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Effect of Puffing in the Extraction of Active Ingredients from the Roots of *Paeonia lactiflora* and *Astragalus membranaceus*

Hyojin Lee[1] and Kyoung Won Jang[1]*

[1]*Department of Biomedical Sciences, Daewon University College, Jecheon 27135, Korea*

Abstract – In Asia, the roots of *Paeonia lactiflora* and *Astragalus membranaceus* have been used as therapeutic agents for thousands of years. Once the medicinal plants are harvested, they are dried and their ingredients are extracted by heat-mediated reflux extraction. However, the condensed structure of organic products (especially roots) limits the extraction of bioactive components. In this study, we assessed the effect of the puffing method (using high temperature and pressure) before the extraction process in relation to the profile and antioxidant capacity of active ingredients. We demonstrated that the additional puffing process before extraction methods improves the yield of polyphenol concentrations and antioxidant activities from the roots of *P. lactiflora* and *A. membranaceus*.
Keywords – *Paeonia lactiflora*, *Astragalus membranaceus*, Puffing, Polyphenols

Introduction

In Asia, the roots of *Paeonia lactiflora* and *Astragalus membranaceus* are used as traditional therapeutic agents. *P. lactiflora* roots have anti-inflammatory and antioxidant activity owing to high levels of paeoniflorin and albiflorin.[1,2] Meanwhile, *A. membranaceus* roots improve immune response and reduce oxidative stress with minimal side effects.[3,4] The extraction process is essential in the exploration of these active ingredients. The heat-mediated reflux extraction is a conventional technique widely used in industry and research, as it requires less extraction time and solvent compared to percolation or maceration methods.[5] Additionally, ultrasonic extraction is normally used for natural product extraction with the advantages of high yields with lower energy consumption and can be performed under low temperature.[6] However, it is difficult to extract the ingredients from the condensed plant root through the reflux and ultrasonic mediated extraction. Therefore, the pre-treatment technique is necessary and can be easily coupled with extraction methods to improve extraction yields of bioactive components from roots. In the food industry, puffing (or extrusion) of substrates, such as starch, at high temperature and pressure induces a porous matrix and structural changes, thereby increasing the substrate's water-holding capacity.[7,8] Puffed grain is normally consumed as cereals and snacks such as ready-to-eat food formulations. Some studies reveal changes in the profile of the active ingredient in Korean Ginseng due to several processes involved in its extraction including the puffing method.[9-11] To assess the effect of puffing on the solubility of active compounds from roots, we applied the puffing method and tested the extraction level of Paeoniflorin and Albiflorin from *P. lactiflora*, and calycosin-7-O-β-D-glucoside from *A. membranaceus*. In previous studies, the explosive puffing of natural products including Red Ginseng and *Dendrobium officinale* significantly improved the level of polyphenols and antioxidant contents.[12,13] Therefore, we compared these findings with that from non-puffed samples and found that puffing improves the extraction of total polyphenols, and also enhances their antioxidant activity.

Experimental

Materials – The roots of *P. lactiflora* and *A. membranaceus* (cultivated in Jecheon, Chungbuk province, Republic of Korea and harvested at November to December, 2020) were purchased from Jecheon Korean Medicine Bio Promotion Foundation (Jecheon, Korea). The samples were chopped into 3-cm-long chips before use. Analytical grade of Albiflorin (BP0134, Chengdu Biopurify Phytochemicals Ltd., China), Paeoniflorin (P1876, TCI, Japan), and Calycosin-7-O-β-D-glucoside (BP030601,

*Author for correspondence
Kyoung Won Jang, Department of Biomedical Sciences, Daewon University College, Jecheon 27135, Korea
Tel: +82-43-649-3106; E-mail: brightstar01@daewon.ac.kr

Chengdu Biopurity Phytochemicals Ltd., China) were purchased.

Puffing and Extraction conditions – The roots of *P. lactiflora* and *A. membranaceus* were separately puffed before the ingredients were extracted from them. Puffing was performed using a rotary gun-type cereal puffing machine under 900 kPa followed by the method elaborated from previous studies.[14] During the puffing process, non-uniform medicinal herbs are easily burned, so the solid supplement such as rice, wheat bran, or soil can be combined.[15] In this regards, the additional condition of puffing with rice (roots:rice = 1:3, w/w) was used to protect roots from carbonization during heating.[9,14] Dried root sample group without puffing treatment was used as a non-puffing control. Each sample was powdered and weighted one gram and underwent extraction using 100 ml of 70% methanol (1:100, w/v), followed by extraction. The extraction of reflux and ultrasonication were performed under same solvent condition and 30 min of extraction time.

HPLC condition – Standard materials and root extract samples were analyzed using the high-performance liquid chromatography (HPLC), separated by Capcellpack C18 Type MG II column (4.6 × 150 mm ID, 5 μm, Osaka Soda, Osaka, Japan) in Agilent 1260 series HPLC system (Agilent Technologies, USA). The mobile phase H_2O: acetonitrile (A/B, v/v), 75:15, 0.8 mL/min, UV spectrum 230 nm. The standard materials of albiflorin and paeoniflorin were dissolved in 100% methanol, and calycosin-7-O-β-D-glucoside was dissolved in DMSO (Dimethyl sulfoxide, SAMCHUN Chemical, Korea). All samples and standards were filtrated using a 0.45-μm syringe filter (NYLON filter media, Whatman, USA) before analysis.

Polyphenol analysis – The total polyphenol content analysis was performed using the Folin-Denis method.[16] Briefly, the 1 mL of sample extracts were mixed with 0.5 mL Folin-Denis' reagent (47742, Supelco, USA), 1 mL Na_2CO_3, and 7.5 mL distilled water. After 30 min, the absorbance at 760 nm was measured using a spectrophotometer (Thermo Scientific™, Multiskan™ FC Microplate Photometer, Waltham, Massachusetts, USA). The polyphenol concentration was obtained using the calibration curve with 0.0625–1.00 μg/mL of gallic acid as standard.

DPPH radical scavenging activity – DPPH ((1,1-diphenyl-2-picryl hydrazyl, Sigma Chemical Co., St. Louis, MO, USA) scavenging assay was used to analyze the antioxidant activity of the samples.[17] A total volume of 0.2 mM DPPH solution reacted with the root extracts for 10 min and the absorbance was measured at 517 nm. DPPH radical scavenging activity was calculated using the equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1-\frac{\text{absorbance of the sample}}{\text{absorbance of the control}}\right)\times 100$$

Statistical analysis – All experiments were carried out in independent triplicates and data obtained were presented as mean ± standard deviation (SD). For the significance test for each sample group, a value with $p < 0.05$ after Student's t test was considered as a significant result compared to the control group.

Result and Discussion

Dry roots of *P. lactiflora* and *A. membranaceus* are condensed and hard to rupture, thereby rendering the extraction of active ingredients difficult. Therefore, we employed the puffing process before the extraction and analyzed the amount and antioxidant activity of active ingredients. The active ingredients of the root samples treated by puffing or non-puffing were analyzed using HPLC. Moreover, the puffing process was separated in two different ways, puffed roots and puffed roots with rice, and compared as shown in Table 1. Using the reflux and ultrasonication methods, the total albiflorin extracted from non-puffed *P. lactiflora* was 37.44 ± 0.37 μg/mL and 35.6 ± 0.25 μg/mL, respectively. The extraction level increased to 41.58 ± 0.46 μg/mL and 36.32 ± 0.33 μg/mL after puffing the roots of *P. lactiflora* with rice. These

Table 1. Quantification of albiflorin, paeoniflorin, and calycosin-7-O-β-D-glucoside of non-puffed, puffed, and puffed with rice of *P. lactiflora* and *A. membranaceus*. (RE: reflux extraction, SE: sonication extraction)

pre-treatment		*P. lactiflora*			*A. membranaceus*					
		Non-puffed	Puffed	Puffed with rice	Non-puffed	Puffed	Puffed with rice	Non-puffed	Puffed	Puffed with rice
		albiflorin (μg/ml)			paeoniflorin (μg/ml)			calycosin-7-O-β-D-glucoside (μg/ml)		
extraction method	RE	37.44 ± 0.37	25.59 ± 0.4	41.58 ± 0.46	372.62 ± 0.64	265.32 ± 0.44	279.93 ± 1.22	1.43 ± 0.17	1.51 ± 0.04	2.12 ± 0.02
	SE	35.6 ± 0.25	33.68 ± 0.1	36.32 ± 0.33	350.82 ± 0.56	261.78 ± 0.37	274.79 ± 1.21	1.38 ± 0.20	1.42 ± 0.07	2.20 ± 0.04

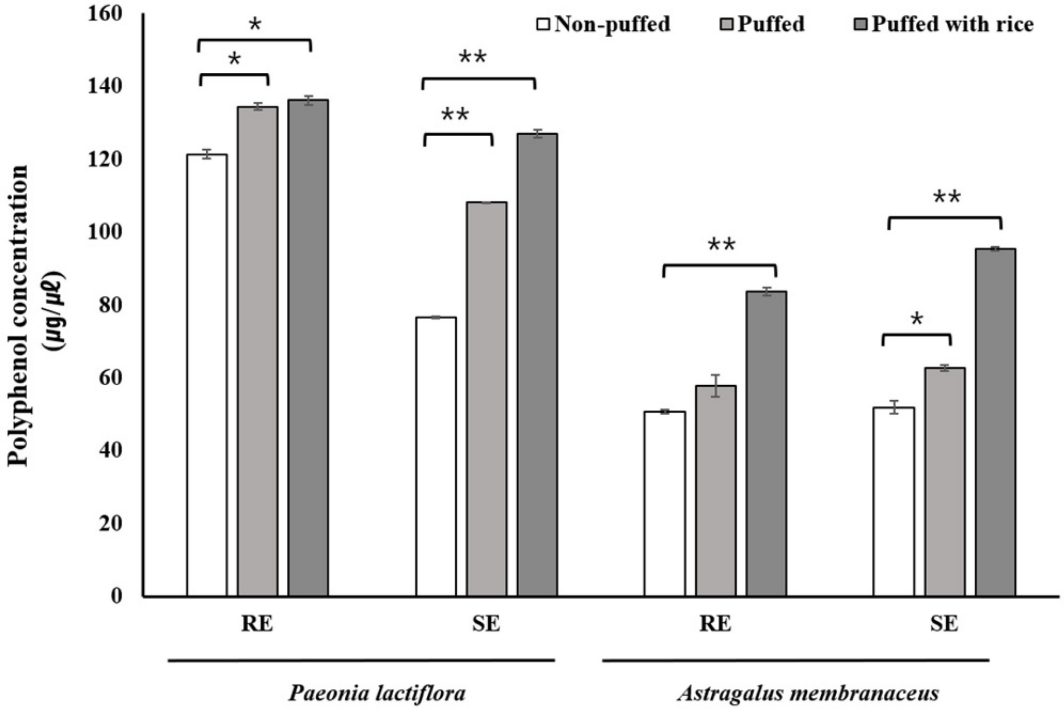

Fig. 1. Total polyphenol content of *P. lactiflora* and *A. membranaceus* root extracts before and after puffing and extracts by reflux extraction (RE) and sonication extraction (SE). Values are presented as mean ± SEM. * indicates $p < 0.05$ and ** indicates $p < 0.001$.

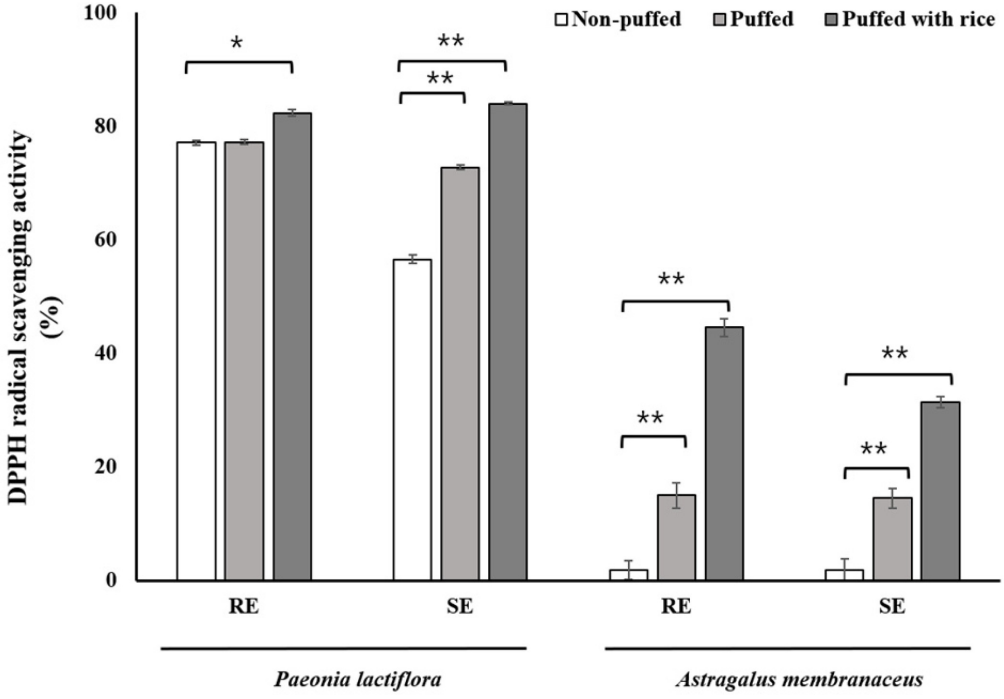

Fig. 2. Antioxidant activities (DPPH radical scavenging activity) of *P. lactiflora* and *A. membranaceus* root extracts before and after puffing obtained with reflux extraction (RE) and sonication extraction (SE). Values are presented as mean ± SEM. * indicates $p < 0.05$ and ** indicates $p < 0.001$.

benefits were observed in puffing the roots of *A. membranaceus*. The level of calycosin-7-O-β-D-glucoside in the extraction products through reflux and sonication significantly increased from 1.43 ± 0.17 μg/mL and 1.38 ±

0.20 μg/mL to 2.12 ± 0.02 μg/mL and 2.20 ± 0.04 μg/mL after puffing their roots with rice. However, the individually puffed roots of *P. lactiflora* and *A. membranaceus* rarely changed ingredient levels as compared to non-puffed samples. The decreased content of paeoniflorin from *P. lactiflora* after the puffing process is due to the thermal instability of paeoniflorin.[18,19] Therefore, the puffing process performed with rice before the reflux and sonication treatment has the beneficial effects when extracting ingredients from root samples.

A comparison of the total polyphenols from puffed or non-puffed roots is displayed in Figure 1. In the root of *P. lactiflora*, the extracted level of total polyphenols using the reflux-mediated method was 121.38 ± 1.2 μg/mL, and it increased to 136.15 ± 1.3 μg/mL after puffing with rice. Moreover, after ultrasonication, the amount of extracted polyphenols was 76.58 ± 0.3 μg/mL, and it increased to 127.03 ± 1.2 μg/mL after puffing the root with rice before the extraction. The puffing process significantly increases the efficiency of sonication extraction in *P. lactiflora*. In the *A. membranaceus*, the total extracted polyphenols from both reflux and sonication methods increased by a minimum of 65% after puffing with rice. Therefore, puffing especially with rice significantly increases the extraction level of polyphenol contents in the roots of *P. lactiflora* and *A. membranaceus*.

The free radical scavenging activity indicate the antioxidant ability of plant extracts measured in their ability to prevent the formation of free radicals that destroy cellular core materials resulting in various diseases including cancer. Here, the DPPH scavenging activity was tested which reflects the antioxidant capacities of natural products.[20] Figure 2 illustrates this activity of root extracts extracted by puffing, puffing with rice, and compared to the non-puffed sample extracts. Using the sonication method, the DPPH scavenging activity of the *P. lactiflora* samples was 56.67% before puffing, increased to 72.78% after puffing, and up to 84.04% after puffing with rice.

In the roots of *A. membranaceus*, a 1.84% radical scavenging activity was observed in non-treated root samples, puffing increased to 14.98% and puffing with rice increased up to 44.61%. These findings reveal that puffing with rice drastically increases the amount of the extracted contents of the DPPH scavenging activity of substances from the roots of *P. lactiflora* and *A. membranaceus*.

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