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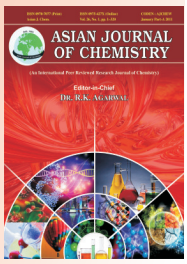

Analysis of Isoliquiritin and Isoliquiritigenin from Insampaedoksan Fermented by *Lactobacillus plantarum* 144 Using RP-HPLC

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Insampaedoksan is a traditional herbal medicine used for antipyretic and antiinflammatory diseases. Thus isoliquiritigenin have bioactivity effects such as antioxidant, estrogenic and anticancer. The level of isoliquiritin and isoliquiritigenin in insampaedoksan were investigated before and after fermentation with *Lactobacillus plantarum* 144, along with isoliquiritin and isoliquiritigenin extraction efficiency with 100 % water, 60 % aqueous MeOH and 100 % MeOH. The characterization of isoliquiritin and isoliquiritigenin were achieved by comparing its retention time (t_R), molecular weight and UV absorption with those of the standard compound. The extraction efficiency of isoliquiritin and isoliquiritigenin were the highest with 100 % MeOH. After fermentation, the amount of 100 % MeOH extract isoliquiritigenin was selectively increased (202.73 %) and isoliquiritin was selectively decreased (82.57 %). The bioconversion rate of isoliquiritin to isoliquritigenin was 39.93 %. Also, the molecular mass of the fermented insampaedoksan fraction (#1) were shown *via* LC-MS and, its high purity was shown to be above > 95 %. Thus, qualitative and quantitative analyses of isoliquiritin and isoliquiritigenin in insampaedoksan and fermented insampaedoksan were conducted.

Keywords: Analysis, Bioconversion, HPLC-DAD, Insampaedoksan, Isolation.

INTRODUCTION

Traditional herbal medicines have been widely used for thousands of years in many Asian countries due to their enhanced efficacy. They are drawing great interest for the prevention and treatment of various illnesses[1]. Among the traditional oriental medicines herbal, insampaedoksan (IS) has been used for the treatment of cold-related symptoms. Insampaedoksan consists of 16 herbs: ginseng, bupleurum root, licorice root, cnidium rhizome, aurantiifructus, plantain, ginger, lonicera flower, forsythia fruit, schizonepeta spike, saposhnikovia root, anthriscussylvestrishoffm, angelica Korea root, aralia continentalis root, hoelen and mentha herb[2,3]. Bioconversion such as fermentation increases the bioactivities of the useful components (*e.g.*, isoflavones, saponins, phytosterols and phenols) of herbs and maximizes their absorption[4-6]. Also, many related literature have demonstrated that fermentation changes the original activities of such components into new treatment effects and enhances their original activities[7,8]. In the field of traditional medicines herbal, the interest in fermented herbal medicines using intestinal microorganisms is increasing. *Lactobacillus*, an intestinal microorganism, inhibited the growth of some harmful bacteria through the production of lactic acid and showed therapeutic effects, including antiinflammatory and anticancer activities[9,10]. The insampaedoksan isoliquiritin and isoliquiritigenin were selected for this analysis. It is were reported to have bioactivity effects such as antioxidant[11], estrogenic[12] and various anticancer effect[13,14].

We investigated the effect of the extraction solvent composition in the isoliquiritin and isoliquiritigenin extraction yield. And compared the bioconversion state of before and after fermentation insampaedoksan with *Lactobacillus plantarum* 144. Also, an isoliquiritigenin fraction was isolation from fermented insampaedoksan *via* HPLC and the high purity (> 95 %) of isoliquiritigenin was identified *via* RP-HPLC. According to the obtained insampaedoksan can be used as a basic material for the development of new drugs in oriental medicine herb.

EXPERIMENTAL

The two standard isoliquiritin and isoliquiritigenin were purchased from Chemfaces (St. Wudong, Wuhan, China). The purity of the standard compounds determined using HPLC was higher than > 98 %. All the oriental medicinal herbs were purchased from Yeongcheon Traditional Herbal Market (Yeongcheon, South Korea). The sample powder

(insampaedoksan; 100 mg) was obtained from Korea Institute of Oriental Medicine (KIOM) KM-based herbal drug research group, South Korea in June 2011. The HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from J.T. Baker, Inc. (Philipsburg, NJ, USA). The analytical grade trifluroacetic acid (TFA) was obtained from Sigma-Aldrich (St. Louis, MO, USA). The triple distilled water was filtered with a 0.45 μm membrane filter (ADVANTEC, Tokyo, Japan) before analysis.

Preparation standard: The standard solution of isoliquiritin and isoliquiritigenin were prepared by dissolving 2 mg in a 10 mL MeOH solution (200 ppm).

Insampaedoksan extraction: The insampaedoksan sample was prepared through the total extraction method. Oriental medicinal herbs were mixed, soaked in 10-volume of water for 1 h and heat-extracted (boiling) for 3 h using an extractor (Gyeongseo, Cosmos-660, Incheon, South Korea). After the lyophilization of the extract, the insampaedoksan was filtered out using standard testing sieves (150 μm, Retsch, Haan, Germany). As a result, whitish insampaedoksan powder was obtained and stored at 4 °C before use.

The sample powder (insampaedoksan; 100 mg) were dissolved in 100 % MeOH, 60 % aqueous MeOH and 100 % water at the concentration of 100 mg/mL, respectively. Each sample was filtered with a 0.2 μm PVDF membrane filter before HPLC analysis.

Fermentation of insampaedoksan: The insampaedoksan sample was adjusted to pH 7 using 1 M NaOH and was then sterilized in an autoclave at 120 °C for 15 min and cooled to 37 °C. The insampaedoksan was incubated with *L. plantarum* 144 obtained from KFRI (Korea Food Research Institute, Seongnam, South Korea) to prepare fermented insamapedoksan. The bacterial strain was incubated in 50 mL MRS broth (Difco TM Lactobacilli MRS Broth, Becton Dickinson, Franklin Lakes, NJ, USA) at 37 °C overnight. The insamapedoksan fermented with *L. plantarum*at 37 °C for 48 h was filtered with a 60 μm nylon net filter (Millipore, Billerica, MA, USA), lyophilized and stored in desiccators at 4 °C. So, the extent of fermentation pH from insampaedoksan and fermented insampaedoksan were 4.02-4.04.

HPLC condition: The experiments in this study were performed using the Waters HPLC 2695 system equipped with a pump, an autosampler, a column oven and a 996 photodiode array UV/visible detector (Waters HPLC system, MA, USA) and the Empower software program was used for data acquisition and processing. The chromatographic column that was used in this experiment was commercially available and was obtained from RS-Tech Optimapak (C_{18}, 4.6 × 250 mm, 5 μm). The column oven temperature was kept at 40 °C. The injection volume was 20 μL and the flow rate of the mobile phase was 1 mL/min.

The wavelength of the UV detector was set at 370 nm. The mobile phase were composed of water (99.9 %) containing 0.1 % triflouroacetic acid (A) and acetonitrile (100 %) (B). The run time was 60 min and the mobile phase program was the step gradient elution, as follows: 20 % (v/v) B at 0-10 min; 20-30 % B at 10-15 min; 30-40 % B at 15-40 min; 40-60 % B at 40-45 min; 60-70 % B at 45-55 min; and 70-20 % B at 55-60 min (Table-1). LC-MS analysis was also carried out to obtain the molecular-mass information and relative response (Agilent 1100+ G 1958, Agilent Technologies, Inc., CA, USA). All the experiments were repeated three times.

TABLE-1
ANALYSIS OF GRADIENT ELUTION CONDITIONS WITH HPLC

UV wavelength (nm)	370	
RP-column	RS-Tech Optima Pak C_{18} (4.6 × 250 mm, 5 μm)	
Column oven temp. (°C)	40	
Injection volume (μL)	20	
Run time (min)	60	
Mobile phase (step gradient elution)	A : Acetonitrile (20 : 70 vol. %) B : Water (80 : 30 vol. %)	
Time (min)	Acetonitrile (%)	Water (0.1 % trifluoro acetic acid) (%)
0	20	80
10	20	80
15	30	70
40	40	60
45	60	40
55	70	30
60	20	80

RESULTS AND DISCUSSION

Isoliquiritin and isoliquiritigenin were qualitatively and quantitatively identified in fermented insampaedoksan by RP-HPLC. Peaks were detected at a wavelength of 370 nm and the retention times (t_R) of isoliquiritin (18.4 min) and isoliquiritigenin (34.5 min) were compared with those of the each standard. For the quantitative analysis of isoliquiritin and isoliquiritigenin, calibration curves were constructed by plotting the injection volume (2 to 20 μL) of each standard *versus* the peak area. The calibration curves showed excellent linearity, with correlation coefficients of $r^2 = 0.9999$ and 0.9994 for isoliquiritin and isoliquiritigenin, respectively.

Fig. 1 shows the chromatograms of the before and after fermentation insampaedoksan by *L. plantarum* 144. The major isoliquiritin of the before fermentation was reduced 83 % after fermentation and another isoliquiritigenin was increased 3.4 times. Generally, various organic acids and amino acids were produced during *lactobacillus* fermentation process, So the pH value was decreasing by these acids. Therefore, the variation of pH not only could be used as the indicator of the fermentation degree but also could know that the fermentation was achieved by *lactobacillus*[15]. Fig. 2 shows the HPLC profiles and LC/MS spectra. Here, the collected isoliquiritigenin fraction (#1) and isolated isoliquiritigenin produced a $[M-H]^-$ ion at *m/z* 255, which corresponded to the molecular mass and relative response of the standard compound. The collected fraction (#1) yielded a highly pure compound (> 95 %) identified. The amounts of isoliquiritin and isoliquiritigenin extracted by 100 % methanol, 60 % methanol and 100 % water before and after fermentation by *L. plantarum* 144 were evaluated (Fig. 3). Fermentation selectively increased isoliquiritigenin and decreased isoliquiritin levels. Here, a comparison of the solvent extraction efficiencies showed that the highest extraction efficiency for each compound was obtained with 100 % methanol. However, water gave the lowest efficiency.

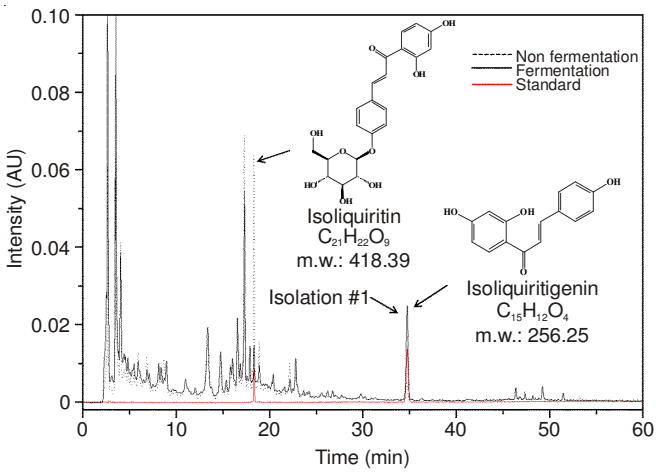

Fig. 1. Analysis of bioconversion from insampaedoksan with *Lactobacillus plantarum* 144

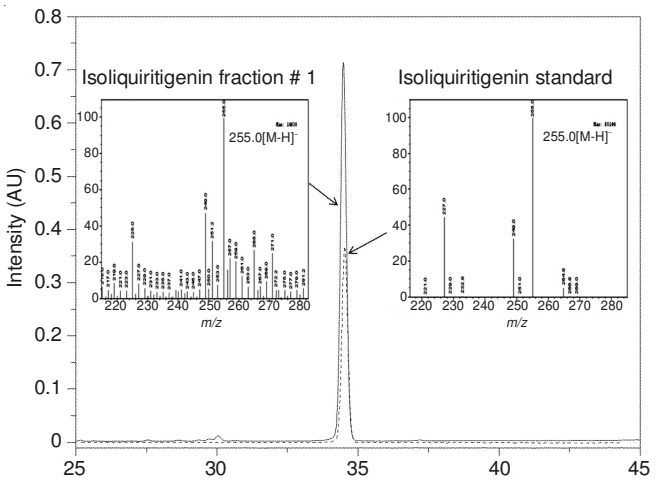

Fig. 2. Isolation of isoliquiritigenin using LC/MS spectra

In addition, the flavonoids and polyphenols compounds in the various Oriental medicine herbs (OMHs) were well soluble enhance in organic solvent methanol, so the bioactivity in methanol extracts were higher than water extracts[16].

Fermentation of insampaedoksan herbs by *L. plantarum* 144 reduced the level of isoliquiritin by 83 % and increased the level of the aglycone isoliquiritigenin by 203 %. In the

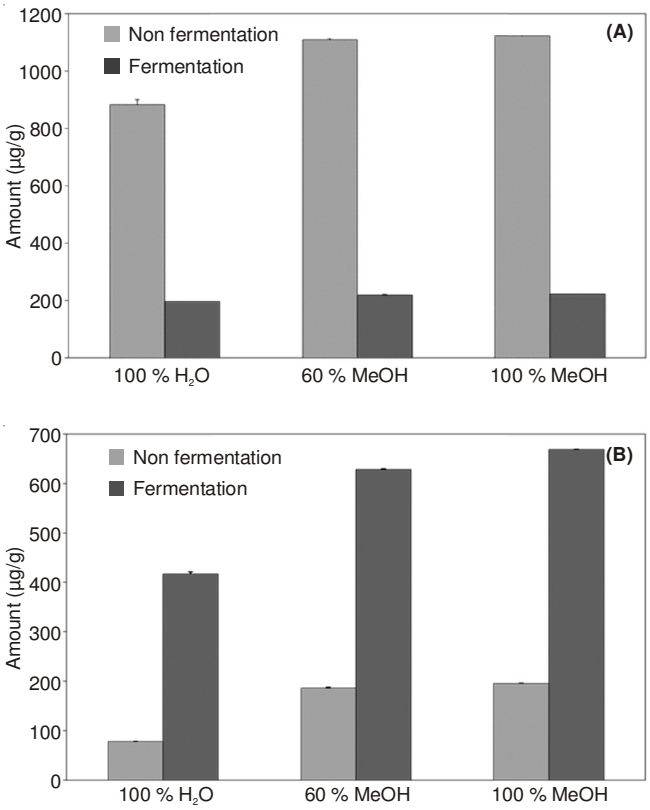

Fig. 3. Extraction efficiency of target compound from insampaedoksan. (A; isoliquiritin, B; isoliquiritigenin)

fermentation process of insampaedoksan, the rate of hydrolysis becomes higher, converts the glycoside isoliquiritin to aglycone isoliquiritigenin, so the amount of isoliquiritigenin was increased. Through the process of bioconversion, fermentation induced by microorganisms can increase the activities of many useful components in medicinal herbs[17-19]. Approximately 40 % of the isoliquiritin was bioconverted to the useful component isoliquiritigenin through fermentation. The extraction efficiency of isoliquiritin was highest before fermentation and fermentation of insampaedoksan herbs by *L. plantarum* 144 reduced the amount of isoliquiritin to one-fifth of the original amount, from 1138 to 221 μg/g. The amount of isoliquiritigenin extracted was lowest before fermentation and increased 3.4-

TABLE-2
AMOUNTS OF ISOLIQUIRITIN AND ISOLIQUIRITIGENIN IN FROM INSAMPAEDOKSAN AND FERMENTED INSAMPAEDOKSAN

	Extraction state	Extraction solvent composition	Peak area 1 (AU)	Peak area 2 (AU)	Peak area 3 (AU)	Peak area average (AU)	[a]S.D.	R.S.D. (%)	Amount (μg/g)
Isoliquiritin	Non fermentation	100 % Water	614,309	603,953	603,762	607,341	6035	0.99	851.25
		60 % MeOH	753,681	756,135	761,429	757,082	3960	0.52	1043.09
		100 % MeOH	834,917	835,034	833,462	834,471	876	0.10	1138.30
	Fermentation	100 % Water	66368	66512	68691	67190	1302	1.94	169.93
		60 % MeOH	107579	105258	106285	106374	1163	1.09	218.11
		100 % MeOH	106901	107432	107499	107277	328	0.31	220.86
Isoliquiritigenin	Non fermentation	100 % Water	27,389	31,392	37,889	32,223	1502	4.66	77.6
		60 % MeOH	147,819	146,263	149,219	147,767	1479	1.00	186.4
		100 % MeOH	157,903	157,569	158,127	157,866	281	0.18	195.4
	Fermentation	100 % Water	376,678	391,692	402,237	390,202	12844	3.29	416.4
		60 % MeOH	617,185	621,193	620,600	619,659	2163	0.35	628.2
		100 % MeOH	655,961	657,861	658,929	657,584	5299	0.81	668.6

[a] Data are expressed as mean (the average value of content) and S.D. (the standard deviation value) of three independent experiments

fold, from 195 to 669 μg/g, following fermentation by *L. plantarum* (Table-2). The results of this study will facilitate the development of new drugs in the field of traditional herbal medicine and enhance the understanding of bioconversion in pharmacological evaluations.

Conclusion

The level of isoliquiritin and isoliquiritigenin in insampaedoksan were investigated before and after fermentation with *Lactobacillus plantarum* 144, along with isoliquiritin and isoliquiritigenin extraction efficiency with 100 % water, 60 % aqueous MeOH and 100 % MeOH. This results, the extraction efficiency of isoliquritin and isoliquiri-tigenin were the highest with 100 % MeOH. After fermentation, the amount of 100 % MeOH extract isoliquiritigenin was selectively increased (202.73 %) and isoliquritin was selectively decreased (82.57 %). Also, separation of fermented insampaedoksan was collected (#1) from the MeOH extraction. Its high purity was shown to be above > 95 %. These results can be applied as the basic data for a study on the constituents of oriental medicinal herbs and traditional herbal medicines and on pharmacological evaluation from fermentation.

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REFERENCES

1. J. Tilburt, *Bull. World Health Organ*., **86**, 594 (2008).
2. J.H. Kim, W.J. Lee, Y.W. Cho and K.Y. Kim, *J. Kor. Soc. Food Sci. Nutr*, **38**, 787 (2009).
3. E.S. Doh, J.P. Chang, K.H. Lee and N.S. Seong, *Kor. J. Medicinal Crop Sci*, **18**, 255 (2010).
4. J.S. Hyon, S.M. Kang, S.W. Han, M.C. Kang, M.C. Oh, C.K. Oh, D.-W. Kim, Y.-J. Jeon and S.-H. Kim, *J. Kor. Soc. Food Sci. Nutr*, **38**, 1310 (2009).
5. D. Nakano, C.J. Kwak, K. Fuji, K. Ikemura, A. Satake, M. Ohkita, M. Takaoka, Y. Ohno, M. Nakai, N. Tomomori, Y. Kiso and Y. Matsumura, *J. Pharmacol. Exp. Ther*., **318**, 328 (2006).
6. Y.W. Lin and B.H. Chiang, *Process Biochem*., **43**, 244 (2008).
7. S. Bose, M.Y. Song, J.K. Nam, M.J. Lee and H.J. Kim, *Food Chem*., **134**, 758 (2012).
8. H.A. Eum, J.H. Lee, M.C. Yang, K.S. Shim, J.H. Lee and J.Y. Ma, *J. Tradit. Complement. Altern. Med*, **8**, 312 (2011).
9. Y.C. Oh, W.K. Cho, J.H. Oh, G.Y. Im, Y.H. Jeong, M.C. Yang and J.Y. Ma, *BMC Complement. Altern. Med*., **12**, 17 (2012).
10. X. Chen, J. Fruehauf, J.D. Goldsmith, H. Xu, K.K. Katchar, H.W. Koon, D. Zhao, E.G. Kokkotou, C. Pothoulakis and C.P. Kelly, *Gastroenterol*., **137**, 914 (2009).
11. H. Haraguchi, H. Ishikawa, K. Mizutani, Y. Tamura and T. Kinoshita, *Med. Chem*., **6**, 339 (1998).
12. C. Hu, H. Liu, J. Du, B. Mo, H. Qi, X. Wang, S. Ye and Z. Li, *J. Steroid Biochem. Mol. Biol*., **113**, 209 (2009).
13. M. Baba, R. Asano, I. Takigami, T. Takahashi, M. Ohmura, Y. Okada, H. Sugimoto, T. Arika, H. Nishino and T. Okuyama, *Biol. Pharm. Bull*., **25**, 247 (2002).
14. M. Kanazawa, Y. Satomi, Y. Mizutani, O. Ukimura, A. Kawauchi, T. Sakai, M. Baba, T. Okuyama, H. Nishino and T. Miki, *Eur. Urol*., **43**, 580 (2003).
15. K.J. Lee, M.J. Gu, J.H. Roh, P.M. Jung and J.Y. Ma, *Yakkak Hoeji*, **57**, 1 (2013).
16. J. Parekh, D. Jadeja and S. Chanda, *Turk. J. Biol*., **29**, 203 (2005).
17. J.M. Halket, D. Waterman, A.M. Przyborowska, R.K.P. Pakel, P.D. Fraser and P.M. Bramley, *J. Exp. Bot*., **56**, 219 (2004).
18. J.A. Marazza, M.S. Garro and G. Savoy de Giori, *Food Microbiol*., **26**, 333 (2009).
19. S.P. Ham, B.L. Lim, J.H. Ka, S.O. Ka, B.H. Park, *Korean J. Orient. Physiol. Pathol.*, **22**, 340 (2008).