Rapid Expansion Supercritical Solution (RESS) Carbon Dioxide as an Environmental Friendly Method for Ginger Rhizome Solid Oil Particles Formation

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Abstract**—**Recently, RESS (Rapid Expansion Supercritical Solution) method has been used by researchers to produce fine particles for pharmaceutical drug substances. Since RESS technology acknowledges a lot of benefits compare to conventional method of ginger extraction, it is suggested to use this method to explore particle formation of bioactive compound from powder ginger. The objective of this research is to produce direct solid oil particles formation from ginger rhizome which contains valuable compounds by using RESS-CO_2 process. RESS experiments were carried using extraction pressure of 3000, 4000, 5000, 6000 and 7000psi and at different extraction temperature of 40, 45, 50, 55, 60, 65 and 70°C for 40 minutes extraction time and contant flowrate (24ml/min). From the studies conducted, it was found that at extraction pressure 5000psi and temperature 40°C, the smallest particle size obtained was 2.22µm on 99 % reduction from the original size of 370µm.

Keywords**—**Particle size, RESS, solid oil particle, supercritical carbon dioxide.

I. Introduction

GINGER rhizome is consumed as a fresh ginger, preserved ginger and also powdered ginger and ginger oil. Fresh ginger is usually used in cooking vegetables, in tea, and also as an ingredient in many other different dishes. Ginger was used in carbonated beverages and bakery products. Traditionally, ginger has been used as a medicine to treat stomach ache, diarrhea, nausea, cholesterol lowering herb, warming remedy and migraine treatments [1].

Research on various clinical trials involving diverse of ginger product have been done by researchers. Ginger is known to contain high antioxidant that can provide additional defence against oxidation [2]. Ginger is a natural dietary component, which has antioxidant and anti-carcinogenic properties [3]-[5]. Some compounds presents in ginger may exert cancer preventive effects by inducing apoptosis in cancerous or transformed cells [1].

Previous studies on ginger extraction used supercritical fluid extraction (SFE) and conventional extraction process such as solvent extraction and steam distillation. These techniques will produce ginger liquid oil that need to undergo conventional technique for particle formation [6]. The disadvantages of conventional technique for particle formation are due to the fact that, extraction product may contaminated with unwanted residue, excessive toxic organic solvents use and dispose, thermal and chemical solute degradation for certain biological substances, change of the solute structure, and difficulties in controlling the solute particle size [7]. In addition, the conventional process for particle formation is time consuming and costly [8]. Therefore, in this study a clean extraction method which is RESS-CO_2 technology was introduced for the extraction of powdered ginger to direct produce solid oil particle of bioactive components. RESS-CO_2 extraction offers a lot of advantageous compare to conventional extraction method such as it is nontoxic, environmental friendly method, easy to separate the solvent, mild operating conditions, short extraction time, eliminate conventional technique for particle formation and offers high quality of final product.

The objective of this research is to explore the potential of RESS-CO_2 extraction method to obtain solid oil particle formation from *Zingiber officinale* Rosc rhizome and to study effect of different extraction pressure and temperature to solid oil particle size produce at constant extraction flowrate.

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II. Material and Methods

A. Material Preparation

Zingiber officinale Rosc rhizome used in this study was purchased from local market. Fresh ginger rhizomes were washed thoroughly with tap water to remove the dirt. The skin of the ginger rhizome was peeled and then cut into cross section slices with 2 to 3 mm thick. An amount of 500 g ginger slices was weighed and undergo oven dried in an oven model Memmert UFE 500 to remove moisture content until it remains in the sample at 10 to 12% [9], [10]. Dried ginger sample then was ground using mechanical grinder Retsch model SM100 for 3 minutes through a plate of 1mm pore size to obtain the ginger powder. The sample was then stored in the refrigerator at 4°C until further use.

B. Moisture Content Determination

The moisture content was expressed as the amount of water that presents in a moist sample. The moisture content determination for ginger rhizome was specified based on the Palm Oil Research Institute of Malaysia (PORIM) test method [11]. An amount of 10 g of ginger slice was weighted accurately in a dried glass dish at 55ºC for 6 to 7 hours. Previously, glass dish undergoes drying process in an oven and cooling at room temperature before the mass of the empty dried dish was weighted. After that, the dish was removed and undergone cooling process. Then, the cooled dish was weighted until it achieved constant weight. Moisture content expressed in percentage of the equivalent ratio of water content with the total mass of the sample was calculated using (1):

$$\text{moisture content, \%} = (m_1\text{-}m_2)/(m_1\text{-}m_0) \quad (1)$$

where; m_0= mass of the dish (g); m_1= mass of the dish with sample before drying (g); m_2= mass of the dish with sample after drying (g).

C. Rapid Expansion Supercritical Solution Carbon Dioxide Extraction

The RESS equipment used was modified from Supercritical Fluid Technologies Model SFT-100 equipment. Each run will be conducted using 8 g sample of ground ginger based on observation on the maximum mass of ground ginger that can be loaded and fitted in 25 ml of the extraction vessel provided by the Supercritical Fluid Technologies Model SFT-100. If the mass of the sample is higher than 8 g, it will cause some loose fitting at the top of the extraction vessel seal that will cause a fault or leaking of CO_2 when the extraction process in progress.

Extraction pressure and temperature was set at the desired value, and ginger sample was inserted in a cotton bag before putting in the extraction vessel. The seal on the top of an extraction vessel was sealed tightly before close the top cover. Dynamic valve was opened and restrictor valve was tightly closed during the extraction time. Glass vial was inserted in the expansion chamber and tightly seal the expansion vessel. When the extraction temperature achieved the desired value, CO_2 pump was run to feed high-pressure liquid CO_2 (99 % purity provided by MOX Linde Gases Sdn Bhd) continuously in the extraction vessel at a fixed solvent flowrate of 24 ml/min. Basically, liquid CO_2 will be converted to a supercritical condition when it is pumped into the extraction vessel (heated zone). In order to achieve the desired pressure, set point, CO_2 pump will continuously actuate.

After 40 minutes of extraction, the restrictor valve was quickly open to depressurize the supercritical solution for the separation of solute from the solvent through the expansion nozzle with external diameter of 2.0 mm and distance of 80 mm from the nozzle to the collection bottle. Depressurized CO_2 at the ambient pressure will convert into gaseous form and purged into the ambient. Extraction product will be collected in collection vials.

The procedure of extraction process was repeated in triplicate under desired operating conditions and the data were given based on average values.

D. Scanning Electron Microscopy (SEM)

Analysis will be carried out to perform analysis on particle size to obtain the smallest solid oil particle size using Scanning Electron Microscopy (SEM), model TM3000 Tabletop Microscope, and brand Hitachi. For the analysis, samples were applied to double sticky carbon tape located on top of the circular aluminium stub [12]. SEM image from different region of the stub were captured

E. ImageJ Program

Image processing and analysis software, ImageJ program were used to perform particle size analysis [13]. Images from SEM analysis were evaluated with the ImageJ program. 100 particles selected randomly in the ImageJ program analysis were used to calculate average solid oil particle size of the ground ginger obtained from RESS processes [14].

III. Results And Discussions

A. Effect of Moisture Contents

In this study, the total moisture content of ground ginger rhizome was found to be 10% as calculated using (1) based on a study done by Mesomo et al. and Nampoothiri et al., on ginger extraction, they used the same value of moisture content in their work [10], [15]. Moisture content of sample plays an important factor in extraction efficiency [16]. According to [17], the moisture content of the sample that was above 18% will negatively affect the extraction process. Moisture content in the sample will affect the solubility and mass transfer kinetic during extraction by reducing supercritical CO_2 and sample contact because the moisture itself will act as diffusion barriers of supercritical CO_2 into the matrix sample and diffusion of the solute out of sample matrices. Therefore, the total moisture content of the dry ginger needs to be determined before conducting the extraction process. Since the total moisture content in this study was below 18%, the sample moisture would not have effects on the extraction process.

B. Effect of Pressure on Particle Size

In this work, the effect of different extraction pressure on the particle size was investigated varies from 3000, 4000, 5000, 6000 and 7000psi while holding constant at a different extraction temperature of 40, 45, 50, 55, 60, 65 and 70°C, respectively for 40 minutes extraction time. The effect of pressure on the particle size at constant temperature is graphically shown in Fig. 1.

Fig. 1 shows at constant temperature, increasing extraction pressure from 3000psi to 4000psi, the average solid oil particle size increased. When extraction pressure increased to 5000psi, particle size produce is reduced. Furthermore, increased the pressure from 5000psi to 6000psi, particle size was decreased except for at the temperature of 40°C. However, continuously increased extraction pressure to 6000psi and 7000psi, will result in average solid oil particle size that tend to increase but the size still smaller compared to the particle size produced at 4000psi. However, when further increases of extraction pressure from 4000psi to 5000psi, a marked reduction of the average solid oil particle size was observed. According to [18], by increasing the extraction pressure, density of solvent enhanced significantly.

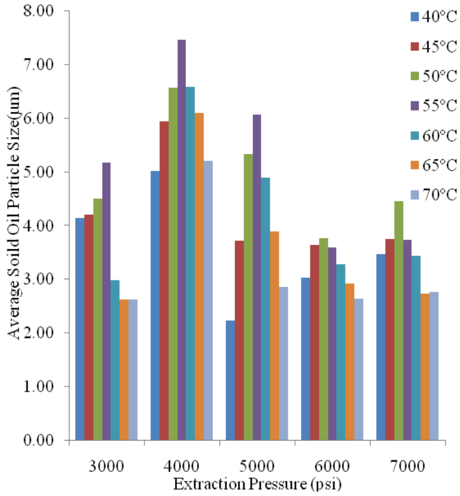

Fig. 1 Effect of Different Pressure on Solid Oil Particle Size at Constant Temperature

As the pressure increased, CO_2 density increased and the intermolecular mean distance of CO_2 molecules decreased, hence the specific interaction between the solute and solvent molecules is increased. As the result of increasing the solvating power, the increase the solubility of ginger solute increased as the pressure increased. Increasing ginger solute solubility results in higher supersaturation in the fluid upon expansion.

The higher supersaturation may result in an increasing nucleation rate, which lead to smaller particle size of ginger solid oil. According to classical theory of nucleation, higher supersaturation causes higher nucleation rate and the particle volume is inversely proportional to the nucleation rate [18]. Referring to [19], in their work regarding effect of RESS parameter such as extraction temperature and extraction pressure on the raloxifene particle size, as the extraction pressure was increased, the velocity of fluid also increased that results to increase in the fluid turbulence, therefore the mass flowrate increase. As a result of increase mass flowrate, particle residence time in the nozzle and expansion chamber decreased which leads to a reduction of particle growth time and particle size. The results appeared to agree with this theoretical prediction and consistent with those reported in previous studies on extraction using RESS technique and supercritical CO_2 as solvent as observed and reported by [18]-[24]. On the other hand, by additional increments of extraction pressure to 6000psi and 7000psi, the average solid oil particle size increased that which may suggest a decoupling of two processes of nucleation and growth [18]. Therefore, the small increase of the particle size begins to produce and an increment in particle size occurred. Similar results were reported by [18]. Based on the experimental data, the smallest particle size is 2.22 µm was achieved at temperature 40°C and extraction pressure 5000psi as shown in Fig. 1 and the SEM image as shown in Fig. 5. Fig. 2 shows the original size of ginger powder before RESS process. Figs. 3-7 shows the SEM image of ginger soild oil particles produced by RESS obtained from constant extraction temperature of 40°C and extraction pressure of 3000, 4000, 5000, 6000 and 7000psi, respectively. These obtained particles were observed to be spherical and which has a particle size range from 2-5 µm.

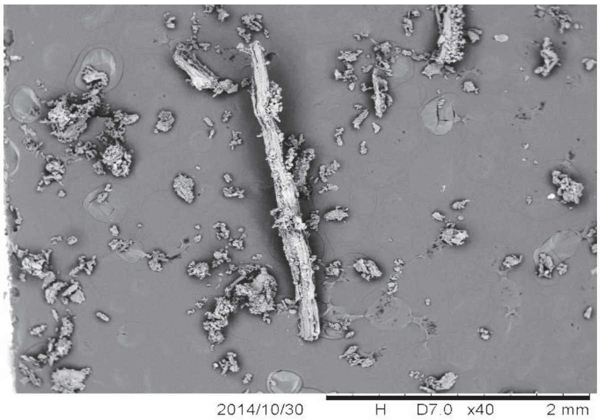

Fig. 2 SEM Image of Unprocessed Ginger Powder Before RESS Process, 370 µm

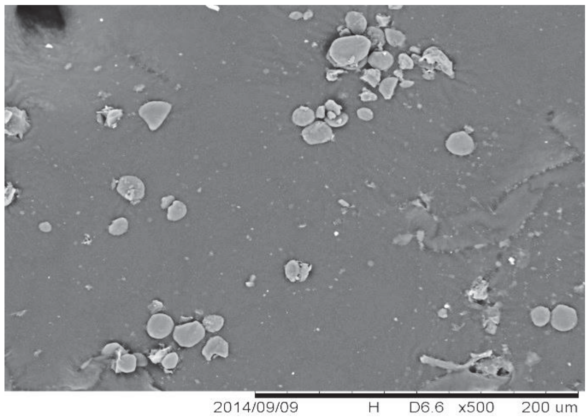

Fig. 3 SEM Image of Ginger Particles Produced by RESS-CO_2 Obtained at Extraction Temperature of 40°C and Extraction Pressure of 3000psi, 4.15 µm

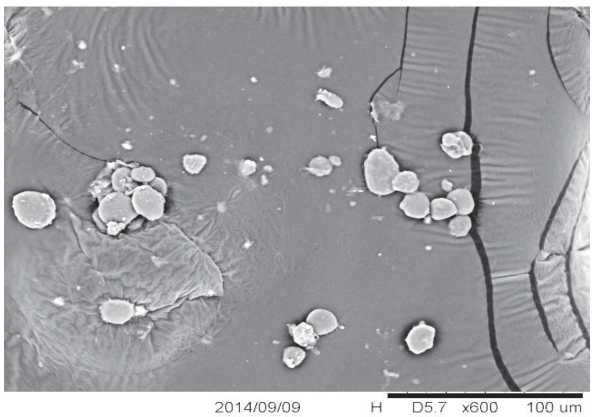

Fig. 4 SEM Image of Ginger Particles Produced by RESS-CO_2 Obtained at Extraction Temperature of 40°C and Extraction Pressure of 4000psi, 5.0 µm

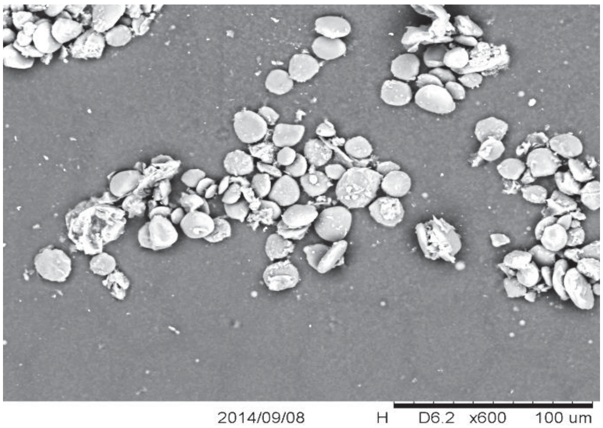

Fig. 5 SEM Image of Ginger Particles Produced by RESS-CO_2 Obtained at Extraction Temperature of 40°C and Extraction pressure of 5000psi, 2.22 µm

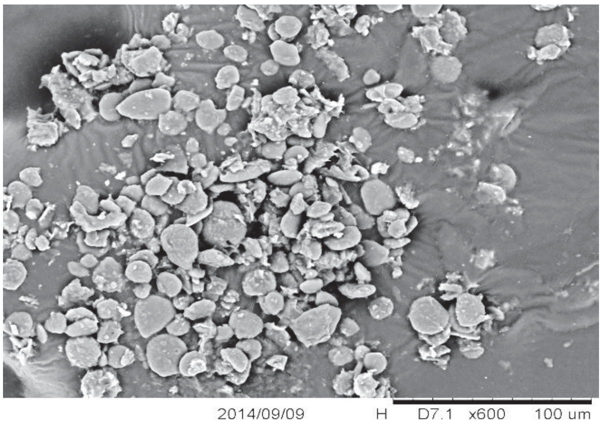

Fig. 6 SEM Image of Ginger Particles Produced by RESS-CO_2 Obtained at Extraction Temperature of 40°C and Extraction Pressure of 6000psi, 3.03 µm

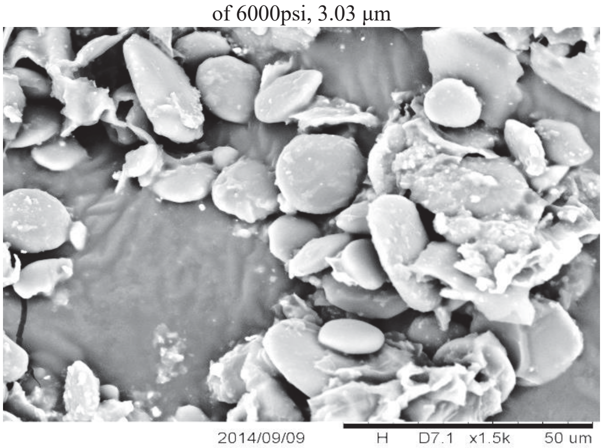

Fig. 7 SEM Image of Ginger Particles Produced by RESS-CO_2 Obtained at Extraction Temperature of 40°C and Extraction Pressure of 7000psi, 3.46 µm

From Table I, Analysis of Variance (ANOVA) test showed significance difference, where all the probability (P) value for the study on the effect of different pressure on particle size at constant temperature, was below than 0.05. This value indicates that the study on the effect of different pressure at constant temperature of 40°C to 70°C give significant effect on the ginger solid oil particle size within the experimental design of extraction pressure range from 3000psi to 7000psi.

C. Effect of Temperatures on Particle Size

In this work, the effect of different extraction temperature on particle size was varied at 40, 45, 50, 55, 60, 65, and 70°C while holding constant at different extraction pressure of 3000, 4000, 5000, 6000 and 7000psi, respectively for 40 minutes extraction time. The effect of temperature on the particle size at constant pressure are shown in Fig. 8.

As illustrated in Fig. 8, from the studies it can be seen at constant pressure, increasing extraction temperature from 40°C to 55°C, average solid oil particle size increased. It may be caused by increasing the extraction temperature, the solubility of the ginger solute in supercritical CO_2 reduced. By reducing the solid solubility, supersaturation is decreased and caused a decrease of nucleation and growth rates similar to the effect of extraction pressure [24]. Subsequently, a reduction of nucleation rate might produce large particle size. In other hand, it may be caused by the saturated supercritical solution is heated before expansion, outside the retrograde region, the resulting unsaturated solution lead to a smaller supersaturation ratio, thus bigger particle produce [23]. However, a further addition of the extraction temperature from 55°C to 70°C, a marked decreased in particle size was detected. This can be explained by an increase in the extraction temperature above 55°C, lead to an increased in the solid solubility. Increased in solid solubility, hence increased in the supersaturation and nucleation rate as a result of increase ginger solute concentration [21]. The improved of nucleation rate leads to reduction in the particle growth time and consequently smaller particle was obtained.

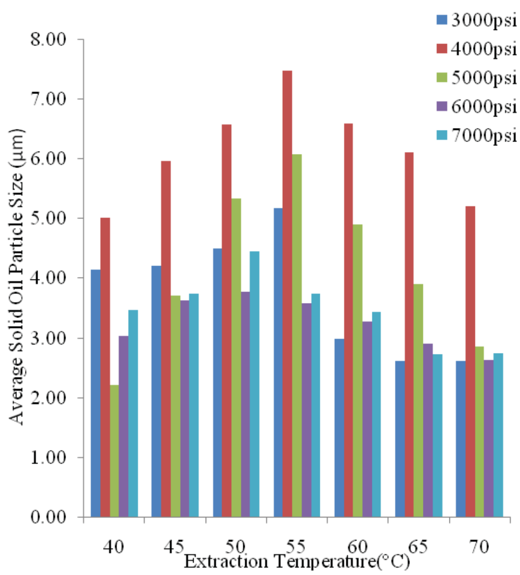

Fig. 8 Effect of Different Temperature on Solid Oil Particle Size at Constant Temperature

IV. Conclusions

From the studies conducted, it was found that RESS-CO_2 extraction method was able to produce solid oil particle of ginger at extraction pressure 500psi and temperature of 40ºC as the smallest oil particle obtained was 2.22µm, 99% reduction from the original size of 370µm. From the studies, it was proven extraction pressure and extraction temperature were important factors that can affect particle size, since increasing the extraction pressure or temperature, will change the size of solid oil particle. Furthermore, the size of precipitate solid oil particle obtained was smaller than the original particle size of ginger.

TABLE I
ANOVA Test for the Effect of Different Pressure on Particle Size at Constant Temperature

Temperature (°C)	Pressure (psi)	Particle Size (µm) Min	Std Deviation	P-Value
	3000	4.15	0.58	
	4000	5.01	0.01	
40	5000	2.22	0.14	4.84E-06
	6000	3.03	0.28	
	7000	3.46	0.08	
	3000	4.21	0.58	
	4000	5.95	0.13	
45	5000	3.71	0.08	1.12E-05
	6000	3.64	0.27	
	7000	3.74	0.11	
	3000	4.50	0.85	
	4000	6.57	0.08	
50	5000	5.33	0.06	5.12E-05
	6000	3.77	0.02	
	7000	4.45	0.17	
	3000	5.18	0.28	
	4000	7.46	0.09	
55	5000	6.07	0.05	4.49E-11
	6000	3.59	0.07	
	7000	3.74	0.02	
	3000	2.99	0.48	
	4000	6.59	0.21	
60	5000	4.89	0.16	6.06E-08
	6000	3.28	0.18	
	7000	3.44	0.15	
	3000	2.62	0.29	
	4000	6.10	0.06	
65	5000	3.90	0.27	2.36E-09
	6000	2.91	0.05	
	7000	2.74	0.11	
	3000	2.62	0.70	
	4000	5.20	0.26	
70	5000	2.86	0.32	4.59E-05
	6000	2.63	0.31	
	7000	2.75	0.19	

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