

The Effect of Fluid Flow Rate and Extraction Time in Supercritical Carbon Dioxide

Open
Access

Noor Aiyah Aris^{1,2}, Nor Faadila Mohd Idrus^{1,2}, Lee Nian Yian¹, Zuhaili Idham¹, Muhammad Syafiq Hazwan Ruslan³, Mohd Azizi Che Yunus^{1,2,*}

¹ Centre of Lipid Engineering and Applied Research (CLEAR), Ibnu Sina Institute for Scientific and Industrial Research, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

² Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

³ Department of Chemical Engineering, Universiti Teknologi Petronas, 32610 Bandar Sri Iskandar, Perak, Malaysia

ARTICLE INFO

ABSTRACT

Article history:

Received 5 June 2019

Received in revised form 4 July 2019

Accepted 12 July 2019

Available online 17 November 2019

Keywords:

Carbon dioxide flow rate; extraction time;
supercritical carbon dioxide; Momordica
charantia

Supercritical fluid extraction (SFE) is the best extraction method for high purity of charantin rich extract from Momordica charantia fruit. It is due to the unique characteristic of transport properties of supercritical fluid such as viscosity and diffusivity. Therefore, the focus of this study was to investigate the effects of carbon dioxide flow rate and extraction time on the Momordica charantia extract yield. The effects of carbon dioxide flow rate and extraction time were investigated using flow rate of 4, 6 and 8 mL/min with constant mean particle size of 0.3 mm. Different temperatures (45 and 65°C) and pressures (10 and 30 MPa) were used to investigate the effect between low and high operating condition. Based on the results, it showed that as the carbon dioxide flow rate increased to 8 mL/min, the extract yield increased to 3.698% at low operating condition. Furthermore, the time taken to reach plateau phase decreased from 150 min to 90 min. Therefore, it is recommended to use high flow rate in this study for highest extract yield in short time.

Copyright © 2019 PENERBIT AKADEMIA BARU - All rights reserved

1. Introduction

Supercritical fluid extraction (SFE) is a clean technology commonly used to extract high purity extract from natural product [1]. Supercritical fluid is a single homogenous fluid, form after the fluid exceed its critical point of temperature and pressure which was first discovered by Baron Cagniard de la Tour in 1822 [2]. Hannay and Hogarth in 1897 then found that the fluid can dissolve the solute and show potential as the alternative for conventional organic solvent [3].

The main advantage of supercritical fluid is the ability of the fluid to change its behaviour by manipulating the operational condition such as temperature and pressure depending on the specific components extracted [4]. Supercritical fluid has low viscosity and high diffusivity, therefore the fluid possesses better transport properties than liquid and easily diffuse into the solute particle which then

* Corresponding author.

E-mail address: r-azizi@utm.my (Mohd Azizi Che Yunus)

enhanced the extraction rates [5]. Carbon dioxide mostly used as the solvent in the supercritical fluid extraction due to its mild critical temperature and pressure (31.1°C, 7.38 MPa), inert, non-flammable, non-explosive, inexpensive, odourless, colourless clean solvent, non-toxic and harmless in pharmaceutical and food [6].

Momordica charantia is one of the natural sources for traditional treatment of Type 2 diabetes mellitus (T2DM). The plant is also known as peria katak (Malaysia), bitter gourd/ bitter melon (English), karela (India), ampalaya (Phillipine) and so on. The plant is a climbing perennial that usually grows up to 5 m, and bears elongated fruit with a range of 9–60 cm long. One of the bioactive component in the Momordica charantia is charantin, a steroidal saponins [7] known to possess a hypoglycemic effect similar with sulfonylurea medicine for T2DM [8].

T2DM is one of the critical disease in Malaysia as well as in the world. World Health Organization (WHO) had estimated that the world population of diabetes patient will likely to increase to 300 million or more by 2025 [9]. Based on Malaysia National Diabetes Registry volume 1 [10], 99.3% of total registered diabetes patient was diagnosed with T2DM and 58.4% from it were women in the age of 45 - 54 years old.

Since SFE is a clean and effective technology to replace conventional method, hence it will be used in this study to extract bioactive component in Momordica charantia. Therefore, the aim of this study was to investigate the effect of carbon dioxide flow rate and extraction time in SFE to increase the extract yield of Momordica charantia.

2. Methodology

2.1 Sample Preparation

Green bitter gourds (*Momordica charantia*) grown in Simpang Renggam (Johor, Malaysia) were purchased from a local market. The fruits were cleaned and cut into small pieces, and then oven dried together with the seeds at 50°C for 24 hours. The dried sample was then grounded using commercial blender (Waring, U.S). The ground sample was sieved using Endecotts Octagon 2000 Digital Sieve Shaker to get mean particle size of 0.3 mm. The sample was then stored in a freezer (-20°C) until further use.

2.2 Supercritical Carbon Dioxide (SC-CO₂) Extraction

A ratio of 1:3 pure ethanol (Merck, Malaysia) was mixed with 5 g of *Momordica charantia* dried sample in the extractor vessel for the SC-CO₂ extraction with addition of co-extractor. While, SC-CO₂ extraction without addition of co-extractor was performed with pure SC-CO₂ solvent only (skipped mixing step of *Momordica charantia* dried sample and pure ethanol).

The supercritical carbon dioxide extraction was carried out at constant pressure and temperature using SFE laboratory apparatus shown in Figure 1, which consists of force ventilation oven (MMM Group, German) fitted with a 50 mL stainless steel extraction vessel. Pure 99.9% CO₂ (Mega Mount Industrial Gases Sdn Bhd, Malaysia) gas was liquidized using a refrigerated (cooler) bath circulator (Daihan Scientific. Co Ltd, Korea) and pumped to extraction vessel using carbon dioxide liquid pump (Tokyo, Japan) with a constant flow rate of 4 mL/min.

Pressure in the extraction vessel was regulated by means of a back-pressure (Tescom Corp.,U.S) valve installed in the line between the extraction vessel and the separator. The depressurization process using control valve will convert the supercritical phase of carbon dioxide into its original phase with the help of water circulation bath (Daihan Scientific. Co Ltd, Korea) at the separator line

which can separate the CO₂ from the extracted sample. The extraction process was done dynamically for total extraction time of 150 min and extract yield collected at every 30 min interval times.

The *Momordica charantia* extract was weighed using the analytical balance (Ohaus, U.S) with an accuracy up to 0.0001 g. The whole process was repeated for the other flow rate of 6 and 8 mL/min using constant 0.3 mm particle size. The pressure (10 and 30 MPa) and temperature (45 and 65°C) used were chosen in order to investigate the effect of low and high operating condition on the *Momordica charantia* extract yield. Temperature higher than 65°C will degrade the thermolabile compound in the extract. While, high pressure than 30 MPa will damage the equipment. The suitable particle size used was determined in the previous study [11].

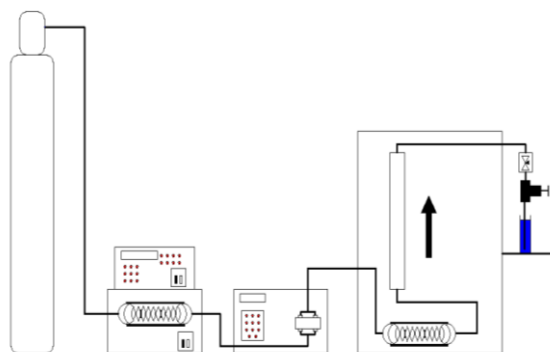


Fig. 1. Supercritical carbon dioxide extraction schematic diagram

2.3 Soxhlet Extraction

Solvent extraction was carried out using Soxhlet apparatus with 20 g of powdered *Momordica charantia*. The sample was extracted with 200 mL of 95% ethanol and hexane (Merck, Malaysia) for 6 hours. The remaining solvent trapped with the extracts was removed via rotary evaporator (Heidolph, German). The extract yield was weighed and then compared with the extract yield from SC-CO₂ extraction before stored in a freezer (-20°C) until further analysis.

2.4 Extract Yield Determination

The percentage of extract yield was calculated using Equation (1). The extract was stored in a freezer (-20°C) until further analysis.

$$\text{Extract yield (\%)} = \frac{\text{Oil extract (g)}}{\text{Initial sample (g)}} \times 100 \quad (1)$$

2.5 Statistical Analysis

Experimental results were expressed as means \pm standard errors. All experiments were carried out in duplicate.

3. Results and Discussion

3.1 Effect of Flow Rate at Constant Low Pressure and Different Temperature

At constant low pressure and different temperature, the extract yield of *Momordica charantia* increases as the flow rate increase as shown in Figure 2. The extract yield increases as the flow rate

increase up to 8 mL/min. Low flow rate of 4 mL/min limits the transport of solute to the bulk solvent due to increase in mass transfer resistance surrounding the solid particle. The solvent then leaving the extractor vessel unsaturated. Hence, the extract yield decreases with decrease in flow rate. In contrast, at high flow rate of 8 mL/min, the mass transfer resistance surrounds the solid particle had been reduced and therefore solute inside solid particle easily transported to bulk solvent. The solvent then leaving the extractor vessel saturated and thus increase the extract yield.

Danlami, *et al.*, [12] suggested that increasing the flow rate enhanced the extraction efficiency. However, when the flow rate was too high it would disturb the process due to low loading of SC-CO₂ and poor solute trapping. Meanwhile, a very low flow rate might plug the restrictors. Ruslan, *et al.*, [13] also agreed that the solvent would pass through the solute with less intimate contact when further increased the flow rate thus decreasing the extraction efficiency and extract yield.

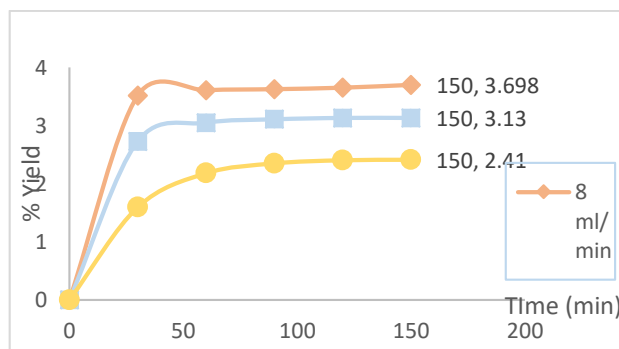


Fig. 2. Effect of flow rate on extraction of *Momordica charantia* (P = 10 MPa, T = 45°C, d_p = 0.3 mm)

However, Figure 3 showed that at 6 mL/min, the extract yield of *Momordica charantia* decreased due to the decreasing of solvent density which apparently decreased the solvent power of SC-CO₂ to extract the solute in solid particle.

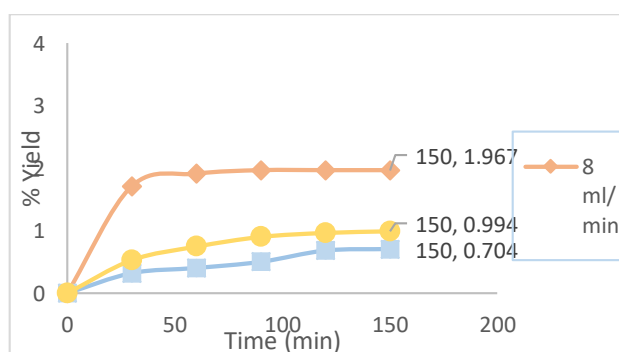


Fig. 3 Effect of flow rate on extraction of *Momordica charantia* (P = 10 MPa, T = 65°C, d_p = 0.3 mm)

At condition of low pressure and high temperature, the increase flow rate of 8 mL/min would help in increasing the extract yield as explained by Maran and Priya [14], when the flow rate increase, the film thickness surround the solid particle reduced and thus reducing the external mass transfer resistance around the solid. Therefore, solute easily escaped to bulk solvent and enhanced the solubility between solute-solvent leads to increase in the extract yield.

Other than that, Özkal and Yener [15] in their study emphasised that increasing flow rate significantly enhanced the grinding efficiency, G due to the increase in the convective effect of SC-

CO₂ consequently causing damages to the weak parts of the solid particle and leading to extra free solute removed from the solid particle during extraction.

3.2 Effect of Flow Rate at Constant High Pressure and Different Temperature

Similarly, the effect of flow rate in constant high pressure and different temperature also indicated that the extract yield of *Momordica charantia* increase as the flow rate increase as shown in Figure 4 and 5.

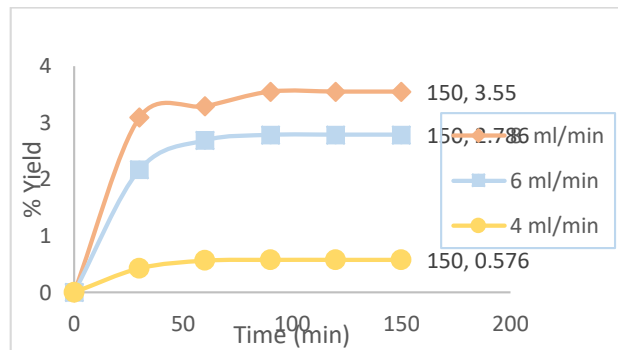


Fig. 4. Effect of flow rate on extraction of *Momordica charantia* (P = 30 MPa, T = 45°C, d_p = 0.3 mm)

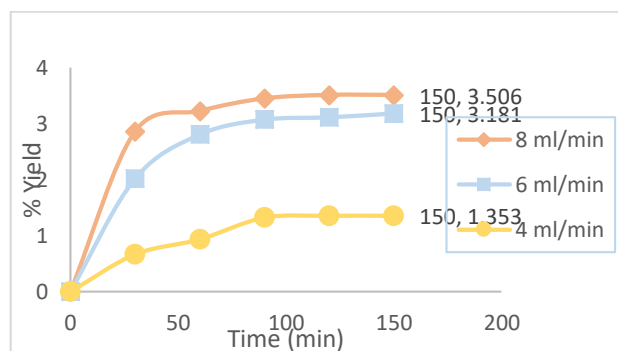


Fig. 5. Effect of flow rate on extraction of *Momordica charantia* (P = 30 MPa, T = 65°C, d_p = 0.3 mm)

Zhao and Zhang [6] demonstrated that at constant high pressure and low temperature, the extract yield was increased with increased of fluid flow rate due to synergistic effect of a mass transfer process and thermodynamic equilibrium state. It appeared that the density of SC-CO₂ increased in high pressure and low temperature conditions and it was possible for the extract to dissolve easily in the SC-CO₂ and thus reached equilibrium before leaving the extractor vessel. It was most likely that mass transfer resistance which control the extraction process rather than solubility.

Meanwhile, in Figure 5, at constant pressure 30 MPa and temperature, 65°C; there was less extract yield of *Momordica charantia* as compared to extract yield shown in Figure 2. The reason is due to solubility dominant that occurred in high pressure and high temperature condition. Increasing the fluid flow rate would not increase the extract yield of *Momordica charantia* because the solubility of solute in solvent was stronger than the effect of fluid flow rate. Rebolleda, *et al.*, [16] agreed that when the extraction condition is high pressure and high temperature, fluid flow rate does not have effect on increasing the extract yield.

3.3 Effect of Extraction Time

Based on the experimental results shown in Figure 2 until Figure 5, the extraction of *Momordica charantia* started to reach plateau phase (equilibrium) at extraction time of 90 minutes. Therefore, for this study, the extraction process should stop at time of 90 minutes instead of 150 minutes as suggested by Lisichkov, *et al.*, [17] and Sodeifian, *et al.*, [18].

According to Klein-Júnior, *et al.*, [19], the determination of extraction time is very important as it will affect the extraction process and might cause incomplete extraction if the extraction time was too short. On the other hand, if the extraction time took too much time, it would lead to wasting time and solvent used as well as degrade the bioactive compound. Salleh *et al.*, [20], agreed that, the increased in extraction time had no significant effect on the extraction yield when the extraction started to reach plateau phase.

However, there was a study done by Belayneh, *et al.*, [21] which needed to increase their extraction time to 510 minutes in order to complete the extraction of omega-3 from *Camelina* seed. Above all, the nature of the plant and other operating condition would also affect the extraction time in order to achieve the plateau phase.

According to Larry [22], Özkal, *et al.*, [23] and Machmudah, *et al.*, [24], the dynamic extraction profiles divided into 3 regions namely fast, intermediates and slow extraction. In fast extraction, most of free oil and bulk solute dispersed onto solid particle and easily dissolved into bulk solvent due to increase in solubility and therefore reduce the time to reach plateau phase. At intermediates region, the extraction process started to decrease due to disruption between solute-solvent interactions before entered the slow extraction region where diffusion process takes place inside solid particle. At slow region, increasing the extraction time would not increase the extract yield since most of the solvent has already reached equilibrium.

Figure 2 shows that the extract yield increased as the solvent flow rate increase. It only took 60 min for the extraction to start reached the plateau phase due to the solubility of solute in solvent depend on the flow rate used. Whereas, when the low flow rate use i.e 4mL/min, the time taken to start reaching the plateau phase had increased to 90 min. Similar trend showed in Figure 3, when the high flow rate 8 mL/min was used, the time taken to reach plateau phase was only 60 min while the flow rate of 6 mL/min and 4 mL/min needs 120 min to reach plateau phase. Figure 4 and 5 also proved that when high flow rate was used, the time to reach plateau phase was reduced.

Undoubtedly, if the low flow rate is chosen, the extraction rate decreases as film thickness surround solid particle and accumulation in the bulk still prevail over intra-particle and solubility demand. The extract oil would likely have a difficult time to dissolve in SC-CO₂ thus reducing the extract yield and longer residence time required for the system to reach equilibrium.

Meanwhile, when the high flow rate was chosen, SC-CO₂ was in excess and increase the operation cost since the SC-CO₂ would only bypass the solid particle with no contact at all and leaved extractor vessel unsaturated. Though the residence time was shorter, however it would reduce the extraction efficiency and gave low quality of extract yield. In summary, a preliminary study needed to be done in order to determine the best extraction time and fluid flow rate to achieve highest extract yield.

3.4 Comparison of Extract Yield between Soxhlet and SC-CO₂ Extraction Method

Figure 6 shows the percentage of *Momordica charantia*'s extract yield obtained using Soxhlet and SC-CO₂ extraction method. From the result, it appeared that extract yield obtained from Soxhlet method (6.802%) was higher than extract yield obtained from SC-CO₂ (3.698%).

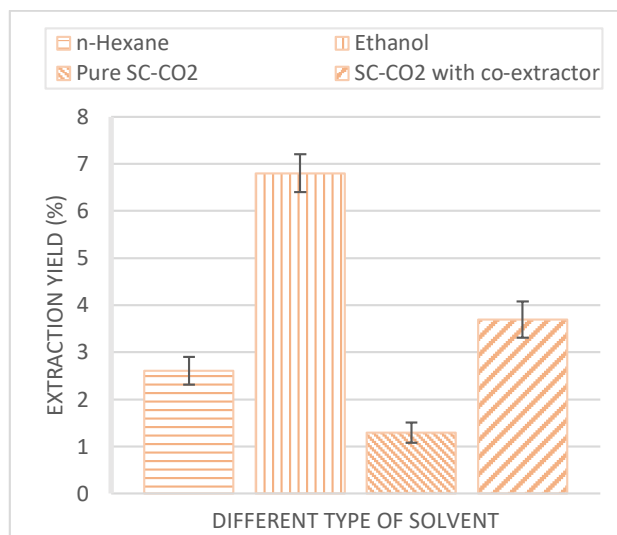


Fig. 6 Comparison on extraction yield of *Momordica charantia* using SC-CO₂ and Soxhlet extraction method

However, the extraction hours of Soxhlet method was longer (6 hours) compared when using SC-CO₂ (150 min) extraction method. The longer extraction time indeed enhanced the extract yield since ethanol (polar solvent) was able to extract both polar and non-polar compound in the extraction process. Nevertheless, longer extraction time might affect the target compound. Thermolabile compound might degrade as the process took longer period and used high temperature so it would decrease the quality of the extract.

Moreover, Soxhlet method used large amount of sample and solvent compared with SC-CO₂ extraction method. For example, 200 mL of ethanol was used for 20 g of sample in Soxhlet method compared to 15 mL of ethanol used for 5 g of sample in SC-CO₂ method.

The type of solvent used also played a major role in extracting the target compound. In Soxhlet method, ethanol gave higher extract yield (6.802%) compared to n-hexane (2.607%). The reason was the solvent polarity of the solvents. Ethanol is more polar rather than n-hexane therefore there will be a limitation on type of compound extracted based on the polarity of solvent.

Similarly, in SC-CO₂ extraction, the extract yield would increase when co-extractor was introduced in the SC-CO₂ extraction process. When only pure SC-CO₂ (no co-extractor in the system) was used as shown in Figure 6, the yield was only 1.294%. Whereas, when co-extractor introduced in SC-CO₂ system (use pure ethanol as a co-extractor), the extract yield increase to 3.698%. The addition of co-extractor was to enhance the transport property of solute in the solvent instead of adding the co-solvent to enhance the polarity of solvent.

Smith, *et al.*, [25] explained in their book, a co-extractor is use by mixing the suitable substance directly with the solid sample containing target compound to facilitate transport of target compound to the co-extractant phase. Then, pure SC-CO₂ is used to perform the whole extraction process. In comparison, the working principle of co-solvent is different because it need to dissolve into SC-CO₂ to form a homogeneous phase prior to extraction. If co-solvent modifies the properties of SC-CO₂ then, addition of co-extractor modifies the transport properties of target compound. Likewise, Yunus, *et al.*, [26] agreed that the extraction of solute is more favourable when the polarity scale of solute and solvent is the same.

4. Conclusion

The effect of fluid flow rate and extraction time indeed influenced the extraction yield in SC-CO₂ extraction method. Free solute outside solid surface would easily dissolved in SC-CO₂ when high flow rate was applied. Besides, high flow rate improved the diffusivity SC-CO₂ to penetrate deep into solid particle and consequently reduced the residence time and helped in increasing the extract yield. Therefore, the higher flow rate, 8 mL/min in this study will be used for further optimization study.

Acknowledgement

The author thanks Universiti Teknologi Malaysia for Research University Grant (Q.J130000.2509.14H23) and Ministry of Higher Education, Malaysia for Fundamental Research Grant Scheme (R.J130000.7809.4F766).

References

- [1] Setapar, S. H. M., L. N. Yian, M. A. C. Yunus, I. I. Muhamad, and M. A. A. Zaini. "Extraction of Rubber (*Hevea brasiliensis*) Seeds Oil Using Supercritical Carbon Dioxide." *Journal of Biobased Materials and Bioenergy* 6 (2012): 1-6.
- [2] Sarker, Satyajit D., and Lutfun Nahar, eds. *Natural products isolation*. New York: Humana Press, 2012.
- [3] Al-Otoom, Awni, Sameer Al-Asheh, Mamdouh Allawzi, Khalid Mahshi, Nahawand Alzenati, Bader Banat, and Bdour Alnimr. "Extraction of oil from uncrushed olives using supercritical fluid extraction method." *The Journal of Supercritical Fluids* 95 (2014): 512-518.
- [4] Yunus, M. C., Nur Husnina Arsad, Salman Zhari, Zuhaili Idham, Siti Hamidah Setapar, and Ana Najwa Mustapha. "Effect of supercritical carbon dioxide condition on oil yield and solubility of *Pithecellobium Jiringan* (Jack) Prain seeds." *Jurnal Teknologi* 60, no. 1 (2013): 45-50.
- [5] Da Silva, Rui PFF, Teresa AP Rocha-Santos, and Armando C. Duarte. "Supercritical fluid extraction of bioactive compounds." *TrAC Trends in Analytical Chemistry* 76 (2016): 40-51.
- [6] Zhao, Suwei, and Dongke Zhang. "A parametric study of supercritical carbon dioxide extraction of oil from *Moringa oleifera* seeds using a response surface methodology." *Separation and Purification Technology* 113 (2013): 9-17.
- [7] Mukherjee, Pulok K., Kuntal Maiti, Kakali Mukherjee, and Peter J. Houghton. "Leads from Indian medicinal plants with hypoglycemic potentials." *Journal of Ethnopharmacology* 106, no. 1 (2006): 1-28.
- [8] Li, W. L., H. C. Zheng, Joshua Bukuru, and Norbert De Kimpe. "Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus." *Journal of ethnopharmacology* 92, no. 1 (2004): 1-21.
- [9] WHO, W. H. O. (2016, 20/3/2016). *10 Facts About Diabetes*. Available: <http://www.who.int/diabetes/en/>
- [10] Feisul, I. M., and A. Soraya. "National Diabetes Registry, 2009-2012." (2013).
- [11] Aris, N. A., Yunus, M. A. C., Yian, L. N., Idham, Z., Ramli, W. N. D., and Aziz, A. H. A. 2016. Effect of Particle Size on Bitter Gourd (*Momordica charantia*) Extract Yield by Supercritical Carbon Dioxide Extraction. in *The 7th International Conference on Postgraduate Education (ICPE-7 2016)*. 2016. 262-266.
- [12] Danlami, Jibrin Mohammed, Agus Arsad, Muhammad Abbas Ahmad Zaini, and Hanizam Sulaiman. "A comparative study of various oil extraction techniques from plants." *Reviews in Chemical Engineering* 30, no. 6 (2014): 605-626.
- [13] Ruslan, Muhammad Syafiq Hazwan, Mohd Azizi Che Yunus, Zuhaili Idham, Noor Azian Morad, and Asmadi Ali. "Parametric evaluation for extraction of catechin from *Areca catechu* Linn seeds using supercritical CO₂ extraction." *Jurnal Teknologi* 74, no. 7 (2015).
- [14] Maran, J. Prakash, and B. Priya. "Supercritical fluid extraction of oil from muskmelon (*Cucumis melo*) seeds." *Journal of the Taiwan Institute of Chemical Engineers* 47 (2015): 71-78.
- [15] Özkal, Sami Gökhan, and Meryem Esra Yener. "Supercritical carbon dioxide extraction of flaxseed oil: Effect of extraction parameters and mass transfer modeling." *The Journal of Supercritical Fluids* 112 (2016): 76-80.
- [16] Rebolleda, Sara, Nuria Rubio, Sagrario Beltrán, M. Teresa Sanz, and María Luisa González-Sanjosé. "Supercritical fluid extraction of corn germ oil: Study of the influence of process parameters on the extraction yield and oil quality." *The Journal of Supercritical Fluids* 72 (2012): 270-277.
- [17] Lisichkov, Kiril, Stefan Kuvendziev, Zoran Zeković, and Mirko Marinkovski. "Influence of operating parameters on the supercritical carbon dioxide extraction of bioactive components from common carp (*Cyprinus carpio* L.) viscera." *Separation and Purification Technology* 138 (2014): 191-197.

- [18] Sodeifian, Gholamhossein, Nadasadat Saadati Ardestani, Seyed Ali Sajadian, and Sajad Ghorbandoost. "Application of supercritical carbon dioxide to extract essential oil from *Cleome coluteoides* Boiss: experimental, response surface and grey wolf optimization methodology." *The Journal of Supercritical Fluids* 114 (2016): 55-63.
- [19] Klein-Junior, Luiz Carlos, Yvan Vander Heyden, and Amelia Teresinha Henriques. "Enlarging the bottleneck in the analysis of alkaloids: A review on sample preparation in herbal matrices." *TrAC Trends in Analytical Chemistry* 80 (2016): 66-82.
- [20] Salleh, Liza Md, Hasmida Mohd Nasir, Harisun Yaakob, and Mohd Azizi Che Yunus. "Determination of Supercritical Carbon Dioxide Extraction Parameters for *Quercus infectoria* Galls and the Effects on Extraction Yield and Antioxidant Activity." *Jurnal Teknologi* 67, no. 2 (2014).
- [21] Belayneh, Henok D., Randy L. Wehling, Edgar Cahoon, and Ozan N. Ciftci. "Extraction of omega-3-rich oil from *Camelina sativa* seed using supercritical carbon dioxide." *The Journal of Supercritical Fluids* 104 (2015): 153-159.
- [22] Larry, T. T. 1996. *Supercritical Fluid Extraction*. John Wiley & Sonc, Inc.
- [23] Özkal, S. G., M. E. Yener, and L. Bayındırlı. "Mass transfer modeling of apricot kernel oil extraction with supercritical carbon dioxide." *The Journal of supercritical fluids* 35, no. 2 (2005): 119-127.
- [24] Machmudah, Siti, Anny Sulaswatty, Mitsuru Sasaki, Motonobu Goto, and Tsutomu Hirose. "Supercritical CO₂ extraction of nutmeg oil: Experiments and modeling." *The Journal of supercritical fluids* 39, no. 1 (2006): 30-39.
- [25] Smith, Richard, Hiroshi Inomata, and Cor Peters. *Introduction to supercritical fluids: a spreadsheet-based approach*. Vol. 4. Newnes, 2013.
- [26] Yunus, Mohd Azizi Che, Salman Zhari, Saharudin Haron, Nur Husnina Arsad, Zuhaili Idham, and Muhammad Syafiq Hazwan Ruslan. "Extraction and identification of vitamin e from *Pithecellobium jiringan* seeds using supercritical carbon dioxide." *Jurnal Teknologi* 74, no. 7 (2015).