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Thesis for the Degree of Master of Engineering

Microparticle Formation and Characterization of Lipid/Polyethylene glycol Complex Using Supercritical Fluids

by

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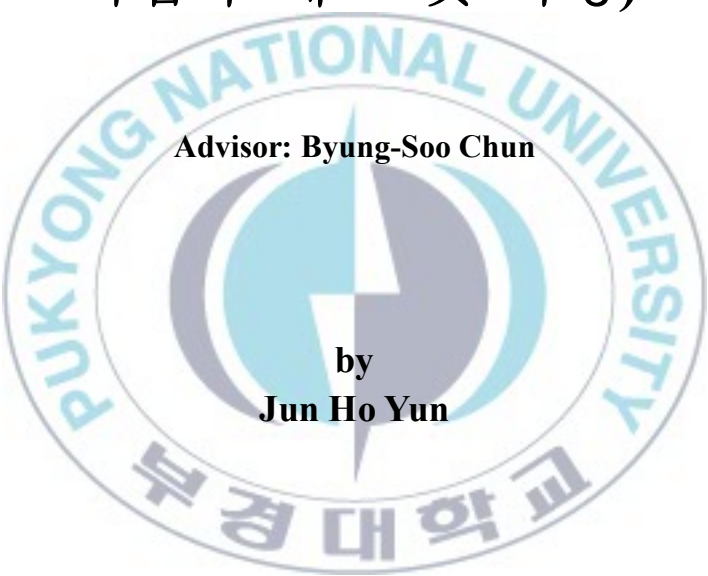
The Graduate School

Pukyuong National University

February 2013

Microparticle Formation and Characterization of Lipid/Polyethylene glycol Complex Using Supercritical Fluids

(초임계 유체를 이용한 지질과 폴리에틸렌글리콜 혼합물의 미립자 제조 및 특성)



Advisor: Byung-Soo Chun

by
Jun Ho Yun

A thesis submitted in partial fulfillment of the requirements
for the degree of

Master of Engineering

in the Department Food Science & Technology, The Graduate School,
Pukyong National University

February 2013

Microparticle Formation and Characterization of Lipid/Polyethylene glycol Complex Using Supercritical Fluids

PUKYONG NATIONAL UNIVERSITY
부경대학교

A dissertation
by
Jun-ho Yun

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February 2013

Contents

초임계 이산화탄소와 폴리에틸렌글리콜을 이용한 지질의 미립자 제조

윤준호

부 경 대 학 교 대 학 원 식 품 공 학 과

요 약

SC-CO_2를 이용하여 고등어 육에서 지질을 제거하였고 지질이 제거된 추잔물로부터 레시틴을 분리, 획득하였으며, 밀배아로부터 오일을 추출하여 기능성 물질로 이용하였다. 레시틴과 밀배아 오일과 식용, 의약 분야에 활용되는 고분자 물질인 폴리에틸렌글리콜(polyethylene glycol : PEG)을 이용하여 기능성 물질 복합체 미립자를 제조하였다. 미립자의 형태 및 크기, 분포는 Scanning electron microscope (SEM)과 Particle size analyzer (PSA)를 이용하여 특성을 파악하였다.

레시틴 미립자에서 주요 인지질인 phosphatidylcholine을 HPLC를 이용하여 정량 분석하였으며, 밀배아 오일 복합체 미립자에서 GC-FID를 이용하여 지방산 조성을 비교하였으며 항산화 물질인 폴리페놀 물질을 분석하였고 항산화 활성을 나타내는 DPPH 라디칼 소거효과를 측정하였다.

레시틴 미립자의 평균 입자 크기는 노즐크기 250 um에서 0.873–1.164 um, 300 um에서 1.180-2.080 um 였다. 압력과 온도가 높아질수록 이산화탄소의 밀도가 높아지면서 입자의 크기가 증가하며, 레시틴의 함량이 증가됨을 알 수 있었다. 밀배아 오일 미립자의 평균 입자크기는 PEG: 오일 혼합비 10:1일 때 0.632-1.350 um, 5:1일 때 1.177-3.767 um였으며 압력이 높을수록 입자의 크기가 작아짐을 알 수 있었다. 점도가 높은 레시틴으로 제조한 복합체 미립자보다 밀배아 오일 미립자의 입자 평균 크기가 작은 경향을 나타냈다. 또한 입자의 크기에 따라 인지질 함량, 항산화 물질의 함량이 증가하였으며 항산화능 또한 유의적으로 변화하는 것을 알 수 있었다.

Introduction

Lecithin is a sticky fatty substance composed mainly of phospholipid mixtures that were found in all living cells, whether of animal or plant origin. The highest concentration of phospholipids occurs in animal products, such as, meat, poultry, fish, eggs, and milk/cheese. In case of plant origin, the major source is from soyabean. Commercial lecithin is a complex mixture. It is comprised of phospholipids containing phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid and phosphatidylinositol, as well as sphingolipids, triglycerides, free fatty acids and glycolipids [1]. Phospholipids are widely used as natural emulsifiers, wetting agents and baking improvers [2]. Moreover, in recent years numerous applications in dietetics, cosmetics and pharmaceuticals have been reported [3]. Pharmacological use of lecithin includes in treatments for hypercholesterolemia, neurologic disorders and liver ailments. Lecithin has also been used to modify the immune system by activating specific and nonspecific defense systems [4-6].

Lecithin is usually recovered as a by-product during the oil production.

Crude lecithin, in general, contains a minimum of 35 wt.% oil, which needs to be reduced to less than 2 wt.% oil before it can be used as an emulsifier [7]. The crude lecithin is conventionally refined for removal of oil by repeated solvent extraction with acetone, as lecithin is 'acetone-insoluble'.

However, removal of oil from crude lecithin by conventional organic solvents has several disadvantages such as a large amount of solvent tedious and time-consuming procedures and a gelatinous lumpy mass formed at the final stage, which reduces the mass transfer efficiency of the process [8].

Wheat germ is a by-product of the wheat milling industry. Germ constitutes about 2-3% of the wheat grain and it can be separated in a fairly pure form from the grain during the milling process. Wheat germ contains about 11% oil [9], and it is used in products such as foods, biological insect control agents, pharmaceuticals and cosmetic formulations [10].

Wheat germ oil has the highest tocopherol content of all vegetable oils, up to about 2500 mg/kg [11], and also the highest content of α-tocopherol, which represents around 60% of the total content. Also, wheat germ oil is

highly valuable due to its highest content of unsaturated fatty acids: it has about 80%, mostly linoleic (18:2) and linolenic (18:3) [12]. These two fatty acids are of great importance in human metabolism and cannot be synthesized by the organism. They are precursors of a group of hormones called prostaglandins, which play an important role in muscle contractions and in the proper healing of inflammatory processes [13]. Furthermore, linoleic acid helps to eliminate cholesterol and is a precursor of cell membrane phospholipids [14].

As an alternative, supercritical carbon dioxide (SC-CO_2) was explored as a solvent for extraction of oil from vegetable seeds followed by extraction of lecithin from the deoiled flakes with supercritical mixture of CO_2 and ethanol [15-17]. This is based on the principle that the polar lecithin is almost insoluble in SC-CO_2 whereas the non-polar oil is soluble in SC-CO_2. It is the most advantageous solvent, as CO_2 is inert, nontoxic, 'generally regarded as safe' (GRAS), and is a gas at the ambient condition, enabling its easy recovery. SC-CO_2 has also been explored as a solvent for removal of oil from crude lecithin without [18-20] and with a co-solvent (e.g. ethanol or n-butanol) [15].

Supercritical CO_2 extraction of vegetable oils from plant materials is an alternative process to solvent extraction. This technique has several well-known advantages: CO_2 is a nontoxic, nonflammable, nonexplosive and low-cost gas. It is also easily removed from the solute by reducing the pressure and has a relatively low critical pressure and temperature [21]. This last property allows extraction of heat-sensitive material, such as flavor and aroma compounds. Another very important advantage of CO_2 is that its solvent power or selectivity can be modified by adjusting the temperature and pressure. This interesting feature has been used to extract volatile oils from solid matrices with minimum co-extraction of triglycerides [22] and to concentrate important nutritional compounds, such as tocopherols and carotenoids [23-24].

Tocopherols are a group of monophenolic antioxidants found in many plant materials. Antioxidants eliminate free radicals, providing in this way primary defense to our body. They accomplish the same task in vegetable oils, preventing the formation of hydroperoxides.

The formulation of natural substances together with a biocompatible or biodegradable carrier material to form composites or encapsulates has a great relevance for pharmaceutical, cosmetic and food industries [25]. Natural substances such as carotenoids, fatty acids, natural antioxidants are being extensively used on a great variety of food products [26].

Particle formations using SC-CO_2, such as RESS, PGSS, and SAS methods have received much attention as alternative precipitation methods to those with organic solvents [27]. Particle formation using SC-CO_2 is important for drug delivery systems that have been successfully used to obtain composites or encapsulates, which comprise an active compound loaded into a matrix of a carrier material, in order to improving product preservation as well as controlling the dissolution rate of the active compound [25].

Small particles of pharmaceuticals with a narrow particle size distribution plays a vital role in the design of conventional drug delivery systems like tablet, capsule, injection; biphasic drug delivery system like suspension and emulsion and the controlled drug delivery systems of implants,

transdermal, microemulsions and nanoparticulate [26-32]. The PGSS process may be used for the production of micro particles with a narrow size distribution and it is vital for food and pharmaceutical industries due to solvent free products [33].

In this study, lecithin has been isolated from de-oiled mackerel and wheat germ oil obtained by SC-CO_2 extraction and optimized a continuous process for the formation of particle with PEG by PGSS process. The shape and size of the particles were analyzed using SEM, PSA, manufacturing particulate particles combined with the functionality of the active substance and characteristics are compared and evaluated by HPLC-ELSD, GC-FID.

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Material and Method

1. Material

Mackerel was purchased from fish market (Kijang, Republic of Korea). Separated muscle, and was collected for dried. Wheat germs were purchased from Young-Nam Flour Mills Company (Nam-Gu, Republic of Korea). The pure carbon dioxide (99%) was supplied by KOSEM, Korea. Standard reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents used in this study were of analytical or HPLC grade.

2. Sample preparation

The mackerel samples were dried in a freeze-drier for about 72 hrs, and the wheat germ samples were dried in a heating-drier for 12 hrs. These dried samples were crushed by a mechanical blender then stored at –40°C until using for SC-CO_2.

3. Method

3.1. Supercritical carbon dioxide (SC-CO_2) extract

SCO_2 extraction was carried out two plants. Plant P1 was a laboratory-scale supercritical fluid extraction process that was used to oil extract from mackerel. The other plant, P2, was pilot-scale extraction process that was used to oil extract from wheat germ.

3.1.1. Mackerel oil extract and lecithin isolate

The flow rate of CO_2 (22 g/min) was constant over the entire extraction period of 2.5 hrs, and extraction parameters were different temperatures (35-45°C) and pressures (15-25 MPa). Mackerel residues providing the highest oil yield by SC-CO_2 extraction were used for lecithin isolation. Lecithin was isolated from de-oiled mackerel obtained by SC-CO_2 extraction according to the method of Wang [34] with modifications. Briefly, 100 mL of ethanol (95%) was added to 30 g of SC-CO_2 extracted mackerel residues and stirred for almost 24 hrs by a magnetic stirrer. The mixture was then centrifuged at 3200 rpm for 10 min. The supernatant containing mainly polar lipids was collected and evaporated in a vacuum rotary evaporator.

3.1.2. Wheat germ oil extracts

Wheat germ oil was extracted by supercritical carbon dioxide for a plant scale. The oil was extracted at temperature of 40°C and pressure of 25 MPa. The total extraction time was 1 hr. The flow rate of CO_2 was kept constant at 54.55 g/min.

3.2. Particle formation

PGSS process was carried out in different temperatures and pressures to measure the optimum condition for the formation of wheat germ oil particle.

3.2.1. Particle formation using PGSS process

The experiments were carried out using PEG 8000 (g/mol), crude lecithin and wheat germ oil with different pressures, temperatures, nozzle size and mixed ratio. The schematic diagram of PGSS process used in this study is shown in Fig. 1. PGSS experiment began by delivering SC-CO_2 to the precipitation chamber until the desired pressure was reached. PEG and Lecithin (10:1), PEG and wheat germ oil (10:1/5:1) in reactor were melted

by SC-CO_2 and mixed by stirred heel. These experiments were carried out at temperatures, ranging from 40 to 50°C and pressures, ranging from 10 to 30 MPa. The lecithin mixture was stirred at 250 rpm and the nozzle size was 250 and 300 μm. The duration for reactions was 1 hr. After reaction, material with PEG were delivered through the nozzle and collected from a separator.

The wheat germ oil particle formations were carried out at one nozzle (300 μm) and two different ratios (10:1) and (5:1) of wheat germ oil and PEG were used during PGSS process for 1 hr. The table of PGSS process conditions used in this study is shown in Table 1.

3.2.2. Analysis of particle by Scanning electron microscope analysis

Scanning electron microscope (SEM) is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan pattern. Samples of the lecithin with PEG on the metallic frit

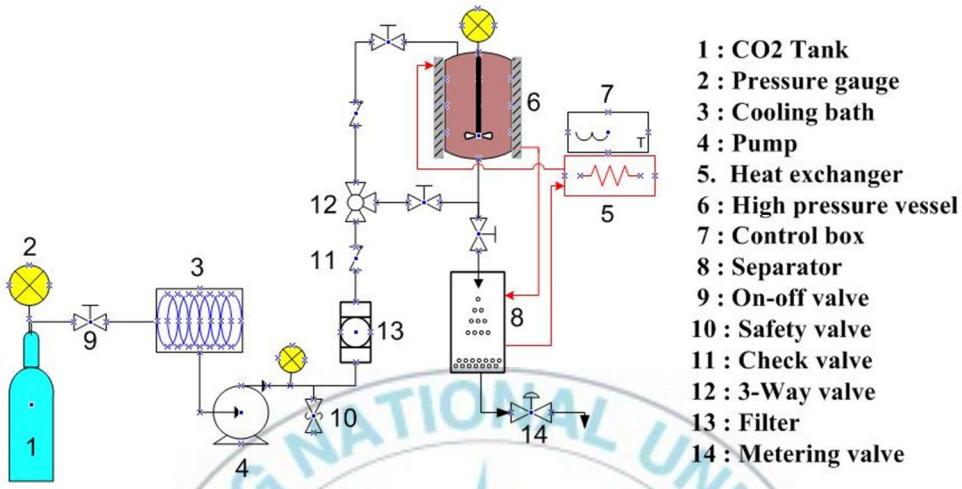

Fig. 1. Schematic diagram of PGSS process.

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Table 1. Condition of PGSS process for manufacturing the microparticle

	Lipid	
	Crude lecithin	Wheat germ oil
Rotor speed	250 rpm	300 rpm
Pressure	15 – 30 MPa	10 – 30 MPa
Temperature	40, 50°C	40, 50°C
Reaction time	1 hour	1 hour
Nozzle size	250, 300 um	300 um
Ratio of mixture (PEG : Lipid)	10:1 (w/w)	10:1, 5:1 (w/w)

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were observed by a scanning electron microscope (S-2400, Hitachin, USA). The SEM samples were covered with gold using a sputter coater. Particles sizes were measured from SEM images using the Sigma Scan Pro image analysis software.

3.2.3. Analysis of particle size by Particle size analyzer

The size distribution of the PEG and lecithin with PEG powder were measured by particle size analyzer (LS 13320, Beckman coulier, USA). The result from the analysis is the relative distribution of volume of particles in the range of size classes. From this basic result, the data on particle size distributions are calculated. The frequency curve is useful for displaying the results to show the peaks in the graph. The peak of the frequency curve gives the modal diameter, the most commonly occurring particle diameter.

3.3. Particle characterization

3.3.1. Phospholipid of lecithin particles measurement by HPLC analysis

In this study, phospholipid (PC) in PEG particle was quantified by a Jasco HPLC equipped with a controller, a 4-line degasser (DG-2080-54), a quaternary gradient unit (LG-2080-04), an intelligent HPLC pump (PU-2080 Plus), an evaporative light scattering detector (ELSD-Softa corporation, Model 400, London, UK) and a silica column (5 μm, 4.6 mm x 250 mm, Waters, Milford, Massachusetts, USA). The analysis was carried out according to the method of Letter [35] with modification of the ELSD operation. Lecithin with PEG was dissolved in chloroform and injected 20 μL via the injector. A gradient program with three mobile phases at a flow rate of 1.25 mL/min was used isopropyl alcohol, hexane and water to the following scheme: t0 min: 58:40:2 (v/v/v), t10 min: 48:50:2, t15min: 42:50:8, t30 min: 48:50:2 and hold up to 35 min. The spray and drift tube temperature of ELSD were set to 70 and 60ºC, respectively. The pressure of nitrogen gas at the nebulizer was 50 psi. The quantification of the phospholipids was performed based on the peak area

of the standard phospholipid, PC (Fig. 2.). Millennium software was used to analyze the data obtained by HPLC.

3.3.2. Characterization of wheat germ oil particle

3.3.2.1. Analysis of fatty acid composition

The fatty acid compositions in the wheat germ oil particle were determined by GC-FID (Agilent 6890N series) with a Supleco Fused silica capillary column (100.0 m × 0.25 um × 0.20 um), operational condition was shown in Table 2. Firstly the fatty acid methyl esters were prepared according to AOCS official method (AOCS, 1998). It was assumed that the ratio of single component peak area to total peak area is the mass fraction of the component. Particle methyl esters were identified by comparison of retention time with Fatty acid methyl esters mixture standard.

3.3.2.2. Analysis of total phenolic content

Total phenolic content (TPC) of wheat germ oil particle was measured with a spectrophotometry according to [39-40]. 1 ml of 50 times diluted (w/v) wheat germ oil particle was mixed with 1 mL of 1:10 (v/v, in deionized water) diluted Folin-Coicalteu reagent (FCR).

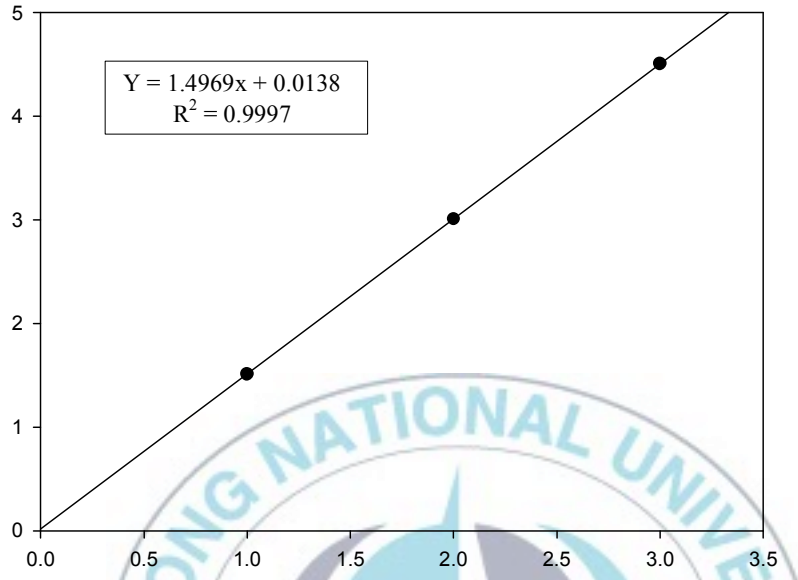

Fig. 2. Total phospholipid standard curve.

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Table 2. Operational condition of gas chromatography for analyzing the composition of fatty acids in wheat germ oil particle

Gas chromategraphy	
Model	Agilent 6890N
Control Mode	Splitless
Injection Temp.	250°C
Detection Temp.	260°C
Carrier Gas/Flow	He, 1 mL/min
Oven Temp.	140°C (5 min) → 4°C/min → 250°C (15 min)
Column	Agilent Supleco fused silica capillary column 100.0 m × 0.25 um × 0.20 um

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After 4 min, 800 ul of sodium carbonate solution (7.5%, w/v) was added into the mixture. Then, the mixture was vortexed for 5 sec and stored at room temperature in dark environment for 2 hrs. Blank was also prepared by replacing 1 ml of particle solution. The absorbance of mixture was measured at 765 nm against blank using UV-spectrometer (UVIKON 933, Kontron Instruments). The measurements were carried out in triplicate and gallic acid was used for calibration of standard curve (Fig. 3.). Results were expressed as mg gallic acid equivalent per 100 g of particle (mg GAE/100 g DW). The calibration equation for gallic acid was $y = 0.0294x + 0.0198$ ($R^2 = 0.9994$)

3.3.2.3. Analysis of DPPH radical-scavenging capacity

The DPPH radical scavenging capacity of particle was determined based on the method described by Wong et al., Miliauskas et al., and Saha et al. [41-43]. 1 ml of 50 times diluted with methanol (w/v) wheat germ oil particle was mixed with 1 mL of methanolic 0.1 mM DPPH, total volume of 2 ml. After thoroughly mixed, they were stored in dark environment at room temperature for 50 min.

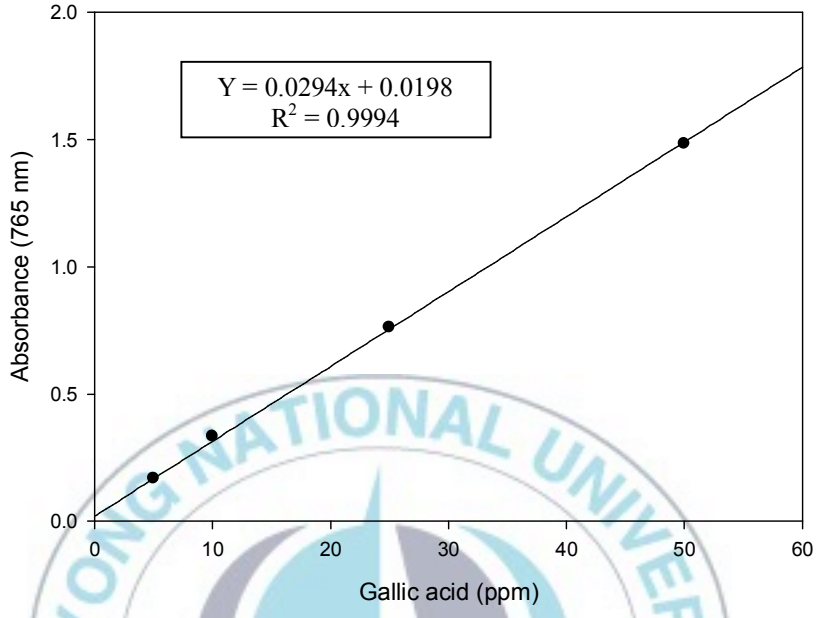

Fig. 3. Total phenolic contents standard curve.

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Subsequently, the absorbance of crude extract and control was measured against methanol (as blank) at 517 nm using UV. The percentage of DPPH radical scavenging effect was calculated as follow equation:

$$\text{Scavenging effect (\%)} = [1-(As/Ac)] \times 100$$

As = absorbance of particle at 517 nm

Ac = absorbance of control at 517 nm

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Results and Discussion

1. Analysis of particle by SEM

Several experiments were performed at different temperatures in supercritical states using two different nozzles, 250 and 300 μm, for lecithin with PEG 8000. The particles produced by PGSS process were characterized by SEM. Fig. 4-1, 2 shows SEM images of micro particles of lecithin with PEG obtained by PGSS process using SC-CO_2. These results can be explained in detail with the help of the various parameters such as the temperature, pressure, nozzle size etc. Almost all particles size was decreased by different temperatures and pressures. Most of the particles obtained by PGSS using SC-CO_2 were agglomerated. Lecithin is sticky, and it had emulsification ability that effect particles aggregation. Agglomerated particles were found by PGSS in biopesticide encapsulation [47]. Fig. 5-1, 2 shows SEM images of micro particles of wheat germ oil with PEG obtained by PGSS process using SC-CO_2. Almost all particles size was decreased by different temperatures and pressures, also.

These results can be explained in detail with the help of the various

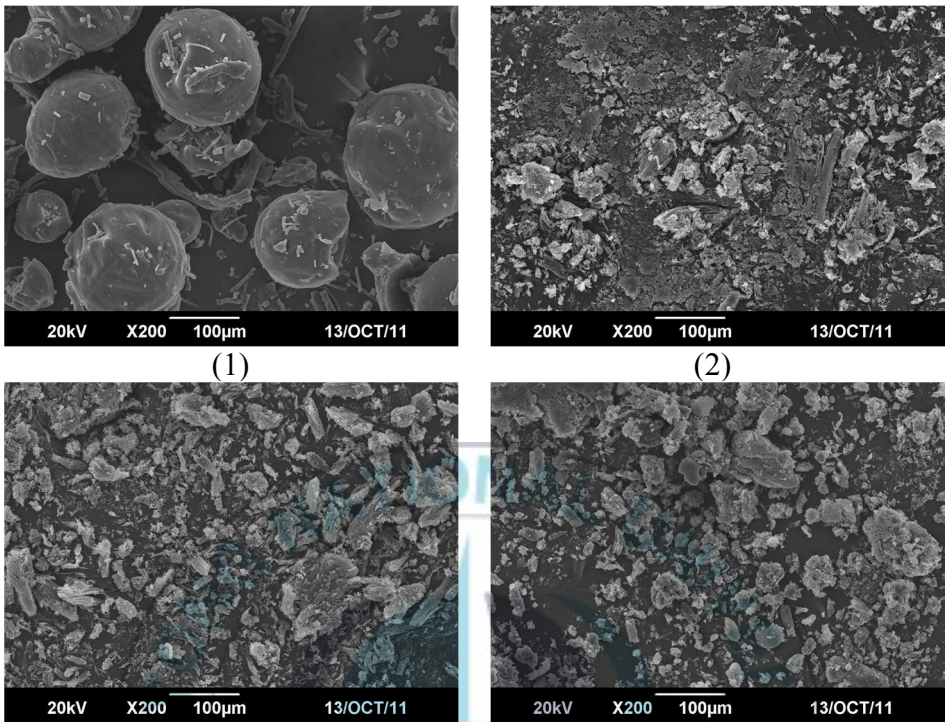

Fig. 4-1. SEM images of the particles obtained by PGSS process using SC-CO_2. (1) Original PEG, (2) 40°C, 15 MPa, (3) 40°C, 30 MPa and (4) 50°C 30 MPa with the nozzle size of 250 μm.

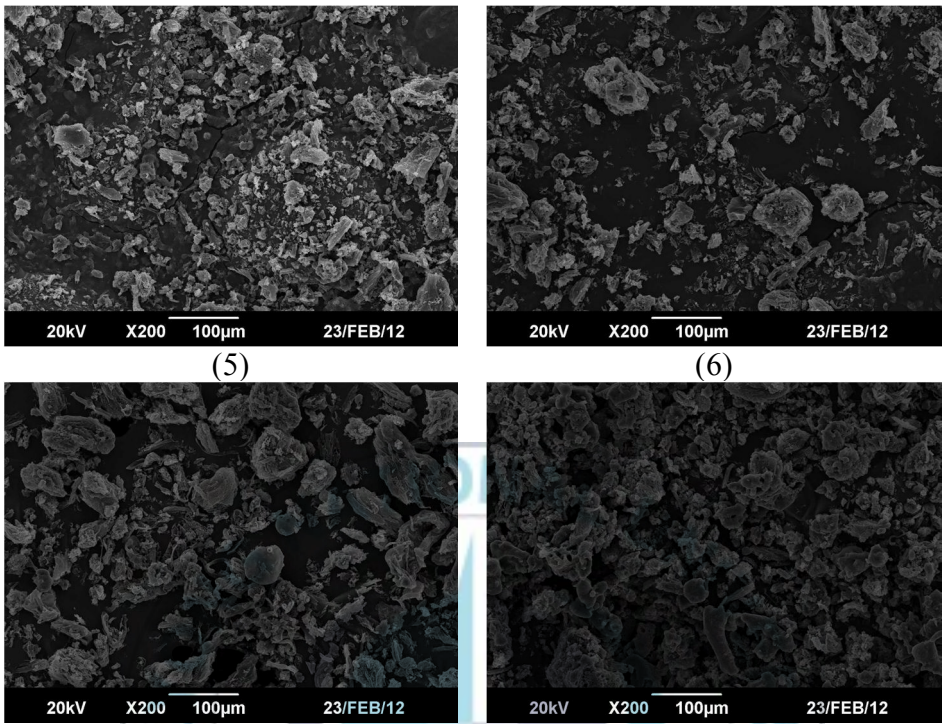

Fig. 4-2. SEM images of the particles obtained by PGSS process using SC-CO_2. (5) 40°C, 20 MPa, (6) 40°C, 30 MPa, (7) 50°C 20 MPa and (8) 50°C 30 MPa with the nozzle size of 300 μm.

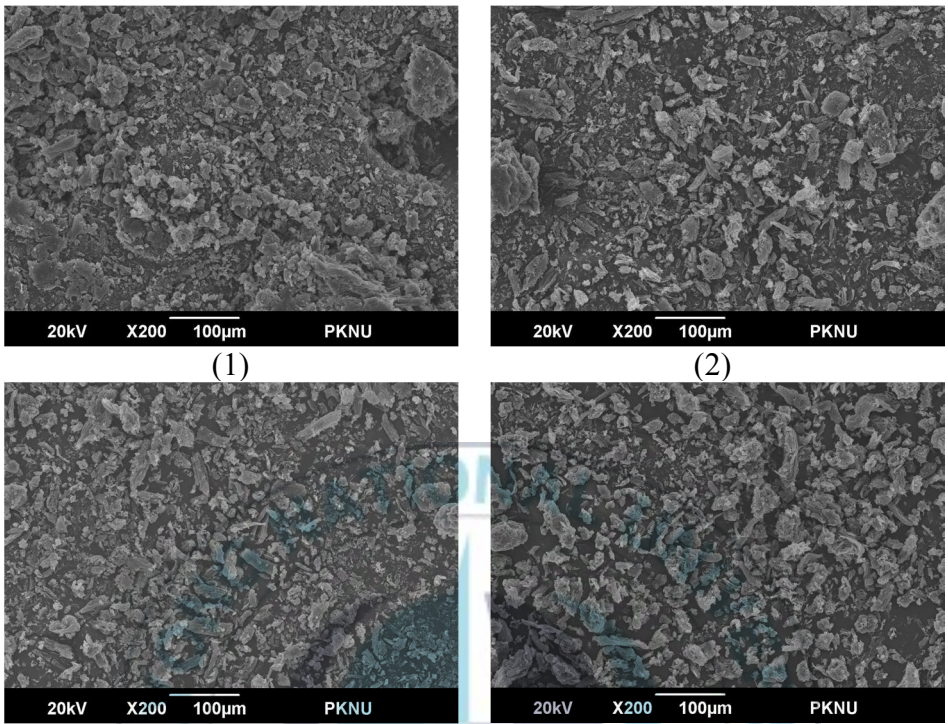

Fig. 5-1. SEM images of the particles obtained by PGSS process using SC-CO_2. (1) 40°C, 10 MPa, (2) 40°C, 30 MPa, (3) 50°C 10 MPa and (4) 50°C 30 MPa with the PEG : Oil ratio (10:1).

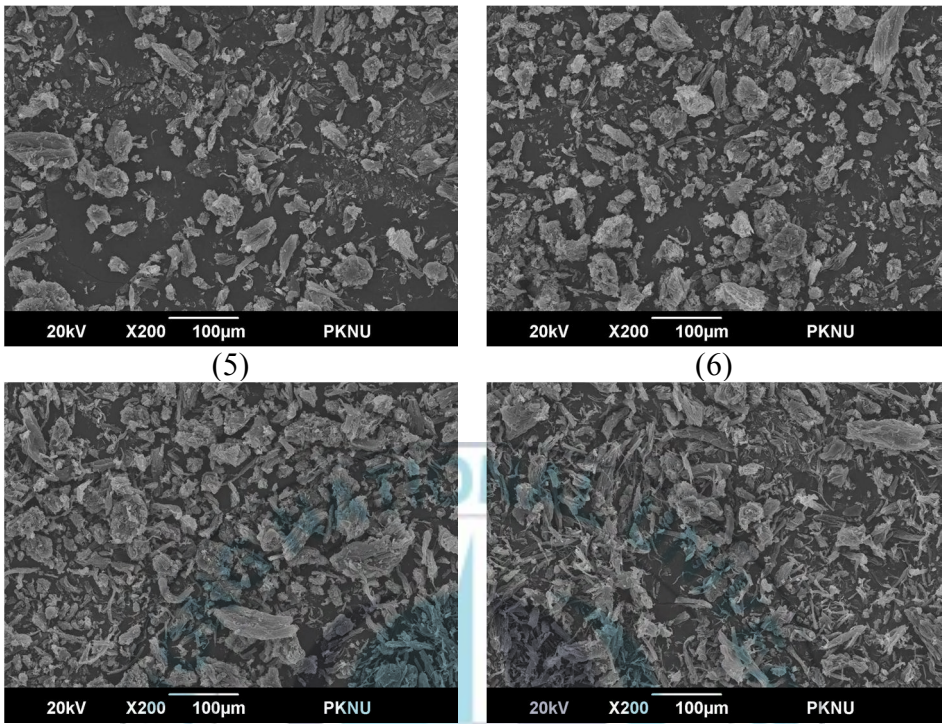

Fig. 5-2. SEM images of the particles obtained by PGSS process using SC-CO_2. (5) 40°C, 10 MPa, (6) 40°C, 30 MPa, (7) 50°C 10 MPa and (8) 50°C 30 MPa with the PEG : Oil ratio (5:1).

parameters such as the temperature, pressure etc. Most of the particles obtained by PGSS using SC-CO_2 were agglomerated.

2. Analysis of particle size by PSA

The size distributions of original PEG and lecithin particles with PEG 8000 obtained by PGSS using SC-CO_2 under different conditions are shown in Fig. 6. In this study, the average particle size of PEG before PGSS process was almost 400 times bigger than that of PEG obtained by PGSS process using SC-CO_2.

The average size of lecithin particles with PEG was found to be ranged from 0.873 to 1.164 μm at nozzle size of 250 μm. On the other hand, the average size of lecithin particles with PEG was 1.180 to 2.080 μm at nozzle size of 300 μm. However, nozzle size also moderately affected the size of particles with PEG. In most cases, the particle size obtained by PGSS process using 300 μm was bigger than that of obtained by PGSS process using 250 μm. Particle size was also remarkably affected by temperatures, pressures (Fig. 6-8.).

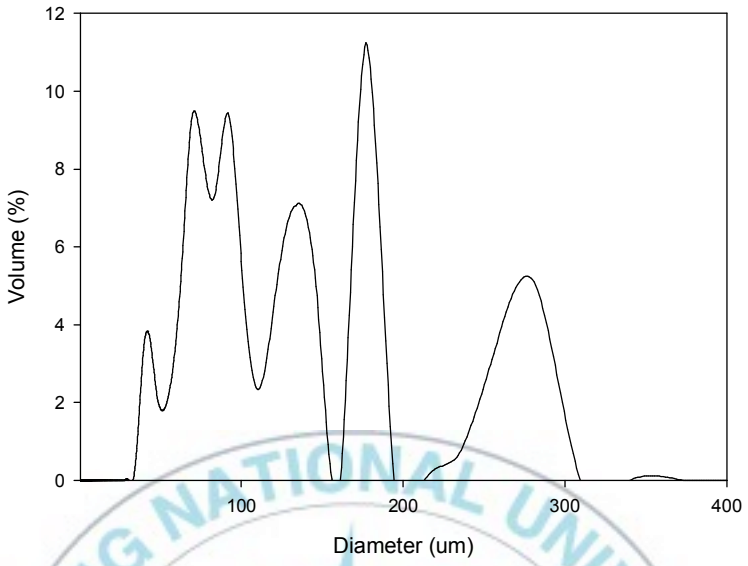

Fig. 6. Particle size and distribute volume of original PEG.

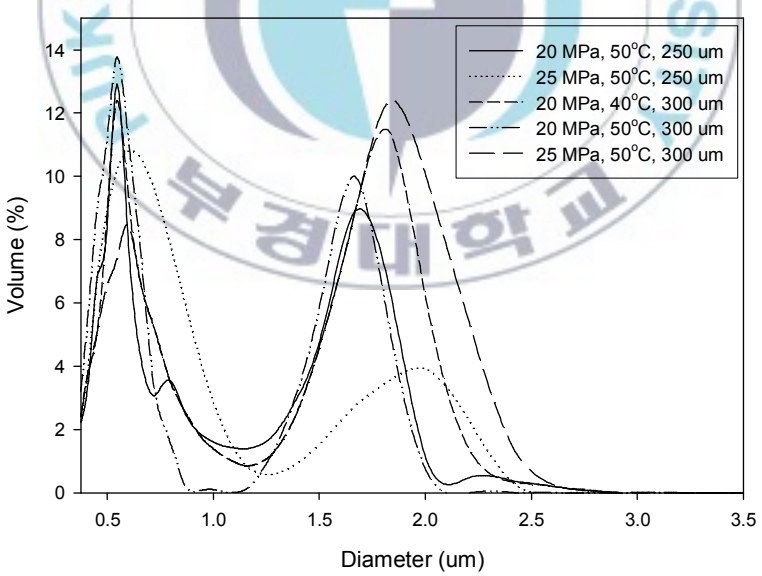

Fig. 7. Particle size and distribute volume of particle formed by PGSS with lecithin.

Table 3. The average diameter of the particles from PEG 8000 with lecithin at different conditions

Nozzle size (μm)	Temperature (°C)	Pressure (MPa)	Average particle size (μm)
250	40	15	1.096
		20	0.873
		25	0.877
		30	0.879
	50	15	1.164
		20	1.026
		25	0.879
		30	1.107
300	40	15	1.180
		20	1.880
		25	1.660
		30	2.088
	50	15	1.320
		20	2.070
		25	1.860
		30	1.760

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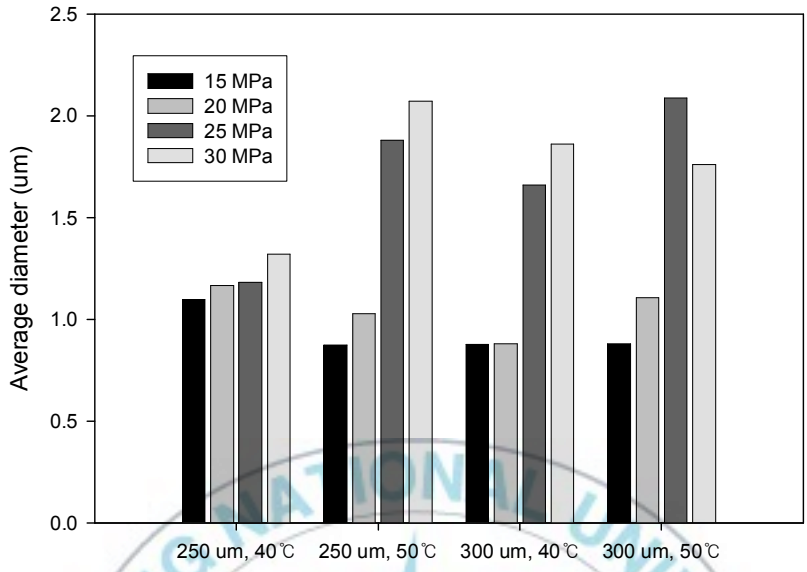

Fig. 8. Compare of lecithin particle sizes by PGSS process condition.

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The size distributions of wheat germ oil particles with PEG obtained by PGSS using SC-CO_2 under different conditions are shown in Fig. 9-10.

The average size of wheat germ oil particles with PEG was found to be ranged from 0.632 to 1.350 μm at PEG/Oil mixed ratio 10:1. On the other hand, the average size of PEG/Oil mixed ratio 5:1 was 1.177 to 3.767 μm.

Temperature and pressure was moderately affected the size of wheat germ oil particles with PEG. In most cases, the particle size obtained by PGSS process using PEG/Oil mixed ratio 5:1 was bigger than that of obtained by PGSS process using PEG/Oil mixed ratio 10:1, it shown in Fig. 11.

CO_2 density depends on pressure and temperature and solubility is increased with higher density and other factors. The solubility of a gas increases as pressure increases, decreases as pressure decreases and as temperature decreases gas solubility increases, as temperature increases the solubility decreases. When the mixture was kept for pressurized a period long enough to reach phase equilibrium, during this time SC-CO_2 was dissolved in the mixture. The high pressure mixture is rapidly depressurized through a nozzle leading to particle formation by precipitation, as the gas goes out of the solution.

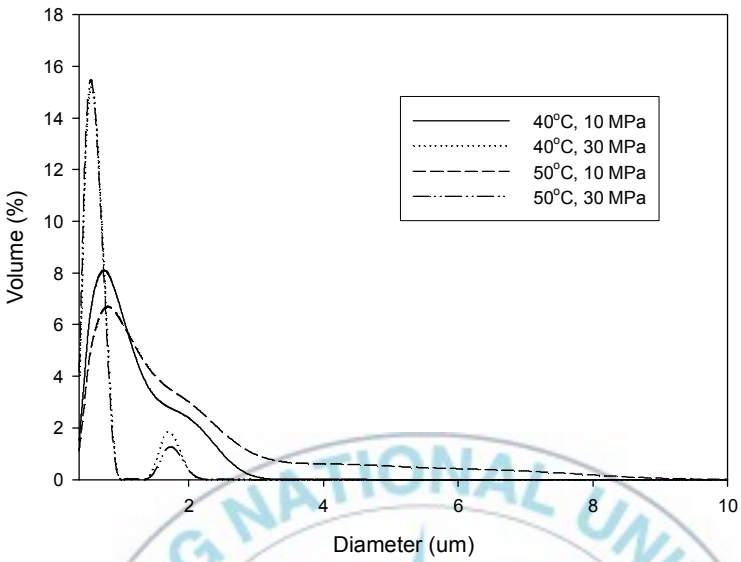

Fig. 9. Particle size and distribution volume of particle formed by PGSS with wheat germ oil (mixed ratio was PEG : Oil = 10:1).

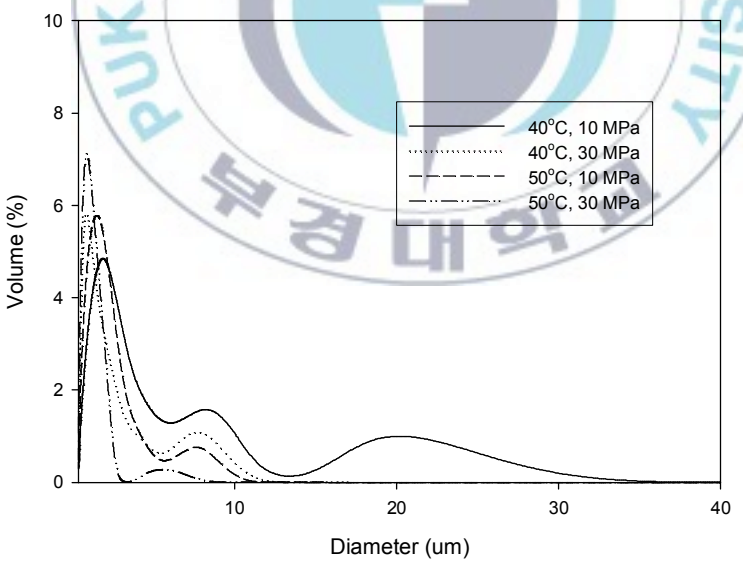

Fig. 10. Particle size and distribution volume of particle formed by PGSS with wheat germ oil (mixed ratio was PEG : Oil = 5:1).

Table 4. The average diameter of the particles from PEG 8000 with wheat germ oil

No.	PEG : Oil	Temperature (°C)	Pressure (MPa)	Average particle size (μm)
1	10:1	40	10	1.006
2			15	0.789
3			20	0.762
4			25	0.686
5			30	0.649
6		50	10	1.351
7			15	1.313
8			20	0.860
9			25	0.709
10			30	0.633
11	5:1	40	10	3.768
12			15	2.114
13			20	2.173
14			25	1.754
15			30	1.800
16		50	10	1.891
17			15	1.840
18			20	1.674
19			25	1.315
20			30	1.178

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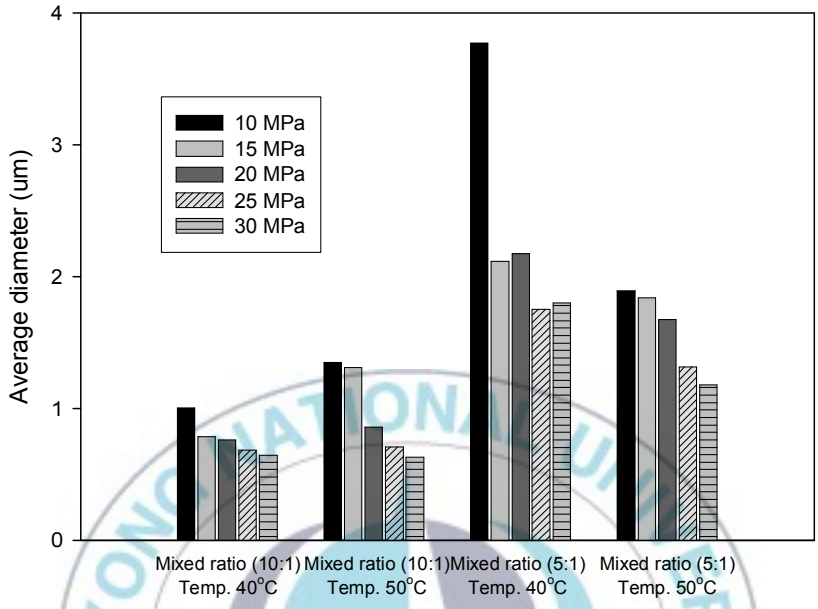

Fig. 11. Comparison of wheat germ oil particle sizes by PGSS process condition.

3. Characterization of lecithin particle

3.1. Lecithin measurement

Fig. 12. shows the amount of lecithin entrapped in PEG by PGSS process under different SC-CO_2 density conditions at the nozzle size of 250 and 300 μm. The amount of lecithin absorbed in PEG was ranged from 4.38 to 8.37 mg/g of lecithin particles at the nozzle size of 300 μm. On the other hand, at the nozzle size of 250 μm, it was 4.24 to 17.49 mg/g of lecithin particles. When the mixture (PEG and lecithin) was kept pressurized a period long enough to reach phase equilibrium, during this time SC-CO_2 was dissolved in the mixture. The high pressure mixture is rapidly depressurized through a nozzle leading to particle formation by precipitation, as the gas goes out of the solution.

Lecithin entrapped in PEG increased with the increase in SC-CO_2 density at nozzle size of 250 μm. At high SC-CO_2 density condition, the solubility of lecithin and PEG was higher and that enhanced the inclusion of lecithin in PEG by PGSS process. Some researchers reported similar effects of pigment solubility in SC-CO_2 from different sources [48, 49].

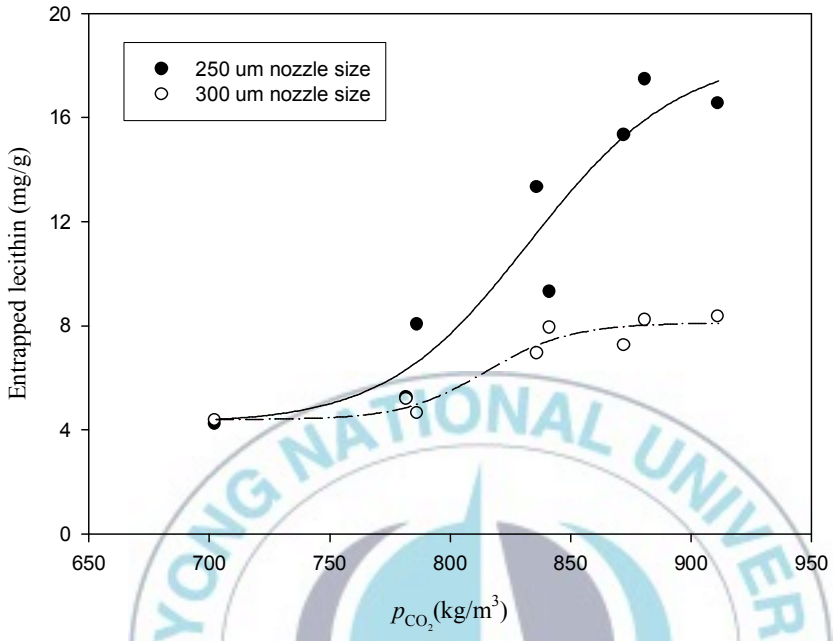

Fig. 12. The amount of lecithin entrapped in PEG by PGSS in different SC-CO_2 density conditions at nozzle size of 250 and 300 μm.

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Table 5. Phospholipid content of lecithin particle at different process conditions

Nozzle size (μm)	Temperature (°C)	Pressure (MPa)	Phospholipid (mg/g)
250	40	15	5.262
		20	9.325
		25	17.489
		30	16.560
	50	15	4.240
		20	8.066
		25	13.341
		30	15.340
300	40	15	5.194
		20	7.941
		25	8.241
		30	8.370
	50	15	4.385
		20	4.654
		25	6.962
		30	7.270

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4. Characterization of wheat germ oil particle

4.1. Fatty acid composition

Fatty acid composition of wheat germ oil particle can be an indicator of its stability, physical properties, and nutritional value. There were almost no differences in fatty acid composition of wheat germ extracted oils. Shown in Table 6-7, a high content of unsaturated fatty acids in particles were identified. The main components are palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2nt), and r-linolenic acid (C18:3). Among them, linoleic acid showed the highest content and its amount represents from 51.60% to 58.48% of total identified fatty acids. Another unsaturated fatty acid, oleic acid was also present in higher percentage ranging from 14.94 to 17.41%. The fatty acid compositions were changed moderately at different PGSS process conditions.

Table 6. Comparison of fatty acid composition[a] of extracted oil and micronized oil (ratio 10:1)

Fatty acid	Extracted oil	1	2	3	4	5	6	7	8	9	10
C14:0	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.92	0.00	0.00
C16:0	19.33	20.69	20.95	18.86	19.54	21.93	20.61	19.91	19.50	22.55	21.88
C16:1	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.59	0.00	0.00
C18:0	0.81	0.00	0.00	0.00	0.00	1.55	0.00	0.00	1.09	0.00	1.07
C18:1n9t	15.55	15.91	15.07	15.26	15.19	17.21	16.21	15.50	16.42	17.76	16.70
C18:1n9c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:2n6	55.59	55.59	56.96	58.48	57.59	53.20	56.74	56.14	53.31	52.59	52.87
C20:1	1.33	1.30	0.00	0.00	0.00	0.00	0.00	1.28	1.61	0.00	1.27
C18:3n3	6.67	6.51	7.03	7.41	7.69	6.11	6.44	7.17	6.55	7.10	6.22

[a] GC area percentage. Footnote (1-10) is shown in Table 4

Table 7. Comparison of fatty acid composition[a] of extracted oil and micronized oil (ratio 5:1)

Fatty acid	Extracted oil	11	12	13	14	15	16	17	18	19	20
C14:0	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C16:0	19.33	17.28	17.95	17.41	16.70	17.98	17.47	17.32	15.83	16.87	15.99
C16:1	0.33	0.00	0.00	1.86	2.45	0.00	0.69	0.00	2.53	0.00	1.92
C18:0	0.81	0.81	0.88	0.80	0.83	0.00	0.98	1.10	0.79	0.87	0.79
C18:1n9t	15.55	16.53	16.84	16.26	16.31	16.70	16.23	16.13	15.64	16.29	15.68
C18:1n9c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:2n6	55.59	56.95	57.37	55.09	54.28	54.89	57.23	57.58	54.04	57.76	56.91
C20:1	1.33	8.43	6.96	8.57	9.43	10.44	7.39	7.86	11.17	8.21	8.71
C18:3n3	6.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a] GC area percentage. Footnote (11-20) is shown in Table 4.

4.2. TPC measurement

TPC of wheat germ oil particle are shown in Table 8. As a result, TPC range from 0.2175 to 1.5977 ug GAE/ 100 mg particle in all conditions and was maximized at 15 MPa and 40^{o}C at the ratio of 5:1. Total phenolic content was varied according to the change of temperature and pressure. Higher temperature and lower pressure increased total phenolic content. Pinelo et al. and Spigno et al. reported that total phenolic content from grape pomace and grape marc was increased up to 60^{o}C [50, 51].

4.3. DPPH radical-scavenging effect

The DPPH radical-scavenging effects of wheat germ oil particles was ranged from 2.00% to 7.54% at the ratio of 10:1 and from 4.42% to 8.09% at the ratio of 5:1, it shown in Table 9. Although the radical-scavenging effects in micronized particles are lower than extracted oil and standard materials, it is enough to be suitable for use as the size of the inhaled sanctions because fine particles are considered superior to its functional expression. The high ratio of oil-micronized particle showed maximum effect, but significant changes couldn't be found.

Table 8. Total phenolic content of wheat germ oil particle at different process conditions

PEG : Oil	Temp. (°C)	Pressure (MPa)	Total phenolic content (μgGAE/ 100 mg)
10:1	40	10	1.354
		15	1.376
		20	1.275
		25	0.599
		30	0.217
	50	10	1.449
		15	1.326
		20	1.348
		25	0.864
		30	0.505
5:1	40	10	1.463
		15	1.598
		20	1.459
		25	1.451
		30	1.533
	50	10	1.566
		15	1.594
		20	1.521
		25	1.399
		30	1.079

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Table 9. DPPH radical scavenging capacity of wheat germ oil particle at different process conditions

PEG : Oil	Temp. (°C)	Pressure (MPa)	DPPH (%)
10:1	40	10	7.54
		15	3.14
		20	4.17
		25	3.31
		30	2.46
	50	10	2.00
		15	4.05
		20	4.00
		25	2.69
		30	4.10
5:1	40	10	6.85
		15	6.71
		20	7.99
		25	7.86
		30	8.09
	50	10	6.84
		15	6.89
		20	6.26
		25	4.41
		30	5.43
Wheat germ oil			60.40
Ascorbic acid			96.25

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Conclusion

In this study, the particles of lecithin and wheat germ oil with PEG were formed by PGSS using SC-CO_2. Most of the aggregated particles prepared by PGSS which average diameter of 1.13 μm. The particles formed by PGSS process at different conditions showed a considerable size reduction with a uniform size distribution volume, and it was due to the unique physical properties of supercritical fluids. It was found that the pressure and temperature had moderate effect on the particle size distribution obtained by PGSS process. The amount of the active compounds (lecithin and wheat germ oil) in PEG mixtures influenced in which higher SC-CO_2 density conditions caused the increase in solvating power of CO_2. Acid value and peroxide value of lecithin was reduced using PGSS process with various conditions. After wheat germ oil particles micronization, TPC showed in all conditions and DPPH activity was small higher at the ratio of 5:1 compared to the ratio of 10:1 which may be valuable effect for food and pharmaceutical industries [52].

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