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# Comparison of Pretreatment Methods for Extraction of Selected Components from *Ganoderma lucidum*

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# 전처리 방법에 따른 영지버섯 추출액의 품질특성 변화

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**Abstract**

This study was conducted to investigate the effects of pretreatment and extraction methods on the water extraction yields of sugar, protein and phenolic compounds, and the antioxidative activity of extract from *Ganoderma lucidum*. The herb was ground, sifted, and treated for 10 min with microwave (2,450 MHz) and ultrasound (20 kHz), and then extracted by boiling underatmospheric pressure and pressured boiling. Particle size (0.8~2.4 mm), extraction time (2 hr) and solvent/solid ratio (20:1) were selected as optimal conditions for extraction. Microwave and ultrasound treatment increased the extraction yields of total sugar, protein and phenolic compounds and the radical scavenging activity of extract. In comparison with microwave treatment and ultrasound treatment, microwave treatment was more effective than that of ultrasound treatment. The yields of all three compounds and the radical scavenging activity of extract were higher in pressured boiling extraction than in boiling underatmospheric pressure. The results showed that microwave radiation and sonication prior to extraction, and pressured extraction could be utilized for improving the extraction efficiency of *G. lucidum*.

Key words : *Ganoderma lucidum*, microwave, ultrasound, extraction

## Introduction

*Ganoderma lucidum* has been widely used as a remedy to promote health and longevity. *G. lucidum* is one of the most famous medicinal fungus and its biological activities and therapeutic effects such as immunomodulation, antitumour, antiinflammation, antioxidation, cholesterol lowing, antihypertension and antiallergy have been reported (1,2). Because of their perceived health-stimulating properties and medicinal effects, today the use of *G. lucidum* is widespread and growing in a health food industry.

Extraction of chemical constituents from the raw materials is a principal unit operation in medicine and food manufacture. The reflux extraction has been used as conventional method for centuries, but it requires long extraction time and has low efficiency. Therefore, new technologies are applied to save the extraction time as well as to increase the extraction yield.

Microwave-assisted extraction (3-5), ultrasound-assisted extraction (6-8) and supercritical fluid extraction (9-11) were developed to improve the conventional extraction method for various herbs. However, they have many disadvantages such as requirement of special equipment, or sometimes the low recovery and the instability of a target component, which limits the wider commercial applications (5,11). Microwave radiation and ultrasonication should be used in combination with the conventional extraction. But, there are no report about

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the effects of both microwave and ultrasound treatment prior to the conventional extraction on extraction property.

This study compared with the effects of both microwave and ultrasound pretreatment in relation to extraction pressure applied to the extractions of *G. lucidum* in order to determine preferable treatment method.

## Materials and Methods

### Materials

*Ganoderma lucidum* was obtained from the herb market in Daegu, Korea. The samples were kept in a sealed plastic bag and stored at 4℃ until use.

Folin-Ciocalteu reagent, gallic acid, diphenyl picryl hydrazyl radical (DPPH) and glucose were purchased from Sigma Chemical Co. (St Louis, USA). All other used chemicals were analytical grade.

### Comparison of sample particle size

Herb samples were ground by a mill (model J-NCM, JISICO, Korea) and sifted in different particle sizes: 0.8~2.4 mm and 2.4~4.0 mm. These samples (10 g) were extracted with 200 mL of distilled water for 2 hr under two extraction pressures: by boiling (100℃) under atmospheric pressure and by boiling (121℃) high pressure. The used ratio of sample and solvent were obtained from preliminary tests to achieve approximately effective conditions.

### Comparison of extraction time

Herb samples (10 g, 0.8~2.4 mm) were extracted with 200 mL of distilled water. Extractions were performed by boiling (100℃) under atmospheric pressure and by boiling (121℃) high pressure during different periods: 0.5, 1, 2 and 3 hr.

### Comparison of pretreatment method

Herb samples (10 g, 0.8~2.4 mm) and distilled water (200 mL) were mixed and treated using a microwave oven (2,450 MHz) or using a sonicator (Sonic Dismembrator F550, Fisher Scientific, USA, 20 kHz) for 10 min prior to extraction. The treated and nontreated samples were extracted for 2 hr under atmospheric pressure (100℃) and high pressure (121℃).

### Component analysis

General extraction efficiency was estimated as content of soluble solids in the water extract. Soluble solids were measured using a refractometer (model N-1E, Atago Co., Japan).

Total sugar content was determined using the phenol-sulfuric acid method (12). The extract (1 mL) was added to a 1 mL of 5% phenol solution plus 5 mL of conc sulfuric acid. The mixture was shaken and left for 20 min. The absorbance of the resulting solution was measured with a spectrophotometer (model UV1601, Shimadzu, Japan) at 470 nm. Concentration of total sugar was determined by comparison with the absorbance of glucose used at different concentration as standard. Yield of total sugar was expressed in grams of total sugar extracted per gram of raw materials.

Protein content was determined using the method provided by the Association of Official Analytical Chemists (13). Yield of protein was expressed in grams of protein extracted per gram of raw materials.

Content of total phenolic compounds was determined using the Folin-Ciocalteu method (14). The extract (5 mL) was transferred into volumetric flask. Folin-Ciocalteu reagent (5 mL) was added and the contents of the flask mixed thoroughly. After 3 min, 5 mL of 10% $Na_2CO_3$ solution was added and left for 1 hr. The absorbance of the resulting solution was measured with a spectrophotometer (model UV1601, Shimadzu, Japan) at 760 nm. Concentration of total phenolic compounds was determined by comparison with the absorbance of gallic acid used at different concentration as standard. Yield of total phenolic compounds was expressed in grams of total phenolic compounds extracted per gram of raw materials.

### Radical scavenging activity

Radical scavenging activity was determined using diphenyl picryl hydrazyl radical (DPPH) (15). The water extract (0.2 mL) was added to a 0.8 mL of $4\times10^{-4}$M ethanolic solution of DPPH radical. The mixture was shaken vigorously and left for 10 min. The absorbance of the resulting solution was measured at 525 nm with a spectrophotometer (model UV1601, Shimadzu, Japan). Radical scavenging activity was calculated using the formula: percentage inhibition = [1 - (absorbance of sample/absorbance of DPPH)] × 100.

### Statistical analysis

Statistical analysis was conducted with the SPSS software (version 12.0, SPSS Inc., USA) for replicated test data. Analyses of variance were performed by an ANOVA procedure. Significant differences ($\alpha$=0.05) between means were determined by Duncan's multiple range test.

## Results and Discussion

### Comparison of sample particle size

Yields of total sugar, protein and phenolic compounds extracted by using atmospheric boiling and high pressure boiling from the different particle sizes of *Ganoderma lucidum* are compared in Table 1. Yields of three components of the *G. lucidum* were higher in high pressure extraction than in extraction under atmospheric pressure as particle having same size was used. The herbs between 0.8 to 2.4 mm exhibited the higher yields except for the yield of phenolic compound extracted by high pressure extraction, when same extraction pressure was applied. From this result, the samples having 0.8~2.4 mm in diameter were used for other experiment in this study. It was well known that the rate of mass transfer was directly proportional to the surface area, so reductions in particle size increase the rate of extraction up to certain limits (16).

**Table 1. Yields of total sugar, protein and phenolic compounds extracted from different particle sizes of *Ganoderma lucidum***

| Extraction method | Particle size (mm) | Total sugar (mg/g) | Protein (mg/g) | Phenolic compounds (mg/g) |
|---|---|---|---|---|
| Nonpressured | 0.8-2.4 | 17.8$^{b}$ | 22.8$^{b}$ | 0.61$^{a}$ |
| | 2.4-4.0 | 14.6$^{a}$ | 19.2$^{a}$ | 0.66$^{a}$ |
| Pressured | 0.8-2.4 | 25.6$^{d}$ | 34.6$^{c}$ | 0.80$^{b}$ |
| | 2.4-4.0 | 20.6$^{c}$ | 24.8$^{b}$ | 0.87$^{c}$ |

$^{a-d}$Values with the same letter are not significantly different at the 5% level.

### Comparison of extraction time

Yields of total sugar, protein and phenolic compounds extracted from *G. lucidum* for different times by using under atmospheric pressure boiling and high pressure boiling are presented in Table 2. In case of *G. lucidum*, the yields of all three compounds increased rapidly up to a peak level during 2 hr of extraction and afterwards it downed slowly. From these results, 2 hr was selected as optimal period for other extraction experiments.

**Table 2. Yields of total sugar, protein and phenolic compound extracted for different extraction times from *Ganoderma lucidum***

| Extraction method | Extraction Period (hour) | Total sugar (mg/g) | Protein (mg/g) | Phenolic compounds (mg/g) |
|---|---|---|---|---|
| Nonpressured | 0.5 | 13.2$^{a}$ | 17.8$^{a}$ | 0.61$^{a}$ |
| | 1 | 13.4$^{a}$ | 19.4$^{a}$ | 0.67$^{ab}$ |
| | 2 | 15.6$^{b}$ | 23.8$^{b}$ | 0.73$^{bc}$ |
| | 3 | 12.6$^{a}$ | 19.0$^{a}$ | 0.70$^{bc}$ |
| Pressured | 0.5 | 16.0$^{b}$ | 32.8$^{c}$ | 0.68$^{ab}$ |
| | 1 | 16.4$^{b}$ | 37.4$^{d}$ | 0.73$^{bc}$ |
| | 2 | 20.8$^{c}$ | 40.8$^{e}$ | 0.86$^{d}$ |
| | 3 | 13.2$^{a}$ | 35.0$^{cd}$ | 0.75$^{c}$ |

$^{a-e}$Values with the same letter are not significantly different at the 5% level.

### Comparison of pretreatment method

Soluble solids of water extracts of *G. lucidumin* relation to the extraction conditions are shown in Fig. 1. Effects of the extraction methods and the pretreatment on the level of soluble solids were almost not different in the *G. lucidum*. Pressured boiling extraction considerably increased soluble solids in the extract as compared with the atmospheric boiling extraction, when same pretreatment was used. This effect could be explained because the extraction temperature was higher. It was reported that higher temperatures increase both the rate at which solutes dissolve in the solvent and the rate of diffusion into the bulk of the solvent (16). Pretreatments using a microwave oven or a sonicator prior to extraction increased soluble solids of extract isolated from the *G. lucidum* regardless of the extraction pressure. These results showed that the efficiency of hot water extraction could be improved by microwave radiation or sonication.

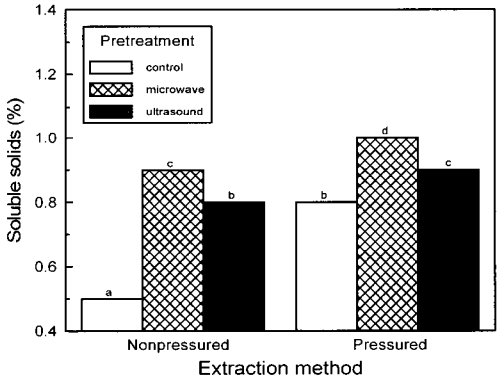


**Fig. 1. Soluble solids of extract isolated from *Ganoderma lucidum* in relation to extraction conditions. Values with the same letter are not significantly different at the 5% level.**

Yield of total sugar extracted from *G. lucidum* with different pretreatment and extraction pressure is presented in Fig. 2. In the *G. lucidum*, the yield of total sugar was increased by pretreatment and pressured extraction. The yields from treated with microwave prior to extraction under atmospheric pressure were significantly higher than that treated with ultrasound, but this effect did not show in case of pressured extraction. This may be due to pretreatment effect was compensated by high temperature during extraction. Yield of protein extracted *G. lucidumwith* with different

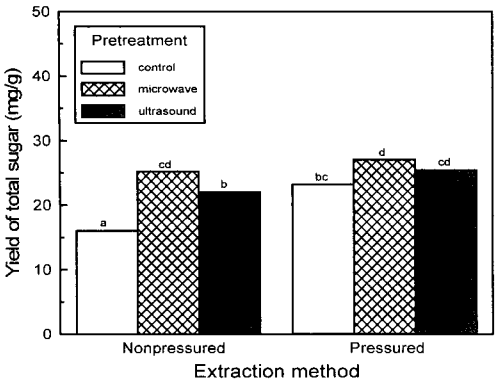


**Fig. 2. Yield of total sugar extracted from *Ganoderma lucidum* in relation to extraction conditions. Values with the same letter are not significantly different at the 5% level.**

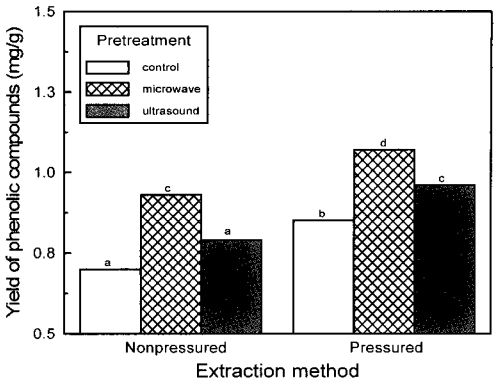


**Fig. 4. Yield of phenolic compound extracted from *Ganoderma lucidum* in relation to extraction conditions. Values with the same letter are not significantly different at the 5% level.**

pretreatment and extraction pressure is compared in Fig. 3. The yield showed significant differences according to extraction conditions. In the *G. lucidum*, the yield was increased by pressured extraction regardless pretreatment methods. And microwave pretreatment did significantly increase the yield by nonpressured and pressured extraction.

Yield of total phenolic compounds extracted from *G. lucidum* in relation to extraction conditions is presented in Fig. 4. The yield was increased by pressured extraction when same pretreatment was used, enhanced by the treatment of microwave or ultrasound prior to both nonpressured and pressured extractions. Between the two pretreatments, microwave was more effective on the isolation of phenolic compounds from the herbs. This effect was significantly shown higher in the pressured extraction. On the other plant, it was known that phenolic compounds could be easily extracted with microwave-assisted extraction (17). However, there was no significant difference between ultrasound treatment and control in terms of the nonpressured extraction.

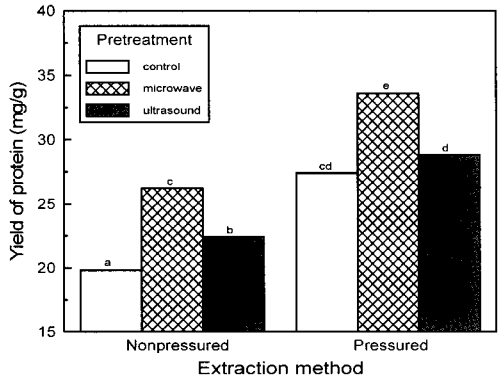


**Fig. 3. Yield of protein extracted from *Ganoderma lucidum* in relation to extraction conditions. Values with the same letter are not significantly different at the 5% level.**

DPPH radical scavenging activity was investigated to evaluate the antioxidative activity of water extracts isolated from *G. lucidum* at different extraction conditions and its result is presented in Fig. 5. In general, it was known that radical scavenging activity is positively related to the level of phenolic compounds (3). Result of this study could be explained because phenolic compounds from *G. lucidum* have a strong radical scavenging activity. Extraction condition affect the radical scavenging activity of the extract of *G. lucidum*. In *G. lucidum*, microwave treatment prior to nonpressured or pressured extraction increased significantly the radical scavenging activity of extracts, and there was no difference between the both extraction pressures.

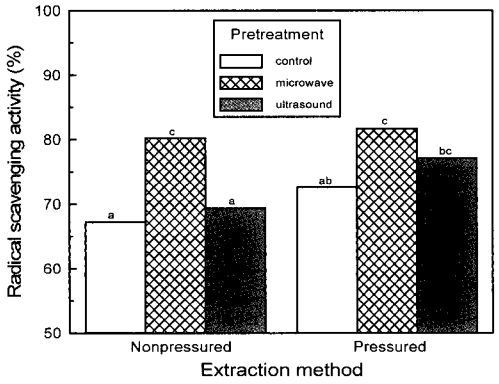


**Fig. 5. Radical scavenging activity of extract isolated from *Ganoderma lucidum* in relation to extraction conditions. Values with the same letter are not significantly different at the 5% level.**

The study showed that the both microwave and ultrasound treatments and the pressured extraction had increasing effect on the extraction efficiency and the antioxidative ability of extract. Therefore, microwave radiation, ultrasonication and pressured extraction could be recommended as a suitable

method to isolate chemical components from *G. lucidum*.

## 요 약

영지버섯(*Ganoderma lucidum*)으로부터 영양 및 기능성 성분의 열수추출 수율을 증대시킬 수 있는 방법을 개발하기 위하여 추출 전처리 및 방법의 효과를 비교, 검토하였다. 영지를 분쇄하여 사별하고 초음파와 마이크로파 처리를 10분 동안 실시한 후 물을 용매로 상압 및 가압추출을 각각 2시간 동안 실시하였다. 추출전 전처리를 행함으로서 총당, 단백질함량 및 페놀성물질의 추출수율을 증가시켰고, 유리기 소거능이 증가하는 경향을 보였으며 마이크로파 전처리가 초음파처리 보다 우수한 것으로 나타났다. 또한 상압추출보다 가압추출에 의해 가용성 고형물, 총당, 단백질 및 페놀성 물질의 추출수율이 증대되는 경향을 보였다. 따라서 초음파나 마이크로파 전처리와 가압추출은 영지버섯의 추출효율 향상에 유효한 방법인 것으로 판단되었다.

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