

Effect of Drying Method on Antioxidant Activity of *Jiwhang* (*Rehmannia glutinosa*)

Jong-Whan Rhim*, Yang Xi, Won-Chul Jeong, Kyung-Sik Ham, Ha-Sook Chung¹, and Eun-Sil Kim¹

Department of Food Engineering, Mokpo National University, Muan, Jeonnam 534-729, Korea

¹Department of Food and Nutrition, Duksung Women's University, Seoul 132-714, Korea

Abstract *Jiwhang* (*Rehmannia glutinosa*), one of the most widely used medicinal herbs, was dried with various methods such as sun drying, hot air drying, vacuum drying, and freeze drying methods, and their effects on the antioxidant capacity in relation with the content of total phenolic compounds were studied with a steamed-and-dried rehmannia (*sookjiwhang*) for comparison. Generally, total phenolic contents decreased significantly by all of the drying treatments except the steamed-and-dried rehmannia, in which total phenolic contents increased 2.4 fold compared with fresh rehmannia. Content of verbascoside, a functional phenolic compound, was the highest in the freeze-dried rehmannia (177.97±0.02 µg/g d.m.) followed by vacuum-dried (105.55±0.07 µg/g d.m.), hot air-dried (23.01±0.02 µg/g d.m.), and sun-dried (4.89±0.13 µg/g d.m.) ones comparable to the fresh rehmannia (80.15±1.26 µg/g d.m.). Antioxidant capacity determined by both 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethyl-benzthiazoline-6-sulphonic acid) (ABTS) methods agreed with the result of total phenolic contents, that is, the antioxidant capacity was the highest in the steamed-and-dried rehmannia followed by fresh rehmannia, vacuum-dried, hot air-dried, sun-dried, and freeze-dried ones. Conclusively, the total phenolic contents and antioxidant capacity of rehmannia were greatly affected by the drying methods used.

Keywords: *Rehmannia glutinosa*, drying method, antioxidant activity, phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethyl-benzthiazoline-6-sulphonic acid) (ABTS)

Introduction

Rehmannia (*Rehmannia glutinosa*; *jiwhang* in Korean) is one of the most widely used medicinal herbs in traditional Oriental medicine (1). The roots of rehmannia have antibacterial, antiseptic, cardiac, diuretic, haemostatic, hypoglycemic, and tonic functions (1,2). They are usually used to replenish vitality, to strengthen the liver, kidney, and heart, and to treat a variety of ailments such as diabetes, constipation, anemia, urinary tract problems, and dizziness (1). *Rehmannia* is frequently prescribed in Oriental medicine for the healing of diseases caused by yin deficiency (2). The concept of yin-yang balance has existed in traditional Oriental medicine for more than 2000 years. Some researchers (3,4) have shown that the effective components of the yin tonic herbs are mainly flavonoids which are phenolic compounds with strong antioxidant activity. According to them the clear trend of antioxidant activity supported the hypothesis that yin in traditional Oriental medicine refers to antioxidant process, whereas yang relates to oxidation process. In modern western medicine, the balance between antioxidation and oxidation is believed to be critical for maintaining a healthy biological system.

Rehmannia is generally used in 3 different forms depending on the preparation method: 1) the fresh root of rehmannia (*saengjiwhang*), 2) the dried, usually by sun-drying method, rehmannia (*gunjiwhang*), and 3) the

steamed, usually in rice wine, and dried rehmannia (*sookjiwhang*). The fresh root is used to treat thirst, the rash of infectious disease, and bleeding caused by pathological heat. The dried root is used to treat bleeding due to blood deficiency and to nourish the body to be vital, and the steamed-and-dried rehmannia is used to stop bleeding and tonify the spleen and stomach (1).

Functional ingredients of rehmannia include iridoid glucosides such as catalpol, leonuride, aucubin, and melittoside; as well as rehmannoside A, rehmannoside B, rehmannoside C, and rehmannoside D (1,5). The contents of these functional ingredients change during processing and storage (6). When the rehmannia is dried or steamed-and-dried, the contents of sucrose, raffinose, stachyose, and catalpol decrease, whereas the contents of fructose and mannitriose increase (7,8), resulting in a different medicinal effect (8).

Traditionally, dried rehmannia has been produced using a sun drying method. However, this technique is extremely weather dependent and may lead to contamination and a decrease in product quality during the prolonged drying period. Therefore, modern drying technology such as hot air drying, vacuum drying, freeze drying, osmotic dehydration, or vacuum impregnation can be applied to produce high quality products.

Recently, phenolic compounds in fruit and vegetable sources have gained much attention, owing to their antioxidant activities and their potential benefits in human health (9,10) and such antioxidant capacity of plant sources has been known to be greatly influenced by processing methods (6,11-13). Therefore, it is important to evaluate the degree of change in antioxidant capacity of the product depending on the processing methods.

*Corresponding author: Tel: +82-61-450-2423; Fax: +82-61-454-1521

E-mail: jwrhim@mokpo.ac.kr

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Though several methods have been proposed to evaluate the antioxidant potential of natural sources, evaluation of the total antioxidant capacity of plant products based on any single method is not recommended due to the complex nature of phytochemicals (14,15). Because multiple reaction steps would be involved, no single assay could accurately reflect all antioxidants in a biological system. At least 2 different methods should be employed in order to evaluate the total antioxidant capacity of the products of interest (16,17).

Recently, the effect of drying temperature on drying rate and apparent color characteristics of rehmanna has been reported (18), but no information is available on the effect of drying method on the antioxidant capacity of rehmanna product.

The main objectives of this study were to determine the total phenolic contents in rehmanna prepared by different drying methods and to compare the antioxidant capacity of these samples applying 2 commonly used organic radical scavenging methods, i.e., DPPH and ABTS methods in relation with the content of total phenolic compounds of the steamed-and-dried rehmanna (*sookjiwhang*), in order to determine the optimum drying method of rehmanna.

Materials and Methods

Materials Fresh rehmanna was obtained from a local farm at Jangheung, Jeonnam, Korea. The fresh rehmanna was washed with tap water to remove dust, rinsed, and then drained completely before further treatment. All the fresh and dried rehmanna samples were packed in airtight polyethylene bags and stored in a refrigerator until used. The Folin-Ciocalteu reagent, tannic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ), and 2,2'-azobis dihydrochloride (AAPH) were purchased from Sigma-Aldrich (St Louis, MO, USA). All other reagents used were reagent grade.

Sun drying About 5 kg of fresh rehmanna was spread in 1 layer on a bamboo mat, placed on a table in a lab room at 20±3°C, and dried for 30 days.

Hot air drying About 5 kg of fresh rehmanna was placed in a drying oven (HB 502M; Hanbaek Co., Ltd., Seoul, Korea) at 60°C for 24 hr.

Vacuum drying Vacuum dried samples were obtained by drying fresh rehmanna (about 5 kg) in a vacuum oven (Eyela, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 60°C and 760 mmHg vacuum (0 mmHg) for 24 hr.

Freeze drying Samples frozen at -80°C were dried using a freeze-drier (Eyela FDU-1100; Tokyo Rikakikai Co., Ltd.). The cold trap temperature of the dryer was set at -40°C with the chamber pressure of 760 mmHg vacuum (0 mmHg), and then the shelf temperature was started from -30°C up to 40°C with the increase of 10°C/every 2 hr and remained for 3 more days.

Steaming and drying Dried rehmanna was steamed after immersion in a turbid rice wine (*makgeolli*) for 24 hr and then dried using a hot air drying method as above. This

process was repeated 9 times to obtain steamed-and-dried rehmanna, which was used as a control.

Proximate analysis Moisture, crude protein (N×5.70), crude lipid, and crude ash contents of fresh and dried rehmanna samples were determined following AOAC methods (19).

Preparation of rehmanna extract Each rehmanna sample (2 g) was homogenized and extracted by refluxing 100 mL of 3 different solutions, i.e., 30%, 80% methanolic aqueous solutions and distilled water, for 2 hr at room temperature. The extracts were filtered through Whatman No. 41 paper and rinsed with the same solution. Then the filtrates were combined with the extracts, filtered through a membrane filter (pore size: 0.45-µm), and then used for further analysis.

Total phenolic contents Total phenolic contents of rehmanna samples were determined according to the Folin-Ciocalteu's procedure with minor modification (20). The rehmanna extract (0.1 mL) was mixed with 2 mL of 2% Na₂CO₃, and then 0.1 mL of 50% Folin-Ciocalteu phenol reagent was added and mixed thoroughly. After 30 min, the absorbance of the mixture was read at 750 nm, and the total phenolic contents was determined using a calibration curve obtained using tannic acid as a standard (10-400 mg/L). Results were expressed as mg of tannic acid equivalents (TAE)/g of rehmanna. All samples were analyzed in triplicate.

Verbascoside content Each rehmanna samples (100 g) were extracted with 200 mL of 100% methanolic aqueous solution at room temperature for 48 hr and evaporated solvent using a vacuum drying oven at 30±2°C, 760 mmHg vacuum for 2 weeks to prevent thermal destruction of verbascoside. The residue was dissolved in 1 mL of methanol, filtered through a 0.45-µm polytetrafluoroethylene (PTFE) filter into an high performance liquid chromatography (HPLC) vial, and capped. HPLC analysis was performed with a Hitachi 2000 series HPLC with an YMC C₁₈ RP column (10×250 mm) (Hitachi High-Technologies Corporation, Yokohama, Japan). The analysis conditions were as follows: injection volume 5 µL; run time 45 min; acetonitrile (AcCN):H₂O=25:75 for 10 min; AcCN:H₂O=33:67 for 45 min; flow rate 2 mL/min; column temperature 30°C; UV detector at λ=230 nm.

Antioxidant activity Antioxidant activity of rehmanna samples was analyzed using 2 independent methods, DPPH and 2,2'-azinobis (3-ethyl-benzthiazoline-6-sulphonic acid) (ABTS) methods. In the DPPH method, the free radical scavenging ability of rehmanna sample extracts was measured according to the methods of Blois (21) with some modification. An aliquot of 0.8 mL of 0.2 mM DPPH methanolic solution was mixed with 0.2 mL of the rehmanna extract. The mixture was shaken using a vortex mixer for 10 sec, centrifuged for 5 min at 5,000×g, and then left to stand for 25 min in the dark place. The absorbance was measured at 520 nm and the DPPH radical scavenging activity (electron donating activity; EDA, %) was calculated as:

Radical scavenging activity (%) = $(1 - A_{\text{sample}}/A_{\text{control}}) \times 100$
 where A_{sample} and A_{control} are absorbances with and without sample, respectively.

In the ABTS method, the scavenging ability of extracts on the ABTS^{•+} radical was measured by the method described by Jara and Fulgencio (22). Phosphate-buffered saline (PBS) (3.4 mM Na₂HPO₄-NaH₂PO₄ 0.15 M NaCl; pH 7.4) containing 1 mM of AAPH with 2.5 mM of ABTS was incubated at 70°C for 30 min, and then the solution was diluted with distilled water to obtain a final absorbance of 0.7±0.02 at 734 nm. Diluted ABTS^{•+} solution (980 µL) was added to 20 µL of ascorbic acid standard solution, the rehmannia extract sample, or distilled water and incubated for 30 min at 37°C. Then absorbance was determined at 734 nm using a spectrophotometer. The ABTS radical scavenging activity was expressed in terms of ascorbic acid equivalent antioxidant capacity (AEAC) as mg of ascorbic acid equivalents (AA eq)/g of sample, which was calculated as follows (23):

$$\text{AEAC} = (\Delta A_{\text{sample}} / \Delta A_{\text{aa}}) \times C_{\text{aa}} \times V \times (1000 / W_{\text{sample}})$$

where ΔA_{sample} and ΔA_{aa} are the changes in absorbances after the addition of sample or ascorbic acid standard solution, respectively, C_{aa} is the concentration of the ascorbic acid standard solution (mg/mL), V is the volume of the extract (mL), and W_{sample} is the weight of sample used for extraction (g). All extracts were analyzed in triplicate.

Statistical analysis Differences among drying methods of rehmannia samples were determined by one-way analysis of variance (ANOVA) using statistical analysis package, SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA). Mean values were separated ($p < 0.05$) with the Duncan's multiple range test.

Results and Discussion

Proximate composition The moisture content of rehmannia samples decreased from 68% of the fresh rehmannia to 2.0-15.2% depending on the drying methods (Table 1). Except the sun dried sample, the moisture contents of all dried rehmannia samples were below the monolayer moisture content of rehmannia root (8.0 g water/100 g dry solid: 7.4% wet basis) reported by Rhim *et al.* (18). However, the moisture content of sun dried rehmannia (15.19±0.38%) was still higher than the monolayer

moisture content of rehmannia root. This indicates more prolonged time is needed for the sun drying to attain safe storage level of moisture content. As the moisture content of the fresh root was reduced, the volume and surface color of rehmannia was also changed accordingly. Surface color of fresh rehmannia is yellowish red due to carotenoid pigments in the fresh rehmannia (24,25). Except for the freeze-dried samples, all the rehmannia samples have been shrunk and darkened considerably with progress in drying. The freeze-dried rehmannia maintained its shape even after drying; however, the surface color changed from yellowish red to creamy white. Change in the content of crude protein, crude lipid, and ash of dehydrated rehmannia may be attributed to the change in the moisture content during dehydration.

Total phenolic contents The plant phenolic compounds are aromatic secondary metabolic products that include a considerable range of substances possessing an aromatic ring bearing one or more hydroxyl substituents, which have an antioxidant effect through interactions with the phenol ring and its resonance stabilization effect (26-29). The phenolic contents of the rehmannia samples changed significantly depending on the drying methods (Table 1). Generally, the phenolic contents of rehmannia decreased from 5.34±0.05 mg/g of the fresh rehmannia to 3.19 mg/g depending on the drying method except the steamed-and-dried rehmannia whose total phenolic contents were 12.56 mg/g. The comparison was based on the solid basis. These results are in good agreement with the reported values by Woo *et al.* (32). They reported the total phenolic contents of dried rehmannia and a steamed-and-dried rehmannia were 5.09 and 13.90 mg/g, respectively. It is interesting to note that the phenolic contents of the steamed and dried rehmannia increased dramatically even though it received very severe thermal treatment like steaming and drying repeated 9 times. Seemingly this result contradicts with the general belief that nutritional quality degrades through food processing like high heat treatment. However, numerous reported results support the present study. Increase in phenolic content has been frequently observed in the thermally processed fruits and vegetables such as rehmannia (6), sweet corn (20), *shiitake* mushroom (23), ginseng (31), garlic (32), onion (33), and tomato (34). Most phenolic compounds in plants are naturally present in conjugated forms (35), and they are usually stored in pectin or cellulose networks and accumulated in the vacuoles in bounded forms. Heat treatment can break supramolecular

Table 1. Proximate composition, total phenolics, and verbascoside contents of the rehmannia samples dried with various methods

Rehmannia	Moisture (%)	Crude lipid (%)	Crude protein (%)	Ash (%)	Total phenolics (mg/g dry matter)	Verbascoside (µg/g dry matter)
Fresh rehmannia	67.74±0.68 ¹⁾	0.17±0.02 ^a	1.48±0.05 ^c	2.43±0.02 ^b	5.34±0.05 ^b	80.15±1.26 ^c
Sun-dried	15.19±0.38 ^b	0.36±0.03 ^a	6.17±0.45 ^{ab}	3.22±0.04 ^a	4.19±0.08 ^c	4.89±0.13 ^e
Hot air-dried	5.13±0.05 ^d	0.38±0.03 ^a	6.60±0.20 ^a	2.78±0.01 ^b	4.01±0.85 ^c	23.01±0.02 ^d
Vacuum-dried	7.40±0.04 ^c	0.69±0.06 ^a	6.00±0.23 ^{ab}	3.18±0.65 ^a	5.75±0.13 ^b	105.55±0.07 ^b
Freeze-dried	2.00±0.08 ^e	0.79±0.06 ^a	6.93±0.38 ^a	3.09±0.29 ^{ab}	3.19±0.00 ^d	177.97±0.02 ^a
Steamed & dried	7.04±0.09 ^c	0.68±0.00 ^a	6.87±0.03 ^a	2.85±0.04 ^b	12.56±1.06 ^a	ND

¹⁾ Values are mean±SD ($n=3$); Any 2 means in the same column followed by the different letter are significantly different ($p < 0.05$) by Duncan's multiple range test; ND, not determined.

structure, resulting in the release of the glycosidic bonded phenolic sugar (20,34). The phenolic sugar reacts better with the Folin-Ciocalteu reagent (36), resulting in the increased polyphenols in the thermally processed fruits and vegetables. This result provides a good example of a benefit of processing of fruits and vegetables. However, such phenomenon of the increase of total phenolic compounds by thermal processing is not universally recognized. It depends on the vegetables, the processing methods, the bioavailability, temperature, and the stability of the structure to heat (37).

Verbascoside content Verbascoside (or acteoside), one of a functional phenolic compounds, is known to have various functional and medicinal effects. Verbascoside has antioxidant activity and is associated with accelerating the wound-healing function (38). The verbascoside content in the rehmannia samples was determined using reverse phase (RP)-HPLC. The optimal mobile-phase composition for the analysis was selected by performing several RP-HPLC runs with various concentrations of AcCN in water. The retention time of verbascoside was 8.70 min. The verbascoside content in the rehmannia samples was determined from the peak area of the chromatograms and was expressed in $\mu\text{g/g}$ of sample in moisture free basis (Table 1). Verbascoside content changed significantly depending on the drying method. The verbascoside concentration decreased from $80.15 \pm 1.25 \mu\text{g/g}$ of the fresh rehmannia to 4.89 ± 0.13 and $23.01 \pm 0.02 \mu\text{g/g}$ after dehydration by the sun and hot air drying, respectively, whereas its concentration increased up to 105.55 ± 0.07 and $177.97 \pm 0.02 \mu\text{g/g}$ after dehydration by the vacuum and freeze drying, respectively. The decrease of verbascoside content in the sun-dried rehmannia may be mainly due to the prolonged exposure (30 days) to the unfavorable conditions such as oxidation and photo-catalytic reactions. The increase in the verbascoside concentration of vacuum- and freeze-dried samples is possibly due to less severe conditions for the dehydration resulting in less destruction of the cell structure and the material itself, and structurally more favorable extraction condition for verbascoside after dehydration.

Antioxidant activity The radical scavenging activity of the rehmannia samples extracted with 3 different solvents (distilled water, 30 and 80% aqueous methanolic solution) toward the stable free radical DPPH was evaluated at 10 mg/mL of the extract and the results are shown in Fig 1. The DPPH scavenging activity of the rehmannia samples was greatly influenced not only by the drying methods but also by the extraction solvents. Generally, the antioxidant activity of the rehmannia samples expressed as EDA decreased by drying process except the steamed-and-dried rehmannia. The mean EDA of the fresh rehmannia was 32% and decreased to 9.3, 20.6, 35.2, and 15.4% for the sun-dried, the hot air-dried, the vacuum-dried, and the freeze-dried rehmannia, respectively. However, that of the steamed-and-dried rehmannia increased up to 81%. This result was in good agreement with the previously reported results of Woo *et al.* (6,30), who found that the antioxidant activity of steamed-and-dried rehmannia determined by the EDA increased significantly and the degree of increase was more profound as the number of steaming increased up to

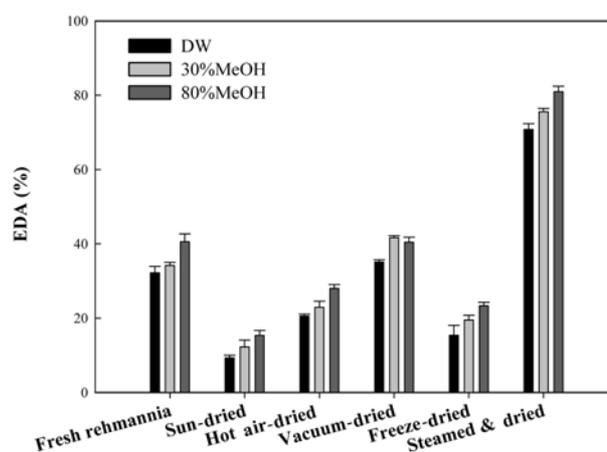


Fig. 1. DPPH radical scavenging activity (electron donating ability; EDA%) of rehmannia samples dried with various methods.

14 times. They also reported that the EDA determined at 1 mg/mL of rehmannia extract was increased from 19.44 % for dried rehmannia up to 75.6% for the 14 times steamed-and-dried rehmannia. The enhancement of the antioxidant properties of the steamed-and-dried rehmannia is mainly due to the naturally occurring compounds such as phenolics or the formation of novel compounds such as Maillard reaction products that have antioxidant activity (6,39).

Recently, Jiménez-Monreal *et al.* (37) found that some vegetables like green bean, celery, and carrot showed the increased antioxidant activity after various cooking methods such as boiling, pressure-cooking, baking, microwaving, griddling, and frying. They suggested 4 possibilities for the increase in antioxidant activity of some vegetables after cooking: i) the liberation of high amounts of antioxidant components due to the thermal destruction of cell walls and sub-cellular compartments; ii) the production of stronger radical scavenging antioxidants by thermal reaction; iii) suppression of the oxidation capacity by thermal inactivation of oxidative enzymes; and iv) production of new non-nutrient antioxidants or formation of novel compounds such as Maillard reaction products with antioxidant activity. Such possibilities may explain the difference in the antioxidant activity of the dried rehmannia samples in the present study.

Total antioxidant activity of the rehmannia samples was also determined by the ABTS cation radical scavenging activity and expressed as the AEAC (mg AA eq/g sample) as shown in Fig. 2. In a similar way to the antioxidant activity determined by the DPPH method, the AEAC of the rehmannia samples was also affected by the drying methods and extraction solvents. The AEAC of the fresh rehmannia was 6.02 mg AA eq/g and decreased to 3.4, 4.6, 5.5, and 2.9 mg AA eq/g for the sun-dried, the hot air-dried, the vacuum-dried, and the freeze-dried rehmannia, respectively, and increased up to 12.15 mg AA eq/g of the steamed-and-dried rehmannia. Both antioxidant activity test results showed that the antioxidant activity for all the rehmannia samples changed depending on the extraction solvents, i.e., the 80% methanolic aqueous solvent exhibited the highest activity followed by 30% methanolic solution, and distilled water. This is presumably due to the

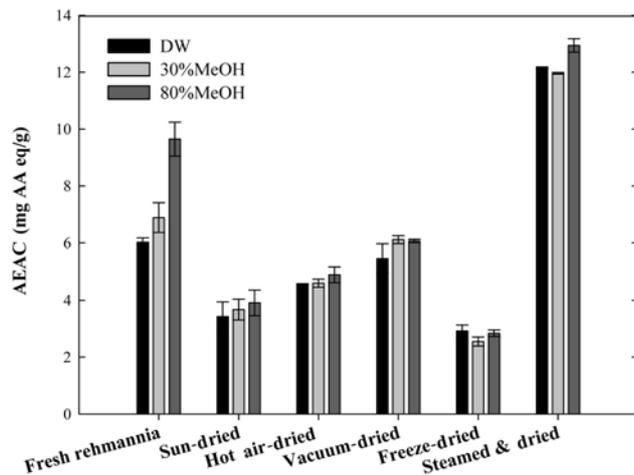


Fig. 2. ABTS cation radical scavenging ability of rehmannia samples dried with various methods.

higher amount of alcohol-soluble phenolic compounds in the rehmannia.

Among the drying methods tested, the vacuum dried rehmannia retained the highest antioxidant activity determined by both DPPH and ABTS methods, followed by hot air-dried, freeze-dried, and sun-dried rehmannia. It is interesting to note that the antioxidant activity of rehmannia samples determined by both DPPH and ABTS methods is consistent with the result of total phenolic contents as shown in Table 1. It is well known that phenolic compounds not only in rehmannia but also in other plants have a strong antioxidant activity (6,29). Woo *et al.* (6) found a highly significant ($p < 0.001$) correlation with high correlation coefficient ($r = 0.9763$) between the EDA (%) of thermally treated rehmannia and total phenolic contents of rehmannia. Velioglu *et al.* (29) also reported a strong correlation between antioxidant activity and total phenolic content in several fruits, vegetables, and grains.

Unexpectedly, freeze-dried rehmannia exhibited the least antioxidant activity with the least phenolic contents among the drying methods tested. Presumably, heat treatment of hot-air and vacuum drying methods caused increase in the free form of phenolic compounds compared to the freeze drying method, resulting in lower total phenolics with lower antioxidant activities in the freeze-dried rehmannia sample, however, this needs to be further studied.

In conclusion, the drying methods had significant effects on the contents of total phenolic compounds and antioxidant activity of dried rehmannia. Among the drying methods tested, the sun drying and the freeze drying resulted in significant losses of total phenolics and antioxidant activity, whereas the vacuum drying method appeared to be the most appropriate for retaining the total phenolic contents and antioxidant activity.

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