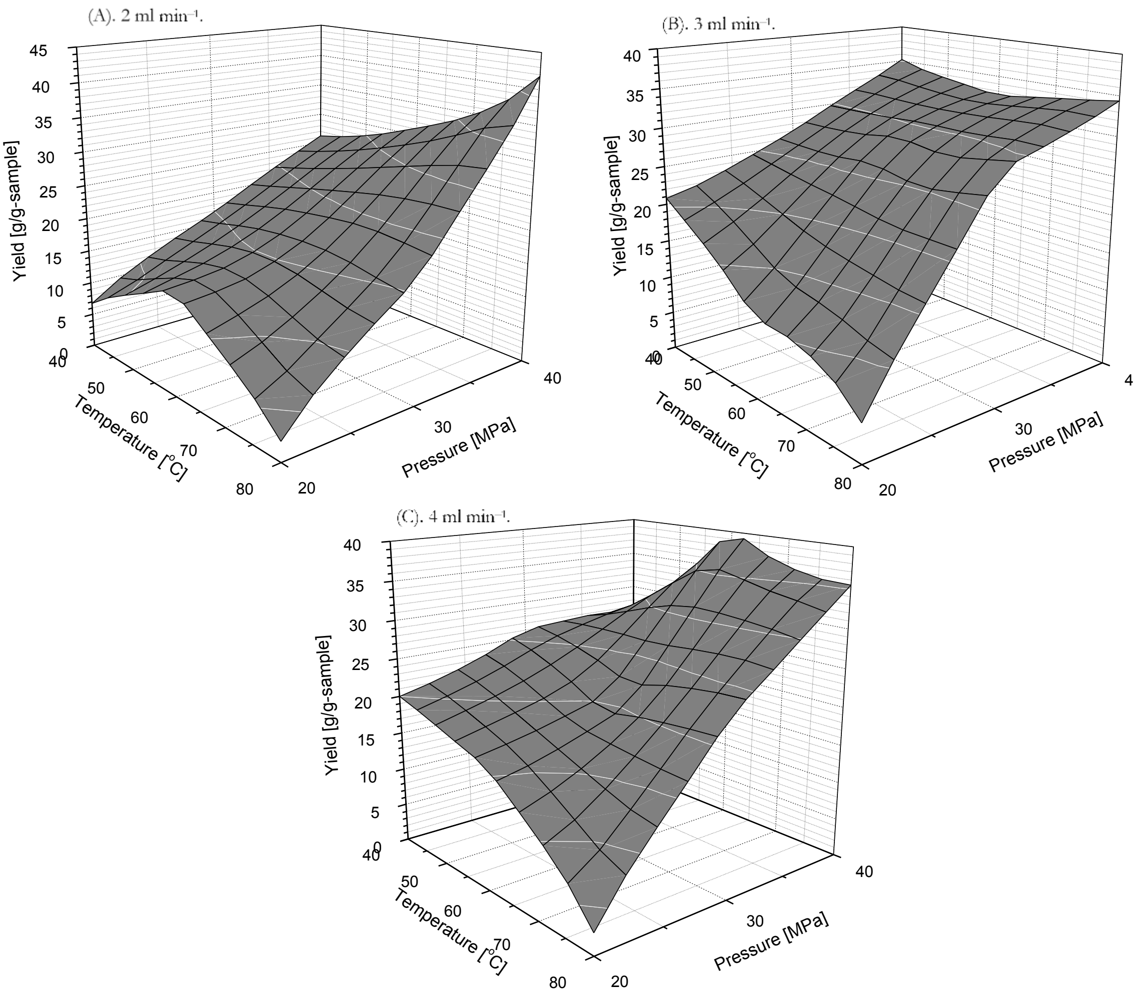

Fig. 7. Prediction of the *Vitellaria paradoxa* Gaertn seeds extraction yield using the empirical correlation.

Figure 6 illustrates the effects of main variables on the *Vitellaria paradoxa* Gaertn seeds extraction yield. This figure reveals that pressures and temperatures as extraction parameters seem to be the dominant variables to affect the yield of *Vitellaria paradoxa* Gaertn seeds extraction, with the significance degree of *p*–value <0.05 (see Table 5). It seemed that applying low extraction temperature and middle extraction pressure were needed to reach the highest extraction yield at the flow rate of CO_2 in middle level. Table 5 lists the significance degree of each factor which was represented by the *p*–value; if the *p*–value factor is less than 0.05, it affects the extraction process in a significant effect with 0.95 confidence level. This table depicts the extraction yield of *Vitellaria paradoxa* Gaertn seeds which was affected by the relation between extraction temperature and extraction pressure, while the relation between flow rate of CO_2 and extraction temperature or extraction pressure does not influence the yield of *Vitellaria paradoxa* Gaertn seeds extraction (*p*–value > 0.05). From the relations among variables, the experimental design correlation of empirical was proposed.

Equation (1) is correlation between variables that affect the *Vitellaria paradoxa* Gaertn seeds extraction process with supercritical CO_2:

$$Y = 24.16138 - 1.54293T + 2.24035P + 0.037256TP + 0.003264T^2 - 0.05694P^2 \quad (1)$$

where Y is the extraction yield of *Vitellaria paradoxa* Gaertn seeds (g/g–sample), T is temperature of extraction (°C), P is pressure of extraction (MPa), and 0.85 is the correlation coefficient. From the fitting the experimental data using solver in Excel 2016, Eq. (1) was determined. Figure 7 illustrates the plot of equation 1 in three–dimensional graph with a parabolic surface appearance. Similar to findings above, the yield of extraction declines with raising temperature of extraction at low and middle of extraction pressures, but rises with raising temperature of extraction at high pressure of extraction. Next, it is very likely that the high extraction yield of *Vitellaria paradoxa* Gaertn seeds may be obtained at extraction pressures of 30 – 40 MPa and at extraction temperatures of 40 – 80 °C, especially at high extraction pressure and temperature.

Table 4. Experimental design and result of extraction yield.

Run	T	P	F	Extract [g/g–sample]
1	–	–	–	0.068
2	–	–	0	0.208
3	–	–	+	0.200
4	–	0	–	0.167
5	–	0	0	0.269
6	–	0	+	0.263
7	–	+	–	0.262
8	–	+	0	0.352
9	–	+	+	0.258
10	0	–	–	0.158
11	0	–	0	0.100
12	0	–	+	0.160
13	0	0	–	0.195
14	0	0	0	0.285
15	0	0	+	0.194
16	0	+	–	0.312
17	0	+	0	0.323
18	0	+	+	0.410
19	+	–	–	0.028
20	+	–	0	0.049
21	+	–	+	0.028
22	+	0	–	0.179
23	+	0	0	0.294
24	+	0	+	0.231
25	+	+	–	0.418
26	+	+	0	0.344
27	+	+	+	0.354

Table 5. Analysis of the experimental design for process optimization.

Variables	T	P	F	$T \times P$	$T \times F$	$P \times F$
p–value	0.6165	<0.0001	0.6051	0.0002	0.6148	0.5726

Figure 8 shows the solubility of *Vitellaria paradoxa* Gaertn seeds lipids in supercritical CO_2 as an extraction pressure function with 3 ml min^{-1} flow rate of CO_2. It shows that the solubility of *Vitellaria paradoxa* Gaertn seeds lipids in supercritical CO_2 increased with increasing extraction pressure at each extraction temperature. Solvent density and the vapor pressure appeared to have a high influence on this phenomenon. Reverchon and De Marco 2006 [29] reported that extraction pressure and extraction temperature were the most dominant parameters that influences the solvent power and selectivity of supercritical CO_2 extraction medium which in turn determine the target component yield. However, Ghoreishi *et al.* 2012 [30] maintained that the extraction temperature effect in the system of supercritical CO_2 extraction is more complicated since the competition between density of solvent and solute vapor pressure. The decline in the density of solvent may decrease the solubility of solute, while the solute vapor pressure increases with environment temperature, leading an improvement in solubility. Their prominent effects are influenced by the type of extraction process. This figure also shows that increasing solubility with increasing extraction pressure was more clear at 80 °C (higher tendency) and less pronounced at 40 °C (lower tendency). The solubility isotherms intersection is also examined at 25 MPa, showing that the solubility is not temperature dependence at this pressure environment, shifting the behavior of solubility with temperature. The higher values of solubility are found at lower temperature environments at below 25 MPa. On the contrary, the increasing temperature promoted to improve in solubility at above 25 MPa. A specified crossover pressure between the solubility isotherms was general for solubility pure compounds data in supercritical CO_2, for instance a crossover pressure of 25 MPa for squalene [31], 17 MPa for coumarin [32], 18 MPa for celecoxib [33], and near point of intersection for related compounds mixtures, such as mixture of triacylglycerol with values of 30 MPa for rice bran oil [16], and from 25 to 30 MPa for fish oil [34].

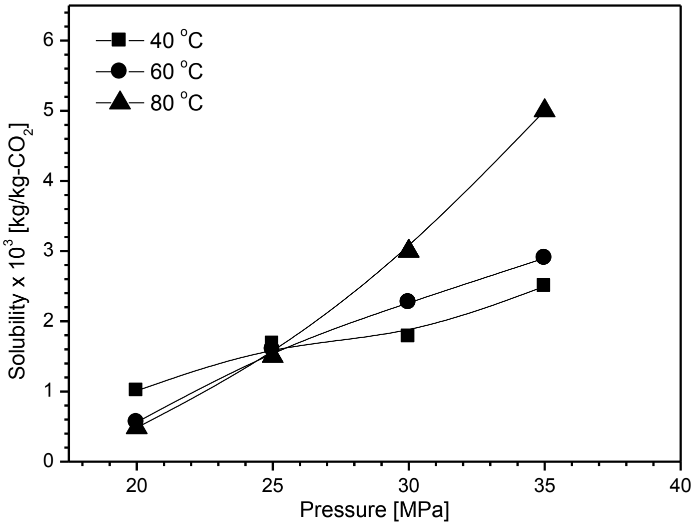

Fig. 8. Solubility of extracted lipids from *Vitellaria paradoxa* Gaertn seeds.

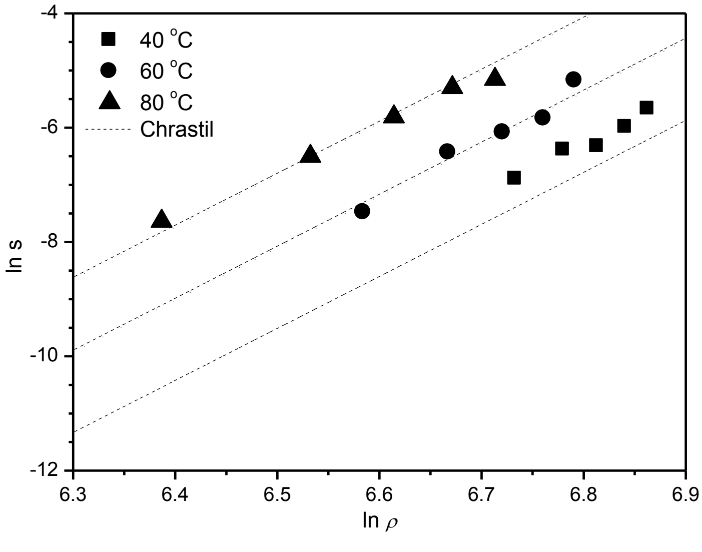

Fig. 9. Equations plot of extracted lipids from *Vitellaria paradoxa* Gaertn seeds.

In this work, the Chrastil equation [35] is employed to correlate apparent solubility between *Vitellaria paradoxa* Gaertn seeds lipids and supercritical CO_2. The Chrastil equation can be expressed as:

$$\ln(s) = k\,ln(\rho) + \frac{a}{T+b} \tag{2}$$

with

$$a = \frac{\Delta H}{R} \text{ and } b = -\ln\left[\frac{{M_\mathrm{C}}^{k}}{M_\mathrm{S} + kM_\mathrm{C}}\right] + q$$

where s was the solubility, ρ was density of solvent, T was operating temperature, k was number of association, ΔH was reaction total heat, q was a constant, and M_S and M_C are the molecular weights of solute and solvent, respectively. Figure 9 shows the apparent solubility of *Vitellaria paradoxa* Gaertn seeds lipids and supercritical CO_2 satisfy the Chrastil equation. The log s can be stated from extraction temperature as a linear function of $1/T$ at constant density, and possess a slope expressed by the constant a value. In order to reduce the deviation of equation from experimental data, the selected constant b value might be applied. By applying the Chrastil equation on the experimental data which was obtained from all experimental conditions studied, the values of constants were obtained as follows: $k = 10.1$, $a = -7500$, and $b = -44.7$. As shown in Fig. 9, the isotherm determined from Chrastil equation by using the aforementioned constants was a good prediction with the experimental points with error below 1%. It could be pointed out that the calculation was a good estimation of the values of experimental and proves that the predicted parameter gives a good depiction of the experimental data.

4. Conclusions

Supercritical CO_2 was employed as a media for extraction of lipids from *Vitellaria paradoxa* Gaertn seeds in semi–batch process. The extraction processes were performed at 40 – 60 °C and 20 – 40 MPa with 2, 3 and 4 ml min^{-1} CO_2 flow rates. The FT–IR spectra showed that the substances from *Vitellaria paradoxa* Gaertn seeds were extracted by supercritical CO_2. The SEM images illustrated that the changes of physical in the surfaces of *Vitellaria paradoxa* Gaertn seeds happened after treatment by supercritical CO_2 extraction. The optimum amount of extracted lipids could reach 0.47 g/g–sample when the experiment was performed at 80 °C and 40 MPa with CO_2 flow rate of 3 ml min^{-1}. The GC–MS indicated that the lipids consisted of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) as main components. The parity plot showed that the calculation gave an accurate estimation of the experimental values and verified that the predicted parameters gave a good depiction of the experimental data. In addition, our experiment has revealed that supercritical CO_2 extraction method is an appropriate method for extracting lipids from *Vitellaria paradoxa* Gaertn seeds.

References

[1] IPGRI, INIA, "Descriptors for Shea tree (*Vitellaria paradoxa*)," International Plant Genetic Resources Institute, Rome, Italy; Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria, Madrid, Spain, 2006, pp. 3.

[2] P. Byakagaba, G. Eilu, J. B. L. Okullo, S. B. Tumwebaze, and E. N. Mwavu, "Population structure and regeneration status of *Vitellaria paradoxa* (C.F.Gaertn.) under different land management regimes in Uganda," *Agric. J.*, vol. 6, no. 1, pp. 14–22, Jan. 2011.

[3] K. Aleza, K. Wala, J. Bayala, G. B. Villamor, M. Dourma, W. Atakpama, and K. Akpagana, "Population structure and regeneration status of *Vitellaria Paradoxa* (CF Gaertner) under different land management regimes in Atacora department, Benin," *Agroforest. Syst.*, vol. 89, no. 3, pp. 511–523, Jun. 2015.

[4] M. O. Israel, "Effects of topical and dietary use of shea butter on animals," *Am. J. Life Sci.*, vol. 2, no. 5, pp. 303–307, Oct. 2014.

[5] M. Goto, H. Kanda, Wahyudiono, and S. Machmudah, "Extraction of carotenoids and lipids from algae by supercritical CO_2 and subcritical dimethyl ether," *J. Supercrit. Fluids*, vol. 96, pp. 245–251, Jan. 2015.

[6] R. Marr and T. Gamse, "Use of supercritical fluids for different processes including new developments – A review," *Chem. Eng. Process.*, vol. 39, pp. 1, pp. 19–28, Jan. 2000.

[7] Q. Lang and C. M. Wai, "Supercritical fluid extraction in herbal and natural product studies – A practical review," *Talanta*, vol. 53, no. 4, pp. 771–782, Jan. 2001.

[8] A. Meireles and M. Angela, "Supercritical extraction from solid: process design data (2001-2003)," *Curr. Opin. Solid State Mater. Sci.*, vol. 7, no. 4–5, pp. 321–330, Aug. – Oct. 2003.

[9] K. Ghafoor, J. Park, and Y. K. Choi, "Optimization of supercritical carbon dioxide extraction of bioactive compounds from grape peel (*Vitis labrusca* B.) by using response surface methodology," *Innov. Food Sci. Emerg. Technol.*, vol. 11, no. 3, pp. 485–490, Jul. 2010.

[10] H. Nerome, M. Ito, S. Machmudah, Wahyudiono, H. Kanda, and M. Goto, "Extraction of phytochemicals from saffron by supercritical carbon dioxide with water and methanol as entrainer," *J. Supercrit. Fluids*, vol. 107, pp. 377–383, Jan. 2016.

[11] S. Machmudah, Widiyastuti, S. Winardi, Wahyudiono, H. Kanda, and M. Goto, "Sub– and supercritical fluids extraction of phytochemical compounds from *Eucheuma cottonii* and *Gracilaria* sp," *Chem. Eng. Trans.*, vol. 56, pp. 1291–1296, Apr. 2017.

[12] G. Brunner, "Supercritical fluids: Technology and application to food processing," *J. Food Eng.*, vol. 67, no. 1–2, pp. 21–33, Mar. 2005.

[13] L. A. Conde–Hernandez, J. R. Espinosa–Victoria, and A. Trejo, "CO_2–supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary (*Rosmarinus officinalis*)," *J. Food Eng.*, vol. 200, pp. 81-86, May 2017.

[14] I. Michalak, K. Chojnacka, and A. Saeid, "Plant growth biostimulants, dietary feed supplements and cosmetics formulated with supercritical CO_2 algal extracts," *Molecules*, vol. 22, no. 1, pp. E66, Jan. 2017.

[15] M. Goto, H. Kanda, Wahyudiono, and S. Machmudah, "Extraction of carotenoids and lipids from algae by supercritical CO_2 and subcritical dimethyl ether," *J. Supercrit. Fluids*, vol. 96, pp. 245–251, Jan. 2015.

[16] S. Machmudah, A. Martin, M. Sasaki, and M. Goto, "Mathematical modeling for simultaneous extraction and fractionation process of coffee beans with supercritical CO_2 and water," *J. Supercrit. Fluids*, vol. 66, pp. 111–119, Jun. 2012.

[17] K. Tomita, S. Machmudah, Wahyudiono, R. Fukuzato, H. Kanda, A.T. Quitain, M. Sasaki, and M. Goto, "Extraction of rice bran oil by supercritical carbon dioxide and solubility consideration," *Sep. Pur. Technol.*, vol. 125, pp. 319–325, Apr. 2014.

[18] R. Halim, M. K. Danquah, and P. A. Webley, "Extraction of oil from microalgae for biodiesel production: A review," *Biotechnol. Adv.*, vol. 30, no. 3, pp. 709–732, Jun. 2012.

[19] S. Machmudah, T. Izumi, M. Sasaki, and M. Goto, "Extraction of pungent components from Japanese pepper (*Xanthoxylum piperitum* DC.) using supercritical CO_2," *Sep. Pur. Technol.*, vol. 68, no. 2, pp. 159–164, Aug. 2009.

[20] M. E. Leblebici, S. Machmudah, M. Sasaki, and M. Goto, "Antiradical efficiency of essential oils from plant seeds obtained by supercritical CO_2, soxhlet extraction, and hydrodistillation," *Sep. Sci. Technol.*, vol. 48, no. 2, pp. 328-337, Aug. 2012.

[21] R. S. Sonale and U. S. Kadimi, "Characterization of gingerol analogues in supercritical carbon dioxide (SC CO_2) extract of ginger (*Zingiber officinale*, R.,)," *J. Food Sci. Technol.*, vol. 51, no. 11, pp. 3383–3389, Nov. 2014.

[22] M. Ono, Y. Kawamoto, C. Uemori, Wahyudiono, H. Kanda, and M. Goto, "Extraction of phytochemicals from grains of paradise using supercritical carbon dioxide," *Engineering J*ournal, vol. 21, no. 4, pp. 53–64, Jul. 2017.

[23] S. Kodama, T. Shoda, S. Machmudah, Wahyudiono, H. Kanda, and M. Goto, "Enhancing pressurized water extraction of β–glucan from barley grain by adding CO_2 under hydrothermal conditions," *Chem. Eng. Process. Process Intensif.*, vol. 97, pp. 45–54, Nov. 2015.

[24] H. Sovova, "Rate of the vegetable oil extraction with supercritical CO_2–I. Modelling of extraction curves," *Chem. Eng. Sci.*, vol. 49, no. 3, pp. 409–414, Jan. 1994.

[25] S. Machmudah, S. Winardi, M. Sasaki, M, Goto, N. Kusumoto, and K. Hayakawa, "Lycopene extraction from tomato peel by–product containing tomato seed using supercritical carbon dioxide," *J. Food Eng.*, vol. 108, no. 2, pp. 290–296, Jan. 2012.

[26] J. A. Egydio, A. M. Moraes, and P. V. T. Rosa, "Supercritical fluid extraction of lycopene from tomato juice and characterization of its antioxidation activity," *J. Supercrit. Fluids*, vol. 54, no. 2, pp. 159–164, Aug. 2010.

[27] S. L. Holdt and S. Kraan, "Bioactive compounds in seaweed: functional food applications and legislation," *J. Appl. Physiol.*, vol. 23, no. 3, pp. 543–597, Feb. 2011.

[28] S. Machmudah, Y. Kawahito, M. Sasaki, and M. Goto, "Supercritical CO_2 extraction of rosehip seed oil: fatty acids composition and process optimization," *J. Supercrit. Fluids*, vol. 41, no. 3, pp. 421–428, Jul. 2007.

[29] E. Reverchon and I. De Marco, "Supercritical fluid extraction and fractionation of natural matter," *J. Supercrit. Fluids*, vol. 38, no. 2, pp. 146–166, Sep. 2006.

[30] S. M. Ghoreishi, H. Kamali, H. S. Ghaziaskar, and A. A. Dadkhah, "Optimization of supercritical extraction of linalyl acetate from lavender via Box - Behnken Design," *Chem. Eng. Technol.*, vol. 35, no. 9, pp. 1641–1648, 2012.

[31] H. A. Martinez–Correa, D. C. A. Gomes, S. L. Kanehisa, and F. A. Cabral, "Measurements and thermodynamic modeling of the solubility of squalene in supercritical carbon dioxide," *J. Food Eng.*, vol. 96, no. 1, pp. 43–50, Jan. 2010.

[32] R. F. Rodrigues, A. K. Tashima, R. M. Pereira, R. S. Mohamed, and F. A. Cabral, "Coumarin solubility and extraction from emburana (*Torresea cearensis*) seeds with supercritical carbon dioxide," *J. Supercrit. Fluids*, vol. 43, no. 3, pp. 375–382, Jan. 2008.

[33] C. C. Tsai, H. M. Lin, and M. J. Lee, "Solubility of niflumic acid and celecoxib in supercritical carbon dioxide," *J. Supercrit. Fluids*, vol. 95, pp. 17–23, Nov. 2014.

[34] B. L. Lopes, A. P. Sanchez-Camargo, A. L. Ferreira, R. Grimaldi, L. C. Paviani, and F. A. Cabral, "Selectivity of supercritical carbon dioxide in the fractionation of fish oil with a lower content of EPA+ DHA," *J. Supercrit. Fluids*, vol. 61, pp. 78–85, Jan. 2012.

[35] J. Chrastil, "Solubility of solids and liquids in supercritical gases," *J. Phys. Chem.*, vol. 86, no. 15, pp. 3016–3021, Jul. 1982.