

Subcritical Water Extraction of Xanthone from Mangosteen (*Garcinia Mangostana* Linn) Pericarp

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Abstract

Subcritical water extraction of phenolic compounds from mangosteen pericarps was examined at temperatures of 120-180°C and pressures of 1-5 MPa using batch and semi-batch extractor. This method is a simple and environmentally friendly extraction method requiring no chemicals other than water. Under these conditions, there is possibility for the formation of phenolic compounds from mangosteen pericarps from decomposition of bounds between lignin, cellulose, and hemicellulose via autohydrolysis. In both of systems, the total phenolic content inclusive xanthone increased with increasing extraction temperature. In batch-system, the maximum yield of xanthone was 34 mg/g sample at 180°C and 3 MPa with 150 min reaction time. The total phenolic content could approach to 61 mg/g sample at 180°C and 3 MPa with 150 min extraction time. The results revealed that subcritical water 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: Mangosteen; Phenolic compound; Xanthone; Subcritical water; Extraction

Introduction

Phenolic compounds are secondary plant metabolites, which are important determinants in the sensory and nutritional quality of fruits, vegetables and other plants. These compounds, one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants. As a large group of bioactive chemicals, they have diverse biological functions. Phenolics may act as phytoalexins [1,2], antifeedants, attractants for pollinators, contributors to plant pigmentation, antioxidants and protective agents against UV light, amongst others [3]. These bioactive properties made these compounds play an important role in plant growth and reproduction, providing an efficient protection against pathogens and predators [2,4], besides contributing to the color and sensory characteristics of fruits and vegetables [2,5].

Recently, the consumption of fruits which contained high in antioxidant properties has become popular due to the increasing public awareness of the health. Pericarps of the fruit have been used in folk medicine for the treatment of many human illnesses [6]. Mangosteen (*Garcinia mangostana* Linn) is one of the fruits which used as an ingredient in commercial products including nutritional supplements, herbal cosmetics, and pharmaceutical products. This fruit belongs to the family of Guttiferae and is known the queen of the fruit. Mangosteen is a tropical tree and cultivated for centuries in South East Asia rainforests, and can be found in many countries worldwide. The major bioactive compounds found in mangosteens are phenolic acid, prenylated xanthone derivatives, anthocyanins, and procyanidins [6,7].

Xanthone is a kind of polyphenolic compounds that contain a distinctive chemical structure with a tricyclic aromatic ring. This compound had a variety of biological activity, for instance antioxidant, antibacterial, antiinflammatory, and anticancer effects [6,8]. Traditionally, xanthone is commonly obtained by extraction with organic solvents such as ethanol, acetone, hexane and methanol [6,9-12]. This extraction methods had several drawbacks; they are time consuming, laborious, have low selectivity and/or low extraction yields. Moreover, this technique employed large amounts of toxic solvents. In this work, water under subcritical conditions (100 to 200°C; 10 MPa)

would be used to extract xanthone from mangosteen via autohydrolysis. This technique has received much attention in past several years, especially in food, pharmaceuticals and cosmetic industry, because it presents an alternative for conventional processes such as organic solvent extraction, steam distillation and the low temperature separation process prevents the degradation of chemical compounds [11]. Under subcritical 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 [13], and phenolic compounds [14]. This treatment has also been demonstrated by several studies to effectively convert cellulosic [15] and lignocellulosic biomass [16] into useful products.

Experimental Section

Materials

The fruits of mangosteen were purchased from the market in Surabaya, Indonesia. They were cleaned and the pericarps of mangosteen were separated and cut into small pieces by using mechanical device. Then, the pericarps were dried in oven at 60°C for one or two days until it reached a constant weight. Next, the dried of pericarps was ground into fine homogeneous powder (around \pm 0.65 mm) using millser.

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After sieving process, they were stored in refrigerator until next step experiments. Xanthone ($C_{13}H_8O_2$, 98.0%) and methanol (CH_2O , 99.7%) were obtained from Wako Pure Chemical Industries Inc. (Tokyo, Japan). They were used without further purification. The chemical structure of xanthone is shown in Figure 1.

Experimental setup and procedure

In this work, the subcritical water extraction was conducted in two processes: batch and semi-batch process. In batch process, the experiments were carried out with teflon lined autoclaves (Parr Instruments-model 4749) as a reactor. The maximum of temperature and pressure are 200°C and 10 MPa, respectively. 2.5 g of feed and pure water corresponding to 0.89-0.95 g cm⁻³ water density were loaded in the teflon lined. Then, it was placed in an autoclave cover (stainless steel) and closed firmly with screw cup. For operation safety, the stainless steel springs were placed at top and bottom of teflon lined autoclave. The reactor was placed into an electric furnace (Linn High Therm GmbH, model VMK 1600, Germany) and quickly heated to the desired temperature. The temperature in the reactor was measured by a thermocouple (K-type) inserted in the reactor cover. The time required to heat up the reactor from room temperature to the desired temperature was around 12-17 min and after that the reactor temperature was constant. The difference of the furnace temperature and the reactor temperature was around 8°C. Pressures were calculated

from the water densities and steam tables. After 30-150 min (include the heating time about 12-17 min), the reactor was turned out from an electric furnace and quickly quenched in a water bath at room temperature. After cooling, the reactor was opened and then liquid and solid fractions were collected with washing inside the reactor by pure water so that the total volume of the product solution became 30 ml. Each experiment was conducted in duplicates/triplicates.

Figure 2 shows the schematic diagram of subcritical water extraction apparatus. The main apparatus consists of a high-pressure pump (200 LC Pump, Perkin Elmer, Germany), heater (Linn High Therm GmbH, model VMK 1600, Germany), 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. 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 2.5 g of feed 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 mangosteen pericarp; the system was then pressurized to the set pressure of 3 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. When the system reached the desired pressure and a steady state was achieved, the electric heater was applied to heat the water. The reactor temperature was maintained at 120-180°C. 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

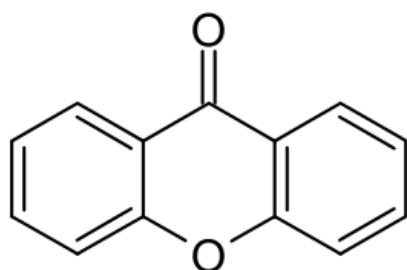


Figure 1: Chemical structure of xanthone.

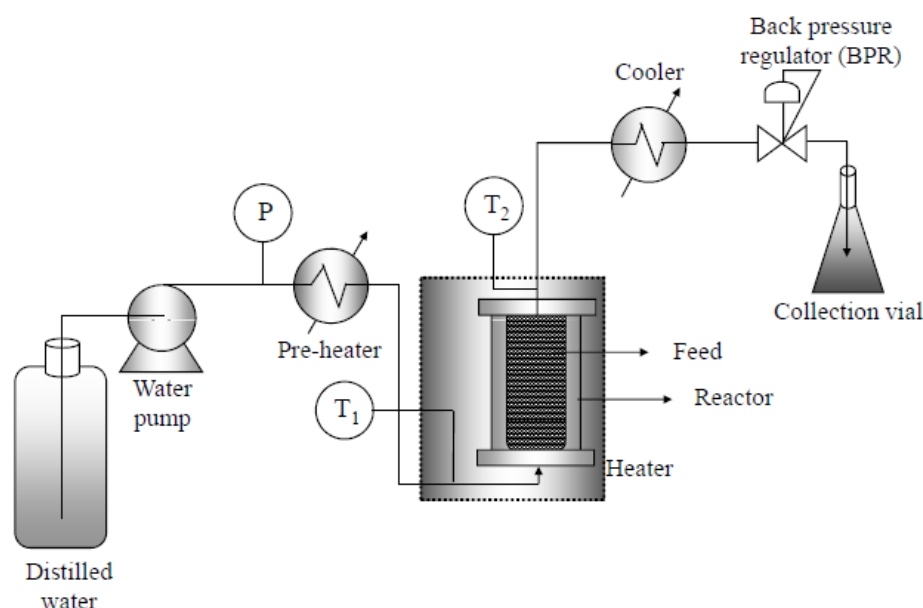


Figure 2: Schematic diagram of subcritical water extraction apparatus.

was 5-8 min, after which the reactor temperature equaled the electric heater temperature. After the temperature at the reactor has reached a preset temperature, the pump was used to feed water at 1.0 ml min⁻¹. Next, the outlet water was passed through the double-tube-type heat exchanger to quench the reaction. The time of experiment was 150 min, which, at 1.0 ml min⁻¹, produced a collected extract volume of 150 mL. Extracted solution was collected every 30 min.

Both of the processes (batch and semi-batch), the solid residues and liquid extracts were totally transferred to a petri dish and sealed bottles, respectively. During extraction process, the light exposure was avoided. The solid residues were dried in an oven at 60°C for 1 day and stored in a desiccator at room temperature. Extracted solution were directly stored in a refrigerator. These processes were maintained until analysis.

Analytical methods

Analysis of phenolic compounds and xanthone content in the extracts was conducted using UV-visible (UV-vis) spectrophotometry V-550 (Jasco Corporation, Japan), allowing spectra of between 190 and 800 nm with 10 nm min⁻¹ of bands in the fast scan mode. Liquid products were analyzed in a quartz cuvette with a 1 cm path length. UV-vis absorption is an effective tool for chemical characterization and may provide important information on the chemical structure of an analyte. 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 subcritical water treatment. The samples were placed directly in the diffuse reflectance attachment sample holder. 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.

Determination of total phenolic contents

The total phenolic content of the extracts was determined using

the Folin-Ciocalteu's reagent. Initially, an aliquot of the extracts (0.1 mL) was diluted to a concentration (2 mL pure water) that was measurable using UV-Vis spectrophotometer prior to the addition of Folin-Ciocalteu's reagent and sodium carbonate. Then the Folin-Ciocalteu's reagent (0.5 mL) was added and mixed thoroughly. After shaking for 1 min, 2.0 ml of sodium carbonate (7.5% w/v) was added and mixed thoroughly. The mixtures were then allowed to stand for 2 h in the dark room before measuring its absorbance in a single beam UV-Vis spectrophotometer. A blank solution was required for initial calibration and it was prepared using methanol (pure solvent). The absorbance values of the extracts were referred to a standard calibration curve produced with five points of known gallic acid concentrations at 0 to 50 ppm to obtain its value in milligrams of gallic acid equivalents (GAE)/g of extract. Measurements were in triplicates.

Results and Discussion

In this work, mangosteen pericarps remained after subcritical water extraction 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. Figure 3 shows the FT-IR spectra of mangosteen pericarps before and after treatment by subcritical water at temperature of 120°C and pressure of 3 MPa in batch process. Based on our previous work [17], the characterization of solid residues was only carried out for solid residue obtained at temperature of 120°C with reaction time 30 and 60 min. Generally, mangosteen pericarps 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 [17-19]. 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-

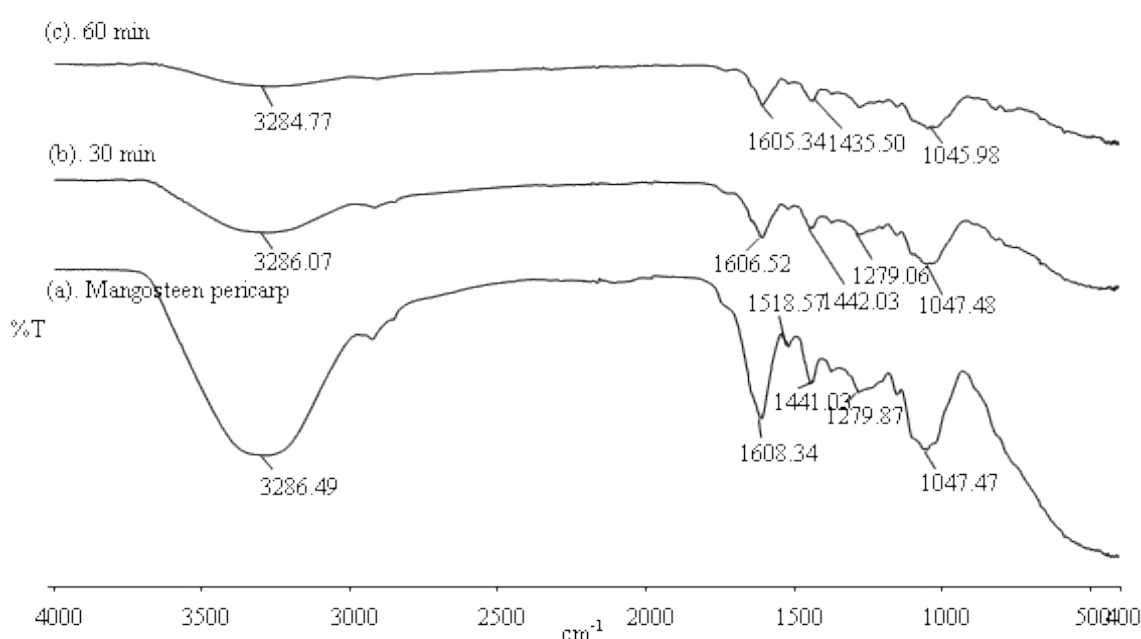


Figure 3: FT-IR spectrum of mangosteen pericarps before and after subcritical water treatment.

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	

Table 1: Main functional groups of the major constituents of plant biomass.

IR spectra due to their structural properties. Absorbance intensity due to hydrogen bonded O-H stretching (3600-3000 cm⁻¹) could be found in each spectrum. This intensity (3284.77-3286.49 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 1045.98-1047.48 cm⁻¹ and 1279.06-1279.87 cm⁻¹ regions are assigned to the stretching and deformation of aromatic C-O groups and the stretching of aryl-alkyl ether linkage C-O-C groups, respectively. In these regions, the peaks in (a and b) were sharper than in (c), showing that the C-O and C-O-C bonds in mangosteen pericarps were more reacted and consumed in (c). The same result also occurred at 1435.50-1442.03 cm⁻¹ and 1605.34-1608.34 cm⁻¹ due to the acid modes of O-H bending and the aromatic stretching modes of C=C, respectively. The intensity of the absorbance in these regions is mostly stable, indicating that methylene groups, syringyl units and ketone groups in mangosteen pericarps have difficulty cleaving under subcritical water conditions. Judging from these results, the extraction of xanthone from mangosteen pericarps by water at subcritical conditions was started at 120°C probably proceeded through hydrolysis reaction.

Although apparently a simple matter, xanthone determination is one of the least satisfactory of the analyses commonly performed on plants biomass. Generally, the methods used involve the solution and hydrolysis of all other plant constituents and the simple assumption that the extract after such treatment is target compound. Here, the determination of xanthone content in an extracts mangosteen pericarp was conducted by using UV-visible spectrophotometry at 243 nm [20,21]. The strong absorption at this wavelength in the UV is typical of a xanthone. Figure 4 shows the effect of extraction temperature on the yield of xanthone when the extraction was carried out at pressure of 3 MPa in batch system. As explained before, autohydrolysis could be applied for lignocellulosic materials lead to the solubilisation of phenolic compounds under subcritical water conditions, leaving a solid phase in both cellulose and hemicellulose. These interactions need to be broken to release of the antioxidant compounds. It was well known that the temperature of the extraction autoclave is a key variable of the extraction process under subcritical water conditions. As shown in Figure 4, the effect of temperature extraction on the yield of xanthone was significant. The yield of xanthone increased with the rise of extraction temperature from 120°C to 160 and 180°C at the same reaction time. The amount of xanthone extract at 160 and 180°C was almost 2-folds of that obtained at 120°C. It could be explained

that xanthone was more soluble in subcritical water at relative higher temperature. When temperature increased from 120 to 180°C, the dielectric constant of water significantly decreases from 50 to 38, which is closed to the dielectric constant of methanol ($\epsilon=33$) or ethanol ($\epsilon=25$). Therefore, the solubility of the compounds inclusive xanthone could be increased due to the decrease in water polarity. Kumar et al. [22] explained that 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. Rangriwong et al. [23] reported that the high amount of gallic acid and ellagic acid extracted with subcritical water at 150 and 180°C could possibly be due to the effect of hydrolysis reaction caused by the increase in the ionization constant of water at subcritical conditions. Nevertheless, they also explained that when the extraction temperature increased to 220°C, the amount of gallic acid and ellagic acid recovered was decreased, possibly due to further degradation at such high extraction temperature.

In subcritical water extraction, the water temperature as an extraction solvent is raised above the atmospheric boiling point, and pressure is applied to maintain the water in liquid state. The viscosity and surface tension of the water decrease, and the solubility and diffusion rate of the target compounds increase. The penetration of the water into the matrix and the transfer of the compounds out from the matrix are faster than in a similar extraction process performed at room temperature. Hence, compared to conventional extraction methods, the subcritical water extraction technique attains more rapid and efficient extraction. That is the main reasons why extraction at elevated temperatures and pressures give enhanced performance compared to extraction at lower temperature and atmospheric pressure [24]. Figure 5 illustrates the effect of extraction pressure on the yield of xanthone in flow type extraction process when the extraction was conducted at temperature of 160°C. As shown in this figure, the yield of xanthone increased with expanding extraction time. The yield of xanthone could approach to 20 mg/g sample at 3 MPa with 150 min extraction time. With the same extraction time, the yield of xanthone was around 18 mg/g sample at 1 and 5 MPa. As seen in this extraction process, the pressure did not result in significantly changes in the yields for the extraction of xanthone from mangosteen pericarps. It was also found that pressure does not have significant effect other than to keep the extraction solvent liquid at these range of temperatures [17,24-26]. Ko

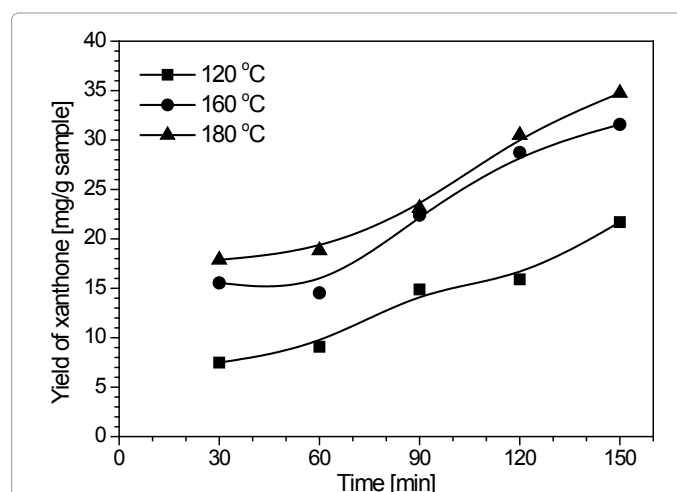


Figure 4: Effect of extraction temperature on the yield of xanthone in batch process.

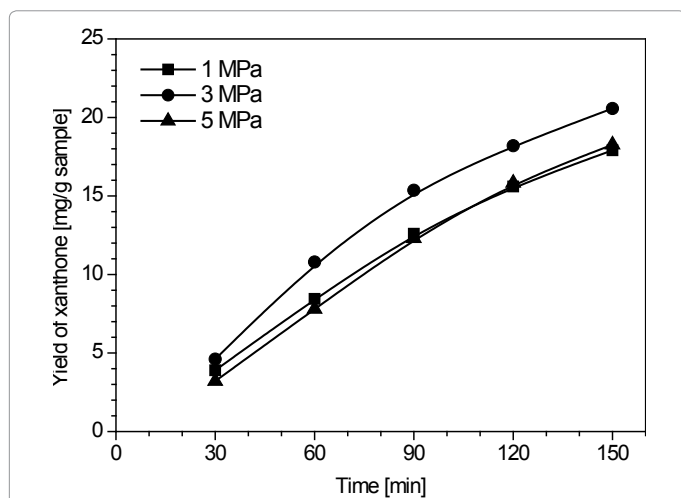


Figure 5: Effect of extraction pressure at 160°C on the yield of xanthone in semi-batch process.

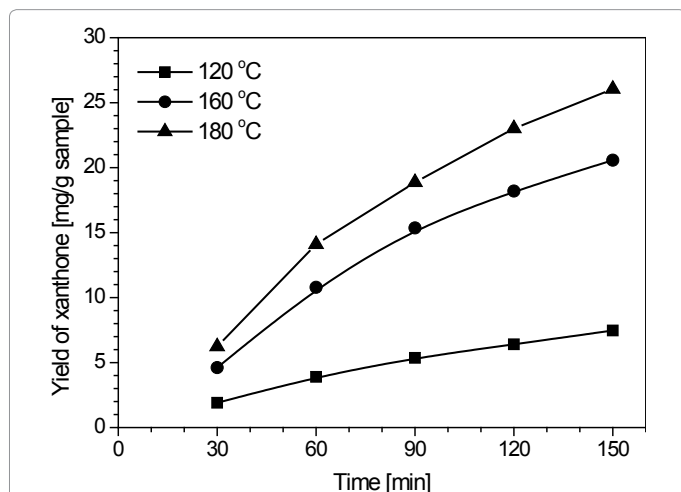


Figure 6: Effect of extraction temperature at 3 MPa on the yield of xanthone in semi-batch process.

et al. [26] performed subcritical water extraction of phenolic compound from onion skin at temperatures of 100-190°C and pressures of 90-131 bars with semi-batch processes. They reported that the changing the pressure has little effect on the extraction efficiency. Based on the result, it could be said that the pressure of extraction process is not a key variable of the extraction process under subcritical water conditions. However, a high pressure can help to enhance the subcritical water extraction efficiency by forcing the organic solvent into the matrix pores.

Figure 6 shows the effect of extraction temperature at a constant pressure on the yield of xanthone in semi-batch process. This figure shows the increase in the cumulative amount of xanthone with the increasing subcritical water extraction temperature from 120 to 180°C at the same extraction time. Similar to subcritical water extraction in batch process (Figure 4), here, subcritical water extraction in semi-batch process has also demonstrated its ability to extract xanthone from mangosteen pericarps depending on the temperature used. The amount of xanthone extract increased significantly when the extraction temperature was increased from 120°C to 160°C or 180°C. The yield of xanthone extract at 160 and 180°C could reach 3-folds of that obtained

at 120°C. Same to previous reasons, the increase in the xanthone content in the extract with the increasing extraction temperature could be explained by the fact that the increasing extraction temperature decreased the polarity or dielectric constant of water and that the decrease contributed to the increase in the solubility of most phenolic acids in the water at subcritical conditions. Even though it is known that increasing the temperature of subcritical water could dissolve several compounds similar to the organic solvents (methanol, ethanol), meanwhile the subcritical water particularly also promotes many reactions, such as hydrolysis and decomposition, which can degrade the compounds in the raw materials. As shown in Figure 6, at 180°C the yield of xanthone increased with longer extraction time and higher than that of 120 and 160°C. It indicated that xanthone was still stable at 180°C and there was no degradation reaction. Empikul et al. [27] carried out subcritical water extraction for defatted rice bran to extract phenolic compounds at temperatures of 120, 160, 200, and 250°C They reported that the subcritical water extraction at 200 and 250°C did not cause degradation of the phenolic substances but it increased the extraction efficiency by 2-3 times for the phenolic substances versus that at 120 and 160°C.

Phenolic compounds are known important due to their antioxidant activities. They possess aromatic structure along with hydroxyl substituents which enable them to protect human tissues from damages caused by oxygen or free radicals, and consequently reduce the risk of different diseases, and offer beneficial effects against cancers, cardiovascular disease, diabetes, and Alzheimer's disease [28]. Pourali et al. reported that there is possibility for the formation of total phenolic content from plant biomass at subcritical water conditions from decomposition of bounds between lignin, cellulose, and hemicellulose via autohydrolysis [28]. The result of phenolic compounds extraction from mangosteen pericarps by water at subcritical conditions would be presented. The Folin-Ciocalteu reagent is used to obtain a crude estimate of the amount of total phenolic content present in extract mangosteen pericarps. Blainski et al. suggested that after optimization of the conditions for the spectrophotometric determination of phenolics using the Folin-Ciocalteu reagent, all parameters analyzed showed adequate results [29]. Figure 7 describes the effect of subcritical water extraction temperature on total phenolic content when the extraction was performed at a constant pressure (3 MPa). At 120°C, the amount of total phenolic content increased with extending extraction time and

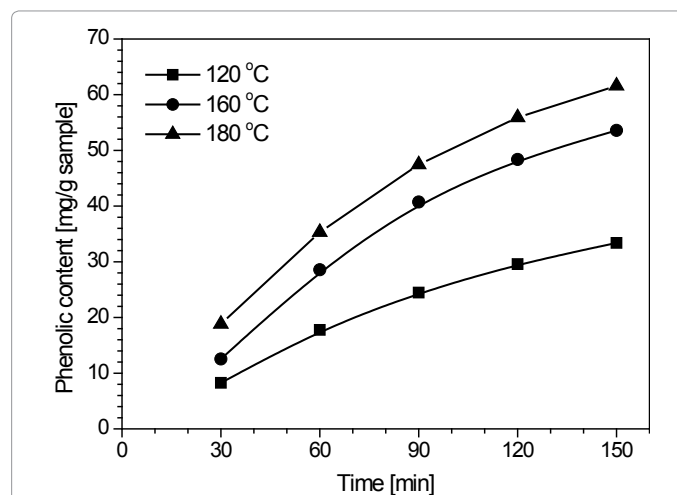


Figure 7: Effect of extraction temperature on total phenolic content of mangosteen extracts.

reached to 30 mg/g sample at 150 min extraction time. At 180°C, it increased significantly to 60 mg/g sample with the same extraction time. Again, this phenomenon could be explained that mangosteen pericarps phenolic compounds were more soluble in subcritical water at relative higher temperature. At such high temperature, phenolic compounds could dissolve in subcritical water as much as they dissolve in the organic solvents [27,14].

Conclusions

Subcritical water extraction of phenolic compounds from mangosteen pericarps was examined at temperatures of 120-180°C and pressures of 1-5 MPa using batch and semi-batch system. Under these conditions, there is possibility for the formation of phenolic compounds from mangosteen pericarps from decomposition of bounds between lignin, cellulose, and hemicellulose via autohydrolysis. In both of systems, the total phenolic content inclusive xanthone increased with increasing extraction temperature. In batch-system, the maximum yield of xanthone was 34 mg/g sample at 180°C and 3 MPa with 150 min reaction time. The total phenolic content could approach to 61 mg/g sample at 180°C and 3 MPa with 150 min extraction time. The FTIR spectrum of solid residues indicated that the liquefaction of mangosteen pericarps was started at 120°C and 3 MPa in batch process. This method could be an excellent alternative medium for extracting phenolic compounds from other types of biomass due to its temperature-dependent selectivity and environmental acceptability.

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