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Phenolic Compounds and Antioxidant Properties of Thai rice Paddy Herb as Affected by Different Drying Temperature

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Abstract The present study investigated the changes in color and the contents and compositions of bioactive compounds in rice paddy herbs as affected by air-drying with different temperature. Phenolic compounds and their antioxidant activities of *L. aromatica* and *L. geoffrayi* dried by hot-air (HA) oven and vacuum (VC) oven at different temperatures (50, 60, 70, and 80 ℃) were evaluated. Overall, changes in the color value (ΔE) were increased with increasing of HA drying temperature, while the increasing temperature of VC drying method led to ΔE decreased. Rice paddy herbs dried with VC drying method had higher contents of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activities, ferric-reducing antioxidant power, total antioxidant capacity, total phenolic content (TPC) and total flavonoid content (TFC), compared with those dried with HA drying method. The TPC and TFC of samples dried by VC, the contents were increased when the drying temperature was increased from 50 to 70 ℃ but decreased at 80 ℃. The most predominant phenolic was sinapic acid for all samples dried with temperature of 50-80 ℃ extract, ranged from 30-50 and 34-53 mg/g in HA dried *L. aromatica* and *L. geoffrayi*, respectively, and the VC dried *L. aromatica* and *L. geoffrayi* were 41-53 and 44-55 mg/g, respectively. Our results have demonstrated that VC drying at 70 ^{o}C should be considered as suitable drying method for rice paddy herbs with respect to preserving its color, antioxidant properties, and phenolic compounds.

Keywords: Hot-air drying, Vacuum drying, *Limnophila aromatica*, *Limnophila geoffrayi*, Antioxidant activity

Introduction

Rice paddy herb is native to Southeast Asia, where it flourishes in hot temperatures and grows most often in watery environments, particularly in flooded rice fields (Do *et al.*, 2014). This plant have been used as a spice and a medicinal plant in Southeast Asia (Gorai *et al.*, 2014). In Thailand, this plant is used in Thai cuisine to add flavor and used as a traditional medicine

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(Siriamornpun *et al.*, 2014). Two particular species, *Limnophila aromatica* Lam. Merr and *Limnophila geoffrayi* Bonati has been found in Thailand, and used as the most extensively utilized in Thai cuisine to add flavor in Thai foods. It contains important flavonoids (Suksamrarn *et al.*, 2003) that also exert antioxidant activities. Previous studies revealed the antioxidant activities of extracts derived from *L. aromatica* (Do *et al.*, 2014; Siriamornpun *et al.*, 2014; Do, 1999) and *L. geoffrayi* (Thongdon-A and Inprakhon, 2009; Jang *et al.*, 2005; Suksamrarn *et al.*, 2003). Many studies have reported antioxidants from *L. aromatica* and *L. geoffrayi* and the effect of extraction solvent on their antioxidant activities but literature is still scanty on the effects of drying temperature as they affect antioxidant properties and phenolic compounds. Therefore, the main attention of this research was to assess the physical quality, antioxidant properties and phenolic compounds of rice paddy herbs by drying temperature. The effects of different temperature (50-80 ℃) were investigated for optimizing the most suitable drying conditions. We hoped to obtain the most appropriate conditions for applying the rice paddy herbs drying method. The present study will provide useful information for industrial use of herbs or spices production.

Materials and methods

Sample preparation

Rice paddy herb samples include two species: *Limnophila aromatica* (Lamarck) Merrill and *Limnophila geoffrayi* Bonati. *L.aromatica* samples were bought from three representative markets in the Kalasin province, Thailand, in March 2017. At each market, 10 kg samples were sampled from three representative outlets. Single composite samples for each representative market, were prepared by combining about 3 kg of homogenized single sample from three representative outlets and then homogenizing again to obtain a uniform single composite sample. *L. geoffrayi* samples were obtained from local farmers markets in the Kalasin, Maha Sarakham and Roi-Et provinces in the northeastern region of Thailand, in March 2017. At each market, 8-10 kg samples were sampled from representative outlets. Single composite samples for each representative market, were prepared by combining about 3 kg of homogenized single sample from three representative outlets and then homogenizing again to obtain a uniform single composite sample. After purchasing, the samples as such were transported as soon as possible to Food Science and Technology laboratory at the Kalasin University. The raw samples were washed and kept at room temperature to drain. Afterwards, the samples

were dried under hot-air (HA) and vacuum (VC) drying. All analytical results were expressed on a dry matter basis and performed in triplicate.

Drying Processes

Samples were subject to two different drying methods, i.e., HA and VC. For each drying method, 100 g of fresh sample was used (in triplicate). In HA drying, the sample was dried by a hot-air drying machine at 50, 60, 70 and 80 ℃ using a hot-air oven (Thermotec 2000 oven, Conterm, New Zealand). For VC drying, the sample was dried in the vacuum dryer at 50 ℃, 60 ℃, 70 ℃ and 80 ℃ using a vacuum oven (VD23, Binder, Germany). Drying time was set to achieve dried samples containing 7% moisture content. After drying, these samples were allowed to cool to ambient temperature before extraction.

Chemicals and analysis instruments

The phenolic compound standards, such as *p*-coumaric acid, benzoic acid, chlorogenic acid, vanillic acid, syringic acid, sinapic acid, protocatechuic acid, ferulic acid, gallic acid and caffeic acid, were purchased from Sigma Chemical Co. (St. Louis, MO). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and Folin–Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany). Other chemicals and solvents were of analytical grade. Absorbance was measured with a Spectronic Genesys 5 UV–vis spectrophotometer. HPLC analysis was conducted using Shimadzu LC-20AC pumps, SPD-M20A Diode array detector. Chromatographic separations were performed on a LUNA C-18 column (4.6mm×250mm i.d., 5 μm).

Colorimetric parameters

Color changes in sample were determined by a Minolta CR-300 Chroma Meter (Minolta, Japan) in *L, a, b* color scale. Parameters *L, a* and *b* determine a three-dimensional color space, in which *L* represents brightness (on a lightness–darkness scale) whereas positive and negative a values determine the redness and greenness, and positive and negative *b* values determine yellowness and blueness, respectively. The instrument was calibrated against a white standard. Measurements were individually taken for 10 samples per treatment and the average of 10 readings was calculated. The color difference *ΔE* was calculated from the *L, a, b* parameters, using the Hunter–Scotfield equation:

$$\Delta E = \sqrt{(\Delta a)^2 + (\Delta b)^2 + (\Delta L)^2}$$

Assessment of antioxidant activity

Sample extraction

Samples were subject to two different drying methods, i.e., HA and VC. For each drying method, 100 g of fresh sample was used (in triplicate). In HA drying, the sample was pretreated by a hot-air drying machine at 50, 60, 70 and 80 ℃ using a hot-air oven (Thermotec 2000 oven, Conterm, New Zealand). For VC drying, the sample was dried in the vacuum dryer at 50 ℃, 60 ℃, 70 ℃ and 80 ℃ using a vacuum oven (VD23, Binder, Germany). Drying time was set to achieve dried samples containing 7% moisture content. After drying, these samples were allowed to cool to ambient temperature before extraction.

DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity of the extracts was measured as described by Wanyo *et al.* (2011) with some modifications. Briefly, sample extract (0.1 mL) was mixed with 3 mL of a 0.1 mM DPPH in ethanol. The mixture was vortexed (1 min), left to stand at room temperature in dark (30 min) and then the absorbance of this solution was read at 517 nm. The percent inhibition activity was calculated as [(Ao-Ae)/Ao]x100 (Ao= Absorbance without extract; Ae = absorbance with extract).

Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce Fe^{3+}–Fe^{2+}. The formation of blue colored Fe^{2+}-TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. The method of Wanyo *et al.* (2011) was used with some modifications. The FRAP reagent was freshly prepared by mixing 100 mL of acetate buffer (300 mM, pH 3.6), 10 mL TPTZ solution (10 mM TPTZ in 40 mM/HCl), 10 mL $FeCl_3 \cdot 6H_2O$ (20 nM) in a ratio of 10:1:1 and 12 mL distilled water, at 37 ^{o}C. To perform the assay, 1.8 mL of FRAP reagent, 180 μL Milli-Q water and 60 μL sample, standard or blank were then added to the same test tubes, and incubated at 37 ^{o}C for 4 min; absorbance was measured at 593 nm, using the FRAP working solution as a blank. The reading of relative absorbance should be within the range 0–2.0; otherwise, the sample should be diluted. In the FRAP assay, the antioxidant potential of sample was determined from a standard curve plotted using the $FeSO_4 \cdot 7H_2O$ linear regression equation to calculate the FRAP values of the sample.

Determination of total antioxidant capacity

The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH (Prieto *et al.*, 1999) and determined by the method described by Dasgupta and De (2004). Aqueous extract (0.3 ml) was added to 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 ^{o}C for 90 min. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against a blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid.

Identification and quantification of phenolic compounds

Determination of total phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent as followed by Abu Bakar *et al.* (2009). Briefly, 300 µL of extract was mixed with 2.25 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min; 2.25 ml of sodium carbonate (60 g/L) solution was added to the mixture. After 90 min at room temperature, absorbance was measured at 725 nm using a spectrophotometer. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).

Determination of total flavonoid content

Total flavonoid content (TFC) was determined using colorimetric method described by Abu Bakar *et al.,* (2009) with slight modification. Briefly, 0.5 mL of the extract was mixed with 2.25 mL of distilled water in a test tube followed by addition of 0.15 mL of 5% $NaNO_2$ solution. After 6 min, 0.3 mL of a 10% $AlCl_3$ $6H_2O$ solution was added and allowed to stand for another 5 min before 1.0 mL of 1M NaOH was added. The mixture was mixed by vortex mixer. The absorbance was measured immediately at 510 nm using spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).

HPLC–DAD system for analysis of phenolic compounds

HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A diode array detection and chromatographic separation were performed on a LUNA C-18 column (4.6 mm x 250 mm, 5 µm). The composition of solvents and gradient elution conditions were described previously by Wanyo *et al.* (2014) with some modifications. The mobile phase consisted of purified

water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5% to 9% solvent B; from 5 to 15 min, 9% solvent B; from15 to 22 min, linear gradient from 9% to 11% solvent B; from 22 to 38 min, linear gradient from 11% to 18% solvent B; from 38 to 43 min, from 18% to 23% solvent B; from 43 to 44 min, from 23 to 90% solvent B; from 44 to 45 min, linear gradient from 90 to 80% solvent B; from 45 to 55 min, isocratic at 80% solvent B; from 55 to 60 min, linear gradient from 80% to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs. Operating conditions were as follows: column temperature, 38 ℃, injection volume, 20 µL and UV-diode array detection at 280 nm (hydroxybenzoic acids) and 320 nm (hydroxycinnamic acids). Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard methods.

Statistical analyses

Analysis of variance (ANOVA) was performed in a completely randomized design, using Duncan's Multiple Range Test. All determinations were done at least in triplicate. The confidence limits used in this study were based on 95 % ($p<0.05$).

Results

Quality assessment results of rice paddy herbs (*L. aromatica* and *L. geoffrayi*) can be obtaied from the different air-drying temperature (50, 60, 70 and 80 ℃) included antioxidant properties, TPC and TFC. Moisture content was fixed at 7% dry basis, according to the Thai industrial standards for dried herbs. The average initial moisture content from fresh samples of *L. aromatica* and *L. geoffrayi* were 89.95% and 85.72%, respectively. The time required to achieve the above levels of moisture content were 8, 4, 3, and 2 h for HA and 24, 10, 5, and 4 h for hot-air drying under vacuum conditions in the respective temperature.

Color parameters

Color parameters of rice paddy herb dried by different temperature compared to fresh samples are shown in Table 1. Overall, when compared with fresh samples, a smaller increase in *L* value of the dried *L. aromatica* than those

of *L. geoffrayi* was observed. Further, *L. geoffrayi* samples showed a larger increase in *L* value. All of dried samples showed a significant increase in *a* value compared with fresh sample, while *b* value was significant decreased. The changes in *L* and *b* value of VC dried paddy herbs were increased with increasing temperature. In addition found that the *L* value of HA dried *L. aromatica* significantly decreased with HA temperature at the temperature of 50 and 60 °C, while a high temperature (70-80 °C) remain unchanged (Table 1).

Table 1. Color parameters of rice paddy herb

Samples	*L*	*a*	*b*	Δ*E*
L. aromatica				
control	40.23 ± 1.41^{d}	-10.21 ± 0.88^{f}	26.54 ± 2.41^{a}	
HA, 50 °C	43.04 ± 1.01^{c}	0.21 ± 0.06^{e}	6.85 ± 0.97^{de}	22.48 ± 0.85^{d}
HA, 60 °C	40.67 ± 0.79^{d}	1.65 ± 0.14^{b}	5.77 ± 0.72^{f}	23.93 ± 0.57^{ab}
HA, 70 °C	40.74 ± 1.49^{d}	2.00 ± 0.15^{a}	7.19 ± 0.48^{d}	22.93 ± 0.37^{cd}
HA, 80 °C	40.84 ± 1.65^{d}	2.21 ± 0.32^{a}	7.57 ± 1.08^{d}	22.75 ± 0.72^{a}
VC, 50 °C	41.19 ± 1.04^{d}	1.67 ± 0.12^{b}	6.07 ± 0.55^{ef}	23.71 ± 0.42^{ab}
VC, 60 °C	45.22 ± 1.11^{b}	0.96 ± 0.10^{c}	10.47 ± 0.83^{c}	20.23 ± 0.72^{bc}
VC, 70 °C	44.63 ± 1.53^{b}	0.77 ± 0.09^{cd}	11.05 ± 0.75^{c}	19.55 ± 0.53^{e}
VC, 80 °C	50.17 ± 1.30^{a}	0.51 ± 0.08^{d}	13.72 ± 0.65^{b}	19.48 ± 0.50^{f}
L. aromatica				
control	34.81 ± 2.95^{f}	-7.69 ± 0.86^{e}	22.62 ± 2.29^{a}	
HA, 50 °C	42.16 ± 1.24^{bcd}	1.04 ± 0.23^{d}	7.44 ± 0.64^{d}	19.04 ± 0.32^{d}
HA, 60 °C	40.90 ± 1.00^{de}	2.10 ± 0.15^{b}	6.42 ± 0.40^{ef}	19.91 ± 0.55^{ab}
HA, 70 °C	41.22 ± 1.63^{cde}	1.41 ± 0.46^{c}	6.87 ± 0.75^{de}	19.36 ± 0.50^{cd}
HA, 80 °C	40.90 ± 1.00^{de}	2.19 ± 0.10^{ab}	6.03 ± 0.47^{ef}	20.28 ± 0.23^{a}
VC, 50 °C	39.97 ± 0.62^{e}	1.93 ± 0.27^{b}	5.90 ± 0.76^{f}	19.99 ± 0.64^{ab}
VC, 60 °C	42.46 ± 1.12^{bc}	2.50 ± 0.10^{a}	7.67 ± 0.82^{d}	19.68 ± 0.75^{bc}
VC, 70 °C	42.97 ± 1.47^{ab}	0.93 ± 0.17^{d}	8.81 ± 0.93^{c}	18.28 ± 0.54^{e}
VC, 80 °C	44.03 ± 1.11^{a}	0.86 ± 0.08^{d}	10.05 ± 0.64^{b}	17.82 ± 0.36^{f}

Values are expressed as mean ± standard deviation (n = 10). Means with different letters in each column indicate statistically significant differences between treatments for the same species according to Duncan's multiple range test ($p < 0.05$).

The results presented the changes in *L*, *a* and *b* value of VC dried rice paddy herbs were small as compared to HA dried samples. The total color difference *ΔE*, which is a combination of parameters *L*, *a* and *b* values, is a colorimetric parameter extensively used to characterize the variation of colors in food during processing. A marginal increase in *ΔE* was observed with HA drying temperature. On the other hand, the change in color (*ΔE*) of VC dried rice paddy herbs were decreased with increasing drying temperature. The

greater changes in color (ΔE) were found when higher VC drying temperatures were applied. The results presented the change in ΔE of VC dried samples were smaller as compared to HA dry samples.

Antioxidant activity

Several methods have been used to determine antioxidant activity of plants. Our present study therefore involved three various established methods to evaluate antioxidative activity of two rice paddy herbs, namely, DPPH radical-scavenging activity, ferric reducing/antioxidant power (FRAP) assay and total antioxidant capacity (TAC). DPPH radical-scavenging activity, FRAP values and TAC of two differently dried rice paddy herbs are shown in Table 2. The results showed that *L. aromatica* had the higher values of DPPH radical scavenging (67–95%) compared to *L. geoffrayi* (61–94 %). Similar results were found in FRAP and Total antioxidant capacity (Table 2).

Table 2. Antioxidant of rice paddy herb

Samples	**DPPH (% inhibition)**	**FRAP (mM $FeSO_4$/g DW)**	**TAC (µg AAE/100g DW**
L. aromatica			
control	92.28 ± 0.12^{c}	28.02 ± 1.30^{a}	23.28 ± 0.05^{a}
HA, 50 °C	78.97 ± 0.03^{e}	5.54 ± 0.11^{d}	7.21 ± 0.04^{e}
HA, 60 °C	67.37 ± 0.13^{i}	4.42 ± 0.24^{e}	5.30 ± 0.08^{g}
HA, 70 °C	73.06 ± 0.05^{g}	5.15 ± 0.05^{de}	6.74 ± 0.07^{e}
HA, 80 °C	71.46 ± 0.04^{h}	2.03 ± 0.02^{f}	7.95 ± 0.30^{d}
VC, 50 °C	74.31 ± 0.06^{f}	7.23 ± 0.19^{c}	6.16 ± 0.19^{f}
VC, 60 °C	86.72 ± 0.03^{d}	16.06 ± 0.00^{b}	12.64 ± 0.59^{b}
VC, 70 °C	95.42 ± 0.03^{a}	16.48 ± 0.17^{b}	10.86 ± 0.09^{c}
VC, 80 °C	95.03 ± 0.14^{b}	16.81 ± 0.32^{b}	12.57 ± 0.43^{b}
L. geoffrayi			
control	94.08 ± 0.06^{a}	25.13 ± 0.20^{a}	12.97 ± 0.12^{b}
HA, 50 °C	63.58 ± 0.02^{f}	3.04 ± 0.06^{e}	3.41 ± 0.02^{g}
HA, 60 °C	63.10 ± 0.09^{g}	2.92 ± 0.05^{ef}	2.99 ± 0.07^{h}
HA, 70 °C	61.34 ± 0.09^{h}	3.05 ± 0.15^{e}	4.44 ± 0.06^{e}
HA, 80 °C	61.26 ± 0.05^{h}	2.51 ± 0.03^{f}	3.73 ± 0.10^{f}
VC, 50 °C	67.00 ± 0.13^{e}	4.53 ± 0.17^{d}	9.51 ± 0.16^{d}
VC, 60 °C	89.65 ± 0.06^{d}	14.24 ± 0.62^{c}	13.61 ± 0.15^{a}
VC, 70 °C	91.89 ± 0.09^{c}	16.88 ± 0.07^{b}	11.23 ± 0.56^{c}
VC, 80 °C	92.40 ± 0.10^{b}	13.95 ± 0.36^{c}	11.26 ± 0.11^{c}

Values are expressed as mean ± standard deviation (n = 3). Means with different letters in each column indicate statistically significant differences between treatments for the same species according to Duncan's multiple range test ($p < 0.05$).

The temperatures used for the drying of rice paddy herbs showed significantly different antioxidant activities (p<0.05). The results showed that the 70 ^{o}C VC dried *L. aromatica* had the highest values of DPPH radical scavenging (95.42%) compared to all dried samples as well as the fresh vegetable control, followed by 80 ^{o}C VC (95.03%), 60 ^{o}C VC (86.72%), 50 ^{o}C HA (77.97%), 50 ^{o}C VC (74.31%), 50 ^{o}C VC (77.97 %), 70 ^{o}C HA (73.06%), 80 ^{o}C HA (71.46%), and 60 ^{o}C HA (67.37%). DPPH radical scavenging activities of HA dried *L. geoffrayi* significantly decreased when HA temperature increased from 50 to 70 ^{o}C and then decreased slightly at 80 ^{o}C. While, DPPH radical scavenging activities of VC dried *L. geoffrayi* significantly increased as the temperature increased. Similar results were found in FRAP (Table 2), TPC and TFC (Table 3). The results from various free radical-scavenging systems revealed that different temperature and methods used for dried of two rice paddy herbs had significant antioxidant activity. The extracts were found to have different levels of antioxidant activity in the systems tested. The antioxidant activities of the *L. aromatica* were: 60 ^{o}C VC > 80 ^{o}C VC > 70 ^{o}C VC > 80 ^{o}C HA>50 ^{o}C HA>70 ^{o}C HA>50 ^{o}C VC>60 ^{o}C HA. The antioxidant activities of the *L. geoffrayi* were: 60 ^{o}C VC > 80 ^{o}C VC > 70 ^{o}C VC > 50 ^{o}C VC>70 ^{o}C HA>80 ^{o}C HA>50 ^{o}C HA>60 ^{o}C HA.

Table 3. Total phenolic content (TPC) and total flavonoid content (TFC) in rice paddy herb

Samples	*L. aromatica*		*L. geoffrayi*	
	TPC (mg GAE/g DW)	**TFC (mg RE/g DW)**	**TPC (mg GAE/g DW)**	**TFC (mg RE/g DW)**
control	30.43±0.18[a]	93.27±3.24[a]	21.72±0.09[a]	63.41±0.37[a]
HA, 50 ^{o}C	12.32 ±0.05[f]	8.48±0.27[h]	1.80±0.03[g]	4.50±0.23[g]
HA, 60 ^{o}C	10.73±0.07[h]	8.43±0.15[h]	1.58±0.03[h]	4.57±0.01[g]
HA, 70 ^{o}C	11.51±0.14[g]	11.35±0.79[f]	1.98±0.03[f]	4.92±0.16[f]
HA, 80 ^{o}C	10.38±0.04[i]	9.14±0.04[g]	1.48±0.05[h]	4.10±0.03[h]
VC, 50 ^{o}C	13.19±0.01[e]	29.75±0.07[e]	4.18±0.01[e]	10.45±0.30[e]
VC, 60 ^{o}C	13.93±0.01[c]	53.65±0.15[c]	12.39±0.23[c]	34.94±0.01[c]
VC, 70 ^{o}C	15.40±0.08[b]	55.15±0.32[b]	14.16±0.02[b]	53.26±0.36[b]
VC, 80 ^{o}C	13.49±0.01[d]	40.39±0.12[d]	9.15±0.80[d]	32.98±0.08[d]

Values are expressed as mean ± standard deviation (n = 3). Means with different letters in the same column were significantly different at the level $p < 0.05$.

Total phenolic content (TPC) and total flavonoid content (TFC)

The polyphenols and flavonoid content in fresh, HA dried, and VC dried of two rice paddy herb extracts were measured, and the results are shown in Table 3. Dried *L. aromatica* contained higher values of TPC and TFC (10.38–15.40 mg GAE/g dry sample and 8.43–55.15 mg RE/g dry sample, respectively) than *L. geoffrayi* (1.48–14.16 mg GAE/g dry sample and 4.10–53.26 mg RE/g dry sample, respectively). The HA drying of two rice paddy herbs contained the lowest amount of TPC and TFC compared to VC dried samples. TPC of HA dried *L. aromatica* and *L. geoffrayi* significantly decreased when the drying temperature increased from 50 to 60 ^{o}C, and then significantly increased when HA temperature increased from 60 to 70 ^{o}C, after that significantly decreased at 80 ^{o}C. TFC of HA dried *L. aromatica* and *L. geoffrayi* remain unchanged when HA temperature increased from 50 to 60 ^{o}C, and then significantly increased when HA temperature increased from 60 to 70 ^{o}C, after that decreased significantly at 80 ^{o}C. This was due to the temperature differences and inactivation of enzyme. During the drying process polyphenoloxydase enzymatic activity remains high for longer periods when the drying temperature is around 50–60 ℃, whereas shorter exposure period are needed to inactivate the enzyme at temperatures of 70 ℃. Therefore, due to low temperature requires a longer drying time compares to high temperature, the resulting TPC and TFC shows down. These findings corroborate our results at 70 ^{o}C the TPC was increased. In the case of VC drying method, TPC and TFC significantly increased when the drying temperature increased from 50 to 70 ^{o}C and then decreased significantly at 80 ^{o}C (Table 3).

Determination of phenolic compounds

RP-HPLC analysis was used to identify the phenolic compounds of rice paddy herb extracts, by comparison with standard compounds. In the rice paddy herb analysed, it was possible to identify 4 hydroxybenzoic acids (HBA): gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanilic acid and 6 hydroxycinnamic acids (HCA): chorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and sinapic acid. The distribution of phenolic acids in all samples is presented in Figure 1. The main phenolic acids found in all samples were sinapic and ferulic acids. Comparing the phenolic acids of two species of rice paddy herb samples, the *L. geoffrayi* contained the highest levels of sinapic acid, with concentrations from 35.83 to77.94 mg/g. In both *L. aromatica* and *L. geoffrayi*, no vanilic, chorogenic, caffeic and *p*-hydroxybenzoic acids were detected.

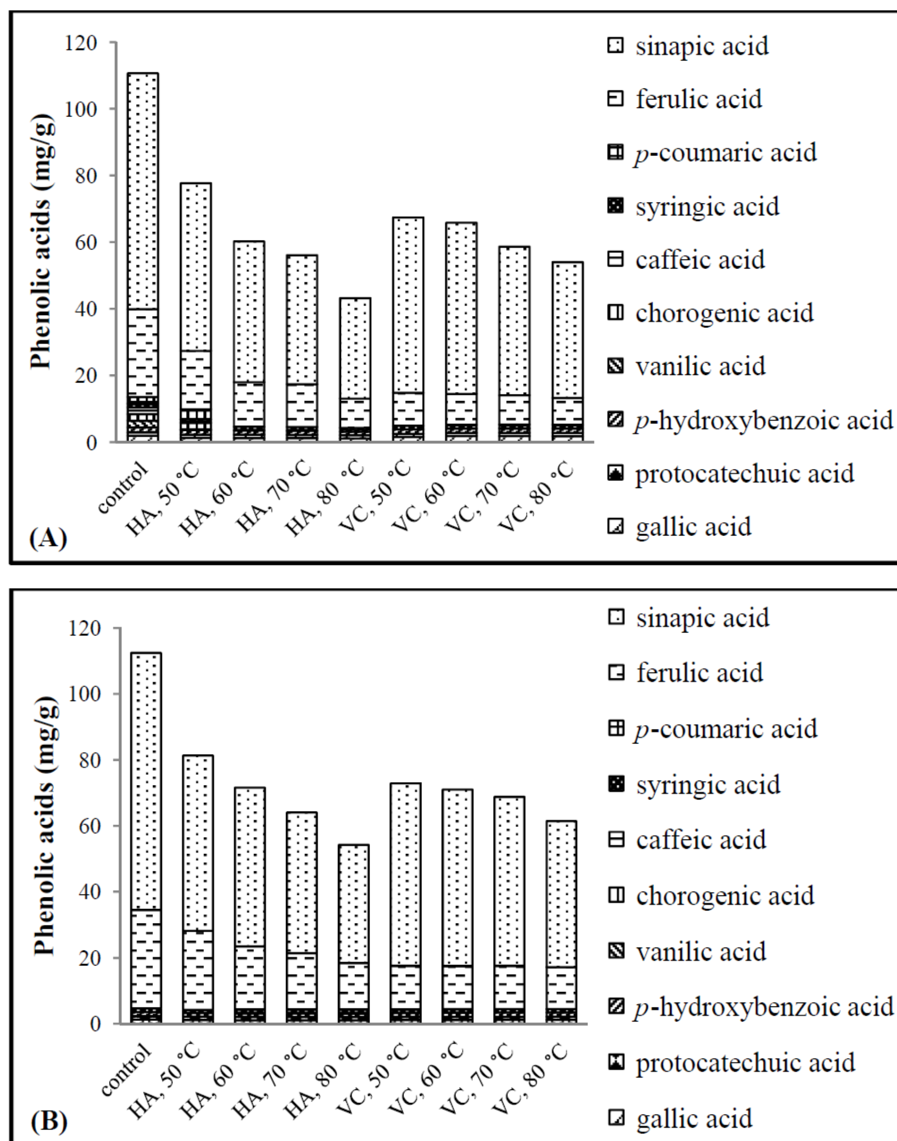

Figure 1. Phenolic acids (mg/g) in *L. aromatica* (A) and *L. geoffrayi* (B)

In the two species of rice paddy herb samples, the total content of the HCA group was higher than the total content of the HBA group. We found that HCA group was ranged from 51 to 109 mg/g in *L. geoffrayi* and 40 to 98 mg/g in *L. aromatica*, while HBA group was ranged from 22 to 30 mg/g in rice bran, 3.10 to 3.56 in *L. geoffrayi* and 3.15 to 4.42 mg/g in *L. aromatica*, respectively. The total content of HCA and HBA of two rice paddy herbs decreased with drying temperature increased (Figure 2).

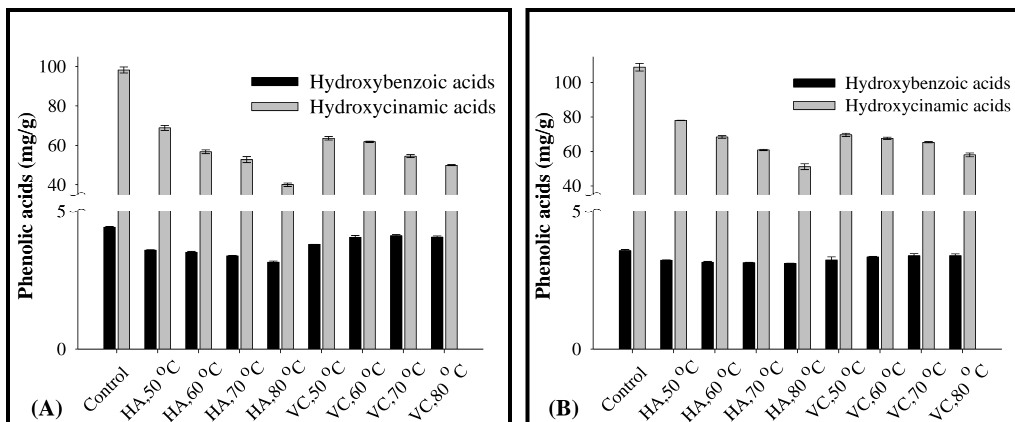

Figure 2. Total hydroxybenzoic acids and hydroxycinamic acids (mg/g) in *L. aromatica* (A) and *L. geoffrayi* (B)

Discussion

The present investigation determined physical quality, antioxidant properties and phenolic compounds of two rice paddy herbs dried under varying HA and VC drying temperatures in order to identify optimum drying conditions that are suitable for preserving its color, antioxidant properties, and phenolic compounds.

In the process of vacuum drying, the heat is usually transferred to the product through conduction and sometimes by radiation. The rapid changes in the direction of the electric field cause the dipolar water molecules in the samples to oscillate many millions of times per second (Lavoie, 2006). This result in heating, due to molecular friction, and thus a rise in temperature and vapour pressure in the sample. While hot-air drying is a slow process relying on heat conduction from the outer surface towards the interior. The use of vacuum drying method resulted in a greater internal mass transfer in a vacuum. The vacuum removes moisture while preventing the oxidation or explosions that can occur when certain materials combine with air (Lavoie, 2006).

Color is a psychological property of food products that effects to the enjoyment of eating. Temperature during drying is one of the causes of color degradation in dehydrated products (Wanyo *et al.*, 2011; Lozano and Ibarz, 1997). In this study, the changes in color were smaller in samples dried by VC than those dried by HA. The color changes in rice paddy herbs caused by the thermal may be due not only the non-enzymatic browning reaction, but also to the destruction of pigments present in the samples. Degradation of certain bioactive compounds in the vegetable tissues might be related to decreasing

bioactivity of the vegetable. In this sense, higher drying temperatures led to lower color degradation, which was attributed to shorter drying times. In addition, lower drying pressures led to lower color degradation too, which was attributed to low oxygen concentration in the drying chamber. An increase in drying temperatures normally increase the mass transfer rates during drying, and in the case of active compounds, elevated temperatures activate competing processes, such as decomposition and epimerization of the compounds (Wanyo *et al.*, 2016; Gertenbach, 2002).

Foods such as vegetables, fruits and grains are reported to contain a wide variety of antioxidant components. Several methods have been used to determine antioxidant activity of plants. DPPH is a stable free-radical compound widely used to test the free-radical scavenging ability of various samples (Sakanaka *et al.*, 2005). The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe^{2+}–TPTZ) (Benzie and Strain, 1996). Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free-radical chain by donating a hydrogen atom (Gordon, 1990). The reduction of Fe^{3+}–TPTZ complex to blue-colored Fe^{2+}–TPTZ occurs at low pH (Benzie and Strain, 1996). Total antioxidant capacity assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The phosphomolybdenum method is quantitative since the antioxidant activity is expressed as the number of equivalents of ascorbic acid (Prieto *et al.*, 1999). The results from various free radical-scavenging systems revealed that different temperature and methods used for dried of two rice paddy herbs had significant antioxidant activity. Phenolic compounds have been widely distributed in fruits and vegetables (Li *et al.*, 2006; Ho *et al.*, 1992), which have received considerable attention, due to their potential antioxidant activities and free-radical scavenging abilities, which potentially have beneficial implications in human health (Li *et al.*, 2006; López-Vélez *et al.*, 2003). Thermal processing has been reported to have both adverse and favorable effects on TPC. Losses in TPC by thermal processing have been reported in many studies, mostly in vegetables (Wanyo *et al.*, 2011; Roy *et al.*, 2007; Chan *et al.*, 2009). Flavonoids are the most common and widely distributed group of plant phenolic compounds, which are characterized by a benzo-y-pyrone structure, which is ubiquitous in fruits and vegetables. TFC of HA and VC dried *L. aromatica* and *L. geoffrayi* significantly decreased when drying temperature increased from 70 to 80 ^{o}C. The decreased possibly due to flavonoids being relatively unstable at higher temperatures.

Phenolic compounds are the most active antioxidant derivatives in plants which mostly found in the outer layers of plants, such as the peel, shell and hull, contain large amounts of polyphenolic compounds to protect the inner components (Bors *et al.*, 2001). A number of phenolic acids are linked to various cell-wall components, such as arabinoxylans and proteins (Hartley *et al.*, 1990). They are known to be good natural antioxidants which not only their ability to donate hydrogen or electrons but also they are stable radical intermediates (Maillard *et al.*, 1996). The main phenolic acids found in all rice paddy herb samples were sinapic and ferulic acids. According to literature, sinapic acid has been reported against various pathological conditions such as oxidative stress (Kikuzaki *et al.*, 2002), infections (Maddox *et al.*, 2009), cancer (Hudson *et al.*, 2000), diabetes (Kanchana *et al.*, 2011), and inflammation (Yun *et al.*, 2008). Moreover, ferulic acid exhibits a wide range of biomedical effects including antioxidant, antiallergic, hepatoprotective, anticarcinogenic, anti-inflammatory, antimicrobial, antiviral, vasodilatory effect, antithrombotic, and helps to increase the viability of sperms (Akihisa *et al.*, 2000; Graf, 1992; Ou and Kwok, 2004; Rukkumani *et al.*, 2004). The total content of HCA and HBA of two rice paddy herbs decreased with drying temperature increased. Generally, thermal treatment has significant effect on the depletion of polyphenols in food products (Kaur and Kapoor, 2001). Our results were similar to the results of Vega-Gálvez *et al.* (2009) who reported loss of phenolic compounds in air dried red pepper in which phenolic content decreased with drying temperature. An increase in drying temperatures normally increases the mass transfer rates during drying, and in the case of active compounds, elevated temperatures activate competing processes, such as decomposition and epimerization of the compounds (Wanyo *et al.*, 2016; Gertenbach, 2002).

Conclusion

The present study has demonstrated that the contents and compositions of phenolic compounds and antioxidant activities of the extracts of rice paddy herb were influenced from various varieties and drying temperatures. Overall, rice paddy herbs dried with VC drying method had higher contents of bioactive compounds, such as phenolic acids, and antioxidant activities, compared with those dried with hot-air drying method. According to the results from our present study, VC drying at 70 ^{o}C should be considered as suitable drying method for rice paddy herbs with respect to preserving its color, antioxidant properties, and phenolic compounds. The present study has provided useful information on drying optimization of bioactive compounds from rice paddy

herbs for industrial use of dried rice paddy herbs or rice paddy herb powders production and further food applications.

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