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Full Length Research Paper

Effects of drying methods on antioxidant properties in *Robinia pseudoacacia* L. flowers

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The effects of different drying methods (sun drying, hot-air drying, freeze drying and microwave-vacuum drying) on the antioxidant properties and phenolic content in *Robinia pseudoacacia* L. flowers (RPF) were investigated. Simultaneously, the correlation between phenolic content and antioxidant capacity was discussed. Results showed that antioxidant properties and phenolic content in dried RPF were affected by the drying methods to different extents. Freeze drying sample possessed the highest antioxidant activity including DPPH radical scavenging, reducing power and hydroxyl radical scavenging ability, and the total phenolic content was 47.30 mg gallic acid equivalents (GAE) /g DW. Microwave-vacuum drying samples had the best iron-chelating ability and its phenolic content (45.52 mg GAE/g DW) was comparable to that of the freeze drying samples (*P*> 0.05). Sun drying was the worst in antioxidant activities and phenolic content was only 29.15 mg GAE/g DW. Additionally, significantly positive correlation was found between the total phenolic content and antioxidant activity (*P*< 0.01). Microwave-vacuum drying was a potential drying technique for RPF.

Key words: *Robinia pseudoacacia* L. flower, drying method, antioxidant property, total phenolics.

INTRODUCTION

Flowers of *Robinia pseudoacacia* L., which was brought to China in 1898, have been commonly used in folk edible material and traditional Chinese medicine for a long time, due to its unique nutrient and subtle flavor, and probably its biological and pharmaceutical properties. According to descriptions of the medical literature, it can be used for treatment of metrorrhagia, hemoptysis, large intestine hemorrhage, rheumatic arthritis, gynecologic disease, and so on. The main bioactive components existed in *R. pseudoacacia* L. flowers (RPF) include flavonoids, phenolics, ascorbic acid, etc., (Jing et al., 2002; Li et al., 2011; Wang et al., 2010; Wang et al., 2011). All above-mentioned compounds play critical roles in preventing ailments and maintaining human health (Uchiyama et al., 2011; Santos et al., 2011; Ghosh, 2009; Graf et al., 2005). There is a growing interest in RPF (Li et al., 2011; Spiteller and Steglich, 2001; Wang et al., 2010, 2011; Xie et al., 2006).

The fresh *R. pseudoacacia* L. flowers contain lots of water, and are perishable plant products, so they are generally dried to low moisture content for long storage. Generally, hot-air drying (HAD) is much frequently used dehydration operation in food and conventional chemical industry. While this method can have adverse effect on the taste, color and nutritional content because of higher temperature and longer drying time (Sharma and Prasad, 2003). Freeze drying (FD) is a better method of moisture removal, and can maintain much bioactive components, while the drying rate is relatively slow (Krokida and Philippopoulos, 2006). Microwave-vacuum drying (MVD) is rapid, energy efficient, and has a high potential for the processing of agricultural products (Han et al., 2008; Vadivambal and Jayas, 2007; Yang et al., 2008). Rapid and efficient drying techniques and methods that could minimize the nutrient loss have acquired considerable

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attention.

Although, some studies have been carried out on the compounds in RPF (Spiteller and Steglich, 2001; Wang et al., 2011; Xie et al., 2006), little information was available about the effect of different drying method on antioxidant capacity and total phenolic content (TPC) in RPF.

Therefore, the objective of this paper is to evaluate the influences of four different drying methods (sun drying (SD), hot air drying (HAD), microwave-vacuum drying (MVD) and freeze drying (FD)) on TPC and antioxidant properties (DPPH free radical scavenging, reducing power, ion-chelating assays, and hydroxyl radicals scavenging ability) of RPF. The results obtained in this paper could help better determine the potential drying methods for final products of higher quality in pharmaceutical or food industry.

MATERIALS AND METHODS

R. pseudoacacia L. flowers, produced in May of 2011, were collected from Hongmen Village, Hongmen Town, Hongqi District, Xinxiang City, Henan Province, P. R. China. Folin–Ciocalteau phenol reagent and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gallic acid and rutin was obtained from Shanghai Tauto Biotech Co., Ltd (Shanghai, China). Ferrozine were from Fluka Chemical (Buchs, Switzerland). All other reagents were of analytical grade.

Drying of *R. pseudoacacia* L. flowers

The fresh flowers of uniform shape and color were selected and dried with the following methods until the weight reached unchangeable: (1) Sun drying: The flowers were placed in the greenhouse with daylight about 27 h. Mid-day temperature in the greenhouse can reach 35°C. The total drying time was 3 days; (2) Hot-air drying.

The flowers were performed in an electric blast oven (101-2A, Tianjin Tongli company, Tianjin, China) at 60°C with the wind speed of 0.4 m/s. The flower were turned once every 30 min during hot-air drying. The drying time was 4 h. (3) Microwave-vacuum drying: The samples were placed in a microwave-vacuum dryer (Jiangnan University MVD-1, Wuxi, China) under the condition of microwave power 1500 W, vacuum 70 kPa, loadage 100 g. And the drying time was 40 min. (4) Freeze drying: The flowers were preceded by freezing at -80°C in a freezer (NBS U410, American), and then carried out in a freeze-dryer (Christ Alpaha 1- 4LSC, Germany) with the condition of pressure 10 Pa (absolute pressure), cryo-temperature -55°C, and heating temperature 65, 55 and 40°C at prophase, metaphase, and anaphase, respectively. The total drying time was 12 hrs. All the dried materials were then powdered in a grinder (RRH-250, OKLIFE (HK) instrument co., Hongkong, China) to 40-mesh size, and stored under -20°C until analysis.

Extraction of dried *R. pseudoacacia* L. flowers samples

Plant powder (3 g) of each dried flowers was extracted by stirring with 45 ml 60% ethanol solution at 25°C at 150 r/min for 12 h and filtering through Whatman No. 1 filter paper. The extraction was repeated again as described earlier. Then the extracts of two times were mixed and filtrated, and diluted to 100 ml with 60% ethanol solution. The extract solution was stored at 4°C in amber bottles and served as the working solution (30 mg/ml) for determination of antioxidant activity and total phenolic content.

DPPH radical scavenging activity

DPPH radical scavenging ability was performed as described previously (Sun and Ho, 2005). The working solution prepared as described earlier was diluted with 60% ethanol solution to obtain different concentrations (0.625 to 20 mg/ml). Each diluted sample (2.5 ml) was mixed with 2.5 ml of DPPH solution (0.2 mmol/L in ethanol). And the reaction mixture was shaken. The absorbance was read at 517 nm using a spectrophotometer (TU-1810PC UV-visible, Purkinje, China) after 30 min in the dark at room temperature. The inhibition of DPPH radical was calculated according to the following formula:

$$S\ (\%) = [1 - (A_i - A_j)/A_o] \times 100$$

where, S is DPPH scavenging activity; A_i is absorbance of control; A_j is absorbance of sample; A_o is absorbance of blank.

Reducing power

The reducing power was evaluated by the method of Oyaizu (1986). Briefly, each diluted sample (2.5 ml) was mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 10 mg/ml potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. After 2.5 ml of 100 mg/ml trichloroacetic acid was added, the mixture was centrifuged at 3000 r/min for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 1 mg/ml ferric chloride, and the absorbance was measured at 700 nm using a spectrophotometer.

Iron-chelating ability

The chelating effect was carried out according to the method of Dinis et al. (1994). Each diluted sample (1.0 ml) was mixed with 3.7 ml of ethanol and 0.1 ml of 2 mmol/L ferrous chloride. The reaction was initiated by the addition of 0.2 ml of 5 mmol/L ferrozine. The absorbance of the reaction mixture was determined at 562 nm after 10 min at room temperature.

Hydroxyl radicals scavenging assay

The hydroxyl radical scavenging activity was determined according to the published method with some modifications (Smirnoff and Cumbes, 1989). Each diluted sample (1.0 ml) was mixed with 1.0 ml of 9 mmol/L salicylic acid-ethanol solution and 1.0 ml of 9 mmol/L ferrous sulfate. The reaction was initiated by the addition of 1.0 ml of 8.8 mmol/L H_2O_2. Then, the mixture was shaken vigorously and incubated at 37°C for 30 min, the absorbance was read at 510 nm against blank. The hydroxyl radicals scavenging activity (Sha) was calculated using the following equation:

$$Sha\ (\%) = [A_0 - (A_X - A_{X0})]/A_0 \times 100.$$

Where, A_0 is the absorbance of blank; A_{X0} is the absorbance of control; A_X is the absorbance of sample.

Total phenolic content

The amount of total phenolics was determined using the Folin-

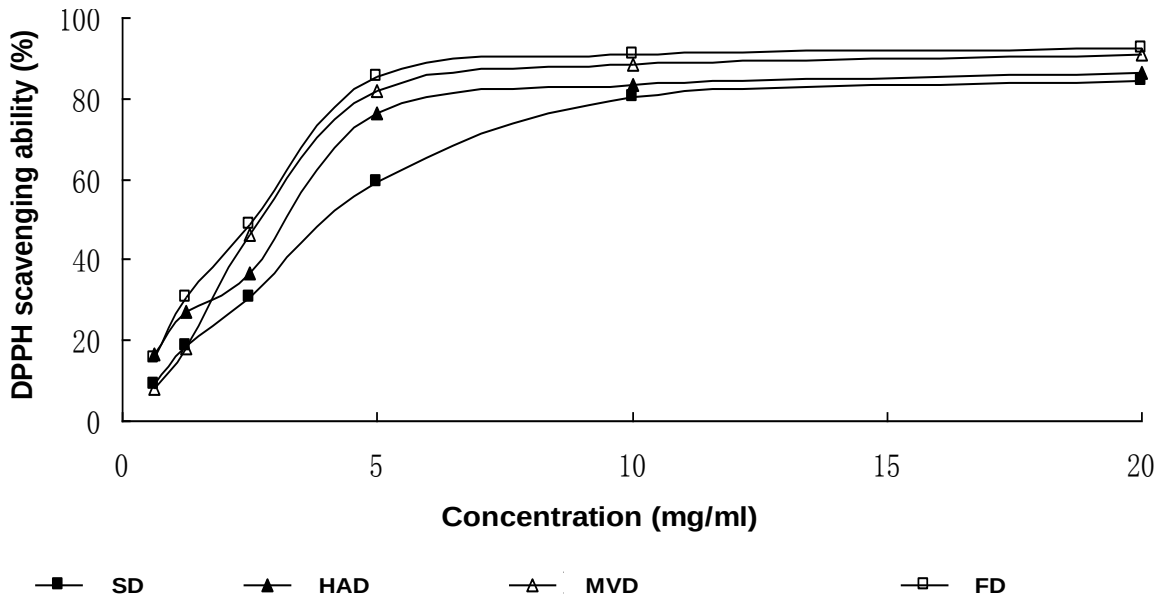

Figure 1. DPPH radical scavenging activity of sun, hot-air, freeze, and microwave-vacuum dried *Robinia pseudoacacia* L. flowers. SD: Sun drying; HAD: Hot-air drying; MVD: Microwave-vacuum drying; FD: Freeze drying.

Ciocalteau phenol described by Taga et al. (1984) with minor modification. The diluted extract (0.3 ml) was added and mixed with 1.7 ml distilled water and 1.0 ml 150 mg/ml aqueous sodium carbonate solution. After 3 min, 50 µl 1 mol/L Folin-Ciocalteau reagent was added to the mixture. The mixture solution was shaken vigorously and then allowed to stand at 25°C for 90 min. The absorbance was recorded at 760 nm. The amount of total phenolics was expressed as gallic acid equivalent (GAE) on dry weight basis.

Statistical analysis

All the data were expressed as means ± standard deviation of three replications, and one factor ANOVA was used for the statistical analysis. Significant differences were calculated according to LSD multiple range test. The values were considered to be significantly different when $P<0.05$. Pearson correlation test was used to determine the correlation among variables.

RESULTS AND DISCUSSION

Effects of drying methods on antioxidant activities of *R. pseudoacacia* L. flowers

DPPH radical scavenging activity

DPPH radical scavenging activity was observed to increase rapidly with sample concentration from 0.625 to 10 mg/ml and then reached a high plateau between 10 and 20 mg/ml (Figure 1). And at the concentration of 5 to 20 mg/ml, the order of DPPH scavenging activity was always in a descending order: FD>MVD>HAD>SD. Additionally, at 10 and 20 mg/ml, DPPH radical scavenging activity of microwave-vacuum dried sample was comparable to that of freeze dried samples ($P>0.05$), and was much higher than that of sun and hot-air dried samples ($P<0.05$).

Reducing power

The reducing power is often considered as the ability of natural antioxidant to donate electrons (Dorman et al., 2003). Reducing power of different dried sample was always in the order: FD>MVD> HAD>SD (Figure 2). At 5 mg/ml, the reducing powers of extract from SD, HAD, MVD, and FD samples were 0.697, 0.939, 1.135 and 1.808, respectively. In addition, at 10 and 20 mg/ml, reducing power of microwave-vacuum dried sample was comparable to that of freeze dried samples ($P>0.05$), and was much higher than that of sun and hot-air dried samples ($P<0.05$).

Iron-chelating ability

Ferrous ions participate in direct and indirect initiation of lipid oxidation (Wettasinghe and Shahidi, 2002). And the iron-chelating ability measures the ability of antioxidants to compete with ferrozine in chelating ferrousion (Elmastas et al., 2006). As shown in Figure 3, increase in iron-chelating ability was observed with increasing concentrations in all samples. Microwave-vacuum dried samples had the highest chelating capacity, while sun dried samples exhibited the lowest capacity. And at 30 mg/ml, the chelating capacity of microwave-vacuum dried

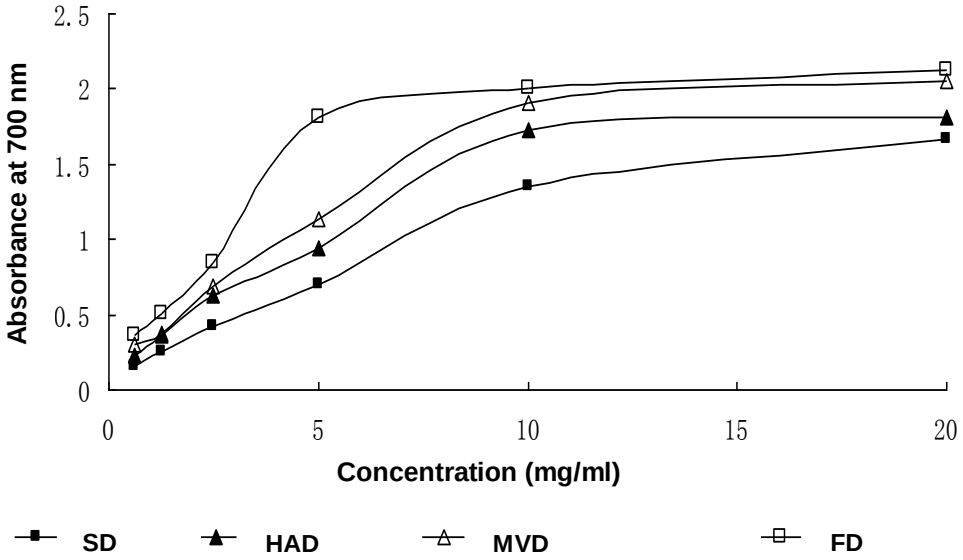

Figure 2. Reducing power of sun, hot-air, freeze, and microwave-vacuum dried *Robinia pseudoacacia* L. flowers. SD: Sun drying; HAD: Hot-air drying; MVD: Microwave-vacuum drying; FD: Freeze drying.

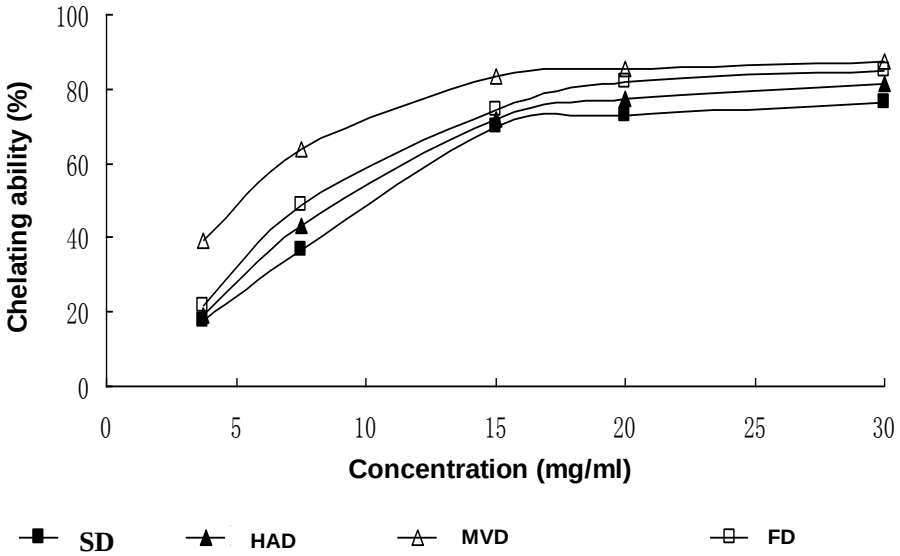

Figure 3. Iron-chelating ability in sun, hot-air, freeze, and microwave-vacuum dried. *Robinia pseudoacacia* L. flowers. SD: Sun drying; HAD: Hot-air drying; MVD: Microwave-vacuum drying; FD: Freeze drying.

samples was as high as 87.2% (Figure 3).

Scavenging ability of hydroxyl radicals

Hydroxyl radicals (•OH) are known to be the most reactive of all the reduced forms of dioxygen, and are capable of damaging almost every molecule found in living cells (Rollet-Labelle et al., 1998). These radicals have the capacity to join the nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity (Moskovitz et al., 2002). Hydroxyl radicals can be formed by the Fenton reaction in the presence of H_2O_2 and reduced transition metals such as Fe^{2+}. It was observed that freeze dried samples had the highest hydroxyl radical

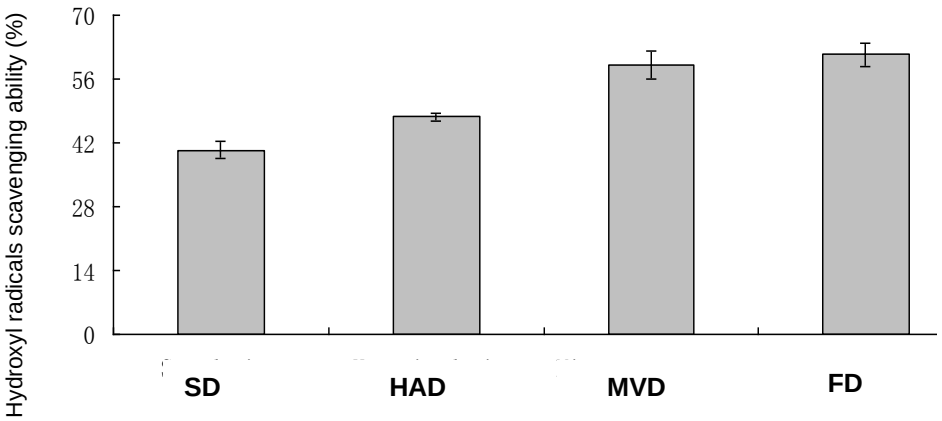

Figure 4. Hydroxyl radical scavenging capacity of sun, hot-air, freeze, and microwave-vacuum dried *Robinia pseudoacacia* L. flowers at the concentration of 20 mg/ml. SD: Sun drying; HAD: Hot-air drying; MVD: Microwave-vacuum drying; FD: Freeze drying.

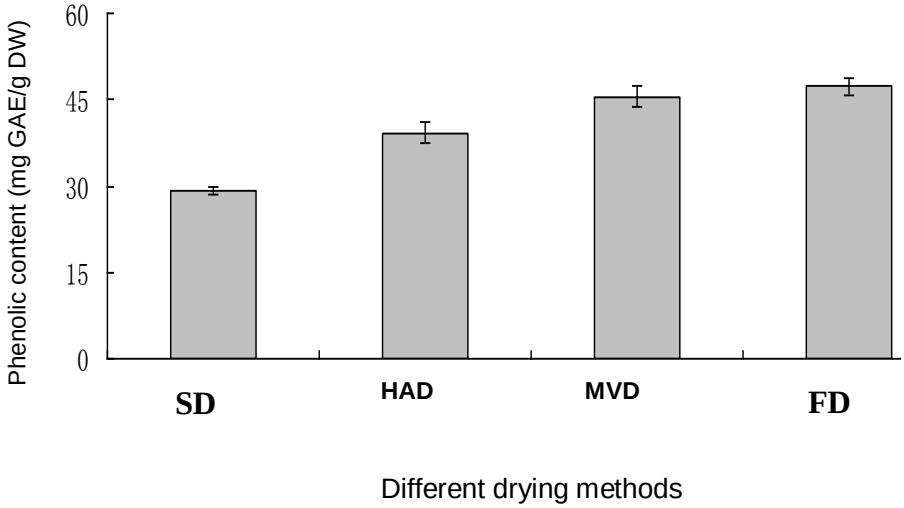

Figure 5. Total phenolic contents in sun, hot-air, freeze, and microwave-vacuum dried *Robinia pseudoacacia* L. flowers. SD: Sun drying; HAD: Hot-air drying; MVD: Microwave-vacuum drying; FD: Freeze drying.

scavenging ability (61.30%) (Figure 4). While sun dried samples exhibited the lowest power (40.40%). Hydroxyl radical scavenging ability of microwave-vacuum dried sample was comparable to that of freeze dried samples (P>0.05). Drying method showed a different influence on hydroxyl radicals scavenging capacity in dried RPF.

Effects of drying methods on total phenolic content of *R. pseudoacacia* L. flowers

The amount of TPC determined in different dried RPF was shown in Figure 5. Freeze dried sample had the highest phenolic content (47.30 mg GAE/g DW). TPC in microwave-vacuum dried sample was as high as 45.52 mg GAE /g DW and was comparable to that of freeze dried sample (P>0.05). While, sun dried sample had the lowest content of phenolic compounds (29.15 mg GAE /g DW) (P<0.05).

With regard to FD, freeze drying may lead to a higher extraction efficiency of total phenolics because freezing could lead to the development of ice crystal within the tissue matrix. Ice crystals could result in a greater rupturing of cell structure, which may lead to better solvent access and extraction (Shih et al. 2009), so the TPC in freeze dried sample was higher. With regard to HAD, hot air is the medium of heat transfer and could rapidly inactivate polyphenol oxidases present in plant

Table 1. Correlation coefficients between antioxidant activities and total phenolic content in dried *Robinia pseudoacacia* L. flower.

	$DPPH^+$ scavenging activity	Reducing power	Iron-chelating ability	Hydroxyl radicals scavenging ability
Phenolics	0.805**	0.890**	0.875**	0.960**

*and **, significant at $P<0.05$ or $P<0.01$, respectively.

materials when temperature was higher than 50°C. However, some of their initial activities may have occurred earlier and caused some polyphenols to be degraded, which may be the reason of the lower content of total phenol in hot-air dried RPF. With regard to SD, the lower TPC in sun dried RPF may be affected with enzymatic processes that occurred during SD. SD did not immediately deactivate degradative enzymes such as polyphenol oxidases; therefore, they are able to degrade phenolic compounds before the plant materials are completely dry. With regard to MVD, the heat generated from microwave is more energy efficient than conventional heating, and it could inactivate degradative enzymes much faster than hot-air heating. Additionally, under the vacuum condition, the intercellular spaces collapsed, which may liberate more phenolic compounds, and also lead to a higher extraction efficiency of total phenols (Di Cesare et al., 2003).

Correlations between phenolic content and antioxidant activity

Good correlations were observed between total phenolic content and antioxidant activities (Table 1). The similar results were found by Shan et al. (2005). The highest correlation was found between phenolic content and hydroxyl radicals scavenging ability (R^2 = 0.960, $P<0.01$). And the correlations coefficient between phenolic content and reducing power was as high as 0.890. Additionally, total phenolic content exhibited a significant positive correlation with ferrous ions chelating ability (R^2 = 0.875, $P< 0.01$) in this study (Table 1). While, Rumbaoa et al. (2009) reported that the total phenolic content was a significantly negative correlation with iron-chelating activity in Philippine sweet potatoes (R^2=-0.800, $P<0.01$). The discrepancy may be due to difference in components contributing to the chelating capacity present in samples caused by different drying methods.

Conclusions

In this paper, freeze drying sample possessed the highest antioxidant activity including DPPH radical scavenging, reducing power, and hydroxyl radical scavenging ability, and the total phenolic content was 47.30 mg GAE/g DW. Microwave-vacuum drying samples had the best iron-chelating ability and its phenolic content (45.52 mg GAE/g DW) was comparable to that the of FD samples ($P>0.05$). Sun drying was the worst in antioxidant activity and content of phenolic compounds. High correlation between phenolic content and antioxidant activity was found in this paper. In addition, microwave-vacuum drying requires only minutes instead of hours for hot-air and freeze drying, or days for sun drying. Considering the antioxidant activity, phenolic content, and drying efficiency, microwave-vacuum drying appears to be a potential drying method for RPF.

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