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Effect of subcritical water technique and ethanolic solvent on total phenolic contents and antioxidant capacity of Thai rice plant (*O. Sativa* cv. Khao Dawk Mali 105)

[1,3]Rodsamran, P. and [1,2*]Sothornvit, R.

[1]*Department of Food Engineering, Faculty of Engineering at Kamphaengsaen, Kasetsart University, Kamphaengsaen Campus, Nakhonpathom 73140, Thailand*
[2]*Center of Advanced Studies in Industrial Technology, Kasetsart University*
[3]*Program of Food Processing Technology, School of Culinary Arts, Suan Dusit University, Bangkok 10700, Thailand*

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Abstract

Thai rice plant cv. Khao Dawk Mali 105 is an interesting sustainable bioresource which can be extracted as a natural antioxidants. The 15 days of cultivating stage and fresh rice plant extracted by absolute ethanol at 60°C for 0.5 h provided the highest total phenolic contents (TPC). Moreover, increasing ethanol concentration from 0% to 80% also increased TPC (from 67.26 to 116.09 mg GAE/ g DW and from 58.20 to 107.07 mg CAE/ g DW for gallic acid and caffeic acid, respectively) and antioxidant capacity (IC_{50} decreased from 1.43 to 0.64 mg DW/ mL) of the extract. Nevertheless, a higher temperature and longer time of subcritical water extraction (SWE) showed the highest TPC of the extract. In contrast, antioxidant capacity increased as increasing time at a low temperature and decreased as increasing time at a high temperature. The extract from SWE at 150°C for 20 min showed higher TPC (143.89 mg GAE/ g DW and 103.79 mg CAE/ g DW for gallic acid and caffeic acid, respectively); however, the organic extraction using 80% ethanol at 60°C for 5 h presented higher antioxidant capacity (0.64 mg DW/ mL). Therefore, SWE requires a shorter extraction time with safe for the environment and the extract is suitable for food components.

Introduction

Nowadays, natural antioxidants, which mainly come from plant extractions, have been used for medical, pharmaceutical, and food products. Tocopherols, flavonoids, and phenolic acids are the most important class of natural antioxidants found in most plant sources (Naczk and Shahidi, 2006). Phenolic acids, such as caffeic, vanillic, ρ-coumaric, ο-coumaric, protocatechuic, sinapic, ρ-hydroxybenzoic and gallic acids, and ferulic and cinnamic acids, are known as hydrophilic phenols (Riachy *et al*., 2011). Natural phenolic compounds potentially show antioxidant, antimutagen, antitumor, antiinflammatory, and anticarcinogenic properties (Lee *et al*., 2003; Budrat and Shotipruk, 2009).

As known, the cultivating stage of the plant affects the total phenolic contents. Immature plants have high phenolic compounds and proanthocyanidin contents and play as deterrent compounds to prevent from insects and herbivores (Parr and Bolwell, 2000). The highest phenolic contents were found in the immature stage as shown in Maoluang (*Antidesma bunius*) fruits (Butkhup and Samappito, 2011) and Maqui (*Aristotelia chilensis*) Chilean native berries (Fredes *et al*., 2012). Furthermore, Chutichudet *et al*. (2011) found that the phenolic contents in lettuce leave decreased as the day of planting increased from 28 to 59 days. However, no research has explored on phenolic compounds of Thai rice plant during the cultivating stage.

Rice (*Oryza sativa*) is the most important cereal crops in Thailand especially the famous variety Khaodawk Mali 105 or known as 'Hommali'. Rice has a life cycle of 80 to more than 200 days from germination to maturity, depending on the variety, ecology and season (Beighley, 2012). During the development, the rice seed uses nutrient in endosperm to produce roots and stems until the first leaf emerges. The leaves capture solar radiation and produce carbohydrates until the development of the rice plant is completed (Wopereis *et al*., 2009). The nutrients in the rice plant at different cultivating stages may be various; therefore, knowing the

*Corresponding author.
Email: *fengrns@ku.ac.th*
Tel: +66 34281098; Fax: +66 34351404

phenolic compounds and their antioxidant activity will identify the utilization of rice plant.

Generally, an organic solvent is a conventional technique to extract antioxidant from fruits, vegetables, and herbs. In order to avoid structural changes to the target antioxidant compounds during extraction, the types and concentrations of organic solvents would be carefully selected (Liang and Fan, 2013). Ethanol is a common organic solvent to extract polyphenols from palm (*Euterpe oleracea*) plants (Pompeu *et al*., 2009), grape wastes (Casazza et al., 2010) and Seabuckthorn leaves (Kumar *et al*., 2011).

Regarding the undesirable organic solvent on volatile organic compounds to environment concern, water at higher temperature is a desirable solvent for extraction (Hassas–Roudsari *et al*., 2009). Subcritical water technique is an alternative process to use water at a high pressure with temperature between 100 and 374°C to obtain the liquid state of water during the extraction (Hassas–Roudsari *et al*., 2009; Plaza *et al*., 2010). Advantages of subcritical water extraction are the high purity of extracts and the efficiency of the process (Ramos *et al*., 2002). Basically, subcritical water extraction at high temperature provides higher yield and antioxidant compounds with a high degree of capacity of extract than the extraction at low temperature (Plaza *et al*., 2010). A powerful subcritical water has been used to extract phenolic compounds from canola meal (Hassas–Roudsari *et al*., 2009), Seabuckthorn leaves (Kumar *et al*., 2011), and bitter melon (Budrat and Shotipruk, 2009; Minh, 2014). Rice plant is an interesting sustainable bioresource and it is needed to determine the extraction of its antioxidant to utilize as a new alternative natural extract. Therefore, the aims of this study were i) to study the effects of cultivating stage and type of Thai rice plant on the total phenolic contents (TPC) and ii) to determine TPC and antioxidant capacity of the extract from Thai rice plant using a subcritical water technique in comparison of ethanolic extraction.

Materials and Methods

Materials

Thai rice seed (*O. sativa* cv. Khao Dawk Mali 105) was purchased from Chonburi Rice seed centre (Rice Department, Chonburi, Thailand). Absolute ethanol (99.8%, v/v) was purchased from Liquor Distillery Organization (Excise Department, Bangkok, Thailand). Folin–Ciocalteu reagent and sodium carbonate (Na_2CO_3) were purchased from Merck KGaA (Darmstadt, Germany) and Ajax Finechem Pty Ltd (New South Wales, Australia), respectively. Gallic acid monohydrate (≥98.0%), caffeic acid (≥98%) and 2, 2'-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) from Sigma–Aldrich was obtained from U&V Holding (Thailand) CO., LTD (Nonthaburi, Thailand).

Plant preparation

Rice plant was cultivated in the simulated field. Briefly, rice seed was soaked in water for 16 h and water was then discarded. Soaked seed was left to sprout in a wetting cloth for 24 h in the dark place. Sprouted rice seed was planted on the plastic tray containing full of soil and heavily watered. During the first three days of growth, rice seed was also extremely watered twice a day, after that, rice plant was watered once a day.

The cultivating stage at 15, 60, 90, and 120 days of rice plants after planting (one plastic tray/ one cultivating stage) was harvested using scissor and washed with tap water to remove soil and dust. Unprocessed rice plant (fresh) was cut into a small pieces and kept in a plastic bag at 4°C. Dry rice plant was prepared by cutting fresh rice plant into a small pieces and drying in a hot air oven at 60°C for 12 h.

Cultivating stage and type of rice plant

In the first study, the effects of cultivating stage (15, 60, 90, and 120 days after planting) and type of rice plant (fresh and dry) were studied. Briefly, 4 g of rice plant was extracted with 80 mL of absolute ethanol at 60°C for 30 min and then filtered through Whatman No.1 filter paper. The cultivating stage and type of rice plant based on the highest total phenolic contents (TPC) was selected to further study on the effect of extraction techniques.

Ethanolic extraction of rice plant

The selected rice plant from the first study was used to determine the effects of ethanol concentration and time of the extraction at 60°C step by step. First, a sample of 4 g rice plant was extracted with 80 mL absolute ethanol at various extraction times from 0.5 to 24 h and the extraction time was selected based on TPC of the extract. Later, rice plant sample was extracted at the selected extraction time with various ethanol concentrations from 0% to 99.8% (absolute) and the appropriate ethanol concentration was chosen based on TPC and antioxidant capacity of the extract.

Subcritical water extraction (SWE) of rice plant

The selected rice plant was extracted using subcritical water extraction technique in a batch–type reactor as described by Khuwijitjaru *et al*. (2012). A stainless steel of high pressure–resistant vessel (100

ml SUS316, Taiatsu Techno, Tokyo, Japan) was heated and the inside temperature was controlled by an aluminum block heater (Applied Scientific Instruments, Bangkok, Thailand). The pressure inside the vessel was depended on the saturated vapor pressure of water at temperature tested.

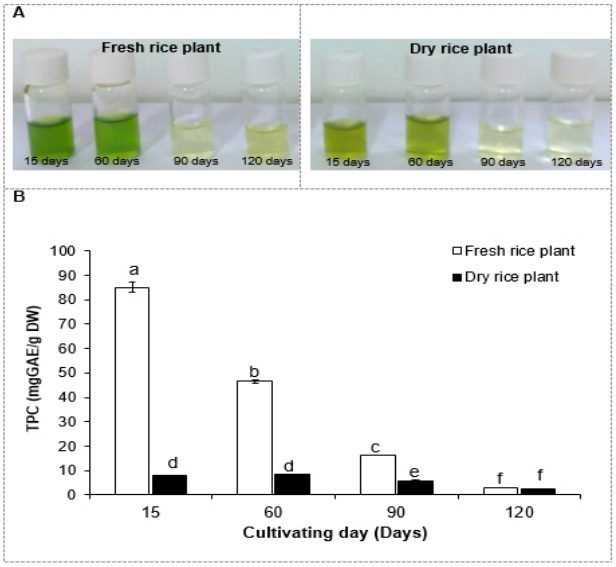
A rice plant sample of 4 g and 80 mL of distilled water were put in the reactor vessel. The temperature was set at 100, 125, 150, and 175°C and held for 10, 20 and 30 min of the extraction. The heat–up time for 100, 125, 150, and 175°C were approximately 6.50, 7.00, 7.50 and 8.50 min, respectively. After reaching the extraction time, the reaction was immediately stopped using cooling water. The extract was taken out from the vessel and filtrated with Whatman No.1 filter paper. Temperature and time of SWE was selected based on TPC and antioxidant capacity of the extract.

Total phenolic contents (TPC)

TPC of the extract was determined by Folin–Ciocalteu method according to the method of Ahmed *et al*. (2010) and Kumar *et al*. (2011) with a slight modification. Briefly, 400 µL of the extract was mixed with 2 mL of 10% Folin–Ciocalteu reagent and reacted for 5 min. Later, 1.6 mL of 7.5% Na_2CO_3 was added, vortexed and incubated the mixture in the dark place at room temperature (28 ± 2°C) for 1 h. The absorbance was measured at 765 nm using a spectrophotometer (UV-Visible 1800, Shimadzu, Tokyo, Japan) and calculated on the basis of the calibration curves of gallic acid and caffeic acid (0–300 mg/L). TPC were expressed as mg gallic acid equivalents per g rice plant on dry weight basis (mg GAE/g DW) or caffeic acid equivalents per g rice plant on dry weight basis (mg CAE/g DW).

Antioxidant capacity

Antioxidant capacity of Thai rice plant extract was measured using DPPH assay according to the method of Hassas–Roudsari *et al*. (2009) with a slight modification. Briefly, the ethanolic and SWE extracts were diluted with ethanol (99.8%) and distilled water, respectively. The 0.5 mL of DPPH• ethanolic solution (0.5 mM) was added into 4 mL of the diluted extract and mixed for 10 s. Each sample was left to stand at room temperature in the dark place for 1 h. The absorbance (A_{sample}) was taken at 515 nm using a spectrophotometer (UV-Visible 1800, Shimadzu, Tokyo, Japan). The spectrophotometer was zeroed with ethanol (99.8%) and distilled water for ethanolic and SWE extractions, respectively. The absorbance of blank (A_{blank}) was obtained from mixing 4 mL of each extract concentration with 0.5 mL of ethanol



[a–f] the same letters were not statistically different (p>0.05).

Figure 1. Appearance (A) and total phenolic contents (TPC) (B) of fresh and dry rice plant extracts from 15, 60, 90 and 120 days by using absolute ethanol (99.8%) as a solvent at 60°C for 30 min.

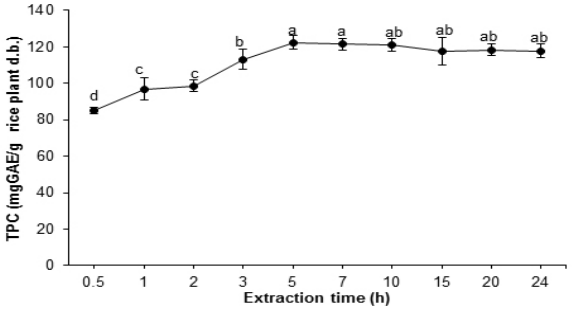
(99.8%) or distilled water. While, the absorbance of control ($A_{control}$) was obtained from mixing 4 mL of ethanol (99.8%) or distilled water with 0.5 mL of DPPH• ethanolic solution. Antioxidant capacity was calculated from equation:

$$Antioxidant\ capacity\ (\%) = \frac{\left[A_{control} - \left(A_{sample} - A_{blank}\right)\right]}{A_{control}} \times 100$$

IC_{50} was calculated from a linear regression of antioxidant capacity curve for all extract concentrations. IC_{50} is the concentration of the extract at a 50% inhibition of the DPPH• radical.

Statistical analysis

The full factorials in completely randomized design (4 levels of cultivating stage and 2 levels of type of rice plant) with three replications were performed to select the highest TPC of rice plant. Regarding ethanolic extraction, a completely randomized design with three replications was done to determine the effect of ethanol concentrations or times on the highest TPC. Moreover, the full factorials in completely randomized design (4 levels of temperature and 3 levels of time) with three replications were performed in SWE. All results were presented as averages along with standard deviation (±SD). The analysis of variance (ANOVA) was analyzed in SPSS software (Version 11.5, SPSS Inc., Chicago, IL). Significant differences among treatment values were tested with Tukey tests ($p<0.05$). Comparison between the best condition of ethanolic extraction and SWE was tested



[a–d] the same letters were not statistically different (p>0.05).

Figure 2. Total phenolic contents (TPC) of 15 days fresh rice plant extracts using absolute ethanol (99.8%) extraction at 60°C for 0.5–24 h.

by independent sample t–test.

Results and Discussion

Effects of cultivating stage and type of rice plant

In this study, rice plant included leave and stem was harvested and used as a raw material. The 15 and 60 days of rice plants consisted of leaves more than stems; hence, these extracts were in green color and the fresh rice plant extracts were in intensive green color than the dry rice plant extracts (Figure 1A). It may probably be due to high chlorophyll contents in fresh leaves at 15 and 60 days of rice plants and a loss of some chlorophyll contents during drying process in the dry samples (Mahanom *et al*., 1999; Rubinskienė *et al*., 2015). However, the extracts obtained from 90 and 120 days of fresh and dry rice plant were in a light brown color. This may be due to a less amount of leaves than stems in these cultivating stages of rice plants resulting in a lower of green color. The interaction between cultivating stage and type of rice plant affected TPC of rice plant extract significantly ($p<0.05$). TPC decreased with an increase in day of cultivating stage in both the fresh and dry rice plant extract (Figure 1B). Generally, the growth periods influence an accumulation of phytochemical compounds. Immature or young plants produce nutrients and bioactive compounds against pests and diseases (Salvador *et al*., 2007). Therefore, the highest TPC was found in the 15 days fresh rice plant extract. Furthermore, as the plant grows, polyphenol synthesis may be interrupted and polyphenol oxidation may be occurred (Shwartz *et al*., 2009) resulting in a lower TPC. The result was similar to the report by Rahman and Wan Rosli (2014). They found that TPC of silk from immature corn was significantly higher than that from mature corn.

The TPC of extract from 15, 60, and 90 days of dry rice plant significantly reduced ($p<0.05$) from

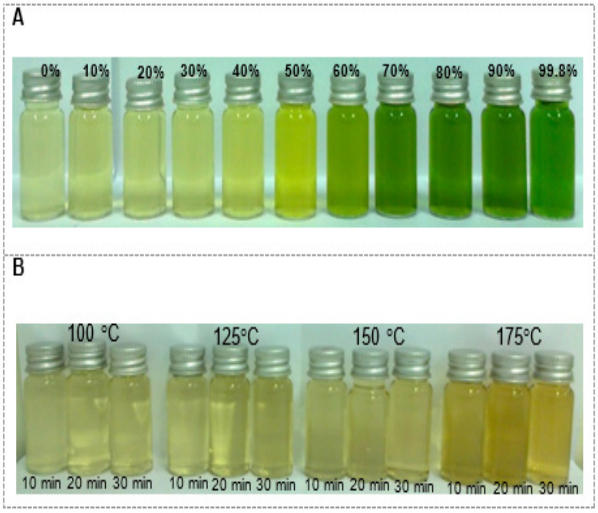


Figure 3. Appearance of extract solutions from 15 days fresh rice plant by using (A) different of ethanol concentrations (0– 99.8%) as a solvent at 60°C for 5 h and (B) subcritical water extraction at different temperatures (100–175°C) and times (10–30 min).

fresh rice plant approximately 11, 6, and 3 times, respectively (Figure 1B). We hypothesized that some active phenolic compounds had been destroyed or evaporated from the dry rice plant during drying process. Meanwhile, there was no significant difference in the TPC of extracts from 120 days in fresh and dry rice plants. Similar results of fresh and dry plant extracts were reported in hazelnut and walnut (Arcan and Yemenicioğlu, 2009) and leave of Datura metel (Alabri *et al*., 2014). Dry sample has an advantage of long term storage but it reduces TPC in raw materials. Thus, the 15 days of fresh rice plant was selected to further use in determination of the ethanolic extraction conditions and the subcritical water extraction conditions on TPC and antioxidant activity of the extracts.

Effect of extraction times in ethanolic extraction

Extraction time significantly affected on TPC of the 15 days fresh rice plant extract ($p<0.05$) (Figure 2). As extraction time increased from 0.5–5 h, TPC also increased. After 5 h of extraction, TPC was slightly constant given approximately 122.47 mg GAE/g DW. It was in agreement with Spigno and De Faveri (2007) who concluded that phenol contents from grape marc did not significantly change from 5 h to 24 h in an absolute ethanolic extraction. This may be explained by Fick's second law of diffusion revealing that the final equilibrium will be attained between the solution concentration in the solid matrix and solvent at a certain period of time (Pinelo *et al*., 2006). Thus, an excessive extraction time was unable to extract more phenolic compounds from rice plant.

Table 1. Total phenolic contents (TPC) and antioxidant capacity (IC_{50}) of extract from 15 days fresh rice plant by using different of ethanol concentrations at 60°C for 5 h.

Ethanol concentration (%, v/v)	Total phenolic content		IC_{50} (mg DW/mL)
	Gallic acid (mg GAE/g DW)	Caffeic acid (mg CAE/g DW)	
0	67.26 ± 5.22^{e}	58.20 ± 5.15^{e}	1.43 ± 0.02^{a}
10	76.30 ± 5.82^{d}	67.19 ± 5.83^{d}	1.31 ± 0.06^{b}
20	83.23 ± 4.14^{cd}	74.11 ± 4.14^{cd}	1.03 ± 0.09^{c}
30	86.01 ± 5.79^{c}	77.04 ± 5.83^{c}	0.86 ± 0.00^{d}
40	96.67 ± 2.92^{b}	87.74 ± 2.81^{b}	0.86 ± 0.05^{d}
50	97.51 ± 7.45^{b}	88.49 ± 7.50^{b}	0.83 ± 0.04^{d}
60	95.63 ± 4.79^{b}	86.77 ± 4.92^{b}	0.85 ± 0.03^{d}
70	114.47 ± 4.88^{a}	105.48 ± 4.84^{a}	0.72 ± 0.03^{e}
80	116.09 ± 3.03^{a}	107.07 ± 2.93^{a}	0.64 ± 0.01^{ef}
90	116.11 ± 4.78^{a}	107.25 ± 4.97^{a}	0.64 ± 0.02^{ef}
99.8	122.47 ± 3.82^{a}	113.67 ± 3.99^{a}	0.54 ± 0.02^{f}

$^{a–f}$ Means in the columns not followed by the same letters are significantly different ($p<0.05$).

Table 2. Total phenolic contents (TPC) of 15 days fresh rice plant extracts using subcritical water extraction at different temperatures (100–175°C) and times (10–30 min).

Temperature (°C)	Time (min)	Total phenolic content		IC_{50} (mg DW/mL)
		Gallic acid (mg GAE/g DW)	Caffeic acid (mg CAE/g DW)	
	10	71.91 ± 1.77^{i}	51.71 ± 1.28^{i}	1.90 ± 0.09^{a}
100	20	75.23 ± 1.72^{i}	54.12 ± 1.24^{i}	1.16 ± 0.05^{b}
	30	77.68 ± 2.66^{i}	55.88 ± 1.93^{i}	1.21 ± 0.08^{b}
	10	93.89 ± 7.40^{h}	67.61 ± 5.36^{h}	1.20 ± 0.03^{b}
125	20	106.13 ± 4.67^{g}	76.46 ± 3.33^{g}	1.00 ± 0.02^{cd}
	30	110.01 ± 5.08^{g}	79.27 ± 3.68^{g}	0.94 ± 0.03^{d}
	10	118.78 ± 2.60^{f}	85.62 ± 1.88^{f}	1.16 ± 0.06^{b}
150	20	143.89 ± 3.32^{e}	103.79 ± 2.40^{e}	0.80 ± 0.05^{e}
	30	155.26 ± 1.77^{d}	112.02 ± 1.27^{d}	1.15 ± 0.07^{b}
	10	221.05 ± 2.56^{c}	159.62 ± 1.85^{c}	0.97 ± 0.08^{c}
175	20	252.27 ± 6.04^{b}	182.21 ± 4.37^{b}	1.11 ± 0.02^{b}
	30	288.01 ± 3.60^{a}	208.07 ± 2.61^{a}	1.22 ± 0.06^{a}

$^{a–i}$ Means in the columns not followed by the same letters are significantly different ($p<0.05$).

Therefore, the 5 h of extraction was chosen to further determinate the effect of ethanol concentrations on TPC and antioxidant capacity.

Effect of ethanol concentrations in ethanolic extraction

Increase in ethanol concentration showed an intensity of green color in the extract (Figure 3A). The green color of extract was due to the green pigment of chlorophyll in several varieties of plant leave. The concentration of solvent is one of the main parameter of the ethanolic extraction. Kamal *et al*. (2006) suggested that the minimum of ethanol concentration to extract the chlorophyll must be at least 70%. Correspondingly, the fresh rice plant extract at 70% to 99.8% ethanolic extractions showed the intensive green color.

TPC of the 15 days fresh rice plant extract increased when increasing from 0% to 70% ethanol and leveling off from 70% to 99.8% in both gallic acid and caffeic acid (Table 1). As known, higher concentration of ethanol lowers viscosity of solvent thus the extraction rate increases due to a higher solvent wetting on rice plant (Kamal *et al*., 2006). In addition, an increase in ethanol concentration results in the reduction of dielectric constant; therefore, this makes an appropriate solubility of less–polar compounds such as phenolic compounds (Cacace and Mazza, 2003). Similarly, the TPC of Euterpe oleracea fruit increased when extracted with approximately 70% ethanol (Pompeu *et al*., 2009).

Furthermore, addition of a small amount of water into organic solvent could improve the efficiency of phenolic compound extraction as compared with single organic solvent system (Spigno *et al*., 2007; Chew *et al*., 2011a; Chew *et al*., 2011b). Moreover, lower ethanol concentration extraction takes an advantage of lower cost in economic and safer for human health. Thus, we selected 70% ethanol to extract TPC in this study since there was no significantly difference in TPC from using absolute ethanol. However, the selection of ethanol concentration to extract natural antioxidants depends on the variety of phenolic compounds in different plants; for example, the optimum ethanol concentrations for phenolic extraction from mangosteen and rambutan peel were 60% and 80%, respectively (Samuagam *et al*., 2013).

The DPPH• test is easy and accurate method to test the antioxidant capacity of extracts. A 50% scavenging of DPPH• (IC_{50}) are used to represent the antioxidant capacity of rice plant extracts. A low IC_{50} value is associated with a stronger DPPH• scavenging capacity (Samuagam *et al*., 2013). Similar to the result of TPC, when ethanol concentration increased, IC_{50} of the extract decreased (Table 1) resulting in an increase in antioxidant capacity. IC_{50} values of the extract at 80% and 90% ethanol were no significantly differences from using absolute ethanol. This may be due to such a higher ethanol concentration enough to extract both polar and less–polar phenolic compounds with higher antioxidant capacity (Chirinos *et al*., 2007). In contrast, without ethanol (0%) was unable to extract less–polar phenolic compounds due to the high polarity of water resulting in a lower antioxidant capacity. The similar results were observed in the extracts of *Orthosiphon stamineus* (Chew *et*

al., 2011a *Centella asiatica* (Chew *et al*., 2011b), and rambutan and langsat peel (Samuagam *et al*., 2013) showing that using 80% ethanol provided the highest antioxidant capacity. Thus, the best ethanolic extraction condition of rice plant was using 80% ethanol at 60°C for 5 h in this study.

Effect of subcritical water extraction (SWE)

The interaction between temperature and time of SWE affected TPC and antioxidant capacity of rice plant. An increase in temperature and time provided higher TPC in both gallic acid and caffeic acid (Table 2). The extracts from SWE at higher extraction temperature (175°C) had higher TPC than at lower temperature at the same extraction time (221.05–288.01 mg GAE/g DW and 159.62–208.07 mg CAE/g DW). This may be due to the semi–polar nature of the phenolic compounds in rice plant that would render them more soluble in SWE at high temperature than at low temperature. The result was in agreement with a higher TPC of canola meal extract from SWE at 160°C than that at 110°C (Hassas–Roudsari *et al*., 2009). Moreover, Baek *et al*. (2008) showed that TPC from licorice roots using SWE increased with increasing in temperature (50–300°C) and time (10–60 min) of extraction. However, rice plant extracts from SWE at 100°C for 10, 20 and 30 min of the extraction time showed similar TPC.

The extract obtained from SWE at 100°C was a light brown color (Figure 3B). At temperature higher than 100°C, when temperature and time of extraction increased, color intensity of the extract also increased. The change of color may be due to the Maillard reaction in the extract at high temperature. The color of the extract became intense corresponding to the increase in TPC at higher temperatures and longer times of extraction. This observation was in agreement with previously report by Singh and Saldaña (2011) who found that the phenolic compounds from potato peel using SWE was directly corresponded to the color extract as the temperature increased from 100°C to 180°C.

Rice plant extract from SWE at 100°C for 10 min provided the highest IC_{50} (1.90 mg DW/mL, Table 2). At 100°C and 125°C, longer extraction time decreased IC_{50} of the extracts. This was possible due to a higher TPC of the extract resulting in a higher degree of antioxidant capacity. IC_{50} of rice plant extract from SWE at 150°C extremely decreased when extraction time increased from 10 to 20 min. However, a longer extraction time increased IC_{50} of rice plant extract at 175°C. This was possible explained by the degradation of some phenolic compounds. In this study, TPC was determined in terms of gallic acid and caffeic acid by Folin–Ciocalteu method. Generally, gallic acid is lower degradability than the other bound form phenolics (protocatechuic acid and ρ-hydroxyl benzoic acid) and free form phenolics (chlorogenic acid and caffeic acid) during extraction at higher temperature of SWE (Singh and Saldaña, 2011). Meanwhile, the higher caffeic acid values can be explained by the fact that the Folin–Ciocalteu reagent is not specific to any form of phenolic compounds (Šeruga *et al*., 2011). In summary, using SWE at higher temperature and longer time showed higher TPC but lower antioxidant capacity of the extract (Table 2). Therefore, the best condition for the extraction from rice plant with SWE was at 150°C for 20 min based on the highest antioxidant capacity and acceptable TPC.

Comparison of ethanolic extraction with SWE

There was a significant difference in TPC and antioxidant capacity of the extract obtained from 80% ethanol extraction at 60°C for 5 h and SWE at 150°C for 20 min. The 15 days fresh rice plant extracted by SWE at 150°C showed higher gallic acid and lower caffeic acid (143.89 mg GAE/ g DW and 103.79 mg CAE/ g DW, respectively) than using 80% ethanol extraction at 60°C (116.09 mg GAE/ g DW and 107.07 mg CAE/ g DW, respectively). In addition, an extraction time of SWE (20 min) was shorter than solvent extraction (5 h). As known, the derivatives of hydroxybenzoic acid (bound form phenolic, gallic acid) are more soluble in SWE, while the derivatives of hydroxycinnamic acid (free form phenolic, caffeic acid) are more soluble in organic solvent (Singh and Saldaña, 2011). Similar result was reported that TPC in terms of gallic acid from potato peel extracted with SWE at 180°C for 60 min was higher than that obtained by 50% ethanol extraction at 65°C for 3 h (Singh and Saldaña, 2011). Moreover, Kumar *et al*. (2011) found that higher TPC (gallic acid) from Seabuckthorn leaves extract was found in SWE at 150°C for 15 min compared with using 70% ethanol extraction at 80°C for 6–10 h.

Meanwhile, the extract using 80% ethanol was lower IC_{50} value (0.64 mg DW/mL) than using SWE (0.80 mg DW/mL) showing a higher antioxidant capacity. This may be due to more hydroxycinnamic acid derivatives extracted using 80% ethanol than using SWE and the free form phenolics exhibit higher antioxidant activity compared with the bound form phenolics (Andreasen *et al*., 2001). However, the solvent extraction may have undesirable effects on the environment, human health, and food components. Furthermore, this method is a time consuming process to remove the solvent out. Thus, subcritical water

extraction is an alternative substitution of ethanolic extraction if the equipment is available.

Conclusion

The total phenolic contents from rice plant were successfully extracted by using ethanol and subcritical water as a solvent. Higher yield of total phenolic content was observed from the 15 days of fresh rice plant at 60°C for 5 h using absolute ethanol. The total phenolic contents of rice plant extract increased as increase in ethanol concentration from 0–70%. Above 70%, the total phenolic contents were nearly constant. When the ethanol concentration increased, IC_{50} of the ethanolic extract decreased. The interaction between temperature and time significantly affected the total phenolic contents and antioxidant capacity. Increasing in temperature and time, total phenolic contents increased. However, antioxidant capacity of the extract decreased as longer extraction time at high temperature. Rice plant extract using subcritical water at 150°C for 20 min provided higher total phenolic contents than using 80% ethanol as a solvent at 60°C for 5 h. Nonetheless, ethanolic extraction exhibited higher antioxidant capacity than subcritical water extraction. However, subcritical water extraction takes less extraction time and water is a friendly environmental extraction than ethanolic extraction. Particularly, Thai rice plant showed a potential bioresource as natural antioxidant compounds.

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