An Innovative Method for the Extraction of Phenolic Compounds from *Polygonum Cuspidatum*

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ABSTRACT— ***Supercritical carbon dioxide ($SCCO_2$) extraction of resveratrol, rutin, and quercetin from Polygonum cuspidatum (P. cuspidatum) was performed in a laboratory scale extraction system with ethanol or water as a co-solvent. In this work, the effect of operating parameters such as an entrainer concentration, pressure, and temperature on extraction yield were investigated. Extractions were carried out at temperatures of 40 − 80 ^oC and pressures of 10 − 40 MPa with a fixed CO_2 flow rate (3 mL min^{-1}) and extraction time (3 h). The results showed that the presence of ethanol or water as a co-solvent could enhance the solvent power or the selectivity of $SCCO_2$ to extract resveratrol, rutin, and quercetin. The increasing concentration of ethanol and water effected the extraction yields of them. By changing the temperature or the pressure, the vapor pressure enhancement of extractable compounds increase analyte solubility and CO_2 in water caused easy penetration of both CO_2 and water into P. cuspidatum to extract the target compounds. These results revealed that $SCCO_2$ extraction with co-solvent is applicable method for extraction of bioactive compounds of plant and may lead to an advanced plant biomass components extraction technology.***

Keywords— Supercritical carbon dioxide, Co-solvent, Resveratrol, Rutin, Quercetin

1. INTRODUCTION

P. cuspidatum is a perennial species with spreading rhizomes and numerous reddish-brown, freely branched stems. In general, *P. cuspidatum* called Japanese knotweed or bamboo, is a famous Chinese traditional medicinal herb. This perennial plant widely spread in China, Japan and Korea and also found growing throughout North America and Europe. The dried root of *P. cuspidatum* is well-known used for folk medicine in Korea and Japan. It is used as an analgesic, antipyretic, diuretic, expectorant, and anti-tussive agent and also used for treatment of chronic bronchitis, infectious hepatitis, diarrhea, cancer, hypertension, atherosclerosis, hyperlipidemia, leucorrhoea, dysmenorrhea, trauma with blood stasis, burn, snake bites, and allergic inflammatory diseases [1,2].

One of the most important bioactive compound in the *P. cuspidatum* roots is resveratrol (3, 5, 4–trihydroxystilbene). This compound is a naturally occurring phytoalexin produced by some spermatophytes in response to injury. Recently, resveratrol has become a popular nutritional supplement used by humans all over the world. Resveratrol was classified as a polyphenolic compound with multiple therapeutic effects and pharmacological activities such as antibacterial, lipotropic, hepato-protective, and anti-tumor function [3]. Detailed research has been conducted to determine the efficacy of its use both in preventive and therapeutic dimensions [4]. Even, Kaeberlein *et al*. [5] reported that resveratrol represents the first efforts to translate anti-aging interventions from the laboratory to the clinic. He also reported that resveratrol could increase life span and slow the progression of age–related diseases in multiple model systems. Other important polyphenolic compounds contained in the *P. cuspidatum* roots are rutin and quercetin. Rutin is a quercetin-3-rutinoside with antioxidant, anti-inflammatory and anticarcinogenic effects, and can also reduce the fragility of blood vessels related to haemorrhagic disease and hypertension in humans [6]. This compound has shown the capability to antagonize the increase of capillary fragility associated with hemorrhagic disease and show antioxidant and lipid peroxidation activities [7]. Quercetin is a flavonol that occurs widely in plants which have a common flavone nucleus composed of two benzene rings linked through a heterocyclic pyrone ring. As a dietary polyphenolic compound,

quercetin has potentially beneficial effects on health [8]. Several biological actions of quercetin including protection of LDL cholesterol against oxidation and promotion of endothelial vasorelaxation have been reported [9]. Quercetin also protects the organism against coronary diseases, lung cancer and asthma [4].

In this work, $SCCO_2$ (T_c = 31.1 ^oC and P_c = 7.4 MPa) would be employed to extract bioactive compounds such resveratrol, rutin, and quercetin from *P. cuspidatum* roots. Extraction is a very important step in the isolation, identification and use of phenolic compounds and there is no single and standard extraction method [10]. Usually, extraction of the polyphenols is performed using conventional techniques, such as soaking and stirring of the raw material with a solvent (typically, different mixtures of water and ethanol, depending on the method used. These conventional extraction methods have several drawbacks, including the degradation of sensitive compounds, long processing times, low selectivities (i.e., low quality extract) and low extraction yields [11–13]. Bleve *et al*. [14] conducted the purification of anthocyanins from grape skin extracts as liquid matrix, by using CO_2 under liquid and sub–critical conditions. They demonstrated that the purification process allowed to eliminate ethyl alcohol from the anthocyanin extract without any thermal or chemical degradation, obtaining a high added value product which maintains its anthocyanin content and antioxidant activity unchanged. Due to its low polarity and its inability to extract compounds with high molecular weight such as anthocyanins (MW = 600 g/mol), they prepared liquid matrix by the extraction of anthocyanins from grape skins using a water/ethyl alcohol solution acidified with 0.2% trifluoroacetic acid before treatment by CO_2. For this reason, the suitable co–solvents was used to improve the solubility of the target compounds and/or to increase the extraction selectivity and solvating power of $SCCO_2$ [2,11,15–18].

Benova *et al*. [2] isolated the selective compounds such as piceid, resveratrol, and emodin based compounds from Japanese knotweed roots by using $SCCO_2$ with acetonitrile, ethanol, and methanol as solvent modifiers. They reported that the optimal condition was found when the extraction was carried at 40 MPa and 100 ^oC with extraction time 45 min by using $SCCO_2$–acetonitrile. Serra *et al*. [16] demonstrated the use of fractioned high pressure process to recover powerful anticancer ingredients from the cull of a traditional Portuguese cherry. By using a $SCCO_2$ (50 ^oC and 25 MPa) extraction in the first step followed by enhanced solvent extraction with different mixtures of CO_2 and ethanol, it was possible to obtain different extract fractions with different composition, antioxidant and antiproliferative activities. They concluded that a pre–treatment with $SCCO_2$ is required to obtain a more concentrated extract with antiproliferative activity. Next, Serra *et al*. [17] reported that when fractioned high pressure extraction, using CO_2 99.99% and ethanol 96% as extraction solvents, was applied, the antiproliferative effect was significantly improved by 16 fold. Recently, Campomanes *et al*. [11] determined the economic feasibility of large-scale operations of $SCCO_2$ extraction for the recovery of phenolics using grape bagasse from Pisco residues. Experimental data were obtained using supercritical CO_2 containing 10% ethanol (w/w) at 313 K and 20 – 35 MPa. They summarized that an evaluation of the economics of the process indicated the feasibility of an industrial $SCCO_2$ extraction plant with a capacity of 0.5 m3 for producing an extract with an expected phenolics concentration of approximately 23 g/kg of extract. The compounds identified in the extracts were syringic, vanillic, gallic, *p*–hydroxybenzoic, protocatechuic, and *p*–coumaric acids.

2. EXPERIMENTAL SECTION

2.1 Materials

Dried roots of *P. cuspidatum* were provided by Futaba Chinese medicine pharmacy (Okayama, Japan). Prior to extraction, the roots were ground with a coffee grinder into certain particle size (< 2 mm) and passed through 16-mesh sieves; the sample was then refrigerated at < 278 K. Resveratrol ($C_{14}H_{12}O_3$, 98.0%), rutin ($C_{27}H_{30}O_{16}$, 99.9%), quercetin ($C_{15}H_{10}O_7$, 99.9%), acetic acid (CH_3COOH, 99.9%), and ethanol (C_2H_5O, 99.9%) were obtained from Wako Pure Chemical Industries Inc. (Tokyo, Japan). They were used without further purification.

2.2 Experimental Setup and Procedure

Figure 1 showed the schematic diagram of $SCCO_2$ extraction apparatus which used in these experiments. The apparatus includes a high-pressure pump for CO_2 (PU–2086; Jasco, Hachioji, Japan), a heating chamber (WFO–400; EYELA, Tokyo, Japan), a 10 mL extraction cell (Thar Technologies, Inc., PA, USA) and back pressure regulator (AKICO, Tokyo, Japan). In this work, the extraction of resveratrol, rutin, and quercetin from *P. cuspidatum* by $SCCO_2$ was conducted at temperatures of 40 – 80 ^oC and pressures of 10 – 40 MPa using a semi-continuous flow-type system with CO_2 flow rate of 3 mL min^{-1}. In each experiment, 4.0 g of dried roots of *P. cuspidatum* sample was loaded into the extraction vessel, filled with glass beads at the bottom and top of the extraction vessel. The extraction vessel was placed in the heating chamber to maintain the operating temperature. The extraction process can be described briefly as follow. Initially, the dried roots of *P. cuspidatum* were loaded into the extraction vessel and placed in the chamber. After the temperature at chamber heater reached to the desired temperature, CO_2 from a cylinder was firstly liquefied and then pumped into the extraction vessel. In all experiments, the extraction products were collected for 3 h, weighed and directly stored in the refrigerator at –10 ^oC. The bottles used for the collection of extracts were wrapped in aluminum foil. These processes were maintained until analysis. Unfortunately, it was found that resveratrol, rutin, and quercetin were not extracted during $SCCO_2$ extraction process. The main reason is probably the high polarity of these compounds. As

explained before, CO_2 is non-polar fluid and it is the main disadvantage in its use for the isolation of antioxidants. Therefore, to promote extraction of them via solubility enhancement, water and ethanol as co-solvents were introduced to increase the polarity of CO_2 allowing operation at a lower pressure. These co-solvents are generally recognized as safe, and environmentally benign, and can thus be used in food and nutraceutical-related extraction processes. Figure 2 depicted the schematic diagram of $SCCO_2$ extraction apparatus after modification with solvent modifier or co-solvent. Simultaneously, ethanol or water was introduced via high–pressure pump (PU–980 Jasco, Japan) to the mixer at various flow rates. To reduce the dissolved oxygen, they were sonicated in flask that cover by parafilm at room temperature for 15 min prior use.

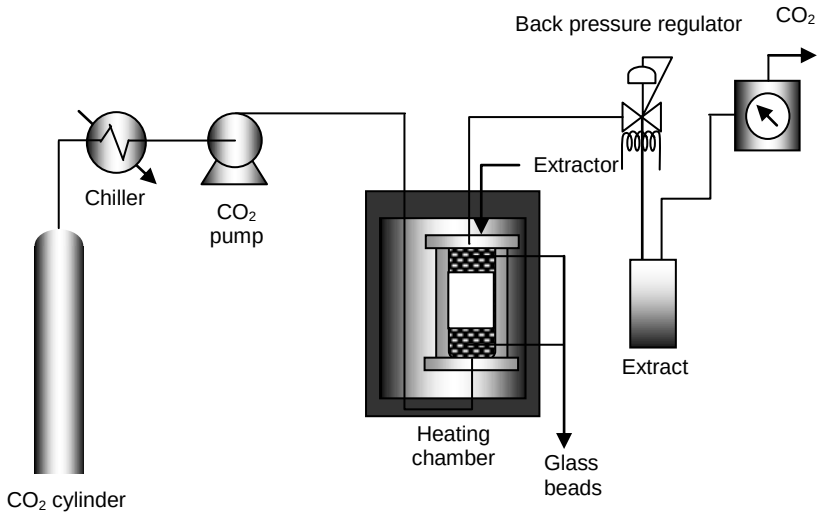

Figure 1: Schematic diagram of $SCCO_2$ extraction apparatus.

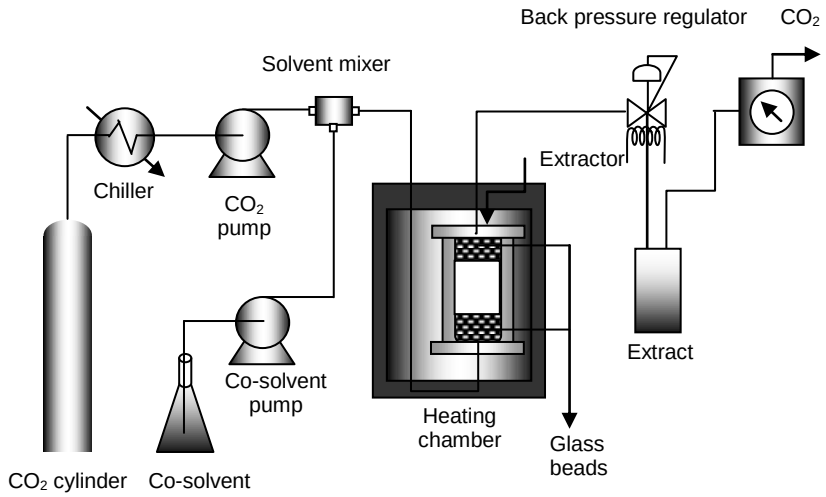

Figure 2: Schematic diagram of $SCCO_2$ extraction modified apparatus.

2.3 Analytical Methods

There are many polyphenolic compounds could be extracted from plant material. However, in this work, resveratrol, rutin, and quercetin were subjected as the target of polyphenolic compounds recovery from *P. cuspidatum*, and determined quantitatively by using HPLC (high-performance liquid chromatography). The organic components in extracts were recovered with 2 mL of ethanol, and all the solutions were filtered using a disposable filter of 0.45 μm pore size prior to HPLC analysis. Initially, the pure compound of resveratrol or rutin or quercetin dissolved in ethanol as a standard was injected in the HPLC system to construct calibration curve in 5 point. After separation process in the HPLC column, the amount of each standard leaving the column will determine the intensity of the signal produced in the detector. By comparing the time it takes for the peak to show up (the retention time) with the retention times for the

standard, the amount of resveratrol or rutin or quercetin in the extract can be identified. This analysis can be performed with good precision; therefore, other techniques analysis was not conducted. The HPLC instrument used was Diode Array Detector SPD–M10A (Shimadzu, Japan) equipped with a STR ODS II column (5 μm; 4.6 x 250 mm; Shinwa Chemical Industries, Ltd., Japan) and operated at room temperature. 20 μl of extract dissolved in ethanol was injected by SIL–10AF auto–sampler (Shimadzu, Japan). Ethanol/water/acetic acid (40/58/2 v/v) were used as mobile phases at flow rate of 0.5 mL/min. Resveratrol was detected at wavelength of 306 nm, and rutin and quercetin were detected at wavelength of 254 nm [19].

3. RESULTS AND DISCUSSION

In this work, to obtain the maximum yield of the extracted compounds (resveratrol, quercetin, and rutin) in *P. cuspidatum*, the soxhlet extraction method was carried out with ethanol as a solvent. It was well known that the extraction of organic compounds using a range of organic solvents from matrices (soils, sewage sludges, vegetables, and plants) has historically been carried out by using this method. It has also been a standard technique and a reference to the performance of other extraction methods during more than one century and, at present [20]. During soxhlet extraction process, the heating mantle temperature was set at 80 ^{o}C (in fact 78 – 82 ^{o}C) for 12 h. The amount of dried *P. cuspidatum* and ethanol used in flask were 6 g and 200 mL, respectively. The flask was then removed from the mantle, and the liquid extracts were transferred to evaporator flask. Afterwards, ethanol was separated from the *P. cuspidatum* extract using a rotary evaporator R–210, Buchi at 50 ^{o}C and immediately analyzed by HPLC. The results showed that the maximum yields of resveratrol, rutin, and quercetin contained in the roots were 1.33, 3.09, and 0.445 mg/g sample, respectively. These results are in good agreement with the results reported by previous researchers [21–23].

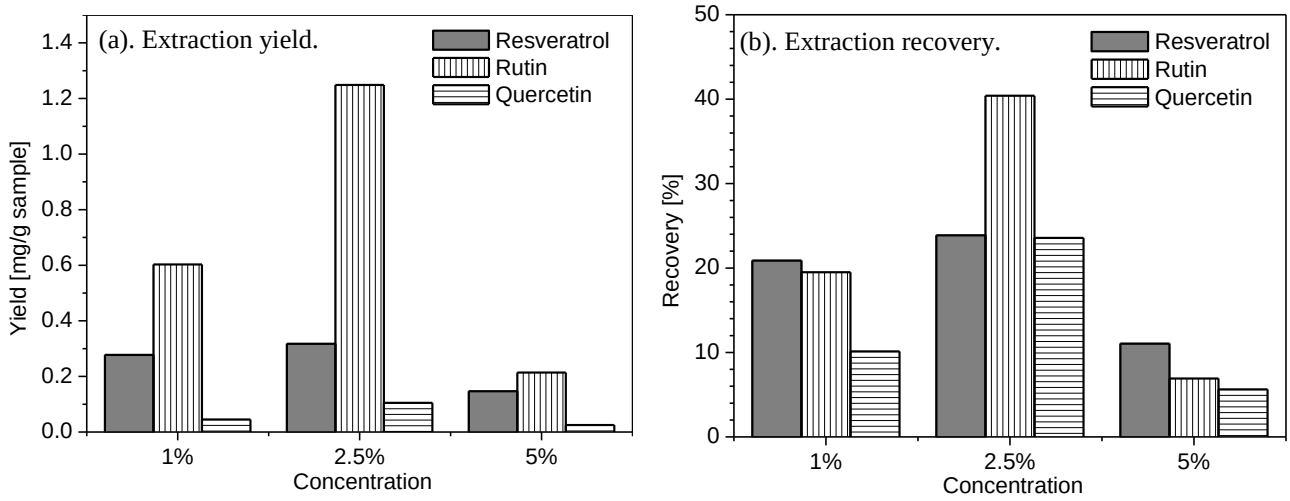

Figure 3: Effect of ethanol as an entrainer on the extraction yield (a) and recovery (b) of resveratrol, rutin, quercetin at 60 ^{o}C and 20 MPa, respectively.

As described in the experimental section, when the extraction of resveratrol, rutin, and quercetin were conducted by using $SCCO_2$ without co–solvent, they could not be extracted out from *P. cuspidatum*. Therefore, the data for these is not presented. Figures 3(a) and 3(b) showed the cumulative extracts of resveratrol, rutin, and quercetin which extracted by $SCCO_2$ at 60 ^{o}C and 20 MPa with various ethanol concentrations, respectively. It is evident that the existence of ethanol as co-solvent could enhance the solvent power or the selectivity of $SCCO_2$ to extract polyphenolic compounds. This is due to the influence of ethanol on the solubility of the solute, and therefore on the extraction polyphenolic compounds process. As shown in these figures, the extraction yields and recovery of them increased with increasing ethanol concentration up to 2.5%, then decreased with increasing of 5% ethanol. This phenomenon is due to the increase in the number of soluble components in the mixture, reducing the selectivity and enhancing the yield. Also, the increase in the ethanol concentration enhances the yield due to proportional changes in the solvent mixture characteristics [24–27]. In addition, high concentration of ethanol may undermine the advantages of $SCCO_2$ extraction and may lead to ethanol saturation resulting the decreasing of the extraction yields and recovery of resveratrol, rutin, and quercetin from *P. cuspidatum* respectively. de Campos *et al*. [24] conducted $SCCO_2$ extraction with ethanol as co–solvent in concentrations of 10, 15 and 20% w/w to obtain grape pomace extracts from Cabernet sauvingnon (*Vitis vinifera*) at 150 bar and 40 ^{o}C. The results showed that the yield of extract increased with increasing ethanol concentrations up to 15%. On the contrary, the solvent mixture with 20% ethanol decreased the extraction yield by 30% (yield of 6.3% w/w), compared to 15% ethanol. In detail they explained that (i) the increase in ethanol concentration may induce the saturation of CO_2 with ethanol, with consequent formation of two phases, for the specific conditions of temperature and pressure of the system; (ii) the ethanol effect, i.e., ethanol molecules form hydrogen bonds between hydrogen from one molecule with oxygen from other molecule, but also, the polar compounds from the solute form hydrogen bonds as well. In spite of that, for

solute solubilization, the hydrogen bonds must be formed between ethanol (solvent) and solute. Therefore, when ethanol is in high concentration in the mixture, there is not enough energy to separate ethanol molecules, causing the non–availability of ethanol molecules and therefore less solute molecules to be solubilized, reducing extraction and decreasing the process yield. Yilmaz *et al*. [25] also reported for extraction of proanthocyanidins from grape seed (*Vitis Vinifera*) using $SCCO_2$ with ethanol as co–solvent (5, 10, 15, 20% in weight). They informed that increasing the percentage of ethanol increased the amount of the extracted proanthocyanidins generally, however, at 250 bar and 30 ^oC increasing the percentage of ethanol to 20% did not cause an increase at the amounts of proanthocyanidins, which in fact lead to a decrease. The reason for that could be that the polarity of the solvents (CO_2 + ethanol) was not favorable for the extraction of the proanthocyanidins. Based on the results, it could be said that $SCCO_2$ extraction of resveratrol, rutin, and quercetin from *P. cuspidatum* was improved greatly by using ethanol modifier. However, the use of much ethanol modifier also negates much of the advantages of $SCCO_2$ extraction.

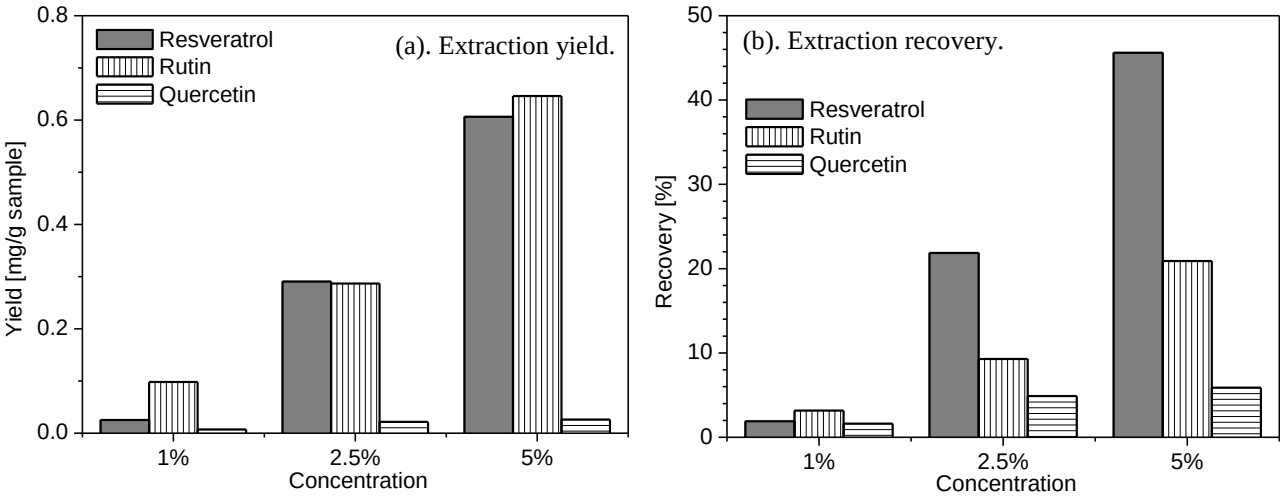

Figure 4: Effect of water as an entrainer on the extraction yield (a) and recovery (b) of resveratrol, rutin, quercetin at 60 ^oC and 20 MPa, respectively.

It was well known that water is always present when processing vegetal materials. Instead of ethanol, water was used as a co–solvent of $SCCO_2$ to extract resveratrol, rutin, and quercetin from *P. cuspidatum*. Water is inflammable, tasteless, cheap, environmentally benign, and less restrictive in terms of residual solvent, as the process remains totally clean. Therefore, water is acceptable for food–grade and pharmaceutical products. Figures 4(a) and 4(b) showed the extraction yields and recovery of resveratrol, rutin, and quercetin, respectively, at 60 ^oC and 20 MPa with water as a co–solvent. As expected, resveratrol, rutin, and quercetin have been extracted from *P. cuspidatum* at these conditions. It showed that the addition of water had great impact on the solvating power of the $SCCO_2$ and thereby improve the extraction yields and recovery of them. Similar enhancing extraction yields and recovery of them with an addition of ethanol, water might also swell the *P. cuspidatum* matrix, leading to enhancement of the solute diffusion out of the *P. cuspidatum* tissue. These figures also showed that the increase in water concentration resulted the increasing extraction yield and recovery of them. At 1% water concentration, the recovery of resveratrol, rutin, and quercetin were less than 5%, and, when the concentration of water increased in 5%, the recovery of them increased significantly and could approach to 47%, 21%, and 6%, respectively. As explained by Reinoso *et al*. [28] that separation (extraction) could be enhanced by using a modifier able to interact with the target compounds. $SCCO_2$ extraction is affected by polar forces, the limited polarizability of CO_2 limits the solubilities of solid aromatic compounds, which are several orders or magnitude lower than in a conventional liquid solvent. The addition of small amounts of a hydrogen bond acceptor results in large solubility enhancements. Next, the presence of modifiers increases the interaction of solutes with the solvent by increasing density, allowing specific chemical interactions and possibly by altering the structure of the vegetal matrix, and/or by breaking polar interactions of the solute and the matrix. Kim *et al*. [29] demonstrated that caffeine and epigallocatechin gallate from green tea can be selectively extracted using $SCCO_2$ with water as a co-solvent at a fixed temperature of 40 ^oC and a fixed pressure of 400 bar. They reported that small amounts of caffeine (2.3%) and epigallocatechin gallate (0.6%) were extracted with $SCCO_2$ – 4 wt% water. At higher water content, the extraction yield of caffeine and epigallocatechin gallate increased significantly. The extraction yield of caffeine increased from 25.9% to 59.8% and that of epigallocatechin gallate increased from 13.8% to 29.9% when water contents increased from 5.8 wt% to 7 wt%. However, Durante *et al*. [30] noted that the excess of water might detrimental for $SCCO_2$ extraction of target compounds and polarity of water could reduce the solvating power of $SCCO_2$ towards relatively non–polar compounds; besides it often causes mechanical problems due to ice formation.

In principle, the separation of compounds by solubility from the supercritical fluid can be carried out by modifying the thermodynamic properties of the supercritical solvent. The solvent power is modified by manipulating the operating

temperature and/or pressure. In $SCCO_2$, temperature and/or pressure during the extraction process are the critical factors that affect the efficiency and selectivity. The use of high temperatures improved the efficiency of the extraction as it helps the disruption of analyte–sample matrix interactions caused by van der Waals forces, hydrogen bonding and dipole attraction. An increased temperature also decreases the viscosity of a liquid solvent, thereby enhancing its penetration inside the matrix particle, which results in an improved extraction process [31]. Figure 5 showed the effect of extraction temperature on the extraction yield of resveratrol, rutin, and quercetin at 20 MPa and water concentration of 5%. The maximum extracts of them except quercetin was found by changing the temperature from 40 to 60 ^oC. At 60 ^oC, the extraction yields of resveratrol, rutin, and quercetin were 0.61, 0.65, and 0.03 mg/g sample, respectively. It could be explained that at constant pressure, the increase of temperature reduces the density of $SCCO_2$ thus reducing its solvating power, but it enhances the vapor pressure of extractable compounds, consequently increasing analyte solubility and extraction yield. Icen and Guru [32] performed $SCCO_2$ extraction of caffeine from tea plant wastes at pressure of 250 bar with extraction time of 3 h and a fixed ratio of 5.23 g co–solvent/100 g CO_2 (10 g/min CO_2 flow rate). They reported that the temperature giving the maximum caffeine extraction at these conditions was found by changing the temperature between 50 and 70 ^oC. The caffeine yield increased with increasing temperature exponentially up to 65 ^oC. In other words, the increase of temperature up to 65 ^oC was effective to dissolve caffeine molecules and to increase caffeine yield. On the contrary, the extraction yield of resveratrol, rutin, and quercetin decreased significantly when the temperature was increased in 80 oC. Mezzomo *et al*. [33] reported that at 100 bar, raising extraction temperature produced a decrease in the peach almond extraction yield (from 14.1 ± 0.3% to 3.80 ± 0.08%), due to the reduction in solvent density which varied from 0.772 g CO_2/cm^3, at 30 ^oC, to 0.385 g CO_2/cm^3, at 50 ^oC. On the other hand, at higher pressures (above 200 bar), the increase in the extracting temperature provided an increase in the yield, despite the reduction in solvent density. This behavior was caused by the enhancement in the solute vapor pressure with temperature, which was more significant than the reduction in the solvent density, increasing consequently the overall extraction yield. It showed that the effect of temperature is complex as there was a competing solubility effect caused by the increase in vapor pressure and the decrease in density upon the increase of temperature. It could be said that the density of CO_2 is reduced at constant pressure with increasing temperature and leading to reduction of fluid solvent power [27,33].

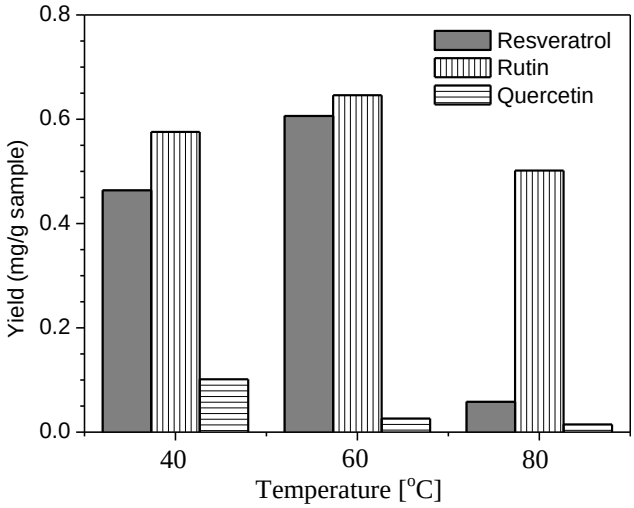

Figure 5: Effect of extraction temperature on the extraction yields of resveratrol, rutin, and quercetin at 20 MPa and water concentration of 5%.

Supercritical fluid extraction is traditionally defined as a high pressure technology and this variable is in fact of supreme importance for many technical and economic aspects of this process. The most direct property prone to be affected by pressure variations is density, which can be used to perceive how close $SCCO_2$ reaches a liquid–like solvent power. As pressure is increased, the CO_2 density increases and the intermolecular mean distance of CO_2 molecules decreases, leading to an enhanced specific interaction between solute and solvent molecules [27,28,34,35]. Figure 6 showed effect of extraction pressure on the extraction yields of resveratrol, rutin, and quercetin at 60 ^oC and water concentration of 5%. The maximum extracts of resveratrol and quercetin were 0.61 and 0.026 mg/g sample at pressure of 20 MPa, respectively. It is clear that increase in extraction pressures facilitate solvent penetration through the interior of the sample matrix to the outer solvent surface thereby increasing the mass transfer rate [26,34,35]. At this condition, the faster extraction kinetics of resveratrol and quercetin observed was occurred. Machmudah *et al*. [34] investigated the effect of pressure on the recovery of compounds in $SCCO_2$ and water phases at a temperature of 40 ^oC, CO_2 flow rate of 3 mL/min, and water volume of 30 mL with 4 g of raw coffee beans. They explained that increasing pressure promoted increasing recovery of caffeine in the $SCCO_2$ phase due to the increasing $SCCO_2$ density. The increasing pressure also caused easy penetration of $SCCO_2$ into the coffee beans, thus increasing the mass transfer of caffeine and resulting in

increasing solubility of caffeine in $SCCO_2$. The increasing pressure also promoted increasing recovery of both caffeine and chlorogenic acids in the water phase. High pressure of CO_2 in water caused easy penetration of both CO_2 and water into the coffee beans to extract both caffeine and chlorogenic acids. Zarena *et al*. [26] carried out optimisation of solvent modified $SCCO_2$ on the extract yield and antioxidant activity from *Garcinia mangostana* L. They reported that at pressure range from 23 to 37 MPa and temperature range of 45 – 58 ^oC the extraction yield increased to 12 wt.% but below or above this range the extract yield decreased. The possible reason may be that the solubility of the compounds depends on the balance between $SCCO_2$ density and solute vapor pressure which are controlled by fluid pressure and temperature. The increase in pressure from 180 to 380 bar results in an increase in CO_2 density, increasing the solvating power of supercritical fluid. Thus higher pressure is responsible for quantitative yields and stronger interaction between the fluid and the matrix. However, except rutin, when the operating pressure increased to 40 MPa, the yields of resveratrol and quercetin decreased to 0.15 and 0.02 mg/g sample, respectively, and the extraction yield of rutin could approach to 1.29 mg/g sample. This operating condition might be suitable for rutin extraction but not suitable for the extraction of resveratrol and quercetin from *P. cuspidatum*. da Porto *et al*. [36] conducted experiments for water and ethanol as co–solvents in $SCCO_2$ extraction of proanthocyanidins from grape marc. They reported that at 10 MPa the extraction of phenols (average value 403.5 mg equivalent gallic acid/100 g dried matter) is higher than at 20 MPa (average value 272.4 mg equivalent gallic acid/100 g dried matter). They explained that the low mass-transfer rates at the high pressure may be partially due to the low dispersion coefficient of the modified $SCCO_2$ which accounts for the axial and radial diffusion mechanisms and to the high porosity of the extraction bed which may have caused an irregular compaction of the extraction bed, giving problems of channeling and reducing the contact between the solvent and the compounds to be extracted thus causing a loss of process efficiency. Next, they suggested that 40.15 ^oC and 10 MPa operating conditions are the most suitable conditions than 40.15 ^oC and 20 MPa to extract grape marc phenols by $SCCO_2$ with 15% water as a co–solvent.

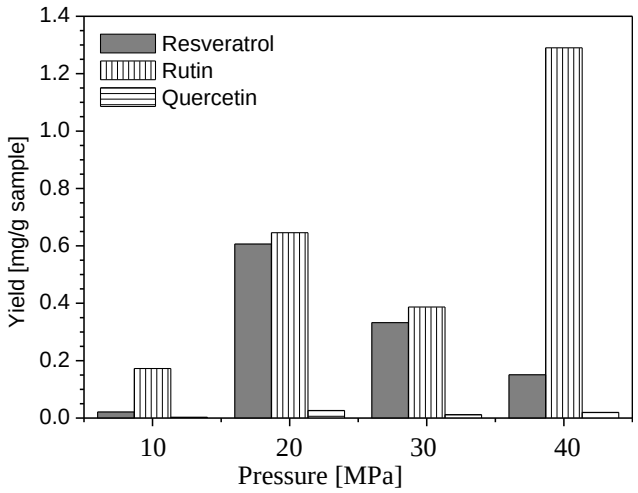

Figure 6: Effect of extraction pressure on the extraction yield of resveratrol, rutin, and quercetin at 60 ^oC and water concentration of 5%.

4. CONCLUSIONS

$SCCO_2$ extraction of polyphenolic compounds from *P. cuspidatum* was studied at temperatures of 40 – 80 °C and pressures of 10 – 40 MPa using a semi–batch system with CO_2 flow rate of 3 mL min^{-1}. Due to the polarity of resveratrol, rutin, and quercetin, they were not extracted at these conditions. In order to improve extraction of them, ethanol and water were employed as co–solvents. The results showed that the presence of ethanol and water as co–solvents could enhance the solvent power or the selectivity of $SCCO_2$ to extract polyphenolic compounds. The increasing concentration of ethanol and water effected the extraction yields of resveratrol, rutin, and quercetin significantly. At 20 MPa and water concentration of 5%, the extraction yields increased clearly by changing the temperature from 40 to 60 ^oC due to the vapor pressure enhancement of extractable compounds, consequently increasing analyte solubility and extraction yield. At constant temperature, the increasing pressure of CO_2 in water caused easy penetration of both CO_2 and water into *P. cuspidatum* to extract the target compounds. Based on the results, it could be said that $SCCO_2$ with water or ethanol as a co–solvent are the most suitable method for extraction of bioactive compounds of plant.

5. ACKNOWLEDGEMENT

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