Effect of Hydrothermal Extraction Condition on The Content of Phenolic Compound Extracted from Rind of Mangosteen (*Garcinia mangostana)* and Its Antioxidant Efficiency

Qifni Yasa' Ash Shiddiqi[1], Achmad Dwitama Karisma[1], Siti Machmudah[1], Widiyastuti[1], Tantular Nurtono[1], Sugeng Winardi[1], Wahyudiono[2], and Motonobu Goto[2]

***Abstract*—Xanthone is an antioxidant compound contained in the rind of mangosteen. There are some methods to extract Xanthone from rind of mangosteen, and one of them is hydrothermal extraction. Hydrothermal extraction is a method to obtain xanthone from rind of mangosteen using water at teIn this work, the effect of extraction temperature, pressure, and mode (batch and continuous), and particle size of starting material on the yield and recovery of extracted xanthone and phenolic compounds was investigated. Extraction was carried out at various temperatures (120, 150, and 180^{o}C) and pressures (1, 3, and 5 MPa) and constant flow rate of 1 mL/min. The xanthone content and antioxidant efficiency of extract was examined by using spectrophotometer, while the concentration of total phenolic compounds in the extract was determined with folin ciocalteu reagent and examined using spectrophotometer In order to confirm the extracted phenolic compounds, the extract residue was analyzed by using FTIR. The experimental result showed that the increase in pressure and temperature caused an increase in total phenolic compound concentration and yield of xanthone. In addition, the result also showed that antioxidant activities were observed in the extract of mangosteen rind.**

Keywords—**Hydrothermal Extraction, Magosteen Rind, Xanthone, Total Phenolic Compounds**

***Abstrak*— *Xanthone ($C_{13}H_8O_2$) merupakan salah satu senyawa antioksidan yang terkandung dalam kulit buah manggis (Garcinia mangostana). Untuk mendapatkan xanthone dari kulit manggis secara optimal perlu dilakukan suatu upaya, salah satunya adalah ekstraksi secara hydrothermal. Ekstraksi secara hydrothermal adalah ekstraksi yang menggunakan air sebagai pelarut pada kondisi subkritis. Ada beberapa hal yang harus diperhatikan untuk mendapatkan kandungan ekstrak xanthone yang tinggi, seperti kandungan xanthone dalam kulit manggis, ukuran feed kulit manggis, suhu dan tekanan operasi, serta metode ekstraksi secara batch dan semi batch. Pada penelitian ini, variabel bebas yang digunakan adalah variasi temperatur (120, 150, dan 180^{o}C) dan tekanan operasi (1, 3, dan 5 MPa). Metode analisa kandungan total phenolic compound dan ekstrak xanthone yang digunakan adalah metode spektrofotometri, sedangkan untuk menganalisa efisiensi antioksidan, metode yang digunakan adalah DPPH assay. Peralatan yang digunakan dalam penelitian ini adalah High Performance Liquid Chromatography (HPLC) pump, Ekstraktor, Furnace, Cooler, Autoclave Stainless Steel Reactor, Back Pressure Regulator (BPR), Collection Vial, Spektrofotometer UV-Vis, dan FTIR.Hasil penelitian menunjukkan bahwa kenaikan tekanan operasi menyebabkan peningkatan konsentrasi total phenolic compound dan yield xanthone. Dan kenaikan temperatur operasi menyebabkan peningkatan konsentrasi total phenolic compound dan yield xanthone, selain itu penelitian juga menunjukkan bahwa terjadi aktivitas antioksidan yang berasal dari ekstrak kulit manggis.*

Keywords—***Subcritical Water, Temperatur, Tekanan, Spectrophotometry***

I. INTRODUCTION

Indonesia is an archipelagos country traversed by the equator with tropic climate. Due to high temperature and rainfall throughout the year, Indonesia has a diversity of ecosystems and it is known as the world's largest mega-biodiversity country after Brazil. Moreover, the diversity is caused by Indonesia's strategic location between two continents, Asia and Australia, and two oceans, the Indian Ocean and the Pacific Ocean. Thus, many kinds of plants and animals that exist in Indonesia are a mixture between the two continents, and about 30.000 plant species grow in Indonesian forest. Many plants in Indonesia have been widely used in the medical area.

The use of plants in traditional medicine has been going on for many generations. One of the plants used as traditional medicine is mangosteen fruit (*Garcinia mangostana*).

This is reinforced by scientific research which found that the rind of the mangosteen contains antioxidant compounds, which can be used as a medicine. The antioxidant compounds consist of xanthone, tannin, phenolic acid, and anthocyanin. Among them, the highest level of antioxidant compound is xanthone. Xanthone possess a good and beneficial for body, such as anti - inflammatory, anti-diabetic, anti-cancer, anti-bacterial, anti-fungal, anti-plasmodial, and it is able to boost immunity and also hepatoprotective.

Traditionally, xanthone was obtained from mangosteen rind by boiling, and then drink the extract in water. This method is associated with lower yields and requires long extraction time. To obtain the optimal yield of xanthone, it is necessary to isolate xanthone from mangosteen rind.

[1]Qifni Yasa' Ash Shiddiqi, Achmad Dwitama Karisma, Siti Machmudah, Widiyastuti, Tantular Nurtono, Sugeng Winardi are with Department of Chemical Engineering, Sepuluh Nopember Institute of Technology, Kampus Sukolilo, Surabaya 60111, Indonesia. Email: machmudah@chem-eng.its.ac.id.

[2]Wahyudiono, Motonobu Goto are with Department of Chemical Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

Hydrothermal extraction or pressurized hot water extraction is one such alternative. Water under pressurized conditions is known as a 'natural and green' method for product extraction and has received increased attention as an important alternative to the conventional separation methods. Water under pressurized conditions can be applied to extract polar organic compounds or to decompose lignocellulosic materials to produce valuable compounds such as saccharides and aromatic organic acids. The method has been applied to recover protein, amino acids, and phenolic compounds [20]. Pressurized hot water treatment has also been demonstrated by several studies to effectively convert cellulosic [18] and lignocellulosic biomass [28] into useful products.

In this work, hydrothermal extraction was carried out at various temperatures and pressures to extract xanthone and Total Phenolic Compounds (TPC) from mangosteen rind. The effect of pressure, temperature, and extraction method (semi-batch and batch) on the yield of xanthone and TPC was investigated. The antioxidant efficiency of extract was also investigated.

II. METHOD

Mangosteen (*Garcinia mangostana*) rind used for starting material was purchased from local market in Surabaya. Prior the extraction, mangosteen rind was dried at 60^{o}C for one day, milled, and sieved into a certain size (35 mesh). Standard xanthone and 1,1 diphenyl-2-picrylhidrazil (DPPH) was supplied by Wako, Japan. Methanol and Folin Ciocalteu reagent was purchased from Merck, Germany.

Hydrothermal extraction was carried out in a semi-batch and a batch reactor at 120 – 180^{o}C. For semi-batch extraction, the extraction was done at various pressures of 1 – 5 MPa.

A. Semi-Batch Hydrothermal Extraction Process

In semi-batch hydrothermal extraction process, initially, 7 g of starting material was loaded into the extractor among glass beads put on the both side of extractor inlet and outlet to prevent channeling. Then the extractor was installed in the furnace. Water as a solvent was then pumped at a flow rate of 1 mL/min into the extractor using an HPLC (High Performance Liquid Chromatography) pump to reach an operating pressure. The pressure was controlled by adjusting a back pressure regulator (BPR). Furthermore, the water is heated using a preheater and furnace to achieve an operating temperature. To ensure the temperature in the extractor according to the desired temperature, temperature in the inlet and outlet of extractor were measured by thermocouples (T1 and T2). The extract solution was cooled by a cooler and then passed the filter and collected in a collection vial for each 30 min. Extract solution was then stored in a refrigerator. The schematic diagram of semi-batch hydrothermal extraction apparatus is shown in Figure 1. The extraction was carried out at temperatures of 120 – 180^{o}C and pressures of 1 – 5 MPa for 3 h.

B. Batch Hydrothermal Extraction Process

The hydrothermal extraction was also conducted in a batch extractor. For the batch hydrothermal extraction, 2 g of starting material was loaded into a Teflon beaker with volume of 15.45 mL, and then 12 mL of water was added to the beaker to fill 80 % of the beaker volume. After that, the beaker was put into an autoclave stainless steel reactor (batch extractor). Figure 2 shows the schematic of batch extractor. The autoclave extractor was closed tightly to prevent a leakage. The extractor was then inserted to the furnace and heated to reach an operating temperature. The extraction was done at temperatures of 120, 150, and 180oC for 30, 60, 90, and 120 min. After that, the extract was filtered using filter.

C. Xanthone Content Analysis

Xanthone content in the extract was analysized using spectrophotometer at a wavelength of 236 nm. Initially, calibration curve was prepared by measuring a standard solution xanthone at concentration of 100 to 500 mg/L. The extract solution was diluted with aquadest prior to analysis. Xanthone content in the extract was obtained based on the absorbance of the extract compared with the calibration curve [4].

D. Total Phenolic Compound Content Analysis

The content of TPC in the extract was determined with Folin Ciocalteu reagent. 0.1 mL of extract was diluted into 2 mL of solution with aquadest. 0.5 mL of Folin Ciocalteu reagent was added to the solution followed by the addition of 2 mL of 7.5 % Na_2CO_3 solution. And then the mixed solution was leaved for 2 h at room temperature (in a dark room). Furthermore, the absorbance of the solution was measured with spectrophotometer at wavelength of 779 nm. TPC content in the extract was determined based on tannic acid standard curve at concentration of 0-200 mg/L. TPC was expressed as mg equivalent tannic acid/g sample [19].

E. Antioxidants Efficiency Analysis

The efficiency of antioxidant was analyzed using DPPH Assay method. DPPH assay is an easy and accurate method to measure the antioxidant capacity of vegetables, fruits and extracts. DPPH is one of the organic nitrogen (free radical) that available commercially. The antioxidant efficiency of extract was determined by adding 40 mL of extract into 2 mL of 25 ppm DPPH in methanol solution, and mix it perfectly. Absorbance of the solution was measured using spectrophotometer at wavelength of 516 nm every minute until a constant absorbance was obtained. Percentage of the remaining DPPH was calculated with the following equation:

$$\%DPPH_{rem} = 100 \times \frac{[DPPH]_{rem}}{[DPPH]_{t=0}} \quad (1)$$

$[DPPH]_{rem}$ is the absorbance of the extract at a certain time, and $[DPPH]_{t=0}$ is the initial absorbance of DPPH. The efficiency of radical/antioxidant was calculated by the following equation:

$$AE = \frac{1}{EC_{50} \times t_{EC50}} \quad (2)$$

EC_{50} is the concentration of extract that caused 50% decrease in initial DPPH absorbance, and t_{EC50} is time needed to reach steady state at EC_{50} concentration

III. Result and Discussion

A. Semi - Batch Hydrothermal Extraction

1) Effect of Pressure on the Content of Extract

Figures 3 and 4 show the effect of extraction pressure on the content of Total Phenolic Compounds (TPC) as Tannic Acid Equivalent (TAE) and the content of xanthone in the extract, respectively, at 120°C. Figure 3 shows that the concentration of TPC at 120°C significantly increased with an increase in pressure from 1 to 5 MPa. As well as for Figure 4, the content of xanthone in the extracts also increased with the increasing pressure, where the highest yield of xanthone is around 16% of the mass of xanthone in the starting material (0.07852 g xanthone/g mangosteen rind (dry basis)). The higher pressure enhanced the interaction between the solute and solvent. In other word, the solubility of solute (xanthone and phenolic compound) in liquid increased with pressure. It was due to an increase in solvent density (subcritical water). The increase in extraction pressure.

2) Effect of Temperature on the Content of Extract

Figure 5 and 6 show the effect of temperature on the content of TPC as tannic acid equivalent (TAE) and xanthone in the extract at a pressure of 1 MPa, respectively. As shown in Figure 5, the TPC extracted from mangosteen rind increased with the increasing extraction temperature from 120 to 180°C. As well as in Figure 6, the yield of xanthone also increased with an increase in temperature. This method is using water at subcritical condition as solvent, which has physical properties of liquid remain in the temperature range of 100 to 374ºC and in pressurized condition. Water has two unique properties, first, the high ion product at elevated temperature. This fact indicates that the water can act as an acid or alkaline catalyst. Another property is low dielectric constant. Dielectric constant of subcritical water at a constant temperature of 200 to 300°C is almost the same as acetone and methanol at ambient temperature. This indicates that the water may be used as a solvent for extracting substances contained in mangosteen rind [1].

From Table 1, the highest TPC content in extract was obtained at temperature of 180°C and pressure of 5 MPa with the maximum concentration of TPC is 50.66 g TAE/100 g sample. In Table 1, it is also known that the maximum yield of xanthone is 27.74 % (mass of extracted xanthone/mass of xanthone in the starting material) obtained at temperature of 150°C and pressure of 5 MPa. While the highest antioxidant efficiency was obtained at temperature of 150°C and pressure of 1 MPa with the value of 0.62 min^{-1}.

B. Batch Hydrothermal Extraction

1) Effect of Temperature and Extraction Time on the content of Total Phenolic Compound (TPC)

Figure 7 shows the effect of operating temperature and extraction time of batch hydrothermal extraction on the content of TPC. In this figure shows that temperature dramatically affected TPC content in the extract. The higher extraction temperature, the higher TPC concentration was obtained. TPC concentration markly increased with increasing extraction time at 180°C. This is due to the increase in TPC solubility in water at high temperature. Moreover, the longer extraction time, the larger water molecules were provided to extract TPC, and the longer water molecules contacted with the samples.

2) Effect of Temperature on the Content of Xanthone

Figure 8 shows the effect of temperature of batch hydrothermal extraction on the content of xanthone for extraction time of 120 min. In Figure 8, temperature significantly affects the yield of xanthone in the extract. It has been explained previously, the increase in temperature causes an increase in ion products in water that cause an increase in solubility of solute in the solvent.

Table 2 shows the calculation result of the antioxidant efficiency in batch hydrothermal extraction at various temperatures and extraction times. The result indicates that for longer extraction time, the extract was faster reducing the levels of free radicals (DPPH concentration). The higher extraction temperature caused the shorter t_{EC50} that also affected the antioxidant efficiency of extract. At temperature of 120°C, the antioxidant efficiency significantly increased for extraction time of 30 and 60 min, and then tended to decrease thereafter, both at extraction time of 90 and 120 min. At temperature of 150°C, the antioxidant efficiency of extract increased with increasing extraction time. At temperature of 180°C, the antioxidant efficiency of extract has similar pattern with temperature of 120°C that significantly increased when extraction time reached 60 min, and gradually decreased with the increasing extraction time. Besides t_{EC50}, the antioxidant efficiency is also affected by the concentration of the extract added into DPPH. The increased in antioxidants efficiency can be explained by the increase in the concentration of TPC acts as an antioxidant extract. However, with increasing extraction time, there is the possibility that extracted substances was also including contaminant that caused the decrease in antioxidant efficiency.

IV. Conclusion

From the experimental results it can be concluded that temperature and pressure have some effects on the content of TPC and yield of xanthone in the mangosteen rind extract. The increasing temperature and pressure of hydrothermal extraction caused the increasing TPC content and yield of xanthone in the extract. Mangosteen rind contains antioxidant components. The antioxidant efficiency increased with the increasing in extraction pressure and temperature. The optimum condition to obtain maximum TPC was at temperature of 180°C and pressure of 5 MPa. While, the maximum yield of xanthone was obtained at temperature of 150°C and pressure of 5 MPa. The content of TPC, yield of xanthone, and antioxidant efficiency of batch hydrothermal extraction were better than those of semi-batch hydrothermal extraction.

References

[1] Adachi, Shuji, *Properties of subcritical water and its utilization*, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University. (2009).

[2] Amashukeli, X., Pelletier, C.C., Kirby, J.P. , Grunthaner, F.J. *Subcritical water extraction of amino acids from Atacama Desert soils.* Journal of geophysical research, vol. 112. (2007).

[3] Ersam, T., *Senyawa Kimia Mikromolekul Beberapa Tumbuhan Artocarpus Hutan Tropika Sumatra Barat*, Disertasi, ITB, Bandung.(2001).

[4] Harborne, J.B., *Metode Fitokimia*, Penerbit ITB,Bandung. (1987),

[5] Jung, H.A.,Bao Ning *S*,Keller, W.J, Mehta, R.G.,Kinghorn, A.D, *Antioxidant Xanthones from the Pericarp of Garcinia mangostana (Mangosteen)*. Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio.(2006).

[6] Kitada , K., Machmudah , S., Sasaki , M., Goto , M., Nakashima , Y., Kumamoto, S., Hasegawa,T.: *Antioxidant and Antibacterial Activity of Nutraceutical Compounds from Chlorella vulgaris Extracted in Hydrothermal Condition*, Separation Science and Technology, 44:5, 1228-1239.(2009).

[7] Kruse , A., Dinjus, E., *Hot compressed water as reaction medium and reactant Properties and synthesis reactions*. Institut fur Technische Chemie, Chemisch-hysikalische Verfahren, Forschungszentrum Karlsruhe.(2007).

[8] Machmudah, S., Sulaswatty, A., Sasaki, M. Goto, M., Hirose, T.. *Supercritical CO2 Extraction of Nutmeg Oil: Experiments and Modeling.* The Journal of Supercritical Fluids, 39, 30-39.(2005).

[9] Magadula, J.J., Tewtrakul, S. *Anti-HIV-1 protease activities of crude extracts of some Garcinia species growing in Tanzania*. African Journal of Biotechnology Vol. 9 (12), pp. 1848-1852, 22 March, 2010.(2010).

[10] Mahabusarakam, W.. Wiriyachitra, P. *Chemical constituent of Garcinia mangostana.* Journal of Natura1 Products Vol. 50, No. 3, pp. 474-478, Maydun.(1987).

[11] Mariela, G., Bernardo G., Roxana, R. Elida, R. , María, A.A.M. *Spectrophotometric Determination of Phenolic Compounds in Propolis.* Facultad de Bioquímica, Química y Farmacia. Universidad Nacional de Tucumán. San Miguel de Tucumán. República Argentina. (2003).

[12] Möckel, H. J., Welter, G., and Melzer, H.. *Correlation between reversed-phased retention and solute molecular surface type and area. I. Theroretical outlines and retention of various hydrocarbon classes.* J. Chromatogr. A, 388, 255-266. (1987).

[13] Pitojo, Setijo. *Budidaya Manggis.* Semarang : Aneka Ilmu.(2007).

[14] Richter, B. E., Jones, B. A., Ezzell, J. L., and Porter, N. L. Accelerated solvent extraction: A technique for sample preparation. *Anal. Chem.*, **68**, 1033-1039. (1996).

[15] Rogalinski, T., Herrmann, S., and Brunner, G. *Production of amino acid from bovine serum albumin by continuous subcritical water hydrolysis.* J. Supercrit. Fluids, **36**, 49-58. (2005).

[16] Rogalinski, T., Liu, K. Albrecht, T. Brunner. G.. *Hydrolysis kinetics of biopolymers in subcritical water*. J. of Supercritical Fluids 46, 335–341. (2008).

[17] Saim, N., Osman, R., , Rozita Osman, Md Yasin, W.H.A., Dol Hamid, R. *Subcritical water extraction of essential oil from coriander (Coriandrum sativum L.) seeds.* Fakulti Sains Gunaan, Universiti Teknologi MARA, Shah Alam, Selangor.(2008).

[18] Sasaki, M., T. Adschiri, K. Arai. Bioresour. Technol., 2003; 86, 301–304.

[19] Schmid G .*Nanoparticles: Theory to application*. Weinheim: Wiley-VCH) (2004).

[20] Sereewatthanawut, I., S. Prapintip, K. Watchiraruji, M. Goto, M. Sasaki, A. Shotipruk. Bioresour. Technol., 2008; 99, 555–561

[21] Singh, P.P, Saldaña. M.D.A. *Subcritical water extraction of phenolic compounds from potato peel.* Journal Food Research International 44 2452–2458. (2011).

[22] Stevi, G.D., Dewa, G.K., Vanda ,S.K. *Aktivitas antioksidan Ekstrak Fenolik dari Kulit Buah Manggis (Garcinia mangostana L.).*Journal MIPA Unsrat online 1 (1) 11-15.(2012).

[23] Sudarmadji S, Suhardi. *Analisis Bahan Makanan dan Pertanian*. Yogyakarta: Liberty. (1996).

[24] Sudjadi.*Metode Pemisahan*, Penerbit Kanisius, Yogyakarta.(1988).

[25] Sutriani L .*Ekstraksi* . online at http://medicafarma.blogspot.com/2008/11/ekstraksi.(2008).

[26] Tambunan, R.M. *Telaah Kadungan Kimia dan Aktivitas Antimikroba Kulit Buah Manggis*. Tesis. Institut Teknologi Bandung, Bandung .(1998).

[27] Wagner, W. and Pruß, A. *The IAPWS formulation 1995 for the thermodynamic properties of ordinary water substance for general and scientific use*. J. Phys. Chem. Ref. Data, **31**, 387-535.(2002).

[28] Wahyudiono, T. Kanetake, M. Sasaki, M. Goto. Chem. Eng. Technol., 2007; 30, 1113–1122

[29] Wiboonsirikul, J., Adachi s. *Extraction of Functional Substances from Agricultural Products or By-products by Subcritical Water Treatment*. Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University. Kyoto .(2008).

[30] Zarena, A.S. Udaya Sankar. K. *Xanthones enriched extracts from mangosteen pericarp obtained by supercritical carbon dioxide process.* Journal of Separation and Purification Technology 80 (2011) 172–178 .(2011).

[31] P. Wilhelm, dan D. Stephan, Photodegradation of rhodamine B in aqueous solution via SiO_2·TiO_2nano-spheres. Technische Universitat Munchen, Garching, Germany, 2007.

[32] S. Bakkialakshmi, P. Selvarani, S. Chenthamarai, *Fluorescence quenching of Rhodamine B base by two amines*, Department of Physics, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India, 2013.

[33] J. Alarcon, *Fiber Optic Devince For Sensing Analysis and Metode Of Making Same*, GooglePatent US 7787923 B2, 2010.

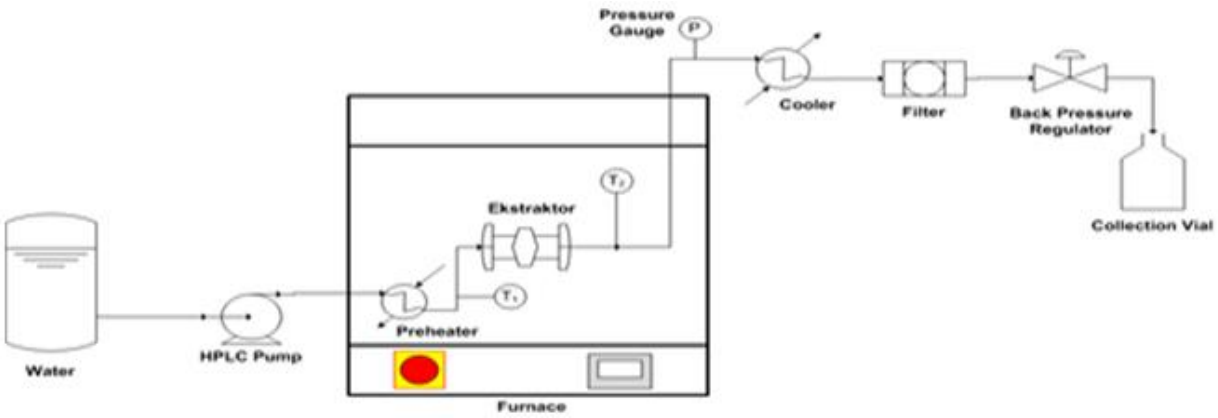

Figure. 1. Apparatus for Semi Batch Hydrothermal Extraction Process

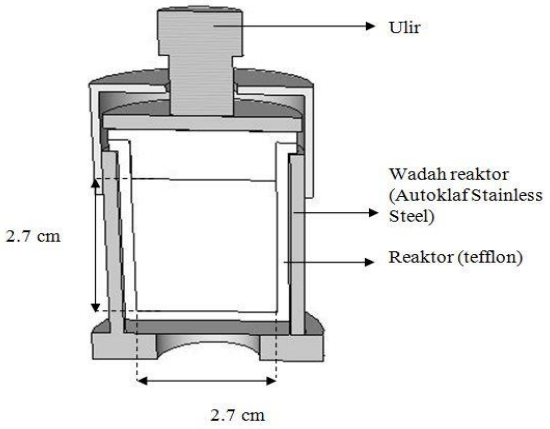

Figure. 2. Cross sectional area of Batch Hydrothermal extraction reactor

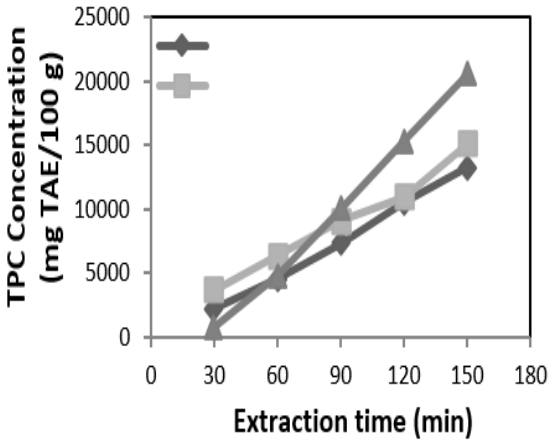

Figure 3. Effect of Operating Pressure on Total phenolic compound concentration at T = 120°C

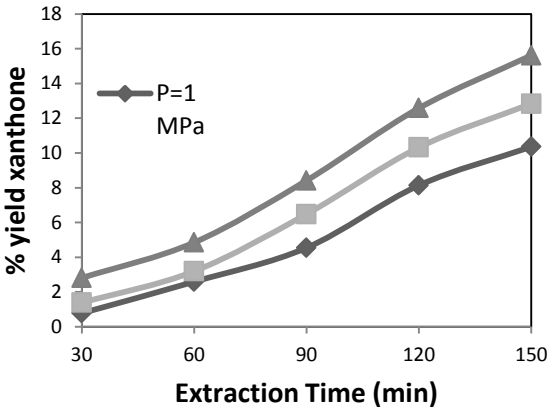

Figure 4. Effect of Operating Pressure on Yield xanthone at T = 120°C

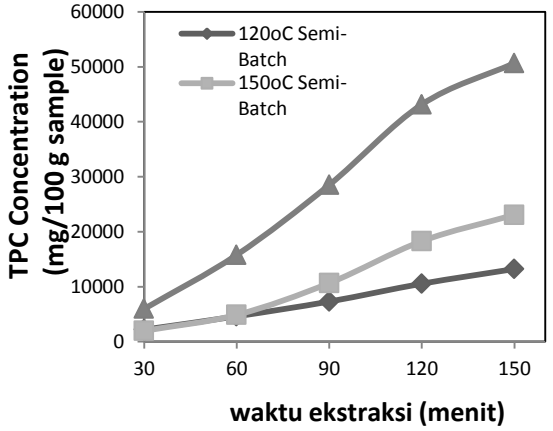

Figure 5. Effect of Operating Temperature to the concentration of TPC at P = 1 MPa

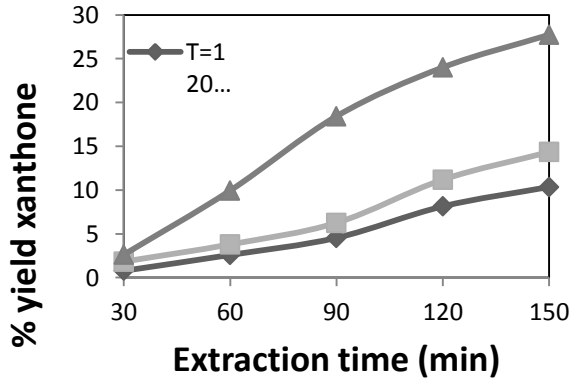

Figure 6. Effect of Operating Temperature to yield xanthone at P = 1 MPa

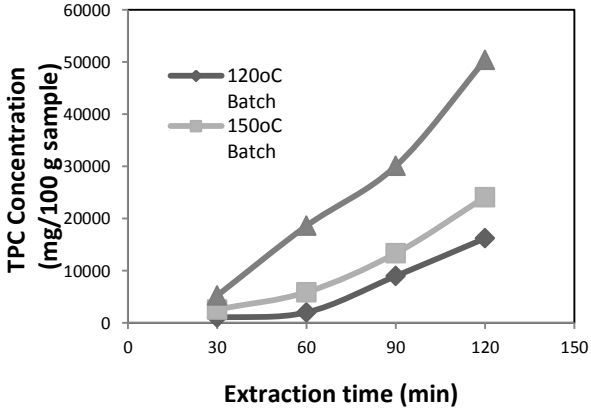

Figure 7. Concentration of Total Phenolic Compound Every Extraction Time

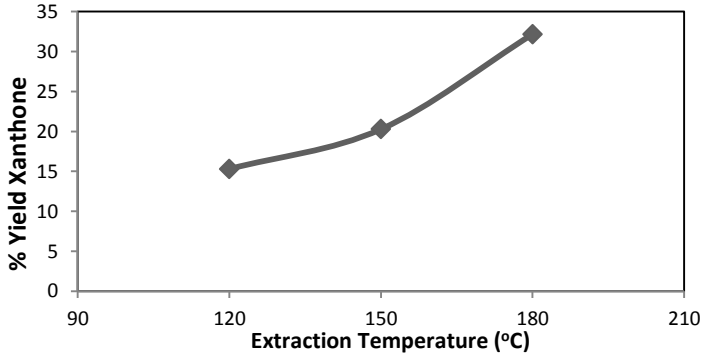

Figure 8 Effect of Operating Temperature on the % Yield of Xanthone

TABLE 1.
THE ANALYSIS RESULTS OF TPC CONTENT, YIELD OF XANTHONE AND ANTIOXIDANT EFFICIENCY IN DIFFERENT OPERATING CONDITION OF SEMI-BATCH HYDROTHERMAL EXTRACTION

P(MPa)	T(°C)	TPC (mg TAE/100g sampel)	% *Yield Xanthone*	Efisiensi Antiokidan (min^{-1})
1	120	13221.32	10.35%	0.14
3	120	15093.02	12.84%	0.23
5	120	20562.03	15.62%	0.47
1	150	10455.01	14.35%	0.62
3	150	23072.44	31.42%	0.09
5	150	25323.68	23.13%	0.18
1	180	39319.44	27.74%	0.20
3	180	41964.96	30.16%	0.22
5	180	50664.28	27.44%	0.23

TABLE 2.
EFFECT OF OPERATING TEMPERATURE AND EXTRACTION TIME ON THE ANTIOXIDANT EFFICIENCY

T(°C)	Waktu Ekstraksi (min)	t_{EC50} (min)	EC_{50}	AE (min^{-1})
120	30	34.5	0.49	0.059
120	60	9.6	0.51	0.21
120	90	8.42	0.58	0.20
120	120	5.33	0.98	0.19
150	30	34.67	0.52	0.05
150	60	9.6	0.57	0.18
150	90	3.67	0.85	0.32
150	120	1.83	1.47	0.37
180	30	24.5	0.67	0.06