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Effects of multi-stage dehumidified-air drying on the polyphenol content of *Hydrocotyle bonariensis*

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Abstract

Traditional drying methods involve high temperatures that degrade heat-sensitive compounds. Dehumidified-air drying, an alternative to traditional drying methods, is suitable for heat-sensitive compounds; however, it consumes a large amount of energy and is comparatively expensive. In this study, a multi-chamber dehumidified-air dryer was designed to dry Hydrocotyle bonariensis, and the retention of the polyphenol content of Hydrocotyle bonariensis under various drying conditions was examined. Multi-chamber dehumidified-air drying involves two chambers; each chamber was operated at temperatures of 30, 40, and 50°C with air volumetric flow rates of 30 and 50 L/min. The results indicated that the highest retention of total phenolic content and total flavonoid content, 24.67 mg of GAE/g dry weight (DW) and 2.204 mg of catechin/g DW, respectively, was obtained at 50°C with a 50 L/min air flow rate in the first drying chamber. Multi-stage dehumidified-air dryers have the potential to dry heat-sensitive products with reduced energy consumption.

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Introduction

Plants typically contain high concentrations of antioxidant compounds (Wojdyło *et al*., 2007) that often are useful for the prevention of various degenerative diseases (Hu and Willett, 2002). The three major groups of natural antioxidants that are available in fruits and vegetables are vitamins, phenolic substances and carotenoids (Thaipong *et al.*, 2006). The most well-known antioxidant components in herbs are phenolic substances. These phenolic compounds can be classified into three groups: simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids (Pandey and Rizvi, 2009). In food, phenolics may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability of the products. Additionally, non-nutritional and preventative health properties are important to producers, processors and consumers (Shahidi and Naczk, 2003). In recent years, the benefits of using natural products that are rich in these antioxidant compounds have led to a growing interest in the pharmaceutical, food and cosmetic industries.

Hydrocotyle bonariensis, known locally in Malaysia as Pegaga Embun, is normally grown in tropical or subtropical regions. Hydrocotyle bonariensis is well-known for the treatment of tuberculosis, providing increased brain capacity, pain relief for rheumatism and arthritis and increased longevity (Vimala *et al.*, 2003). Studies have shown that *Hydrocotyle bonariensis* contains various bioactive components, such as alkaloids, tannins, flavonoids, saponins, and phenolic compounds. These phytochemical compounds have protective or disease preventive properties (Thamaraiselvi, 2012). Among bioactive components, phenolic acids and flavonoids are the primary phenolics that possess antioxidant activity (Wojdyło *et al*., 2007). The total phenolic and flavonoid contents of Hydrocotyle bonariensis have been reported as 9.25 and 3.32 mg/g dry weight (DW), respectively. The concentrations of antioxidant compounds in Hydrocotyle bonariensis are high compared to those present in other herbs, such as *B. racemosa, K. galanga, P. sarmentosum* and *C. caudatus* (Sumazian *et al*., 2010). Moreover, *Hydrocotyle bonariensis* has no mutagenic effect on *Salmonella typhimurium* strains; thus, it has potential for use in traditional medicine (Florinsiah *et al.*, 2013). In a previous study, *Hydrocotyle bonariensis* was proven to provide protection against galactose-induced cataract and reduced cataract progression with no reverse cataractogenesis (Ajani *et al*., 2009). These health benefits of *Hydrocotyle bonariensis* provide strong motivation for developing means to convert the plants into a shelf-life-stable product with high retention of the naturally occurring antioxidant properties.

A number of drying methods are commonly used to reduce the moisture content of food products and produce a shelf-life-stable product.

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However, commercial drying methods, such as hot air drying, drum drying and spray drying, involve high temperatures, which can potentially degrade antioxidant compounds. Thus, a multi-chamber dehumidified-air dryer was developed as a non-thermal drying method, where dehumidified air is continuously generated by air recirculation through desiccants (Woo *et al*., 2014). This dehumidification process presents an alternative that is more cost effective and energy effective than high-temperature drying. To measure the effectiveness of the proposed multi-chamber dehumidified-air drying method, the retention of the antioxidant components in Hydrocotyle bonariensis, primarily total phenolic content (TPC) and total flavonoid content (TFC), was used as an evaluation parameter, i.e., TPC and TFC were compared for different drying conditions.

Materials and Methods

Dehumidified-air drying

A continuous dehumidified-air dryer, as shown in Figure 1, was used in this study. Before beginning the actual drying process, the dryer was run for 30 min to reach the steady state at the desired operating condition. An air compressor (SVP 202, Swan, Taiwan) was used to supply air into two chambers filled with desiccants (molecular sieve, 3A), which adsorbed the moisture from incoming compressed air to produce dehumidified air (relative humidity of 0-1%). The adsorbent was regenerated through pressure cycling at intervals of 2 min. Dehumidified air was continuously supplied and controlled by solenoid valves (2W030-08, Airtac, Taiwan). The flow rate of dehumidified air was controlled manually by adjusting a needle valve and was measured with a volumetric flow meter (822-2-OV1-PV1-V1MP-CRWM, Sierra, USA). A heater, temperature controller (CH402, RKC, Japan) and thermocouple were used to adjust the temperature of the dry air. For safety purposes, a pressure switch (AP-C30W, Keyence, Japan) was incorporated, which would disable the heater if the air pressure were not sufficiently high. The drying chambers (100 mL for each chamber) were connected in series with lids on both ends and were covered with an insulator to prevent heat loss. Each drying chamber was loaded with the fresh sample, Hydrocotyle bonariensis (30.00 ± 1.00 g), which was cut into 1 × 1 cm^2 portions in a basket. The baskets, including the samples, were weighed every 10 min during the initial drying period. When a decrease in weight was not obvious, the drying period was extended to 30 min or 1 h, as necessary. Drying was ended once the moisture content of the

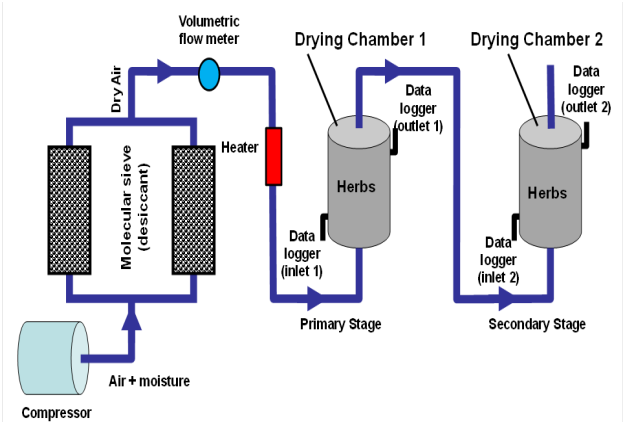

Figure 1. Schematic diagram of the continuous dehumidified-air dryer

samples was less than 10%. The moisture content was measured using a halogen moisture analyzer (Precisa, model XM-120, Switzerland) with three replications. The inlet and outlet of each drying chamber were instrumented to record and monitor the drying temperature and relative humidity of the dehumidified air using data loggers (EL-USB-2-LCD, Lascar, UK). The experiments were conducted under a variety of conditions: air flow rates of 30 and 50 L/min and at temperatures of 30, 40, and 50°C; each condition was tested in triplicate (Woo *et al.*, 2014).

Chemicals

All the chemicals used in this study were analytical grade. Methanol (purity>99.8%), sodium carbonate (purity>99.5%) and sodium hydroxide (purity>99%) were purchased from Systerm, Malaysia. Folin-Ciocalteu reagent and sodium nitrate (purity>99.5%) were purchased from Merck, Germany. Gallic acid (purity>98%) was purchased from Acros Organics, Belgium. Aluminum chloride (purity>97%) was purchased from Friendemann Schmidt, United States. Catechin (purity>99%) was purchased from Sigma-Aldrich, Malaysia.

Sample extraction

The extraction process was based on the method proposed by Ismail *et al.* (2004) with modifications. The leaves of Hydrocotyle bonariensis were crushed into a powder using a blender (HR 2100, Philips, China). Extraction began by mixing 2 g of each sample with 20 mL of 80% methanol (v/v) in an incubator shaker (C24, Edison, NJ, USA), which was agitated overnight (16 h) at room temperature (25°C). The slurries were centrifuged (Kubota 5800, Japan) for 15 min at 3,500 rpm (2380 × g) at 4°C. The supernatant was filtered and collected using a Buckner funnel and a Whatman No. 1 filter. The crude

extracts were dried under reduced pressure using a vacuum rotary evaporator (Heidolph Laborota 4000, Schwabach, Germany) at a temperature of 40°C and a speed of 60 rpm (Ismail *et al*., 2004). The extract residues were re-suspended with 80% methanol (v/v) to 50 mL to determine the total phenolic content (TPC) and total flavonoid content (TFC).

Determination of the total phenolic content

The TPC of the Hydrocotyle bonariensis extract was measured using the Folin-Ciocalteu colorimetric method (Yahya *et al.*, 2014). Briefly, 100 µL of extract was transferred into a test tube, and 0.75 mL of Folin-Ciocalteu reagent (diluted 10-fold with distilled water) was added and mixed. The mixture was allowed to stand for 5 min at room temperature. Next, 0.75 mL of 6% (w/v) sodium carbonate was added and gently mixed. The mixture solution was allowed to stand for 60 min, and the absorbance was read at 725 nm. A standard calibration curve for gallic acid (0-100 mg/mL) was plotted. The results are expressed as gallic acid equivalent (GAE) mg/g DW. Triplicate measurements were collected for the TPC.

Determination of the total flavonoid content

The TFC was measured using a colorimetric method adapted from Zhishen *et al*. (1999) with some modifications adapted from Zhang *et al.* (2010). First, 0.25 mL of extract was added to a test tube containing 0.75 mL of distilled water. Then, 0.15 mL of sodium nitrate (5%) was added to the test tube and mixed gently. The mixture was allowed to stand for 5 min before the addition of 0.3 mL of aluminum chloride (10%). After 5 min, 1 mL of sodium hydroxide (1 M) was added. The solution was thoroughly mixed, and the absorbance was read at 510 nm. Catechin, with a range of 0.04-0.20 mg/mL, was used as a standard calibration curve. The results are expressed as mg of catechin/g DW. Triplicate measurements were collected for the TFC.

Statistical analysis

The experimental data were analyzed using both one-way ANOVA and two-way ANOVA, which was followed by a post-hoc comparison (Tukey's test) with significant differences among the means at $p<0.05$. This analysis was conducted using the Minitab version 16 computing software (Minitab Inc., USA).

Results and Discussion

The fresh Hydrocotyle bonariensis contained 29.07 mg of GAE/g DW of the TPC and 3.31 mg of catechin/g DW of the TFC. Table 1 illustrates that the drying process reduced the TPC and TFC compared to the fresh leaves. Furthermore, the leaves dried in the 2nd drying chamber had lower TPC and TFC values than the 1st drying chamber. This difference may be due to the lower efficiency of the 2nd drying chamber, which required a longer drying time to withdraw the moisture from the samples. Decreases in the TPC and TFC after drying may be due to the deterioration of heat-sensitive compounds (Vashisth *et al.*, 2011). The longer drying time in the 2nd drying chamber may have resulted in enhanced deterioration of phenolic compounds, leading to lower TPC and TFC values in leaves (Rodríguez *et al*., 2014).

Table 1. Effects of drying temperature and air flow rate on the total phenolic content and total flavonoid content of *Hydrocotyle bonariensis*

Antioxidant Properties	Chamber	Drying Temperature (°C)	Air flow rate (L/min) 30	50
Total Phenolic Content (mg of GAE/g DW)	Fresh leaves		29.066±1.423	
	1	30	19.17±0.377^{Aa}	18.38±0.288^{Ab}
		40	18.90±0.597^{Aa}	22.42±0.294^{Bb}
		50	21.87±1.737^{Ba}	24.67±0.723^{Cb}
	2	30	17.21±1.072^{Aa}	16.30±0.247^{Aa}
		40	16.54±0.676^{Aa}	14.18±1.826^{Bb}
		50	20.68±0.614^{Ba}	18.39±1.147^{Cb}
Total Flavonoid (mg of Catechin/g DW)	Fresh leaves		3.314±1.3911	
	1	30	1.830±0.049^{Ba}	1.533±0.127^{Ab}
		40	2.057±0.064^{Aa}	2.041±0.073^{Ba}
		50	1.991±0.080^{Aa}	2.204±0.110^{Cb}
	2	30	1.789±0.031^{Aa}	1.171±0.121^{Ab}
		40	1.901±0.140^{Aa}	1.607±0.032^{Bb}
		50	1.732±0.169^{Aa}	2.129±0.181^{Cb}

*Data are expressed as the average ± standard deviation for three replicates. Values in the same column within the same chamber having the same letter (A, B and C) for each parameter are not significantly different at a confidence level of 95%. Values in the same row having the same letter (a and b) for each parameter are not significantly different at a confidence level of 95%.

The interactive effects between the drying temperature and air flow rate were analyzed using two-way ANOVA. An interactive effect was significant ($p<0.05$) for the TPC value in the 1st drying chamber but not significant ($p>0.05$) in the 2nd drying chamber. In both drying chambers, both the drying temperature and air flow rate had significant ($p<0.05$) effects on the TPC.

A significant interactive effect ($p<0.05$) between the drying temperature and air flow rate was observed for the TFC in both drying chambers. An air flow rate of 30 L/min yielded no significant effects ($p>0.05$); however, an air flow rate of 50 L/min exhibited a significant effect ($p<0.05$) on the TFC value at constant temperature. The drying temperatures significantly affected ($p<0.05$) the TFC under all conditions, except at 40°C in the 1st drying chamber.

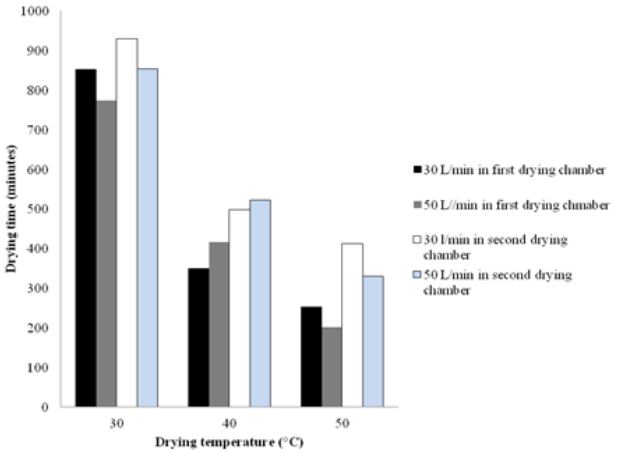

Figure 2. Changes in drying time for different air flow rates and drying temperatures

A post hoc multiple comparison test (Tukey's test) was used to compare differences between TFC and TPC in the 1st and 2nd drying chambers. There was no significant difference ($p>0.05$) in TFC and TPC for both chambers at 50°C and 50 L/min or for TFC at 30°C and 30 L/min. Otherwise, drying in both chambers yielded significant differences in the TFC and TPC. Drying at the highest temperature (50°C) and the highest air flow rate (50 L/min) yielded the highest TPC and TFC, with 84.9% retention of phenolic compounds and 66.5% retention of flavonoid compounds.

The interaction between the temperature and air flow rate led to changes in drying time, as shown in Figure 2. The drying time in the 2nd drying chamber is longer than that in the 1st drying chamber. The drying curves for both drying chambers decreased gradually at an air flow rate of 30 L/min but decreased dramatically at 50 L/min; unsurprisingly, the drying time at 50 L/min was shorter than that at 30 L/min. Operation at 50°C and 50 L/min yielded the shortest drying time, which may be related to the higher retention rates for TPC (1st drying chamber: 84.9% and 2nd drying chamber: 63.3%) and TFC (1st drying chamber: 66.5% and 2nd drying chamber: 64.2%). These results correspond with those presented in a previous study, where a high temperature and short drying time maximized antioxidant activity (Mrkìc et al., 2006). At high temperatures, the water content of the sample was removed at a faster rate compared to lower drying temperatures, which could lead to deformations in the vegetal matrix, which could in turn result in exposure of antioxidant compounds to oxidation reactions (Martínez-Las Heras *et al*., 2014); the glycosides of phenolics are localized in the hydrophilic regions of the cell, and other soluble phenols are found in the cytoplasm and cell nuclei (Sakihama *et al*., 2002). However, due to short drying times, the deterioration rate of these phenolic compounds was lower than for long drying times. Furthermore, high convective forces acting at the air-solid interface can retard heat diffusion into *Hydrocotyle bonariensis* leaves (Vega-Gálvez *et al*., 2012), which helps limit the deterioration of heat-sensitive compounds. The results of the present study are in agreement with previous work indicating high TPC retention for high-temperature drying (Moraes *et al*., 2013; Reis *et al*., 2013). However, another study reported that a higher drying temperature yielded increased deterioration of active compounds (Vashisth *et al*., 2011). Therefore, further study is still needed to confirm the influence of temperature on phenolic retention and to elucidate mechanisms by which phenolics are created or destroyed.

Conclusion

The multi-stage continuous dehumidified-air dryer used in this study has the potential to dry raw materials rich in polyphenol compounds, with up to 84.9% retention of phenolic compounds and 66.5% retention of flavonoid compounds after the drying process. The best drying results were achieved at a temperature of 50°C and an air flow rate of 50 L/min for the shortest drying time. Further examination of the drying efficiency for shorter drying times and preservation of thermal labile phenolic compounds should be conducted.

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References

Ajani, E. O., Salako, A. A., Sharlie, P. D., Akinleye, W. A., Adeoye, A. O., Salau, B. A. and Adebawo, O. O. 2009. Chemopreventive and remediation effect of *Hydrocotyl bonariensis* Comm. Ex Lam (Apiaceae) leave extract in galactose-induced cataract. Journal of Ethnopharmacology 123(1): 134-142.

Florinsiah, L., Zuraina, M. F., Shahirah, N. N., Norfazlina, M., Zaila, C. S. and Rajab, N. 2013. Mutagenicity Effect of Hydrocotyle bonariensis Extracts in *Salmonella*/Microsome Assay. International Journal of Pharmaceutical Sciences Review and Research 20(2): 47-50.

Hu, F. B. and Willett, W. C. 2002. Optimal diets for prevention of coronary heart disease. Journal of

American Medical Association 2888: 2569-2578.

Ismail, A., Marjan, Z. M. and Foong, C. W. 2004. Total antioxidant activity and phenolic content in selected vegetables. Food Chemistry 87(4): 581-586.

Martínez-Las Heras, R., Heredia, A., Castelló, M. L. and Andrés, A. 2014. Influence of drying method and extraction variables on the antioxidant properties of persimmon leaves. Food Bioscience 6: 1-8.

Moraes, I. C. F., Sobral, P. J. d. A., Branco, I. G., Ré, T. B. and Gomide, C. A. 2013. Dehydration of "dedo de moça" pepper: kinetics and phytochemical concentration. Food Science and Technology (Campinas)33: 134-141.

Mrkìc, V., Cocci, E., Rosa, M. D. and Sacchetti, G. 2006. Effect of drying conditions on bioactive compounds and antioxidant activity of broccoli (*Brassica oleracea* L.). Journal of the Science of Food and Agriculture 86(10): 1559-1566.

Pandey, K. B. and Rizvi, S. I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity 2(5): 270-278.

Que, F., Mao, L., Fang, X. and Wu, T. 2008. Comparison of hot air-drying and freeze-drying on the physicochemical properties and antioxidant activities of pumpkin (*Cucurbita moschata* Duch.) flours. International Journal of Food Science and Technology 43(7): 1195-1201.

Reis, R. C., Castro, V. C., Devilla, I. A., Oliveira, C. A., Barbosa, L. S. and Rodovalho, R. 2013. Effect of drying temperature on the nutritional and antioxidant qualities of cumari peppers from Pará (*Capsicum chinense* Jacqui). Brazilian Journal of Chemical Engineering 30: 337-343.

Rodríguez, K., Ah-Hen, K., Vega-Gálvez, A., López, J., Quispe-Fuentes, I., Lemus-Mondaca, R. and Gálvez-Ranilla, L. 2014. Changes in bioactive compounds and antioxidant activity during convective drying of murta (*Ugni molinae* T.) berries. International Journal of Food Science and Technology 49(4): 990-1000.

Sakihama, Y., Cohen, M. F., Grace, S. C. and Yamasaki, H. 2002. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. Toxicology 177(1): 67-80.

Shahidi, F. and Naczk, M. 2003. Phenolics in Food and Nutraceuticals. Florida: CRC Press.

Sumazian, Y., Syahida, A., Hakiman, M. and Maziah, M. 2010. Antioxidant activities, flavonoids, ascorbic acid and phenolic contents of Malaysian vegetables. Journal of Medicinal Plants Research 4(10): 881-890.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Byrne, D. H. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19: 669-675.

Thamaraiselvi, P. 2012. Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.) Solms. Asian Journal of Plant Science and Research 2(2): 115-122.

Vashisth, T., Singh, R. K. and Pegg, R. B. 2011. Effects of drying on the phenolics content and antioxidant activity of Muscadine pomace. LWT - Food Science and Technology 44(7): 1649-1657.

Vimala, S., Adenan, M. I., Ahmad, A. R., Shahdan, R., Adenan, M., Ahmad, A. and Shahdan, R. 2003. Nature's choice to wellness: antioxidant vegetables/ ulam. Selangor: Forest Research Institute Malaysia (FRIM).

Wojdyło, A., Oszmiański, J. and Czemerys, R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry 105(3): 940-949.

Woo, C. L., Yeoh, H. S., Go, S. K. and Chong, G. H. 2014. Green drying: continuous dehumidified-air dryer. Engineering Journal 18(2): 119-126.

Yahya, A.-H., Chong, G.-H. and Tan, C.-P. 2014. The phytochemical properties of a new citrus hybrid (*Citrus hystrix*× *Citrus microcarpa*). Science Asia 40: 121-124.