

Effect of particle size and co-extractant on *Momordica charantia* extract yield and diffusion coefficient using supercritical CO₂

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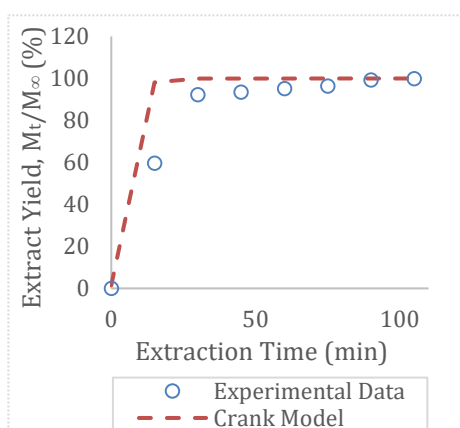
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Graphical abstract



Abstract

Supercritical carbon dioxide (SC-CO₂) is a fluid with high diffusivity and low viscosity which can penetrate deep into the solid particle efficiently compared to liquid or gas. SC-CO₂ is commonly used as a green solvent in modern extraction of bioactive compound from plants. Sometimes, a co-solvent was added in the system to increase solvent polarity or mix a co-extractant with dried sample to improve solute's transport properties. Hence, the aim of this study was to determine *Momordica charantia* extract yield with different mean particle size as well as diffusion coefficient, D_e , in the extraction process with and without co-extractant. Different mean particle sizes used were 0.2, 0.3, 0.5, and 0.7 mm at constant operating condition (20 MPa, 65 °C and 4 mL/min). A feed-to-solvent (1:3) ratio was used in the extraction process with co-extractant. Based on the results, mean particle size of 0.3 mm gave the highest extract yield, 3.32% and 1.34% with and without co-extractant respectively. Whereas, the value of D_e at 0.3 mm mean particle size, with and without co-extractant are 8.820×10^{-12} and 7.920×10^{-12} m²/s respectively. Therefore, 0.3 mm is the best mean particle size to produce the highest *Momordica charantia* extract yield and D_e value in SC-CO₂ extraction with the presence of co-extractant.

Keywords: *Momordica charantia*, co-extractant, supercritical carbon dioxide, particle size, diffusion coefficient.

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INTRODUCTION

Diffusion coefficient, D_e , is one of the important parameters in describing mass transfer in supercritical fluid extraction (SFE) process (López-Padilla *et al.*, 2016). According to Medina (2012), the variations of D_e value from subcritical to supercritical region might due to chemical potential of solute which influence the diffusion flux, increase of density when heavier molecule of solute favours lighter solvent molecule in that region, and solute molecules form a cluster when near critical region forcing the solvent to move towards the region with solute's high concentration. Therefore, it is important to consider the D_e value for the design and efficiency of the SFE process (Liong *et al.*, 1991).

SFE is a modern green technology in extraction process because the end product comply the basic requirement for product's safety (Setapar *et al.*, 2012). Ruslan *et al.* (2015) claimed that the extract from SFE is considered consumable and natural by the World Health Organization (WHO) and European Food and Drug Association (EFDA). Danlami *et al.* (2015) suggested that SFE is a green technology due to its chemical inertness, non-toxic and non-inflammable characteristics as well as less amount of solvent and sample used. Supercritical fluid such as carbon dioxide (CO₂) is commonly used as a solvent in the extraction process.

The main reason is because of its mild operating temperature and relatively low pressure condition. The critical temperature and pressure for carbon dioxide are 31 °C and 7.38 MPa respectively. Furthermore, CO₂ can easily be separated from the end product after depressurized (Yunus *et al.*, 2015). Arsad *et al.* (2016) also agreed that by using SFE, the oil yield from piper beetle leaves possess high percentage of antioxidant activity compared with conventional method. Therefore, SFE is the best extraction method in order to achieve higher quality of extract. Since CO₂ is a non-polar solvent which favours mostly non-polar compound, addition of co-solvent will increase its polarity and enhance the ability to extract more polar compound. Danlami *et al.* (2014) recommended the used of ethanol as a co-solvent due to its high miscibility with CO₂ and allowed to be used in the food, cosmetic and pharmaceutical industries.

Sometimes, due to limitation of equipment, co-solvent cannot be used since it needs personal pump to operate and dissolve in SC-CO₂ before it enters the extraction vessel. Thus, a co-extractant is introduced to improve the transport properties of target compound into SC-CO₂ solvent bulk. Ciurlia *et al.* (2009) described the major difference in percentage yield of lycopene when using hazelnut oil as a co-solvent and co-extractant. Hazelnut oil as a co-extractant managed to extract 70% of lycopene instead of only 30% when it is used as a co-solvent.

Moreover, as co-extractant, hazelnut oil accelerates the reaction to only 4 hours to achieve approximately 70% of lycopene compared to when it is used as a co-solvent—it needs at least 6 hours to extract 30% of lycopene. Therefore, it is better to use co-extractant directly in the solid sample to improve solute transport properties in SC-CO₂ extraction.

Previous researcher used conventional method to extract bioactive compound in natural plants. Yasir *et al.* (2016) used orbital shaker to extract phenolic compound in *Momordica dioica*'s seed with methanol and water as the solvent. Shanmugapriya and Poornima (2014) used a Soxhlet apparatus with ethanol as a solvent to extract charantin from *Momordica charantia*, an active compound known to lower the blood glucose. However, conventional method needs large amount of solvent and sample with long extraction time as well as low selectivity towards valuable target compound (Hadzri *et al.*, 2014).

Momordica charantia L. (Cucurbitaceae) is a scientific name for bitter melon which commonly known among local to treat diabetes mellitus due to its hypoglycemic effects (Zhang *et al.*, 2015). Besides that, the extract from *Momordica charantia* could reduce body weight, improve glucose metabolism, and insulin signalling (Yang *et al.*, 2015).

World of health organization (WHO) has estimated that the world population of diabetes patient will likely increase to 300 million or more by 2025 (Wang *et al.*, 2014). Meanwhile in Malaysia, total diabetes patients has increased from 11.6% in 2006 to 15.2% in 2011 (Feisul & Azmi, 2013). The prevalence percentage is expected to rise to 21.6% by 2020 (Bakar *et al.*, 2015). It is important to choose a suitable extraction method to extract a valuable plant such as *Momordica charantia*. Hence, the aim of this study was to extract *Momordica charantia* with different mean particle sizes, using green technology of supercritical carbon dioxide (SC-CO₂) solvent with and without co-extractant, to determine its extract yield and diffusion coefficient as well as the extraction efficiency of charantin in both conditions. Determination of diffusion coefficient was performed using Crank model. Meanwhile, high performance liquid chromatography (HPLC) was used to determine the extraction efficiency of charantin.

EXPERIMENTAL

Materials

Green bitter gourds (*Momordica charantia*) grown in Simpang Renggam (Johor, Malaysia) were purchased from a local market. The fruits were washed to eliminate any traces of impurities, cut into small pieces and dried in an oven (Memmert UFE 500) at 50 °C for 24 h (Fuangchan *et al.*, 2011). The dried sample was then ground using commercial blender (Waring, U.S). The ground samples were sieved into 0.2, 0.3, 0.5, and 0.7 mm mean particle size using Endecotts Octagon 2000 Digital Sieve Shaker. The sample was then stored in a tight seal container inside the freezer (−20 °C) until the extraction day. Carbon dioxide (purity 99.99%) was supplied by the Kras Instrument & Service (Johor Bahru, Malaysia). Charantin (purity 98%) standard was purchased from Wuhan Chemfaces Biochemical (Hubei, China). Pure (100%) ethanol was purchased from Merck (Darmstadt, Germany).

Supercritical Carbon Dioxide Extraction

A pure ethanol was mixed with 5 g of *Momordica charantia* dried sample with a feed-to-solvent (1:3) ratio in the extractor vessel for the SC-CO₂ extraction with addition of co-extractant. While, SC-CO₂ extraction without addition of co-extractant was performed with pure SC-CO₂ solvent only (skipped mixing step of *Momordica charantia* dried sample and pure ethanol). The ratio of co-extractant and mass of sample were chosen based on limitation of equipment.

The supercritical carbon dioxide extraction was carried out at constant pressure of 20 MPa and temperature of 65 °C using SFE laboratory apparatus shown in Fig. 1, which consisted of force ventilation oven (MMM Group, German) fitted with a 50 mL stainless steel extraction vessel. Pure 99.9% CO₂ gas (Mega Mount Industrial Gases Sdn Bhd, Malaysia) 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 then separate the CO₂ from the extracted sample. The extraction process was done dynamically for total extraction time of 105 min and oil yield were collected at every 15 min of interval times.

The *Momordica charantia* extract was weighed using the analytical balance (Ohaus, U.S) with an accuracy up to 0.0001 g. The percentage of *Momordica charantia* extract yield was then calculated using Equation 1. The extract was later stored in a freezer (−20 °C) until further analysis.

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

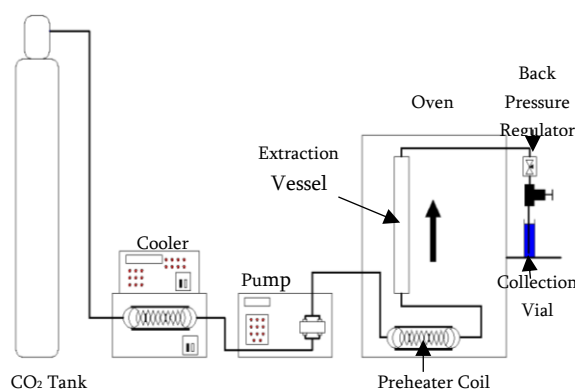


Fig. 1 Supercritical carbon dioxide extraction schematic diagram.

High Performance Liquid Chromatography (HPLC)

HPLC (Agilent 1260 Infinity, U.S) analysis was performed with C-18 Inertsil ODS-3 column (5 μm particle, 4.6 mm × 250 mm ID). The mobile phase used was 100:2 (v/v) methanol-water and deliver at 1 mL/min flow rate. The sample injection volume was 20 μL using UV detection wavelength at 204 nm.

Crank Model

Crank model was used to investigate the effect of particle size in influencing the mass transfer of solute-solvent in the fixed bed under supercritical condition. Crank (1975) has developed an equation to solve the diffusion in sphere when the surface concentration is maintained at constant by substituting diffusivity, D , with diffusion coefficient, D_e (Santos *et al.*, 1996).

Huang *et al.* (2011), Santos *et al.* (1996), Reverchon *et al.* (1993) and Bartle *et al.* (1990) summarized the assumptions for Crank model:

- All the single solid particle was assumed to be solid with constant similar size.
- The solute to be extracted was scattered uniformly inside the single solid particle and assumed to be at the same stage of extraction.
- The flow rate of supercritical fluid was fast enough to penetrate deep inside the solid particle with mass transfer resistance close to zero.
- Intra-particle mass transfer was the controlling factor in the extraction process.
- Axial dispersion is neglected

The total amount of diffusing molecule entering or leaving the sphere in non-steady state is given in Eq. 2.

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-\frac{D_e n^2 \pi^2 t}{r^2}\right) \quad (2)$$

where, M_t is a total amount of solute diffused from sphere at time, t in unit g, M_∞ is a total amount of solute in unit g, n is a number of iterations, D_e is a diffusion coefficient in unit m^2s^{-1} , r is a particle radius in metre and t is time in second.

Statistical Analysis

The deviation between the calculated and experimental value was evaluated. Model with the lowest amount of absolute average relative deviation (AARD) will be the best model to be used for further research. The AARD formula is as stated in Eq. 3.

$$AARD = \frac{1}{N} \sum_{i=1}^N \left| \frac{y_{calculated} - y_{experimental}}{y_{experimental}} \right| \times 100 \tag{3}$$

where, N is a total number of experimental data. While, $y_{calculated}$ and $y_{experimental}$ are the data obtained from model equations and experimental data, respectively at i condition.

RESULTS AND DISCUSSION

Effect of mean particle size in *Momordica charantia* extract yield

Fig. 2 shows a percentage of *Momordica charantia* extract yield for all different mean particle sizes in SC-CO₂ extraction (with and without co-extractant) at constant pressure of 20 MPa and temperature of 65 °C. From the figure, it shows that the SC-CO₂ (with co-extractant) extraction gave highest percentage of *Momordica charantia* extract yield compared with SC-CO₂ (without co-extractant) extraction. In fact, the time taken to reach plateau phase are shorten in SC-CO₂ (with co-extractant) extraction rather than in SC-CO₂ (without co-extractant) extraction.

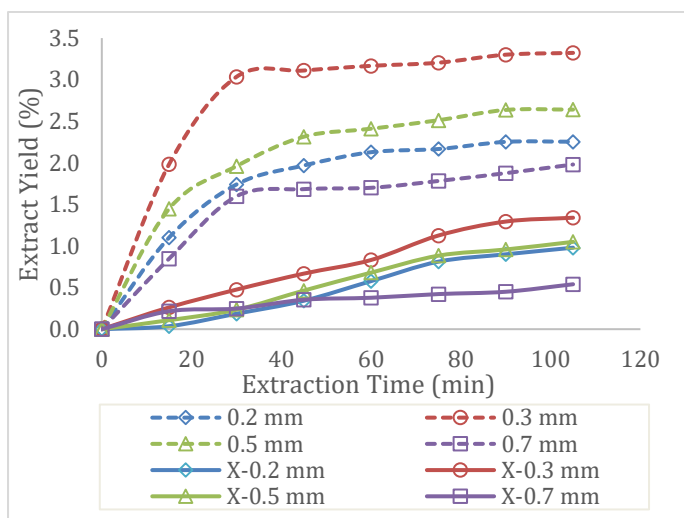


Fig. 2 Percentage of *Momordica charantia* extract yield in SC-CO₂ extraction (with and without co-extractant). X- denoted a SC-CO₂ (without co-extractant) extraction.

In SC-CO₂ (with co-extractant) extraction, mean particle size of 0.3 mm gave the highest *Momordica charantia* extract yield, 3.32% compared with other mean sizes as summarized in Table 2. Higher yield of extract can be obtained from particle size ranged from 0.3 to 0.5; below or above this range may cause a lower yield of extract. This might be due to the diffusion factor, where smaller particle size gives larger surface area and thus shorten the diffusion path for the solute-solvent to travel in comparison with larger particle size. Therefore, it is easier for the SC-CO₂ molecules to diffuse into and out of the smaller solid particle size.

The grinding process broke the cell walls of the solid particle, leading to more free solutes released onto the surface and easily dissolved in the SC-CO₂. Hence, the extract yield increases as the particle sizes were decreased by grinding. Özkal and Yener (2016) stated that the fraction of free solute released is represented by grinding

efficiency, G , whereby more solute will be released when the particle size is decreasing due to more broken solid cells.

However, the smallest mean particle size of 0.2 mm which supposed to obtain the highest amount of oil yield among the other mean particle sizes can only obtain 2.25% extract yield after 105 min of extraction. The reason is because the solid particle is too compact for the SC-CO₂ molecules to penetrate into it. Kluson et al. (2012), pointed out that the decrease in the extract yield for smallest particle size is caused by solid particle compactness, and therefore SC-CO₂ molecules can only remove free solute on the solid surface only.

The same trend also occurred in SC-CO₂ (without co-extractant) extraction as shown in Fig. 2 and Table 1. Mean particle size of 0.3 mm gives the highest *Momordica charantia* extract yield, 1.34% compared with other mean sizes. The bigger particle sizes of 0.5 mm and 0.7 mm could only give a 1.05 and 0.54% of *Momordica charantia* extract yield. Meanwhile, the smallest mean particle size of 0.2 mm can only give a 0.98% of *Momordica charantia* extract yield.

Undoubtedly, mean particle size of 0.3 mm is the best particle for extraction of *Momordica charantia* in SC-CO₂ (with and without co-extractant) extraction in order to get a higher extract yield.

Diffusion coefficient, D_e in SC-CO₂ (without co-extractant) extraction

Table 1 lists the diffusion coefficient, D_e value for different mean particle size used in the SC-CO₂ (without co-extractant) extraction. The D_e value was determined by fitting the graph of extract yield percentage, M_t/M_∞ for both experimental data and Crank model against extraction time as shown in Fig. 3. From the figure, Crank model predicted that the extraction curve will reach plateau phase at 80 min. However, the experimental curve shows the opposite of the prediction. It seems that the extraction of *Momordica charantia* using 0.7 mm mean particle size will take a longer time to reach plateau phase.

Table 1 Summary of diffusion coefficient, D_e and extract yield for different mean particle size, d_p at 20 MPa, 65 °C and 4 mL/min in SC-CO₂ (without co-extractant) extraction.

Mean Particle Size, d_p (mm)	Diffusion Coefficient, $D_e \times 10^{12}$ (m^2/s)	Extract Yield (%)	AARD (%)
0.2	0.72	0.98	61.20
0.3	7.92	1.34	22.80
0.5	5.19	1.05	30.10
0.7	9.95	0.54	10.90

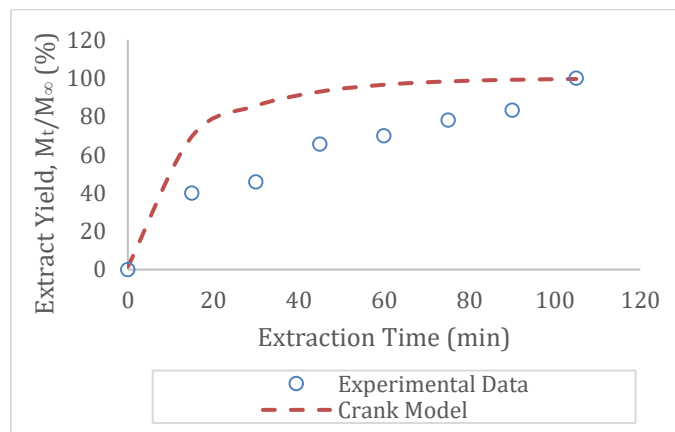


Fig. 3 Crank model for SC-CO₂ extraction of *Momordica charantia* at 20 MPa, 65 °C, 4 mL/min, 0.7 mm; fitted value $D_e = 9.950 \times 10^{-12} m^2/s$.

Furthermore, mean particle size of 0.7 mm gave the lowest percentage of extract yield among other sizes even though it does give the highest D_e value, $9.950 \times 10^{12} m^2/s$ as well as the lowest average absolute relative deviation (AARD), 10.9%. In contrast, mean particle size of 0.3 mm which gave the highest extract yield among other size, has low D_e value, $7.920 \times 10^{12} m^2/s$ and higher AARD, 22.8%. Supposedly, higher D_e value represent higher ability of solvent to penetrate into solid particle which then increase the extract yield.

Unfortunately, the theory does not apply in this case. However, according to Nasir (2017), a diffusion equation does not necessarily gives an accurate description about the mechanism of extraction in natural plants since it is complex and not well defined. In fact, Vladić *et al.* (2016) stated that the fitting model is acceptable when the AARD value is up to 5–10% only. Above 10% indicates poor correlation between experimental data and fitted model. Therefore, Crank model failed to predict D_e value for different mean particle size in SC-CO₂ (without co-extractant) extraction.

Diffusion coefficient, D_e in SC-CO₂ (with co-extractant) extraction

Table 2 lists the D_e value for all different mean particle sizes used in the SC-CO₂ (with co-extractant) extraction. It shows that mean particle size of 0.3 mm gave the highest D_e value with low AARD which are $8.820 \times 10^{-12} \text{ m}^2/\text{s}$ and 5.968 respectively. While, Figure 4 shows the graph of extract yield, M_t/M_∞ for both experimental data and Crank model against extraction time at mean particle size of 0.3 mm.

From Figure 4, Crank model successfully predicted an extraction curve where it will only takes a 15 min to reach plateau phase compared with experimental as shown in Figure 3. Even tough from the experimental data, it took at least 75 min to reach plateau phase. It appears that, the model has successfully fitted with the experimental data and looks reliable. In fact, from Table 2, the D_e value and AARD for other mean particle sizes are within an acceptable range as mentioned by Vladić *et al.* (2016).

Table 2 Summary of diffusion coefficient, D_e and extract yield for different mean particle size, d_p at 20 MPa, 65 °C and 4 mL/min in SC-CO₂ with 1:3 ethanol as co-extractant.

Mean Particle Size, d_p (mm)	Diffusion Coefficient, $D_e \times 10^{12}$ (m^2/s)	Extract Yield (%)	AARD (%)
0.2	2.19	2.25	7.23
0.3	8.82	3.32	5.96
0.5	4.99	2.64	5.17
0.7	8.79	1.98	5.99

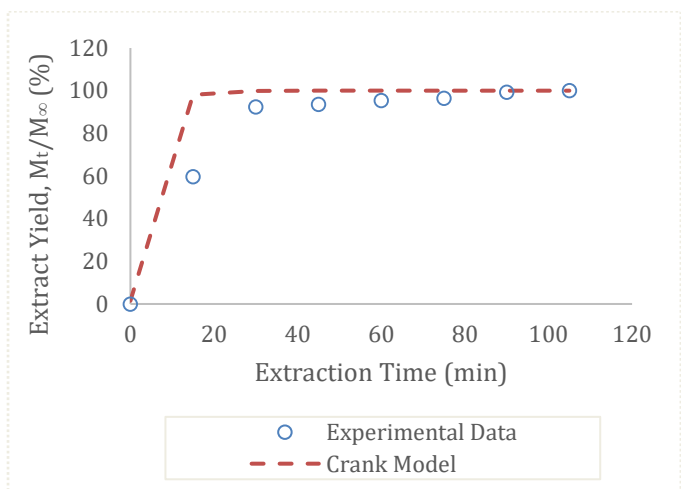


Fig. 4 Crank model for SC-CO₂ (with co-extractant) extraction of *Momordica charantia* at 20 MPa, 65 °C, 4 mL/min, 0.3 mm; fitted value $D_e = 8.820 \times 10^{-12} \text{ m}^2/\text{s}$.

According to Crank model in Eq. 2, particle radius, r is directly proportional to the diffusion coefficient, D_e . Therefore, the smaller particle size will give higher D_e value in the extraction process. On the contrary, mean particle size of 0.2 mm has the lowest D_e value, $2.190 \times 10^{-12} \text{ m}^2/\text{s}$ indicates the difficulty of solvent to penetrate inside solid particle. As a matter of fact, for mean particle size of 0.2 mm, even though it is the smallest particle size but the size is too fine and compact in the extraction vessel. As explained by Samadi and Vaziri (2017), fine particle will create a “channelling” phenomenon due to adhesion effect. Adhesion effect happened when solid particles stick together and form a cake of solid particle, therefore restrict the

interaction between solvent and solute in the extraction vessel and decrease the extract yield and D_e value. Hence, the D_e and extract yield values for mean particle size 0.2 mm are lower than mean particle size 0.3 mm. Meanwhile, the larger mean particle size such as 0.5 and 0.7 mm will increase the mass transfer resistance due to their smaller surface area thereby decreasing the D_e value and extract yield.

As mentioned previously, particle size influences the D_e value in the extraction system. Silva and Delgado (2011) found that particle size is the most influence parameter in the diffusion coefficient in their study. They observed that the models used, deviate significantly from the experimental data in different particle size studied. Indeed, particle size influenced the diffusion coefficient in the extraction process. Hence, Crank model successfully predict D_e value for different mean particle size in SC-CO₂ (with co-extractant) extraction.

Effect of co-extractant in SC-CO₂ extraction

In comparison, extraction process in SC-CO₂ (with co-extractant) extraction is more efficient in extracting *Momordica charantia* due to improvement of solute transport properties compared with the SC-CO₂ (without co-extractant) extraction. Moreover, the D_e value and extract yield in SC-CO₂ (with co-extractant) extraction were higher and reliable compared with the SC-CO₂ (without co-extractant) extraction. Furthermore, the time taken to reach plateau phase for the extraction process is shorter in SC-CO₂ (with co-extractant) extraction rather than in SC-CO₂ (without co-extractant) extraction.

The reason is due to the addition of co-extractant directly in solid sample which probably gives the swollen effect so that the solute easily transported out of solid matrix. Park *et al.* (2007) showed that the swollen effect by addition of co-extractant in green tea solid matrix helps caffeine to be easily transported out of the solid matrix and facilitate through the SC-CO₂ solvent bulk.

Another reason would be that the co-extractant changed the transport properties of solute and at the same time act as a co-solvent to increase solvent strength of SC-CO₂ solvent. Melo *et al.* (2014) stated that ethanol is a polar substance commonly used in food, cosmetic or pharmaceutical industries. It can increase the polarity of SC-CO₂ even with only small amount added. Durante *et al.* (2014) proposed that the oil in the pumpkin seed facilitates the transport of carotenoid from pumpkin flesh and probably improves the polarity of SC-CO₂ by acting as a co-solvent and increase the fluid density as it simultaneously co-extracted in a diffusion-controlled mechanism.

More importantly, with addition of co-extractant in *Momordica charantia* dried sample, it manages to extract target compound in this study, which is charantin as shown in Fig. 5.

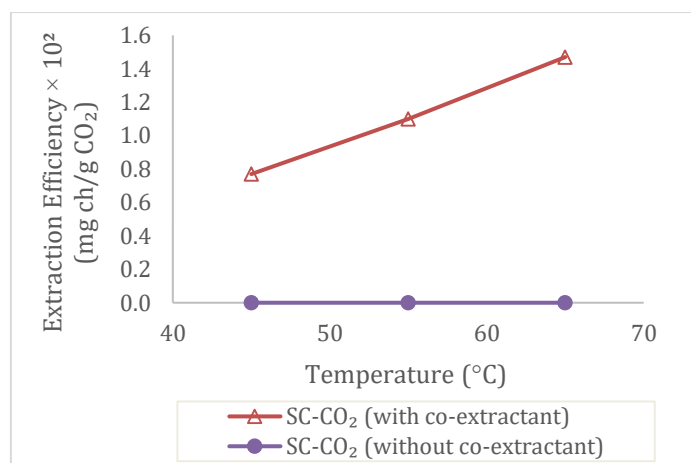


Fig. 5 Extraction efficiency of charantin in the extraction of *Momordica charantia* at various temperature at constant pressure, 20 MPa using SC-CO₂ (with and without co-extractant) solvent.

Charantin is a molecule consists of a mixture (1:1) of two steroidal saponins compounds, sitosterol glucoside, and stigmasterol glucoside. The uniqueness of this molecule is that the steroidal part of it is highly soluble in non-polar solvents such as chloroform and dichloromethane. In contrast, the glucosides tail favours polar solvent such as ethanol or

methanol due to its polarity and bulk structure (MW = 578.494) (Pitipanapong et al., 2007). Therefore, SC-CO₂ (with co-extractant) is the suitable solvent to extract charantin in this study.

Similarly, a study done by Ruslan et al. (2015) and Ruslan et al. (2018) revealed the comparison between pure SC-CO₂ extraction and SC-CO₂ with methanol as the co-extractant in extracting catechin. Catechin can be extracted with the help of 5% (v/v) methanol as a co-extractant compared with no addition of co-extractant (0% v/v methanol). As catechin is a polar compound, it needs a polar solvent to extract the compound. It appears that the extractability of catechin was due to hydrogen bonding and interaction between hydroxyl group of catechin and methanol.

On the other hand, addition of co-solvent or co-extractant might not favour the extraction of target compound. As experienced by Hatami et al. (2012) in their study, the addition of ethanol as a co-solvent gave negative impact in enhancing the recovery of diterpenic from spent coffee grounds due to mild polarity of the compound. The modified (addition of co-solvent) SC-CO₂ has higher affinity towards non-diterpenic compounds (other compound) which then only increase the total extract yield but not the diterpenic yield.

CONCLUSION

In this study, we can conclude that mean particle size of 0.3 mm gave the highest extract yield of 3.32% and 1.34% respectively for with and without co-extractant respectively. Whereas, the value of D_e at 0.3 mm mean particle size, with and without co-extractant are 8.820×10^{-12} and 7.920×10^{-12} m²/s respectively. Therefore, 0.3 mm is the best mean particle size to produce highest *Momordica charantia* extract yield and D_e value in SC-CO₂ extraction with co-extractant. Besides, the addition of co-extractant in *Momordica charantia* dried sample prior extraction increased the extraction efficiency of target compound, charantin from *Momordica charantia*. Above all, the use of SC-CO₂ with co-extractant had proved its efficiency in extracting *Momordica charantia* in terms of extract yield and diffusion coefficient as well as charantin, the target compound.

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