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HYDROTHERMAL EXTRACTION OF PHYTOCHEMICAL COMPOUNDS FROM *Polygonum cuspidatum* ROOTS IN A SEMI- BATCH REACTOR SYSTEM

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ABSTRACT

Hydrothermal extraction is known as a natural and green way for antioxidant compounds extraction. Antioxidant compounds from the roots of traditional Chinese medicinal herb *Polygonum cuspidatum* (*P. cuspidatum*) has been extracted at hydrothermal conditions. The antioxidant compounds were identified as polyphenolic compounds of resveratrol, rutin and quercetin. The effect of temperatures on the extraction yield of antioxidant compounds was studied. Based on the result, extraction yields of resveratrol, quercetin, and rutin significantly increased with increasing temperature at 10 MPa. After 180 min of extraction time, the yields of resveratrol, quercetin, and rutin were 0.95, 0.26, and 6.73 mg/g of feed loaded at 473 K, respectively. These results revealed that hydrothermal extraction is applicable method for the isolation of polyphenolic compounds from other types of biomass and may lead to an advanced plant biomass components extraction technology.

Keywords: *polygonum cuspidatum*, resveratrol, quercetin, rutin, hydrothermal extraction.

INTRODUCTION

Polygonum 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 (Shan et al., 2008).

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 (Du *et al.*, 2007). Detailed research has been conducted to determine the efficacy of its use both in preventive and therapeutic dimensions (Zitka *et al.*, 2011). Even, Kaeberlein (2010) 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 quercetin and rutin. 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 (de Boer *et al.*, 2005). Several biological actions of quercetin including protection of LDL cholesterol against oxidation and promotion of endothelial vasorelaxation have been reported (Careri *et al.,* 2003). Quercetin also protects the organism against coronary diseases, lung cancer and asthma (Zitka *et al.*, 2011). 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 (Baumgertel *et al.*, 2003). This compound has shown the capability to antagonize the increase of capillary fragility associated with hemorrhagic disease and show antioxidant and lipid peroxidation activities (Yang and Ren, 2008).

Extraction is one of the main steps for the recovery and isolation of bioactive phytochemicals from plant materials, before analysis. This process is influenced by their chemical nature, the extraction method employed, sample particle size, as well as the presence of interfering substances. Traditionally, abundant volatile organic solvents, including methanol, ethanol, acetone, chloroform, ethyl acetate and some mixed solvents were utilized to extract polyphenolic compounds from Chinese herb by maceration at room temperature (Yang et al., 2001), heating reflux extraction (Wang et al., 2001), soxhlet extraction (Benova *et al.*, 2010) and microwave-assisted extraction (MAE) (Routray and Orsat, 2012). Supercritical CO_2 extraction technology with modifier has also been applied for extraction of polyphenolic compounds from *P. cuspidatum* (Benova *et al.*, 2010; Wenli et al., 2005). Benova et al. (2010) isolated the selective compounds such as resveratrol and anthraquinone based compounds from Japanese knotweed roots by using supercritical CO_2. They reported that the

optimal condition was found when the extraction was carried at 40 MPa and 100°C with extraction time 45 min. This technique offered good yields of clean extracts and resembles classical Soxhlet extraction but the solvent used is above its critical temperature and pressure, which provides an unusual combination of properties. In addition, supercritical CO_2 is not suitable for more polar compounds such as stilbene groups.

In this work, water under subcritical conditions (423 to 473 K; 10 MPa) was used to extract polyphenolic compounds, such as resveratrol, quercetin and rutin from *P. cuspidatum*. Under these conditions, water–soluble compounds could be extracted from *P. cuspidatum* via autohydrolysis. Generally, autohydrolysis was applied for lignocellulosic materials lead to the solubilisation of hemicelluloses, leaving a solid phase in both cellulose and lignin. Next, the solid product was subjected to further processing for obtaining a variety of commercial products, for example enzymatic hydrolysis and further fermentation of hydrolyzates, allowing an integrated benefit of the raw material. Water under sub-critical condition is known as a "natural and green" way for product extraction, has received increased attention as an important alternative to conventional separation methods, such as hot water extraction conducted at boiling point temperature and atmospheric pressure (Wahyudiono *et al.*, 2013; Matsunaga *et al.*, 2014a; Machmudah *et al.*, 2015). Under sub–critical conditions, water may extract polar organic compounds or decompose lignocellulosic materials to produce valuable compounds such as saccharides and aromatic organic acids. This technique has been applied to recover protein and amino acids (Zhu et al., 2010), and phenolic compounds (He *et al.*, 2012). The hydrothermal treatment has also been demonstrated by several studies to effectively convert cellulosic (Wang *et al.*, 2013) and lignocellulosic biomass (Zhou *et al.*, 2011) into useful products.

EXPERIMENTAL SECTION

Materials

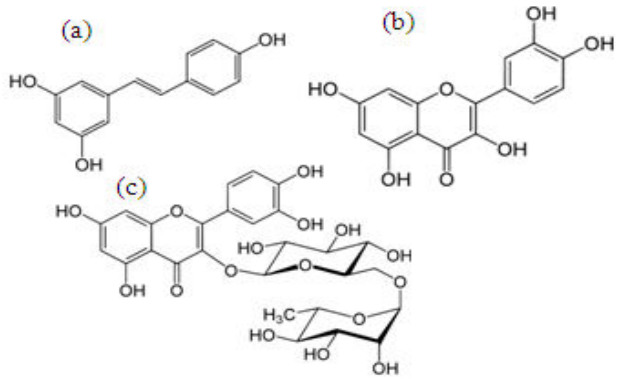


Figure-1. Chemical structures of (a) Resveratrol, (b) Quercetin, (c) Rutin.

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 ($C2H_5O$, 99.9%) were obtained from Wako Pure Chemical Industries Inc. (Tokyo, Japan). They were used without further purification. The chemical structures of resveratrol, quercetin, and rutin are shown in Figure-1.

Methods

The apparatus, whose schematic diagram is shown in Figure-2, consists of a high-pressure pump (PU-980 Jasco, Japan), heater (ESPEC ST-110, Japan), reactor (10 mL in volume; Thar Design Inc., USA) and back-pressure regulators (BPR; AKICO, Japan). Both sides of the reactor were equipped with removable threaded covers included stainless-steel filters (0.1–1.0 μm). The pre-heater was fabricated from 1/8 inch stainless-steel tubing (SUS316) with a volume of 50 mL and was heated using a mantel heater at temperatures of 423-473 K. The 1/16 inch stainless-steel tube was used to introduce hot water from the pre-heater to the reactor, which was located in the heater. After the reactor inclusive of 4.0 g of *P. cuspidatum* was installed to the system, distilled water at room temperature was pumped through the reactor inclusive pre-heater for a few minutes to purge air and completely wet the *P. cuspidatum*; the system was then pressurized to the set pressure of 10 MPa through the back-pressure regulator, monitored by a pressure gauge (P, Migishita, Japan). These pressures are selected to keep the water in the liquid state at temperatures above its normal boiling point. In all experiments, feeds were placed between two layers of glass beads (the bottom and top) in the extraction container. The glass beads were used in order to distribute the solvent flow uniformly and reduce the dead space in the container. Therefore, the residence time was less than about 30 seconds. Glass beads (1.5-2.5 mm in diameter) were obtained from Oshinriko Co. Japan.

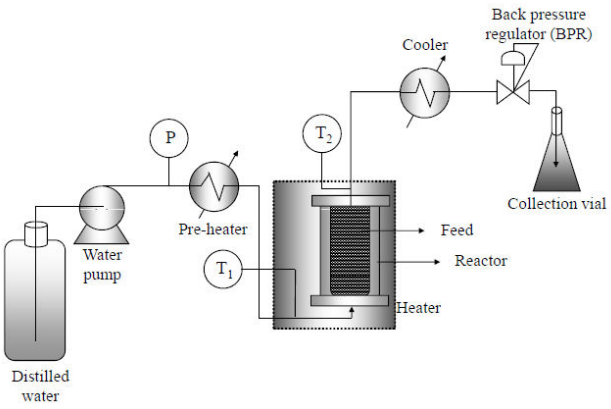


Figure-2. Schematic diagram of hydrothermal extraction apparatus.

When the system reached the desired pressure and a steady state was achieved, the electric heater was applied to heat the water. In this study, the reactor temperature was maintained at 423-473 K. The temperatures of the pre-heater, reactor and the electric heater were measured using K–type thermocouples and monitored using temperature controller (OMRON E5CJ, Japan). The time required to heat the reactor from room temperature to the desired temperature was 5-8 min, after which the reactor temperature equaled the electric heater temperature. After the temperature at the reactor area reached a preset temperature, the pump was used to feed water at 1.0 mL min^{-1}. During the experiment, temperatures of the reactor water inlet (T_1) and outlet (T_2) were monitored; their profile is plotted in Figure-3. Next, the outlet water was passed through the double–tube–type heat exchanger to quench the reaction. The time of experiment was 3 h, which, at 1.0 mL min^{-1}, produced a collected extract volume of 180 mL. Once the temperature in the reactor decreased to about 318 K, cold water was pumped through the system to purge liquid remaining in the reactor. The solid residues and liquid extracts were totally transferred to a petri dish and sealed bottles, respectively. The solid residues were dried in an oven at 333 K for 1 day and stored in a desiccator at room temperature. Extracted solution was collected every 30 minutes and they were directly stored in a refrigerator. To obtain the extraction yield, the extraction solutions were freeze–dried and weighted. These processes were maintained until analysis.

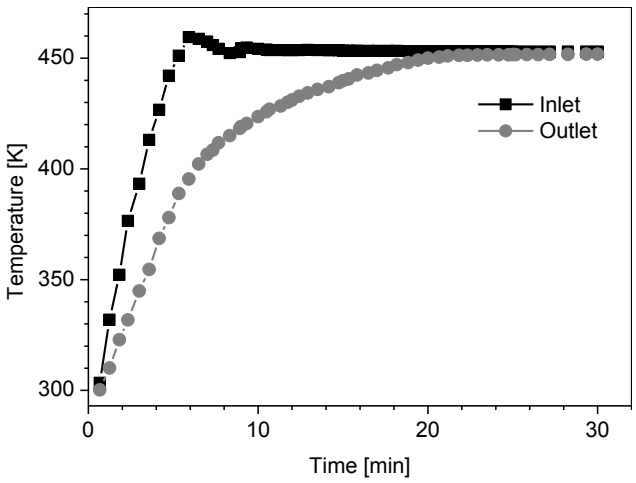


Figure-3. Typical temperature profile of hydrothermal extraction at 453 K, 10 MPa and 1.0 mL min^{-1} water flowrate without feed.

Analytical methods

Figure-4 shows the general working procedure that was used. Extracted solution was analyzed by HPLC LC–10AD equipped with diode array detector SPD–M10A (Shimadzu, Japan). 10 µL of extract dissolved in ethanol was injected by SIL–10AF auto-sampler (Shimadzu, Japan) and separated with an STR ODS II column (5 µm; 4.6 x 250 mm; Shinwa Chemical Industries, Ltd., Japan) at room temperature. 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. The extracted solution was also analyzed by TOC (TOC–5050A Shimadzu, Japan) to determine organic carbon dissolved in water. 50 µL of liquid extract was injected. TOC was calculated by subtracting inorganic carbon (IC) from total carbon (TC). In this work, the antioxidant activity in the extracted solutions were not analyzed. The solid residues collected at each operating temperature were analyzed by a Spectrum One FT-IR spectrophotometer (Perkin-Elmer, Ltd., England) to determine the structure of the solid residues after the hot compressed water treatment. The samples were placed directly in the diffuse reflectance attachment sample holder between two KBr salt plates in a micro-compression cell. Pre-flattening of the sample in a diamond cell was necessary prior to mounting. The spectra were measured in ATR (attenuated total reflectance) mode (golden single reflection ATR system, P/N 10500 series, Specac) at 4 cm^{-1} resolution. The scanning wavenumber ranged from 4000 to 650 cm^{-1}.

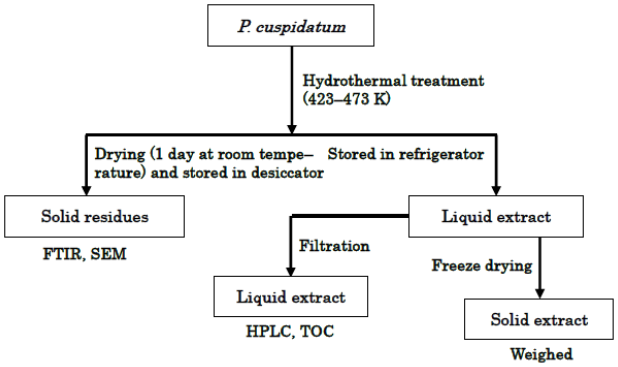


Figure-4. General working procedure.

Soxhlet extraction

In order to obtain the maximum yield of the extracted compounds (resveratrol, rutin and quercetin) 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 (Luque de Castro and Garcia-Ayuso, 1998). In this extraction, the heating mantle temperature was set at 353 K (in fact 351-355 K) 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 323 K and immediately analyzed by HPLC. The results showed that the maximum yields of resveratrol, quercetin, and

rutin contained in the roots were 1.33, 0.445, and 3.09 mg/g–sample, respectively. These results are in good agreement with the results reported by previous researchers (Peng *et al*., 2013).

RESULTS AND DISCUSSIONS

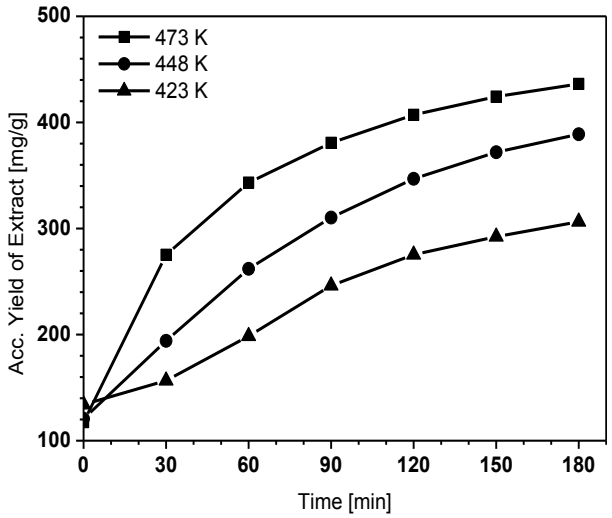


Figure-5. Accumulation yield of extract at various temperatures.

In order to understand the accumulation yield of extract, the liquid extracts of *P. cuspidatum* was treated by using freeze-drying (Eyela FDU-1200, Rikakikai Co. Ltd. Tokyo, Japan). Freeze-drying is considered as one of the best methods to keep the quality attributes of the materials submitted to drying processes since the combination between absence of liquid water and low temperature stop most degradation reactions. In this process, the amount of water in the extracts is removed by dehydration, through sublimation of ice in the materials. This process, generally, recommended for drying of materials containing heat-sensitive antioxidant components such as tocopherols, ascorbic acid, carotenoids and plant phenolics. During freeze-drying treatment, there may be a chance of decline in the content of antioxidants due to degradation of certain compounds. However, due to the absence of liquid water and the low temperatures required for the process, most of deterioration and microbiological reactions are stopped which gives a final product of excellent quality (Santos & Silva, 2008). The accumulation yield of extract was determined by weight of dried extract divided by weight of dry *P. cuspidatum* roots loaded in the extractor. Figure-5 shows the accumulation yield of extract at various temperatures. Due to the axial gradient of the temperature in the extractor system should be small, zero minutes of extraction time was set as the effluent first came out of the system in about 15 min after the pump turned on. As shown Figure–5, because of decomposition of the cell-wall structure due to thermal treatment, the increasing temperature caused increasing extraction yield. When the temperature of extractor system reached 423 K, the accumulated yield products could approach to 306 mg/g of feed loaded. At 473 K, the generation of solubilized products became fast and the accumulated yield reached 436 mg/g of feed loaded. As explained before, one of the main sources for the extraction of antioxidants are plant tissues. They consisted of cellulose, hemicellulose, and lignin which act as barriers to the release of intracellular substances. These interactions need to be broken to release of the antioxidant compounds. At 423-473 K, a low dielectric constant allows liquid water to dissolve organic compounds, while a high ionization constant provides an acidic medium for the hydrolysis of biomass components via the cleavage of ether and ester bonds (Kumar et al., 2011). Hemicellulose compounds solubilized in neutral water at 423 K; and moreover hemicellulose undergoes hydrolysis reactions in the presence of the hydronium ions generated by water auto-ionization, which act as catalysts. Mok & Antal (1992) reported that the hot compressed water technique was able to completely solubilize the hemicellulose from the entire biomass. A fraction of the cellulose and lignin constituents might be also solubilized at 473 K. Hence, 423 to 473 K is the most promising temperature range for extracting *P. cuspidatum* components (hemicelluloses), because in this temperature range a high yield without extensive degradation of the extracted compounds can be obtained (Leppanen et al., 2011). One of the main parameters affecting its extraction efficiency is pressure. It was needed to keep a condensed phase of water during the extraction process. High pressure could also force the penetration of water into the feed pores and enhance the mass transfer from the feed components to the water as a solvent. However, as reported by previous researchers that pressure was found to have no effect other than to keep the solvents liquid at these range of temperatures (Kronholm *et al*., 2007; Matsunaga *et al.*, 2014b). Therefore, the effect of extraction pressure was not presented in this work.

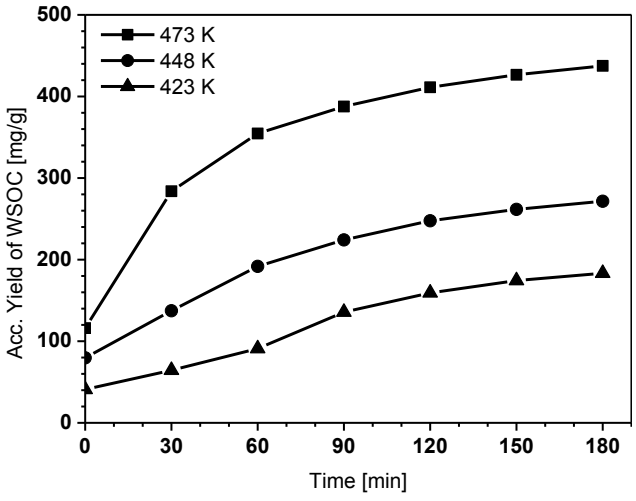


Figure-6. Water soluble organic carbon (WSOC) of extract at various temperatures.

In general, plant carbohydrate macromolecules are mainly constructed from their monomeric components by the formation of ether bridges. The acetyl groups in

hemicelluloses are connected to the pentoses by ester bonds: for the case of lignin, in addition to ether bonds, C-C-bonds also occur between phenyl–propane units. Therefore, plants biomass had stable macromolecular structure. However, after hydrothermal treatment, the color of *P. cuspidatum* roots changed from brown to dark brown and became soft. It indicated that autohydrolysis leads to cleavage of ether and ester bonds which contained in *P. cuspidatum* roots. In order to determine organic compounds in the extract, water soluble organic carbon (WSOC) of extracted solution was analyzed by TOC. Figure–6 shows the accumulation of WSOC at various extraction temperatures. The accumulation of WSOC was defined by the following equation:

$$\text{WSOC} = \frac{TOC \times v}{w} \quad (1)$$

where TOC, v, and w were TOC of the liquid extract (mg/l), volume of the liquid extract (l), and weight of dry *P. cuspidatum* roots loaded (g), respectively. The results showed that the accumulation of WSOC increased with increasing extraction temperature. Initially, WSOC yields were 40.94 and 116.11 mg C/g of dry feed loaded at 423 and 473 K, respectively. After 120 min, they could approach to 159.31 and 411.28. These results indicated that about 15.93% and 41.13% of the organic carbon in dry *P. cuspidatum* roots was recovered into water solution. It also confirmed that temperature is the main parameter influencing the physicochemical properties of water and the compounds to be extracted. It has a great influence on the extraction-rate and efficiency due to the increased solubility of water–soluble organic compounds (Leppanen et al., 2011; Matsunaga et al., 2014b; Askin et al., 2010). Askin et al. (2010) reported that the total amount of WSOC recovery of the *Ganoderma lucidum* extracts increased with increasing temperature, with the highest yields of WSOC at 423 and 473 K being 74.7 and 241.1 mg WSOC/g dry sample, respectively. The highest cumulative extraction percentage yield (57.4%) was achieved in extract obtained at 473 K for a total duration of 130 min.

In this study, *P. cuspidatum* remained after hydrothermal treatment was referred to as solid residue; this residue was characterized by infrared spectroscopy in the wavenumber region of 4000–650 cm^{-1}. Infrared spectroscopy is an analytical technique that allows identification of unknown substances and of the types of chemical bonds the compounds in those substances contain. FT–IR spectra of *P. cuspidatum* as starting material in addition to as solid residues are shown in Figure-7. It can be observed that *P. cuspidatum* consisting of cellulose, hemicellulose and lignin as three components of wood biomass is most likely composed of alkene, esters, aromatics, ketone and alcohol, with different oxygen–containing functional groups observed (Matsunaga *et al.*, 2014b; Xiao *et al.*, 2011). As a reference, the peak positions of all infrared bands and their functional groups are summarized in Table-1. Each molecule is composed of many different chemical bonds which are slightly elastic: they can stretch, bend, or vibrate. Therefore, some differences exist at each FT–IR spectra due to their structural properties.

Table-1. Main functional groups of the major constituents of *P. cuspidatum*.

Wave number (cm^{-1})	**Functional groups**	**Compounds**
3600 – 3000	O–H stretching	Acid, methanol
2860 – 2970	C–H_n stretching	Alkyl, aliphatic, aromatic
1700–1730, 1510–1560	C = O stretching	Ketone and carbonyl
1632	C = C	Benzene stretching ring
1613, 1450	C = C stretching	Aromatic skeletal mode
1470–1430	O–CH_3	Methoxyl–O–CH_3
1440–1400	O–H bending	Acid
1402	C–H bending	–
1232	C–O–C stretching	Aryl–alkyl ether linkage
1215	C–O stretching	Phenol
1170, 1082	C–O–C stretching vibration	Pyranose ring skeletal
1108	O–H association	C–OH
1060	C–O stretching and C–O deformation	C–OH (ethanol)
700–900	C–H	Aromatic hydrogen
700–650	C–C stretching	–

Absorbance intensity due to hydrogen bonded O–H stretching (3600–3000 cm^{-1}) could be found in each spectrum. This intensity (3334.3-3288.6 cm^{-1}) decreased with increasing temperature, possibly due to the loss of alcoholic groups as further decomposition occurs at higher temperatures. The bands in the 1031.1–1016.4 cm^{-1}, 1157.6–1148.3 cm^{-1}, and 1237.8–1230.8 cm^{-1} regions are assigned to the stretching and deformation of aromatic C–O groups, C–O–C stretching vibration groups, and the stretching of aryl–alkyl ether linkage C–O–C groups, respectively. In these regions, the peaks in (a–c) were sharper than in (d), showing that the C–O and C–O–C bonds in *P. cuspidatum* were more reacted and consumed in (d). The same result also occurred at 1449.2–1441.3 cm^{-1} and 1610.4–1606.4 cm^{-1} due to the acid modes of O–H bending and the aromatic stretching modes of C=C,

respectively. The bands in the 779.6–763.7 cm^{-1}, 1316.4–1314.7 cm^{-1}, 1368.9–1364.3 cm^{-1}, 1516.1–1509.3 cm^{-1}, and 2927.4–2925.8 cm^{-1} regions are assigned to the aromatic C–H groups, C–O syringyl units, deformation and vibration of aliphatic C–H groups, C=O stretching of ketone and carbonyl groups, and the stretching of aliphatic and aromatic C–H groups, respectively. The intensity of the absorbance in these regions is mostly stable, indicating that methylene groups, syringyl units and ketone groups in *P. cuspidatum* have difficulty cleaving under this condition.

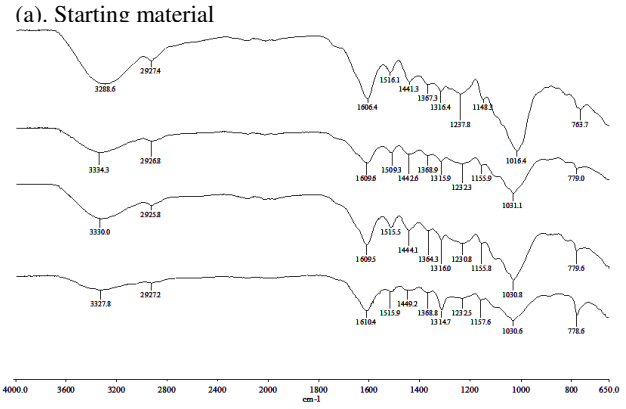


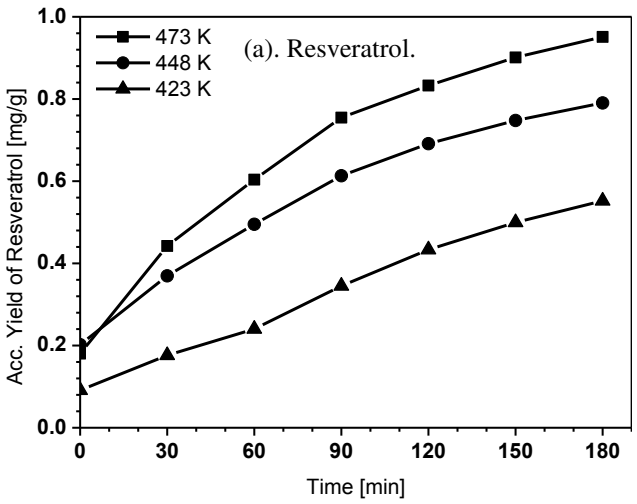
Figure-7. FT–IR spectrum of *P. cuspidatum* roots before and after hydrothermal extraction.

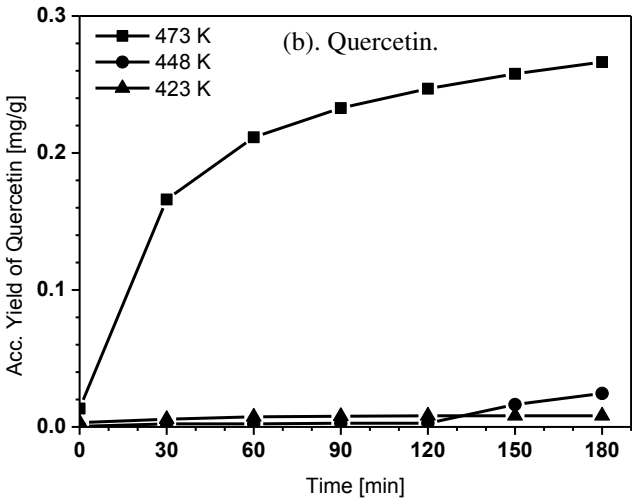
In hydrothermal extraction of lignocellulosic materials, the presence of water as the reactant leads to hydrolysis reaction and cleavage of bonds between hetero–atoms and carbon atoms. The splitting of chemical bonds may be enhanced by the increased in disproportionation of water at elevated temperatures. In order to increase the value-added chemical compounds from lignocellulosic materials, they need to be separated into its components. Autohydrolysis is one of the popular techniques to extract lignocellulosic material components with just compressed–hot water. This technique is attractive because no chemicals must be added and inexpensive process. Therefore, as introduced before, autohydrolysis was employed to extract resveratrol, quercetin, and rutin from *P. cuspidatum*. Figure-8 (a-c) showed resveratrol, quercetin, and rutin extracted at 423-473 K as a function of extraction time, respectively. These polyphenolic compounds are proven to be potent antioxidants and to have important biological, pharmacological and medicinal properties. As depicted in these figures, except quercetin at 423 and 448 K, the yields of them increased significantly with the reaction time at each extraction temperature. Rutin is one of examples of rhamnose in glycosides nature containing a sugar unit combined with a non–sugar unit which contained in hemicelluloses. At zero min reaction time, the yield of rutin was 0.86, 1.84, and 1.64 mg/g of dry sample loaded at temperatures of 423, 448, and 473 K, respectively. This indicates that solubilization of hemicellulose and its derived compounds commenced and occurred in water at these extraction temperatures (Matsunaga et al, 2014b). Sattler et al. (2008) reported that extraction of quantifiable levels of hemicelluloses from oriented strand board wood flakes starts at 393 K. The most promising temperature for industrial implementation of extraction of hemicelluloses is around 413 K. Leppanen *et al.* (2011) explained that at low temperatures (< 433 K), only small amounts of carbohydrates from Norway spruce were dissolved, but the amount of extracted hemicelluloses increased steadily from 433 K. Similar results were also reported by Matsunaga *et al.* (2014b). The experiments were carried out at temperatures of 373-463 K and a pressure of 4.0 MPa using a semi-batch system. They reported that small amounts of hemicellulose from *Ganoderma lucidum* were extracted in water at 413 K. Then, the solubilization of hemicellulose increased significantly at 463 K. In this work, at temperatures of 423 and 448 K, the amounts of extracted rutin increased in the similar trend during extraction process until 180 min. With the same extraction time, the yield of rutin increased significantly at 473 K. It showed that the temperature is the most crucial parameters in autohydrolysis to extract hemicelluloses from lignocellulosic materials, since it affects the hydrolysis rate and selectivity (Maki-Arvela *et al*., 2011). In this case, an increased temperature might decrease the water viscosity, thereby enhancing its penetration inside the *P. cuspidatum* roots, which results in an improved extraction process.

Other polyphenolic compounds, quercetin has also been extracted. As a major representative of the flavonol subclass, this compound has received considerable attention. Figure-8 (b) described the yield of quercetin after treatment by hydrothermal extraction at 423-473 K. At extraction temperatures of 423 and 448 K, it seems hard to extract quercetin at these conditions. At 180 min of extraction time, the yield of quercetin is very low (less than 0.05 mg/g of *P. cuspidatum* roots loaded). On the contrary, the yield of quercetin increased clearly with increasing extraction time when the extraction temperature was 473 K. These results indicated that the efficiency of hydrothermal extraction is greatly affected by the extraction temperature. Ko *et al.* reported that the extraction rate of quercetin from onion skin increased as the extraction temperature increased to 438 K at pressures of 90-131 bar (Ko *et al*., 2011). Due to the combination of longer residence time and temperature, the extracted quercetin was gradually degraded as the temperature increased above 438 K. Therefore, they concluded that the temperatures between 423 and 443 K are the most suitable for hydrothermal extraction of quercetin from onion skin with extraction time consumed was 7-8 min. Vergara-Salinas et al. (2012) explained that temperature and extraction time consumed affected polyphenol extract profiles both quantitatively and qualitatively. Their results showed that the highest total extract yield and antioxidant activity were obtained at 473 K, although maximum polyphenol extraction yields of hydroxycinnamic acids, flavones, flavonols/flavanones, and total polyphenols were detected at 373 K and 5 min. In this work, the residence time was less than 30 seconds; therefore, during the flow-

through extraction process the extracted molecules undergo severe conditions only a short time, which can prevent further degradation. Several groups also reported that the treatment temperature and time are important factors for determining the amount and variety of the biomaterials and bioactive substances because high temperature will enhance the solubility of the less polar substances in the water from different matrices, but a long treatment time may degrade these substances (Herrero et al., 2006; Wiboonsirikul *et al*., 2007; Viriya-Empikul *et al*., 2012).

Due to the changing dielectric constant of water with temperature under moderate pressure, the polarity of water was also change and making the water capable of extracting different compounds. Therefore, besides quercetin and rutin, the liquid products from hydrothermal extraction process of *P. cuspidatum* contain other components (such as low molecular weight polyphenolic compounds or lipohilic compounds). However, in this study, resveratrol as one of polyphenolic compounds was decided as one of main extraction products. Resveratrol are two of the most abundant phenols present in natural products. As shown in Figure-8 (a), the yield of resveratrol increased clearly with extraction time at each extraction temperature. Initially (30 min), the yield of resveratrol was 0.18, 0.37, and 0.44 mg/g of *P. cuspidatum* roots loaded at 423, 448, and 473 K, respectively. After 180 min of extraction time, it could approach to 0.55, 0.79, and 0.95 mg/g of *P. cuspidatum* roots loaded at the same extraction temperature. This increase was attributed to higher bound cleavage rate of lignin/phenolic–carbohydrate complexes of *P. cuspidatum*, and also to the more solubility and consequently extraction of polyphenolic compounds in water with relating lower polarity water. This figure also demonstrated that the yield of resveratrol increased slightly as the extraction time at each extraction temperature. It indicated that resveratrol was produced directly from decomposition of lignin/phenolics-carbohydrate complex part of *P. cuspidatum* (Pourali *et al*., 2010; Gil–Chavez *et al.,* 2013; Ruiz *et al*., 2013).





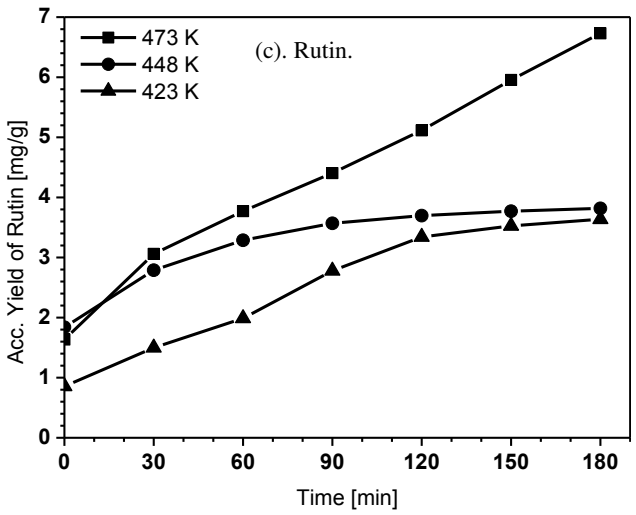


Figure-8. Effect of temperatures on polyphenolic compounds yield, (a) Resveratrol, (b) Quercetin, (c) Rutin, respectively.

Table-2. Selected of water properties at various temperatures.

Fluid	Ordinary water	Present work		
Temperature (K)	298	423	448	473
Pressure (MPa)	0.1	10	10	10
Dielectric constant, ε	78.4	44.4	39.5	34.3
Ion product, pK_w	14	11.26	10.99	10.75

Table-2 shows the physical properties of water (dielectric constants, ε and ion products, pK_w) obtained through temperature changes at the same pressure. As explained before that hydrothermal processing has been considered a cost-effective treatment and in general, the major advantages that this process does not require the addition and recovery of chemicals different from water, limited

equipment corrosion problems, simple and economical operation (Ruiz *et al*., 2013).

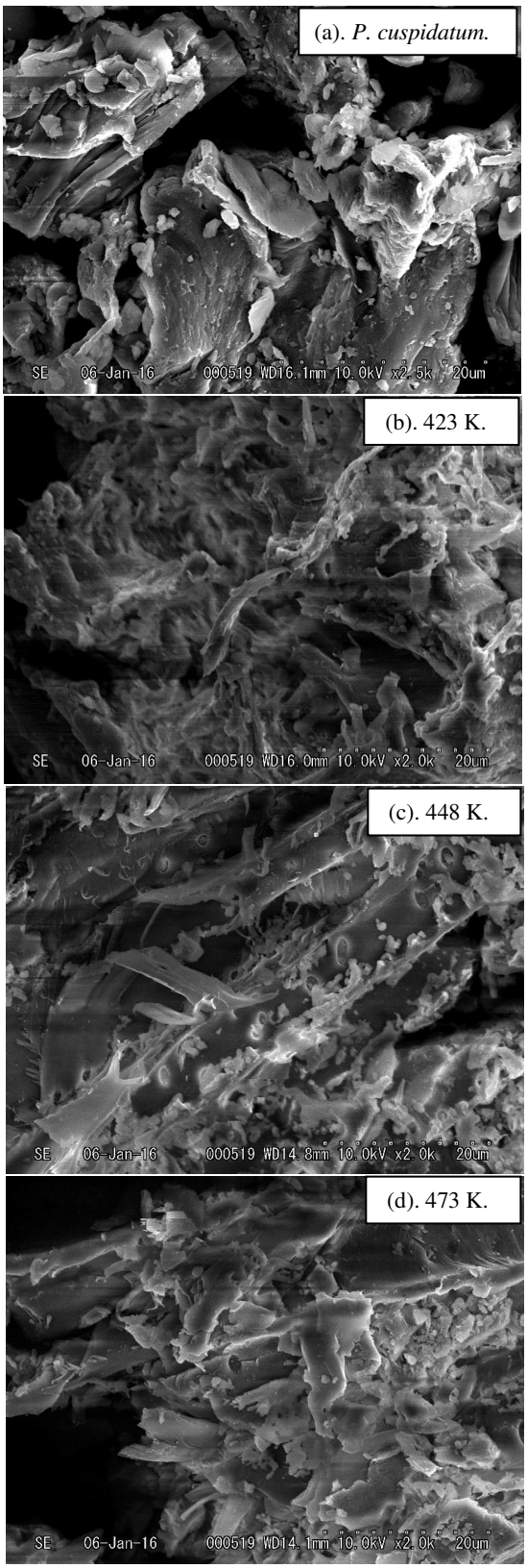


Figure-9. SEM images of *P. cuspidatum* before and after treatment by hydrothermal.

As shown in Table-2, when the temperature of water increases, its dielectric constant and ion product change. The dielectric constant of water, the most important factor when using water as an extraction solvent, decreases from 78 at room temperature (298 K, 0.1 MPa) to 44.4 and 34.3 at 423 and 473 K with 10 MPa of pressure, respectively. The not too low dielectric constant seems to enhance some degradation reactions, e.g. biomass liquefaction via hydrolysis reaction. Decreasing the dielectric constant was followed by increasing the solubility of organic compounds from *P. cuspidatum* (see Figures 5, 6, and 8). As results, the yields of resveratrol, quercetin, and rutin increased with increasing temperature (473 K). Rodriguez-Meizoso *et al.* (38) suggested that the reduction of dielectric constant could increase the solubility of organic compounds such as polyphenols, reduction of the solubility of inorganic compounds and the extraction of specific components with high antioxidant power. Simultaneously, at the same conditions, the ionic product of water ($K_w = [H^+] [OH^-]$) is also relatively high due the increasing of the concentrations of hydrogen and hydroxyl ions. It means that many acid- or base-catalyzed reactions, such as biomass hydrolysis, are accelerated. Therefore, subcritical water has the ability to recover or dissolve substances from natural products which found in the plant biomass.

Figure-9 showed SEM images of *P. cuspidatum* roots and their solid residues after hydrothermal treatment at temperatures of 423-473 K. Obviously, the change of surface morphologies of *P. cuspidatum* roots before and after hydrothermal treatment occurred. Before hydrothermal treatment, the surface morphology of *P. cuspidatum* root seemed flat and smooth with no disruption. The surface morphology also did not show the presence of any pores or surface cracks. After hydrothermal treatment, the physical structures disruption of *P. cuspidatum* roots were found and clearly observed at temperatures of 423-473 K. At the higher temperatures of hydrothermal treatment, the physical structures disruption of *P. cuspidatum* roots occurred extensively. Compared to the *P. cuspidatum* roots starting material, those of treated roots were disrupted and fragmented. The textures of *P. cuspidatum* roots after hydrothermal treatment appeared different from that of the original *P. cuspidatum* roots since some materials melted and resolidified. However, some cracks were also found on the disrupted roots surface. These indicated the cell wall of *P. cuspidatum* roots was crushed, resulting the release of their components to dissolve in water at hydrothermal conditions.

CONCLUSIONS

Hydrothermal extraction of antioxidant compounds from *P. cuspidatum* roots was examined at temperatures of 423-473 K and a pressure of 10.0 MPa using a semi-batch system, a simple and environmentally friendly extraction method requiring no chemicals other than water. Under these conditions, thermal softening of *P. cuspidatum* roots occurred, allowing the extraction of its components such as resveratrol, quercetin, and rutin via

autohydrolysis reactions. The yields of resveratrol, quercetin, and rutin increased with increasing extraction temperature at the same extraction time. After 180 min of extraction time, the yields of them were 0.95, 0.26, and 6.73 mg/g of feed loaded at 473 K, respectively. The amount of WSOC was found to increase with increasing temperature, which indicated that the liquefaction of *P. cuspidatum* also increased. Based on these results, it is proposed that hydrothermal extraction is applicable to isolate polyphenolic compounds from other types of biomass.

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