

Optimization for Subcritical Water Extraction of Phenolic Compounds from Rambutan Peels

Nuttawan Yoswathana and M. N. Eshtiaghi

Abstract—Rambutan is a tropical fruit which peel possesses antioxidant properties. This work was conducted to optimize extraction conditions of phenolic compounds from rambutan peel. Response surface methodology (RSM) was adopted to optimize subcritical water extraction (SWE) on temperature, extraction time and percent solvent mixture. The results demonstrated that the optimum conditions for SWE were as follows: temperature 160°C, extraction time 20min. and concentration of 50% ethanol. Comparison of the phenolic compounds from the rambutan peels in maceration 6h, soxhlet 4h, and SWE 20min., it indicated that total phenolic content (using Folin-Ciocalteu's phenol reagent) was 26.42, 70.29, and 172.47mg of tannic acid equivalent (TAE) per g dry rambutan peel, respectively. The comparative study concluded that SWE was a promising technique for phenolic compounds extraction from rambutan peel, due to much more two times of conventional techniques and shorter extraction times.

Keywords—Subcritical water extraction, Rambutan peel, phenolic compounds, response surface methodology.

I. INTRODUCTION

THE agricultural wastes are produced vast quantity annually. In addition, the disposal of agricultural wastes can have a serious environmental impact. Nowadays, many investigations on waste utilization have been aimed at evaluation of the waste materials in possible value-added applications. Rambutan (*Nephelium lappaceum* L.) is native to Southeast Asia. The fruit canning factory has variety wastes of seeds and peels of fruits such as lychee, longan, and rambutan. The discarded rambutan peels are red and be covered with hairy spines which comprise powerful phenolic antioxidants [1]-[3]. Moreover, various biological properties such as antibacterial and anti-herpes simplex virus type 1 have also been reported [4]-[5].

Phytochemicals, especially phenolic in fruits and vegetables are major bioactive compounds known for health benefits. Plant phenolics are commonly found in both edible and non-edible parts of the plants. Phenolic compounds represent a majority of the natural antioxidants presently identified. The most important classes of natural antioxidants include tocopherols, flavonoids, and phenolics acids [6]. Recently,

there has been an increasing interest in phenolic compounds from fruit that function as free radical scavengers. The potential free radical scavenging property observed in the rambutan fruit was influenced by phenolic compounds, especially ellagitannins, present in its peels [5]. The variables in phenolic compound types and content depended on the types of fruits, cultivars and the stage of fruit development [7]-[8]. Phenolic accumulation in rambutan peels increased gradually in the early and middle stages of growth and then increased rapidly in both cultivars [9]. Several factors that contribute to the efficiency of polyphenols extraction such as type of solvent, pH, temperature, solvent to solid ratio, particle size, and extraction method have been studied [10].

The properties of extracts from plants such as antioxidant activity depend on the extraction solvent and extraction techniques used [11]-[13]. Water and ethanol are commonly used to extract phytochemicals from plants due to absence of toxicity. Extraction with ethanol and water was investigated for isolation of possible antioxidants in the present study. The conventional methods used currently for extraction of compounds from herbal plants are distillation and solvent extraction. Low extraction efficiency and toxic solvent residues in the extracts occurs when using these technologies. Recently, Subcritical water, also called pressurized (hot) water, superheated water or hot liquid water, it refers to water at temperature between 100 and 374°C and at a pressure which is high enough to maintain the liquid state (below the critical pressure of 22MPa [12]. Subcritical water extraction (SWE) exhibits a number of advantages over conventional extraction methods (i.e. maceration, hydro-distillation, soxhlet with organic solvent), mainly including the reduction of extraction time, simplicity, less expensive operation, higher quality of the extracts and environment-friendly [11]-[13]. SWE has been used for the extraction of catechin and epicatechin from green tea [14], anti-cancer damnacanthol from roots of *Morinda citrifolia* [15], lignans from whole flaxseed [16], antioxidant nutraceuticals from star fruit residues [17], antioxidant from red grape marc [18], antioxidants from rosemary [19], and flavonoids, phenolic acids, and anthocyanins from red grape skins [20].

The objectives of this work were to extract and compare the total phenolic content from rambutan peels by subcritical water extraction (SWE) with conventional methods. The optimized conditions of SWE using response surface methodology were investigated the effect of extraction parameters on the total phenolic content (TPC) as following: temperature, extraction time, and percent solvent mixture.

Nuttawan Yoswathana is with Mahidol University, Faculty of Engineering, Department of Chemical Engineering, Salaya, Thailand (corresponding author to provide phone: 0066-28892138-6101; fax: 0066-28892138-6129; e-mail: nuttawan.yos@mahidol.ac.th).

M.N. Eshtiaghi is with Mahidol University, Faculty of Engineering, Department of Chemical Engineering, Salaya, Thailand. (e-mail: mohammand.esh@mahidol.ac.th).

II. MATERIAL AND METHODS

A. Chemicals

All chemical were obtained from Sigma Chemical Co. Ltd. (USA) unless otherwise specified.

B. Preparation of Plant Sample

Rambutan peels were taken from a fruit canning factory at Nakornprathom province, Thailand. They were washed and cut into small pieces. Then they were dried in the oven at 60°C for 6 hours. Lastly, they were ground into particles ranging from 0.2 to 0.5mm in size by a hammer mill and being forced through a sieve.

C. Maceration

The rambutan peel powder (10g) was added into 150ml of hexane in conical flask and 150ml of ethanol in conical flask at solid to solvent ratio of 1:15 in a 500mL flask, respectively. Then, they were mixed on a magnetic stirrer for 2 to 8 hours at room temperature (28°C). The supernatant was passed through Whatman filter paper (no.1). All filtrates were evaporated under vacuum at 60°C using a rotary evaporator (Buchi, Switzerland). The volume of sample is adjusted to 25ml using HPLC grade ethanol and stored in refrigerator until analysis. The experiments were carried out in duplicate.

D. Soxhlet

Soxhlet extractions were carried out in triplicate using 20g (dry weight) of rambutan peel powder with 300ml of 95% ethanol for 2 to 8 hours. Temperature during soxhlet extraction was set at 70°C. The filtrate extracts was evaporated under vacuum at 60°C using a rotary evaporator (Buchi, Switzerland). The extracted sample was evaporated and prepared for analysis same as maceration.

E. Subcritical Water Extraction (SWE)

The Rambutan peel powder (8g) was filled into an extraction column (Type 304 Stainless) 200ml and then added 120ml of solvent with varying water-ethanol concentration into vessel. The extraction vessel was placed in a heating bath to maintain an operating temperature within $\pm 1^\circ\text{C}$ of the set point temperature for each run. Response surface methodology (RSM) was employed to optimize the operating conditions of subcritical water technique to obtain a high extraction yield. The studied parameters and their concentration ranges were: ethanol concentration (X_1) at 5, 50, and 95% mixture, temperature (X_2) at 100, 130, and 160°C, extraction time (X_3) 20, 40, and 60min. After the operation finished, the extracted solution were collected and then prepared sample for analysis as maceration. All the experiments were performed in duplicate and each set of yields was average.

F. Determination of Total Phenolic Content

The total phenolic content of extracts was determined using Folin-Ciocalteu's phenol reagent (modified from Kahkonen et al., 1999) [21]. Briefly, 1ml of extracts was mixed with 1ml of Folin-Ciocalteu's phenol reagent and allowed to react for 3 minutes. Then, 0.8ml of 7.5% (w/v) sodium carbonate was added. The mixture were agitated and allowed to stand for

further 30 minutes in the dark. The absorbance of rambutan peel extracts and a prepared blank were measured at 765nm using a spectrophotometer (UV-Vis model 1601, Shimadzu, Japan). The concentration of total phenolic compounds in rambutan peel extracts was expressed as mg of tannic acid equivalents (TAE) per g dry weight of rambutan peel using a linear equation. All determination was performed in duplicate.

III. RESULTS AND DISCUSSIONS

A. Types of Solvent

The effect of types of solvent (hexane and ethanol) on the extracts yield using maceration technique was studied as shown in Fig. 1.

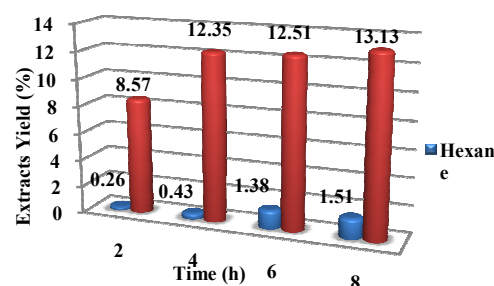


Fig. 1 Effect of solvents on the extracts yield of maceration at solid to solvent ratio (w/v) of 1:15 and various extraction times

For extracts yield (Fig. 1), it increases with increasing extraction time due to contact more times. According to the fact the total extract yield kept on increasing with polarity of organic solvent, the extracts yield in ethanol (polar) was much higher than that of hexane (non-polar) and did experiment 6h to get the highest yield of 12.51%. Hence, it could do experiment only 4h, because of a small amount increasing extracts yield.

B. Comparison of Maceration and Soxhlet techniques

The comparison of maceration and soxhlet techniques at solid to solvent ratio (w/v) of 1:15 and using 95% ethanol was investigated the influence of temperature on extracts yield of rambutan peel at various times as shown in Fig. 2.

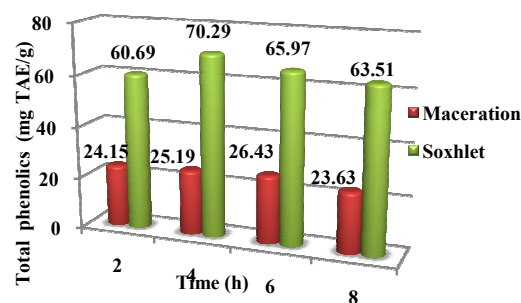


Fig. 2 Comparison of maceration and soxhlet techniques on total phenolic contents

Soxhlet technique showed total phenolics much more than two times of maceration for all times and did experiment only 4h to get the highest total phenolic compounds of 70.29mg TAE/g dry rambutan peel. While maceration technique could extract the highest total phenolics only 26.43mg TAE/g dry rambutan peel for 6h. According to increasing temperature [22], it helps to enhance both the solubility of solute and the diffusion coefficient. Heating also might soften the plant tissue. Thus, the extraction could be developed by increasing temperature.

C. Optimization for Subcritical Water Extraction (SWE) by Response Surface Methodology (RSM)

Response surface methodology was a good tool for optimization of extraction conditions [23]-[25]. The experimental data of total phenolic contents from rambutan peel using SWE were used to calculate the coefficients of the second-order polynomial in (1). The coefficient of determining (R^2) was 0.9252, indicating adequate accuracy. The application of RSM yielded the following regression (1) which was an empirical relationship between total phenolic contents (Y) and the test variables in code units i.e. X_1 , X_2 , and X_3 were temperature ($^{\circ}\text{C}$), concentration ethanol in water (%), and extraction time (min.), respectively:

$$Y = 330 - 3.1887X_1 - 0.1811X_2 - 1.1033X_3 + 0.0268X_1^2 - 0.0031X_2^2 - 0.0295X_3^2 + 0.0045X_1X_2 - 0.0112X_1X_3 - 0.0009X_2X_3 \quad (1)$$

Based on the above findings, an optimization study was performed to surface plot in Figs. 3-5.

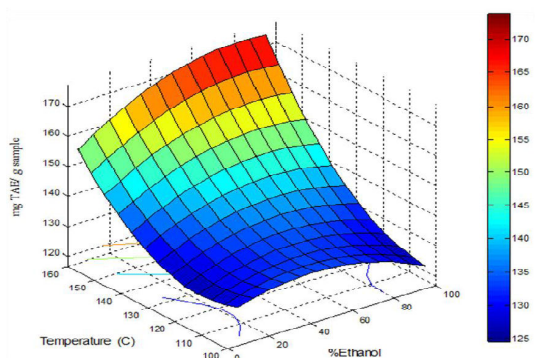


Fig. 3 Surface plot of temperature and % ethanol in water at time 20min for total phenolic contents from rambutan peels

From Fig. 3, temperature and concentration of ethanol in water affected to extraction of total phenolic content from rambutan peel. The best conditions for SWE were 50% ethanol and 160 $^{\circ}\text{C}$ and 20min. and gave 172.47mg TAE/g dry rambutan peel. In addition, the result also demonstrated that total phenolic contents increased as the temperature increases as a result of increased solubility of phenolic compounds in solvent. Meanwhile, concentration of ethanol in water did not have a significant effect on phenolic compounds extraction in

this study. In addition, Heat was known to have the ability in enhancing the recovery of phenolic compounds. [22], [26].

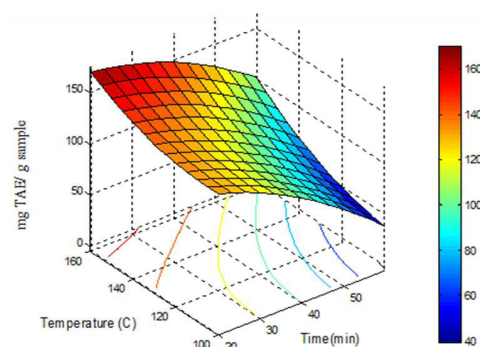


Fig. 4 Surface plot of temperature and extraction times at 50% ethanol for total phenolic contents from rambutan peels

In term of extraction times, it was reported that an extended extraction time favored the extraction of phenolic compounds [27]. This might be due to the time requirement of the exposure of the solute to the release medium where the liquid penetrated into the dried powder, dissolved the solute and subsequently diffused out from the powder. In this study, Fig. 4 showed that it seemed to extending the period of extraction time lead to decreasing extracts yield. This occurrence might be due to the applied extraction high temperature, which decomposed the antioxidant at the extended extraction times i.e., increasing the loss of phenolic by oxidation [22].

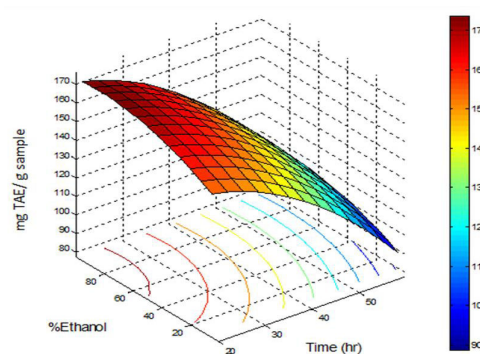


Fig. 5 Surface plot of % ethanol and extraction times at temperature 160 $^{\circ}\text{C}$ for total phenolic contents from rambutan peels

The surface plot in Fig. 5 revealed that the high amounts of phenolic compounds occurred within extraction time 20min. at any concentration of ethanol in mixture. Total phenolic contents decreased inversely with extraction time more than 20min. at any concentration of ethanol in mixture. They increased with increasing % ethanol in water, but did not have a significant effect. As a result, propose statistical model is adequate for predicting phenolic compounds from rambutan peel using SWE.

D. Comparison of Total Phenolic Contents from Various Extraction Methods

The total phenolic contents were extracted from rambutan peel at different extraction methods (maceration, soxhlet and SWE) were presented in Fig. 6.

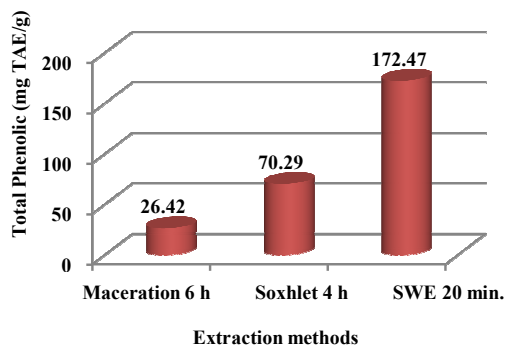


Fig. 6 Comparison of total phenolic contents from different extraction methods

From Fig. 6, the maximum of total phenolic contents from subcritical water extraction, soxhlet and maceration were compared, they showed that phenolic compounds were 172.47, 70.29, 26.42mg TAE/g dry rambutan peel, respectively. Thus, SWE (160°C, 50% ethanol, 20min.) removed higher 2, 5 times of amounts of phenolic compounds and much higher 12, 18 times of extraction time than conventional extraction (maceration 6h and soxhlet 4h, respectively). According to Shi et al. [28], higher temperature and pressure would cause softening of the plant tissue, disrupting the interactions between phenolic compounds and protein or polysaccharides and increasing the solubility of the phenolic compounds, which improves the rate of diffusion, thus giving a higher rate of extraction [25].

IV. CONCLUSION

This study showed that phenolic compounds from rambutan peel were successfully extracted using subcritical water extraction (SWE). The SWE removed high amounts of phenolic compounds from rambutan peels in shorter time and required about 50% less solvent than with conventional extraction. The RSM was used to determine the optimum process parameters that high yield total phenolic contents. The optimal conditions based on both individual (temperature and extraction time) and combination all responses were determined. However, it was suggested that the temperature range could be the optimum for the extraction at single parameters due to this could lead to negative effects, if extended extraction times were applied.

ACKNOWLEDGMENTS

The authors wish to thank the National Research Council of Thailand (NRCT) for financial support of this work. We also

thank Faculty of Engineering, Mahidol University for supported conference, equipment and work place.

REFERENCES

- [1] L.P. Leong, and G. Shui, "An investigation of antioxidant capacity of fruits in Singapore markets," *Food Chem.*, vol.76, pp. 69–75, 2002.
- [2] N. Thitilertdecha, A. Teerawutgulrag, and N. Rakariyatham, "Antioxidant and antibacterial activities of *Nephelium lappaceum* L. extracts," *LWT-Food Sci. Technol. J.*, vol. 41, pp. 2029–2035, 2008.
- [3] U. Palanisamy, H. M. Cheng, T. Masilamani, T. Subramaniam, L.T.Ling, and A. K. Radhakrishnan, "Rind of rambutan, *Nephelium lappaceum*, a potential source of natural antioxidants," *Food Chem.*, vol. 109, pp. 54–63, 2008.
- [4] A. Nawawi, N. Nakamura, M. Hattori, M. Kurokawa, and K. Shiraki, "Inhibitory effects of Indonesian medicinal plants on the infection of herpes simplex virus type 1," *Phytotherapy Res.*, vol. 13, pp. 37–41, 1999.
- [5] N. Thitilertdecha, A. Teerawutgulrag, J.D. Kilburn, and N. Rakariyatham, "Identification of major phenolic compounds from *Nephelium lappaceum* L. and their antioxidant activities," *Molecules*, vol. 15, pp. 1453–1465, 2010.
- [6] M. Naczek, and F. Shahidi, "Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis," *J. Pharm. Biomed. Anal.*, vol. 41, pp. 1523–1542, 2006.
- [7] S. Kondo, K. Tsuda, N. Muto, and J.E. Ueda, "Antioxidative activity of apple skin or flesh extracts associated with fruit development on selected apple cultivars," *Sci. Hort.*, vol. 96, pp. 177–185, 2002.
- [8] M. Skerget, P. Kotnik, M. Hadolin, A. R. Hra's, M. Simonić, and Z.Knez, "Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities," *Food Chem.*, vol. 89, pp. 191–198, 2005.
- [9] N. Thitilertdecha, and N. Rakariyatham, "Phenolic content and free radicals scavenging activities in rambutan drying fruit maturation," *Scientia Horticulturae.*, vol. 129, pp. 247–252, 2011.
- [10] M.T. Escrivano-Bailon, and C. Santos-Buelga, "Polyphenol extraction from foods (Book style with paper title and editor)," In C. Santos-Buelga and G. Williamson (Eds.), "Methods in polyphenol analysis," Cambridge, U.K.: Royal Society of Chemistry, pp. 1–16, 2003.
- [11] J. Kronholm, K. Hartonen, and M.L. Riekkola, "Analytical extractions with water at elevated temperatures and pressures," *TrAC – Trends Anal. Chem.*, vol. 26, pp. 396–412, 2007.
- [12] L. Ramos, E. M. Kristenson, and U.A.T. Brinkman, "Current use of pressurized liquid extraction and subcritical water extraction in environmental analysis," *J. Chromatogr.*, vol. A 975, pp. 3–29, 2002.
- [13] M. Herrero, A. Cifuentes, E. Ibañez, "Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review," *Food Chem.*, vol. 98, pp. 136–148, 2006.
- [14] H. Etoh, N. Ohtaki, H. Kato, A. Kulkarni, and A. Morita, "Sub-critical water extraction of residual green tea to produce a roasted green tea-like extract," *Bioscience, Biotechnology, and Biochemistry*, vol. 74, pp. 858–860, 2010.
- [15] T. Anekpankul, M. Goto, M. Sasaki, P. Pavasant, and A. Shotipurk, "Extraction of anti-cancer damcanthal from roots of *Morinda citrifolia* by subcritical water," *Separation and Purification Technol.*, vol. 55, pp. 343–349, 2007.
- [16] J.E. Cacace, and G. Mazza, "Pressurized low polarity water extraction of lignans from whole flaxseed," *J. Food Eng.*, vol. 77, pp. 1087–1095, 2006.
- [17] G. Shui, G. and L.P. Leong, "Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals," *Food Chem.*, 97, 277–284, 2006.
- [18] C. Negro, L. Tommasi, A. Miceli, "Phenolic compounds and antioxidant activity from red grape marc extracts," *Bioresour. Technol.*, vol. 87, pp. 41–44, 2003.
- [19] A. Basile, M. Jiménez-Carmona, and A.A. Clifford, "Extraction of Rosemary by superheated water," *J. Agri. Food Chem.*, vol. 46, pp. 5205–5209, 1998.
- [20] J.M. Luque-Rodriguez, M. D. Juque de Castro, and P. Perez-Juan, "Dynamic superheated liquid extraction of anthocyanins and other phenolics from red grape skins of wine making residues," *Bioresour. Technol.*, vol. 98, pp. 2705–2713, 2007.

- [21] M.P. Kahkonen, A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala, and M. Heinonen, "Antioxidant activity of plant extract containing phenolic compounds," *J. Agri. Food Chem.*, vol. 47, pp. 3954-3962, 1999.
- [22] M.A. Al-Farsi, and C.Y. Lee, "Optimization of phenolics and dietary fibre extraction from date seeds," *Food Chem.*, vol. 108, pp. 977-985, 2008.
- [23] T. Zhu, H.J. Heo, and K. H. Row, "Optimization of crude polysaccharides extraction from *Hizikia fusiformis* using response surface methodology," *Carbohydrate Polymer*, vol. 82, pp. 106-110, 2010.
- [24] J. Shi, J. Yu, J. Pohorly, C. Young, M. Bryan, and Y. Wu, "Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution," *Food Agri. Envi.*, vol. 1, pp. 42-47, 2003.
- [25] E. Karacabey, and G. Mazza, "Optimisation of antioxidant activity of grape cane extracts using response surface methodology," *Food Chem.*, vol. 119, pp. 343-348, 2010.
- [26] D.R. Pompeu, E.M. Silva, and H. Rogez, "Optimisation of the solvent extraction of phenolic antioxidants from fruits of *Euterpe oleracea* using response surface methodology," *Bioresour. Technol.*, vol. 100, pp. 6067-6082, 2009..
- [27] P.P. Singh, and M.D.A. Saldana, "Subcritical water extraction of phenolic compounds from potato peel," *Food Research International*, vol. 44, pp. 2452-2458, 2011.
- [28] J. Shi, J. Yu, J. Pohorly, C. Young, M. Bryan, and Y. Wu, "Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution," *Food Agri. Envi.*, vol. 1, pp. 42-47, 2003.