ผลของสภาวะการทำแห้งและระยะเวลาแช่ชาต่อปริมาณสารประกอบฟีนอลิกและฤทธิ์การต้านสารอนุมูลอิสระของชากลีบบัว

The Effects of Drying and Steeping Conditions on Phenolic Contents and Antioxidant Activities of Lotus Petal Tea

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บทคัดย่อ

งานวิจัยนี้ศึกษาผลของอุณหภูมิ เวลาในการทำแห้งชากลีบบัว และระยะเวลาแช่ชาต่อปริมาณความชื้น น้ำอิสระ สารประกอบฟีนอลิกทั้งหมด และฤทธิ์การต้านสารอนุมูลอิสระ (วิธี DPPH และ FRAP) โดยจะทำแห้งกลีบบัวที่อุณหภูมิ 50 60 หรือ 70 องศาเซลเซียส เป็นระยะเวลา 1-6 ชั่วโมง หลังจากนั้นจะนำชากลีบบัวมาแช่ในน้ำร้อน (90 องศาเซลเซียส) เป็นเวลา 3 หรือ 5 นาที ผลการทดลองพบว่าปริมาณความชื้นและน้ำอิสระในตัวอย่างจะลดลงเมื่อระยะเวลาในการทำแห้งเพิ่มสูงขึ้น โดยอัตราการทำแห้งของตัวอย่างที่อุณหภูมิ 70 องศาเซลเซียส จะสูงกว่าตัวอย่างทำแห้งที่อุณหภูมิ 50 และ 60 องศาเซลเซียส แต่มีปริมาณสารประกอบฟีนอลิกทั้งหมดและฤทธิ์การต้านสารอนุมูลอิสระน้อยกว่าตัวอย่างที่ทำแห้งอุณหภูมิต่ำกว่า ชากลีบบัวที่เตรียมจากสภาวะการทำแห้งที่อุณหภูมิ 50 องศาเซลเซียส และแช่ชา 5 นาทีจะมีค่าสารประกอบฟีนอลิกทั้งหมดสูงกว่าตัวอย่างอื่น ๆ (16.18 mg/mL) ($p<0.05$) ในชากลีบบัวที่ผ่านการทำแห้งที่อุณหภูมิ 50 และ 60 องศาเซลเซียส ระยะเวลาในการแช่ชาที่นานขึ้นจะส่งผลต่อการเพิ่มขึ้นของสารประกอบฟีนอลิกทั้งหมดและฤทธิ์การต้านสารอนุมูลอิสระ ($p<0.05$) สภาวะที่เหมาะสมในการทำแห้งและแช่ชากลีบบัวคือการทำแห้งที่อุณหภูมิ 50 องศาเซลเซียส ระยะเวลา 5 ชั่วโมง และใช้เวลาแช่ชา 5 นาที

คำสำคัญ: กลีบบัว สภาวะการทำแห้ง สารต้านอนุมูลอิสระ ปริมาณสารประกอบฟีนอลิก

ABSTRACT

This study investigated the effect of drying temperature and time, and steeping duration on moisture content, water activity (a_w), total phenolic content and antioxidant activity using DPPH and ABTS assays of lotus petal tea. The lotus petal was dried at 50, 60, or 70°C for 1-6 hours. Then, the dried petal was steeping in hot water (90°C) for 3 or 5 min. The results showed that moisture content and a_w of lotus petal decreased as increased drying time. The slope of drying curve of lotus petal that dried at 70°C was higher than those of 50 and 60°C. However, drying lotus petal at 70°C presented lower total phenolic content and antioxidant activities than those dried at a lower temperature. The highest total phenolic content (16.18 mg/mL) was detected in the lotus petal tea prepared by dried at 50°C and steeped for 5 min ($p<0.05$). The longer steeping duration significantly increased the amount of total phenolic and antioxidant activities of the lotus petal that dried at 50 and 60 °C ($p<0.05$). The optimum drying and steeping conditions were dried the lotus petal at 50 °C for 5 hours and steeped for 5 min.

Keywords: lotus petal, drying conditions, antioxidant, phenolic content

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INTRODUCTION

Tea is one of the most consumed drink worldwide because of its antioxidant activity which contributes several health benefits [1]. Tea has been previously reported in numerous literatures as a rich source of natural bioactive compounds such as carotenoids, phenolic acids, flavonoids, coumarins, alkaloids, polyacetylenes, saponins and terpenoids [2]. Several plant materials have been used as material for herbal tea production such as chamomile, peppermint, rosehips or ginger, and lime, pomegranate, blueberry, apple has been used for fruit tea [3]. However, there is limited research and information about lotus tea. Lotus (*Nelumbo nucifer*) is also known as Indian lotus which is cultivated in Southeast Asia. Lotus has been used for food and medicine because of its health promotion activities such as antioxidant, anti-obesity, anti-neurodegenerative, psychopharmacological, and anti-inflammatory effects. There were 12 phenolic acids and 89 to 90 flavonoids have been detected in lotus [4]. Moreover, 3 phenolic acids, 8 flavonoids and anthocyanin have been detected in lotus with water extraction. The highest gallic acid content was detected in petal. In addition, luteolin, quercetin, naringenin, isorhamnetin, cyanidin, delphinidin were also detected in the petal [5].

Drying is a crucial factor in tea production. Generally, a tea leaf is dried at 90-140°C and commonly consumed as hot tea infusions. Heat treatment could terminate enzyme reactions and cause the loss of moisture and bioactive compounds. Moreover, new compounds are produced during heat treatment [6]. High temperature or high drying rate cause a case hardening and the loss of bioactive constituents which affect tea quality. The antioxidant activities of herbal tea leaves decreased after increase drying temperature to 80 and 90°C [7]. Therefore, the low drying rate and temperature (50, 60, or 70°C) on antioxidant activities of lotus petal tea is focused on this study. There are some studies avoid heat treatment according to the research of Damiani et al. (2014) [8]. White tea was infused in the water at room temperature for 2 hours and had higher in healthful bioactive compounds compared to hot water infusion. However, it took a long time which was not commonly tea consuming [8]. Moreover, steeping condition is a crucial parameter that affects to health beneficial properties of tea [9]. Therefore, this study aims to investigate the effect of drying temperature (50, 60, or 70°C) and time (1-6 hours), and steeping duration (3 or 5 min) on moisture content, water activity (a_w), total phenolic content and antioxidant activities of lotus petal tea. The optimum conditions such as drying temperature and time and steeping duration for exhibiting greater health benefits of lotus petal tea were also reported.

MATERIAL AND METHODS

1. Materials

Lotus (*Nelumbo nucifer*) was purchased from a local farm in Suphan Buri Province, Thailand.

2. Dried lotus petal preparation

Lotus petal was washed and let it dry at room temperature. The lotus petal was dried by using tray dryer (Model 5302023, Progress electronic, Bangkok, Thailand) at 50, 60 or 70°C for 1-6 hours. Moisture content and a_w of lotus

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petal were measured every hour during drying until the moisture content lower than 10%. The dried lotus petal was packed in a laminated aluminum foil bags kept under vacuum and dry condition at room temperature for 2 weeks before analysis.

3. Lotus petal tea preparation

According to the Notification of the Ministry of Health Thailand, the standard of moisture content of tea products should be lower than 10%. Therefore, the lotus petals that dried at 50, 60, and 70°C for 5 hours were selected for tea preparation and determined total phenolic content and antioxidant activities. The 0.5 g of dried lotus petal was dissolved in 20 mL of hot distilled water (90°C) [8]. Then, the sample was steeped for 3 or 5 min. The extracted liquid was separated and analyzed total phenolic content and antioxidant activities.

4. Moisture content and water activity (a_w) analysis of lotus petal

The moisture content and a_w of lotus petals that dried at 50, 60, and 70°C for 1-6 hours were measured. The lotus petal was randomly taken every hour and cut into small pieces. Then, 2.0 g of lotus petal was used for the moisture content and a_w analysis. Moisture content was performed according to the method of AOAC (2000) [10]. The a_w was measured by using a water activity meter (Model 4TE, Aqualab, Washington, USA).

5. Determination of total phenolic content

The total phenolic content of tea extract was determined by the method of Pereira et al. (2014) with slight modification [11]. The tea extract (1 mL) was mixed with 1 mL of Folin-Ciocalteu reagent and kept at room temperature for 10 min. Then, 10 mL of 7% (w/v) sodium carbonate was added and the total volume was adjusted to 25 mL by using distilled water and kept at the dark in room temperature for 30 min. The reaction was stopped in an ice bath. The absorbance at 750 nm was recorded. Gallic acid was used as a standard and the total phenolic content was expressed as milligrams of gallic acid equivalents (mg GAE/mL tea).

6. Determination of antioxidant activities

The 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the extract was determined by the method of Xu and Chang (2007) with slight modification [12]. A 200 µL of the extract was mixed with 600 µL of DPPH solution and 5.20 mL of ethanol. The mixture was kept in the dark for 30 min. Then, the absorbance of the mixture was measured at 531 nm. The DPPH scavenging activity was calculated by the following equation (1)

$$\text{Scavenging activity (\%)} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ sample} \times 100 \quad (1)$$

Control is the absorbance of the solution absence of any sample.

The 2,2 '-azino-bis (3-ethylbenzthiazoline) 6-sulfonic acid (ABTS) radical scavenging activity was determined by the method of Re et al. (1999) with some modification [13]. ABTS stock solution was prepared by mixing 7.0 mM of ABTS and 2.4 mM of potassium persulfate with a ratio of 1:1 (v/v) for 12-16 hours in the dark. Then, the stock solution was diluted to the

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absorbance of 0.70 ± 0.01 with ethanol at 734 nm. The ABTS working solution (50 µL) was mixed with 10 µL of the extract, following incubation for 10 min in the dark. Then, the absorbance of the mixture was measured at 734 nm. The ABTS scavenging activity was calculated by the following equation (2).

$$\text{Scavenging activity (\%)} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ sample} \times 100 \quad (2)$$

Control is the absorbance of the solution absence of any sample.

7. Statistical analysis

Each experiment was carried out in three replications. All data were analyzed by ANOVA and Duncan's new multiple range test procedures to separate means, and differences were reported as significant at $p < 0.05$, using SPSS version 12.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

1. Effect of drying conditions on moisture content and a_w

The moisture content of lotus petal that treated with different drying temperatures is presented as a drying curve in Figure 1. The moisture content of fresh lotus petal was 85-86% and it decreased as increased drying time. The lowest moisture content was found at 6 hours (4-6%) depending on drying conditions. The moisture content was dramatically decreased with the highest and lowest rate in lotus petal that dried at 70 and 50 °C, respectively. The highest slope of moisture reduction of lotus petal that dried at 50, 60 and 70 °C were dected in 3-5, 2-5, and 1-3 hours of drying time, respectively.

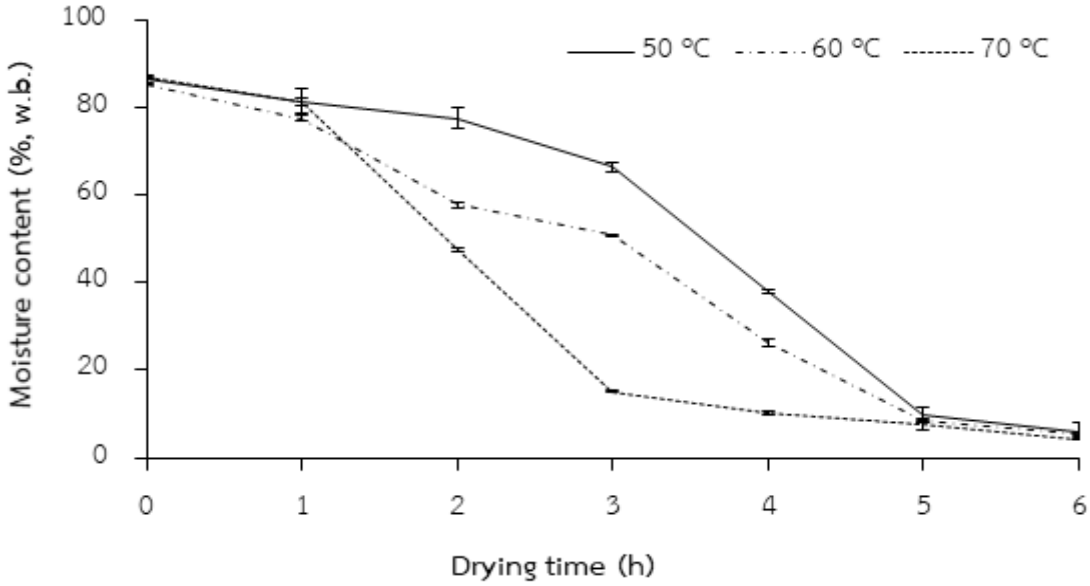

Figure 1 Drying curve of lotus petal that prepared by different drying temperatures.

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The a_w of lotus petal that prepared by different drying temperatures is exhibited in Table 1. The a_w of fresh lotus petal was approximately 0.980. The lowest a_w was found at 6 hours (0.36-0.53) depending on drying temperature. The a_w of lotus petal was gradually decreased as increasing drying time and the a_w was dramatically decreased after drying for 3 hours. The significantly lowest a_w was detected in lotus petal that dried at 70 °C for 6 hours.

Table 1 The water activity of lotus petal that prepared by different drying temperatures.

Drying time (hours)	Drying temperature (°C)		
	50	60	70
0	0.981 ± 0.001 ^{a,A}	0.984 ± 0.007 ^{a,A}	0.989 ± 0.001 ^{a,A}
1	0.982 ± 0.003 ^{a,A}	0.975 ± 0.001 ^{a,B}	0.976 ± 0.001 ^{b,B}
2	0.965 ± 0.004 ^{b,B}	0.971 ± 0.003 ^{a,A}	0.968 ± 0.004 ^{c,B}
3	0.959 ± 0.004 ^{c,A}	0.942 ± 0.001 ^{a,B}	0.938 ± 0.002 ^{d,C}
4	0.914 ± 0.015 ^{d,A}	0.830 ± 0.002 ^{b,B}	0.742 ± 0.003 ^{e,C}
5	0.694 ± 0.012 ^{e,A}	0.488 ± 0.006 ^{c,B}	0.390 ± 0.006 ^{f,C}
6	0.533 ± 0.011 ^{f,A}	0.423 ± 0.006 ^{d,B}	0.361 ± 0.011 ^{g,C}

Capital letter in each drying temperature, different letters represent significant differences ($p<0.05$).
Lowercase letter in each drying time, different letters represent significant differences ($p<0.05$).

The higher drying temperature reduced the moisture content and a_w more than those of drying at lower temperature, resulting in reducing the production time base on similar moisture content. Ismanto et al. (2017) reported that drying surian leaves at 90°C spent a shorter time than those of drying at 50°C [14]. This agreed to the result of Teshome et al. (2014) who reported that the production time of black tea that dried at 130°C was lower than those of 90°C [15].

At the initial drying duration (1-3 hours) of the lotus petal dried at 70°C, the moisture content and a_w were dramatically drop ($p<0.05$). However, the reduction rate of moisture content of lotus petal dried at 70 °C was higher when compared to the reduction rate of a_w. This might be due to drying with high temperature in this study might cause a case hardening that retards the capillary action. Thus, some existing free water in the dried lotus petal could not be removed, resulted in the lower reduction rate of a_w at the first 3 hours of drying. In tea particles, there are 3 categories of moisture. A rapid rate of drying removes free and surface moisture but bonded moisture in the core needs to undergo a slow rate of drying [16]. Drying with high temperature in this study not only caused a case hardening that resulted in the yield inferior tea quality, but it is also affected the color, flavor, nutritional, and bioactive compounds of tea.

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According to the Notification of the Ministry of Health Thailand, the standard of moisture content of tea products should be lower than 10%. In this study, the moisture content of lotus petals that dried at 50, 60, and 70°C for 5 hours were 9.82, 8.46 and 7.54%, respectively. Therefore, the drying duration at 5 hours meet the standard requirement of tea and it was selected for study total phenolic content and antioxidant activities further.

2. Effects of drying conditions and steeping time on total phenolic content and antioxidant activities

The total phenolic content of the lotus petal extract prepared by different drying conditions and steeping time is shown in Figure 2. The total phenolic content of the extract of lotus petal that dried at 70°C was lower than those dried at 50 and 60°C ($p<0.05$). Since, phenolic could be degraded by using high temperature. Cheong et al. (2005) reported that drying with high temperature could reduce bioactive compounds such as catechin of Korean green tea [17]. Moreover, the total phenolic content of herbal tea leaves decreased as increased drying temperature [14]. Besides, the longer steeping time significantly increased the total phenolic content of the extract prepared from lotus petal that dried at 50 and 60°C. The total phenolic content of the extract prepared by lotus petal dried at 50 and 60°C and steeped for 5 min were significantly higher those steeped for 3 min ($p<0.05$). This is explained by the increasing contact time between lotus petals and hot water. Moreover, several compounds from lotus petals were soluble in hot water. These also published in the study of Castiglioni et al. (2015) who found that the longer steeping time (120 min at room temperature) of green and white tea presented the higher total phenolic content than those short time steeping (7 min at 90°C) [9]. However, phenolic contents of the extract prepared by lotus petal dried at 70 °C tend to decrease as increased steeping duration ($p\geq0.05$). This might be due to the case hardening of lotus petal dried at 70°C might retard the water permeability and the oxidation of phenolic compounds during longer steeping duration [18]. This might also occur in the extract of lotus petal dried at 50 and 60 °C. However, the higher amount of phenolic compounds of the extract prepared by lotus petal dried at 50 and 60 °C had greater solubility resulted in the increasing of total phenolic content when increasing the steeping duration.

The radical scavenging activity of the lotus petal extract prepared by different drying temperatures and steeping time using the DPPH and ABTS assays are presented in Figure 3 and 4, respectively. The strongest antioxidant activities from both DPPH (83.22%) and ABTS (82.44%) assays were the extract prepared by dried lotus petal at 50 °C and steeped for 5 min ($p<0.05$). These were higher than those dried at 60°C (DPPH = 77.01%, ABTS = 74.75%) and 70°C (DPPH = 61.17%, ABTS = 54.17%). The longer steeping time had the potential to increase the radical scavenging activity of the extract prepared by dried lotus petal at 50 and 60°C. However, the radical scavenging activities of the extract prepared by dried lotus petal at 70°C

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decreased when increased steeping time ($p<0.05$). This might be due to the loss phenolic compounds during drying and steeping as showed in Figure 2.

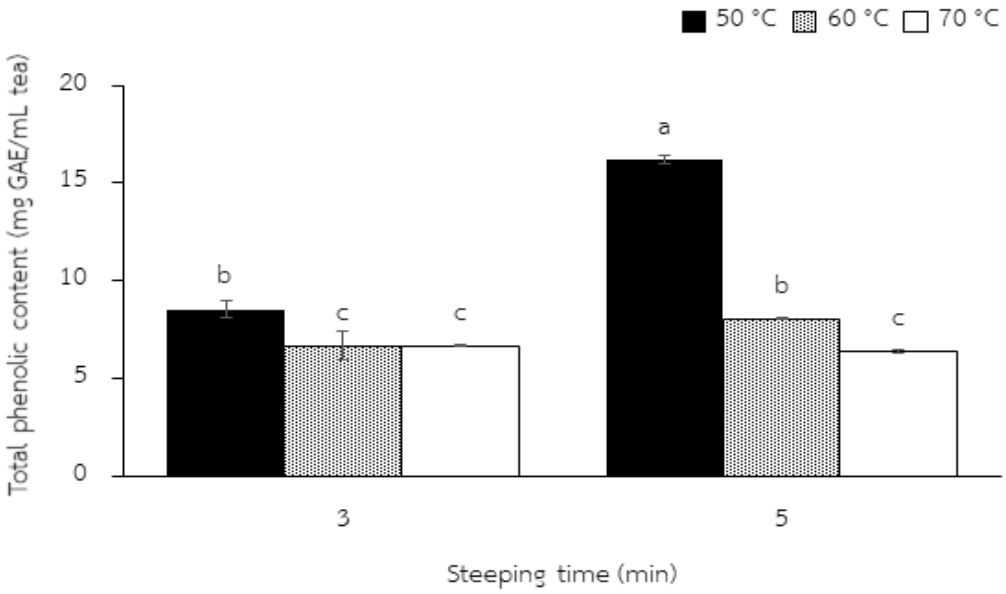

Figure 2 Total phenolic content of the lotus petal extract prepared by different drying temperatures and steeping times.

Different letters represent significant differences ($p<0.05$).

The high total phenolic content of the lotus petal extract contributed to high antioxidant activities from both DPPH and ABTS assays. This agreed to the result of Fu et al. (2011) who reported that the total phenolic content of herbal tea was positively correlated with antioxidant capacities [19]. Yang and Liu (2013) reported that the antioxidant activity of tea infusions was higher than those tea that contains lower total phenolic content. This is because of phenolics are suggested to be the major bioactive compounds responsible for antioxidant profiles [20].

Temviriyanukul et al. (2020) also supported that total phenolic content surpassed antioxidant properties. Moreover, gallic acid was the highest phenolic acids in petal. In addition, flavonoids such as luteolin, quercetin, naringenin, isorhamnetin, cyanidin, delphinidin and anthocyanin were also detected in the petal [5]. These compounds were main bioactive compounds in lotus petal tea and could be a contributor to lotus petal tea's antioxidant activities. However, those bioactive compounds could be destroyed by high temperature process. As a result of the reduction of total phenolic content when increase drying temperature that presented in Figure 2, the total phenolic content of the extract prepared by dried lotus petal at 70°C had significantly lower than those dried at 50 and 60°C ($p<0.05$). This could be supported by the study of Ismanto et al. (2016) who reported

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that the amount of tannin and total polyphenols, and the activity of antioxidants of herbal tea leaves decreased as increasing drying temperature to 80 and 90°C [7]. Moreover, polyphenols and total flavonoids content, and radical scavenging rates of loquat flower tea reduced while increasing the temperature during drying of both vacuum drying (60 °C) and hot-air drying (100°C) [21].

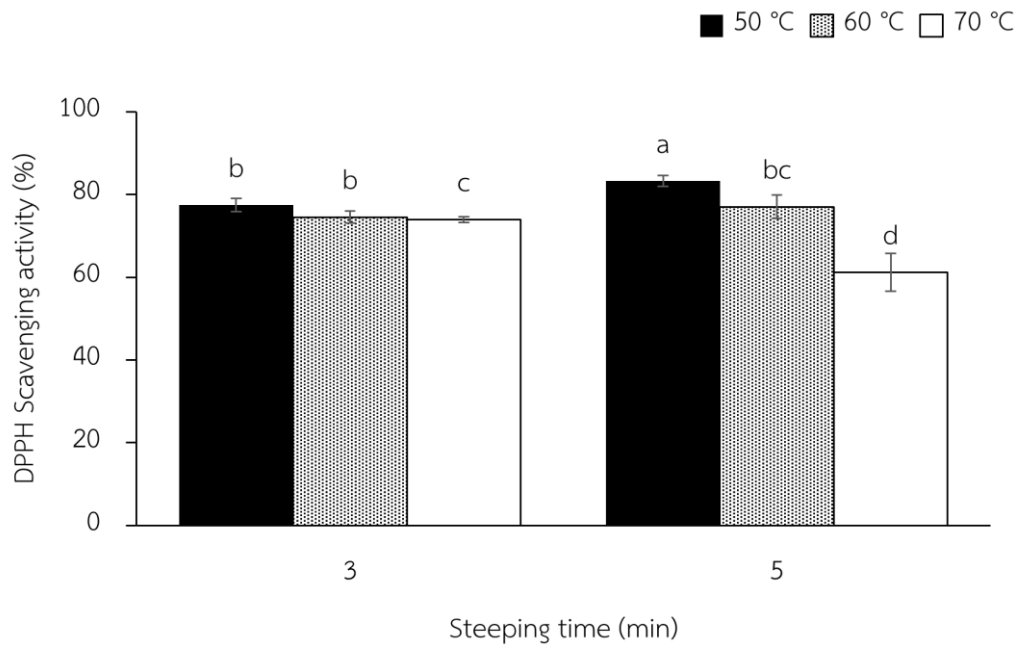

Figure 3 DPPH radical scavenging activity of the lotus petal extract prepared by different drying temperatures and steeping times.
Different letters represent significant differences ($p<0.05$).

The antioxidant activities of the extract prepared by lotus petal dried at 50 and 60 °C increased while increasing the steeping duration. This could be explained by the higher extraction efficiency of 5 min steeping duration resulted in higher amount of phenolic than those steeped for 3 min (as presented in Figure 2). However, total phenolic content of the extract prepared by lotus petal dried at 70 °C and steeped for 3 min was not significantly different from 5 min ($p\geq0.05$), but its antioxidant activities significantly decreased as increasing steeping duration ($p<0.05$). It possible due to phenolic compounds could generally increase soluble, while some main active phenolic compounds might be degraded at high temperature during steeping for a longer duration. Gan et al. (2017) revealed that the high temperature of drying had distinct impacts on different phenolic compounds in mung bean sprouts. The drying at higher temperature (70 and 80°C) reduced rutin and *p*-coumaric acid contents, while increasing the content of caffeic acid [22]. Moreover, this could be explained by

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the loss of some compounds such as flavonoids and anthocyanin during steeping for a longer duration. Those compounds also had an antioxidant property and could be degraded by using high temperature.

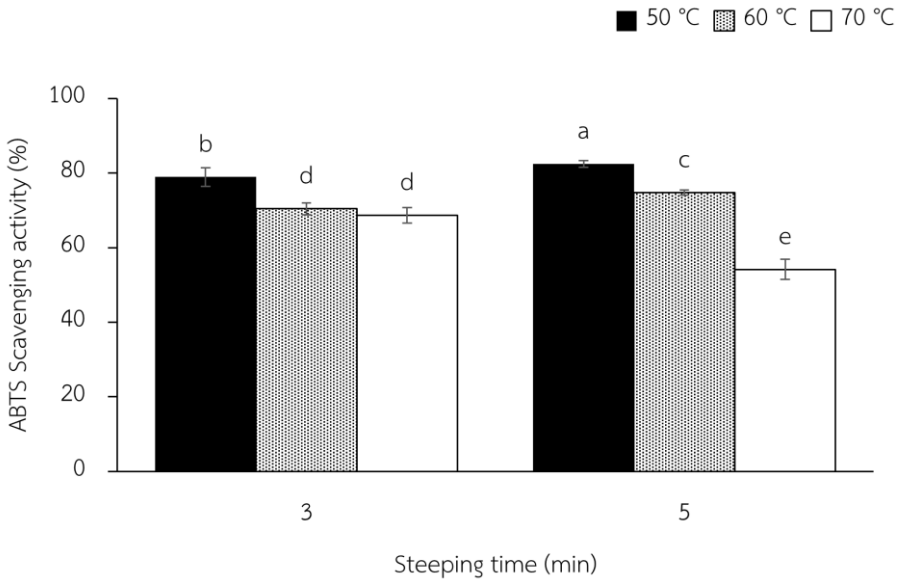

Figure 4 ABTS radical scavenging activity of the lotus petal extract prepared by different drying temperatures and steeping times.
Different letters represent significant differences ($p<0.05$).

CONCLUSIONS

The moisture content and a_w of lotus petal decreased as increased drying time. The slope of the drying curve of lotus petal that dried at 70 °C was higher than 50 and 60 °C. However, drying lotus petal at 70 °C presented lower total phenolic content and antioxidant activities than those dried at a lower temperature. The highest total phenolic content was detected in the lotus petal extract prepared by dried at 50 °C and steeped for 5 min ($p<0.05$). The longer steeping duration significantly increased the amount of total phenolic and antioxidant activities of the lotus petal that dried at 50 and 60 °C ($p<0.05$). These findings suggested that the optimum drying and steeping conditions were dried the lotus petal at 50°C for 5 hours and steeped for 5 min.

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