Natural Product Sciences
11(3) : 123-126 (2005)

Enhanced Yield of Extraction from *Gastrodia elata* Blume by Ultrasonication and Enzyme Reaction

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Abstract – *Gastrodia elata* Blume (Chunma) belongs to *Orchidaceae*, which is a perennial parasitic herbaceous plant and grows in the woods of the central provinces of China, Korea and Japan. Recently, the constituents of the tubers of this plant have been investigated by researchers who have revealed the presence of phenolic compounds including gastrodin as a major constituent, together with 4-hydroxybenzaldehyde, 4-hydroxybenzyl alcohol, parishin, 4,4-dihydroxybenzyl sulfoxide, vanillin, vanillyl alcohol, beta-sitosterol, organic acids and polysaccharides, etc. In this study, we used ultrasonicator and two kinds of enzymes for enhancement of extraction yield. We also used electronic nose for the aroma pattern analysis of Chunma extracts. The concentrations of glucose and functional constituents (gastrodin, vanillin, 4-hydroxybenzaldehyde and 4-hydroxybenzyl alcohol) were measured by biochemistry analyzer and HPLC, respectively. Therefore, we showed that the yield of extraction was increased and discomfortable odor was reduced.
Keywords – *Gastrodia elata* Blume extraction, ultrasonication, α-amylase, glucoamylase, electronic nose

Introduction

Gastrodia elata Blume (Chunma), a perennial herbaceous plant belongs to *Orchidaceae*. It grows together with symbiotic *Armillaria mellea*. We can look up the clinical properties of Chunma in herbal documents like Boncho-gangmok, Donguebogam and they work on not only geriatric diseases such as hypertension, headache, paralysis, neurosis, diabetes but also stress and fatigue recovery. Ancestors have applied Chunma to folk remedies such as headache, giddy, hand and foot paralysis, palsy, or epilepsy (Ku, B. H., 1991; Society of Oriental Medicine, 1993; Chung, H. S., *et al.*, 1996). Refering to the literature of Chunma, these constituents are mostly phenolic compounds such as gastrodin, phenolic glycoside, sulfureous phenolic compound and organic acids, sugars, β-sitosterol. In addition, it contains sterol, cholesterol, p-hydroxylbenzyl alcohol, p-hydroxy benzaldehyde and vanillin (Hayashi, J., *et al.*, 2002; Hsieh, M. T., *et al.*, 1997; Noda, N., *et al.*, 1995; Taguchi, H., *et al.*, 1981; Wu, C. R., *et al.*, 1996; Liu, X. Q., *et al.*, 2002; Zhou, J., *et al.*, 1979). Chunma had been grown on domestic farm areas since 7-8 years. Korea Food & Drug Administration did not approve Chunma untill September, 2000 and its supply exceeded at that time (Shin, C. S., *et al.*, 1999; Lee, B. Y., *et al.*, 2002; Kang, T. S., *et al.*, 2002). Now it is very urgent to find basic data for the commercial use of Chunma as health foods.

In this study, we tried ultrasonic treatment, enzymatic process and condensation process to enhance the yield of extraction and reduce the discomfortable odor.

Materials and Methods

Materials – We used Chunma from the Mu-Ju Health Food Association. After removing soil and dust on Chunma with the pure water, we kept it in refrigerator at 4°C.

Ultrasonication – To increase the yield of functional constituents, we used ultrasonicator (HIELSCHER U200S). First we glinded Chunma kept in cold storage into the slush using common commercial mixer (HANIL Co., Ltd). After adding water with the weight ratio of 10 (water) to 1 (Chunma), we performed ultrasonic extraction. The frequency of ultrasonicator is fixed at 24 kHz and we

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changed the power from 0 to 200 W during the experiments. In addition we combined water bath with agitator to maintain constant temperature and agitation. Ultrasonication conditions were varied by power and elapsed time.

Enzyme reaction – Chunma contains more than 80% starch (dry basis), which causes spoiling, sedimentation, translucency for food processing. So we added α-amylase (Termamyl type LS, NOVOZYMES®, 120 KNU/g) and glucoamylase (AMG 300 L, NOVOZYMES®, 300 AGU/mL) to hydrolyze starch into soluble materials. The optimal temperature range of α-amylase and glucoamylase is 90-95°C, 60-65°C respectively, and optimal reaction time is 1-2 hours. We followed the time span and temperature according to NOVOZYMES® company as reference. Cooling apparatus with reflux including water bath and agitator for enzyme reaction and biochemistry analyzer (YSI 2700, USA) was used to analyze glucose concentration. We filtered the extracted materials using filter paper (ADVANTEC No. 2), then centrifuged filtered materials using high-speed centrifuge (HM-150 IV, HANIL Co., Ltd).

Condensation process – We condensed Chunma extracts to 50 °Brix at 60°C to reduce the discomfortable odor and lengthen storage life using vacuum evaporator (EYELA, NE, Japan).

Functional constituents analysis by HPLC – To analyze functional constituents, we used HPLC (High Performance Liquid Chromatography, WATERS, USA) system, Waters Xterra (5 μm, 15×0.46 cm) column and UV spectrometer (270 nm). Solvents were water and ethanol and we injected 20 μl sample at 0.8 mL/min. We performed the gradient technique at room temperature to increase separation degree (95/95/56/35/0/0/95% MeOH at 0/4/12/16/20/25/27 min). We used HPLC–level standard samples such as gastrodin, p-hydroxybenzyl alcohol, p-hydroxybenzaldehyde, vanillin from Sigma company.

Aroma pattern comparison by electronic nose – We used electronic nose manufactured by KOSMO (4200, USA) to compare the aroma pattern of Chunma extracts with that of standard sample. As seen in Fig. 1, electronic nose is composed of sample position where sample is injected and inject position where sample moves to sensor together with transport gas (He). After passing the inject position, it arrives at SAW crystal sensor and changes its own frequency. The changed frequency degree is different in different materials.

Results and Discussion

Ultrasonication – The main purpose to use ultrasound is to disrupt biological cell walls and help fast extraction of functional constituents out of cell. Ultrasound treated Chunma shows clean milk color and excellent homogeneity comparing with untreated Chunma. Besides, cell tissue of ultrasound treated Chunma shows complete destruction in the picture taken by optical microscope camera (not shown). As we tried various treatment conditions, the optimum condition for ultrasonic treatment was to apply 80 W for 30 minutes.

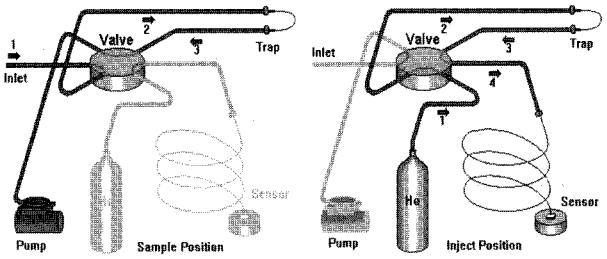

Fig. 1. Schematic diagram of electronic nose.

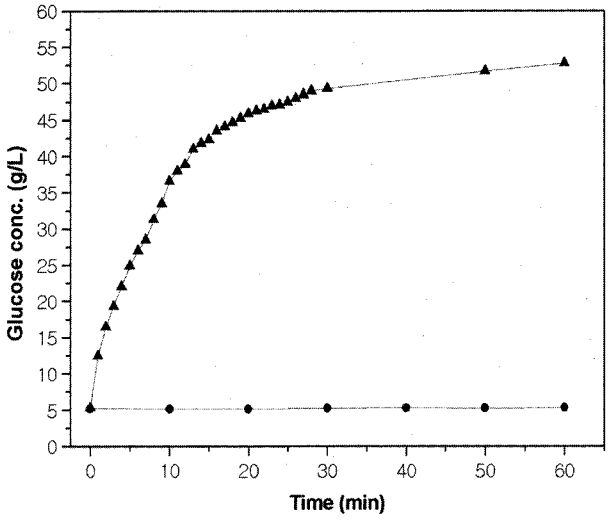

Fig. 2. Profiles of glucose concentration. (- ▲ - : ultrasonic and enzyme pre-treatment, - ● - : untreatment).

Enzyme reaction to reduce starch – Fig. 2 shows the result of enzyme reaction using α-amylase and glucoamylase. As we can see in this graph, the amount of glucose content of pre-treated Chunma is more than 10 times than that of untreated Chunma. From this result, we can find that α-amylase cuts macromolecule chains successfully and glucoamylase helps changes into monosaccharides. We also performed this experiment changing time, and amount of enzyme to search the optimum conditions (data not shown). As a result, the optimum reaction condition of α-amylase is to perform at 90-95°C for 2 hours. On the contrary, the optimum

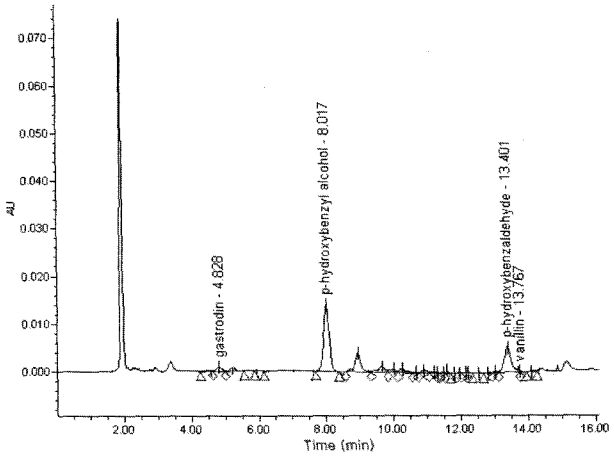

Fig. 3. HPLC chromatograms of functional constituents in pre-treated Chunma. Column: Waters Xterra (5 μm, 15×0.46 cm), gradient technique: 95/95/56/35/0/0/95% MeOH at 0/4/12/16/20/25/27 min, injection volume: 20 μl.

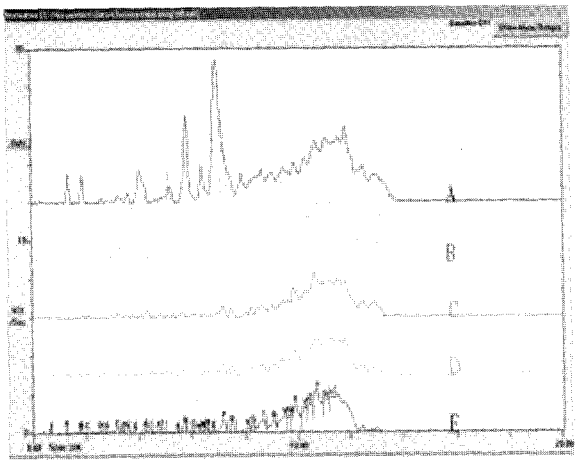

Fig. 4. Aroma patterns of electronic nose. (A: Control (Chunma sample), B: Extract by conventional method, C: Extract by ultrasonic and enzymatic pre-treatment, D: Condensation of sample C, E: Distilled solution).

Table 1. Retention time and concentration of functional constituents in HPLC analysis

Components	Retention time (min)	Concentration (ppm)	
		Untreated sample	Pre-treated sample
Gastrodin	4.828	6.521	8.739
p-Hydroxybenzyl alcohol	8.017	11.590	13.956
p-Hydroxybenzaldehyde	13.401	0.102	0.932
Vanillin	13.767	0.041	0.064
Total concentration		18.254	23.691

reaction condition of glucoamylase is to perform at 60-65°C for 30 minutes. The optimum weight percentages of added enzyme of α-amylase and glucoamylase are 0.3, 0.6% (v/w), respectively.

Analysis of functional constituents – To analyze functional constituents in untreated and pre-treated Chunma, we used HPLC (Fig. 3) and Table 1 shows the results. In conclusion, we could check that the total yield of extraction of functional constituents after pre-treatment process (23.691 ppm) increased more than 30% in comparison with the untreated process (18.254 ppm). After pre-treatment, the concentration of p-hydroxybenzyl alcohol increased about 20% from 11.590 ppm to 13.956 ppm, which was the highest among constituents. Similarly, the concentration of gastrodin increased about 34% from 6.521 ppm to 8.739 ppm. But in case of p-hydroxybenzaldehyde and vanillin, the concentration change was very minimal to ignore.

Aroma pattern of Chunma extracts – Fig. 4,5 gives

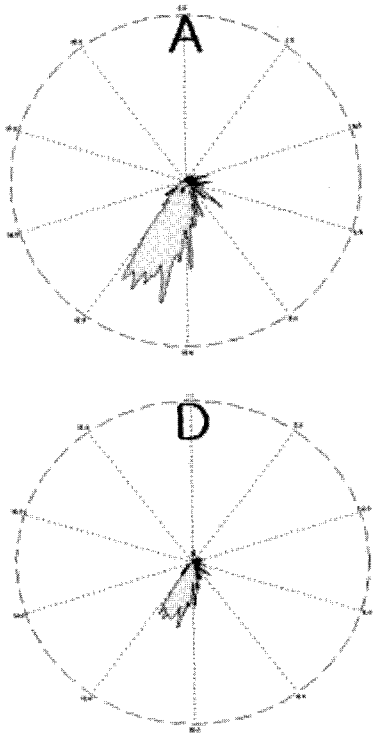

Fig. 5. Finger prints of aroma pattern. (A: Control (Chunma sample), D: Condensation of sample C).

aroma pattern results of pre-treated Chunma constituents. Sample A is raw material of Chunma and sample B is constituents which were extracted by conventional method. As you can see, the graphs of sample A and sample B are very irregular. From these results, we can conclude that volatile constituents which cause the own discomfortable and characteristic odor of Chunma.

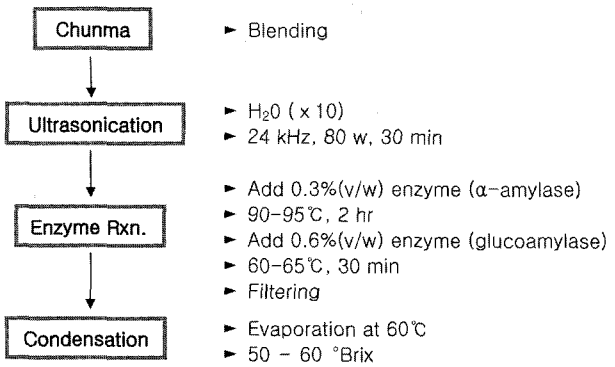

Fig. 6. Flow diagram for production of condensed extracts from Chunma.

Refering to the literature of Chunma, these volatile constituents were acid, alcohol, hydrocarbon, carbonyl, ester etc. (Lee, J. W., *et al.*, 1997; Lee, B. Y., *et al.*, 2002). Sample C is constituents extracted by ultrasonic treatment and enzyme reaction and we got sample D after condensing sample C. The graphs of sample C and D lose the pattern of sample A and B. Sample E is distilled water from concentration process. The graph of sample E shows that volatile condensation were dissolved in distilled water during the condensation process. Finally, we conclude that discomfortable and characteristic odor of Chunma is a little bit disappeared after the pre-treatment and condensation processes.

Flow diagram for production of condensed extracts from Chunma – Fig. 6 shows processing steps in manufacture of Chunma constituents using the pre-treatment and condensation processes. After glinding the low material and adding water with the weight ratio of 10 (water) to 1 (Chunma), we performed ultrasonic extraction at 80 W for 30 minutes. Then, we used cooling apparatus with reflux including water bath and agitator for enzyme reaction using α-amylase and glucoamylase. We filtered the extracted material using filter paper, then condensed it to 50-60 °Brix at 60°C.

In conclusion, in this investigation we report the successful results using ultrasonic treatment, enzymatic process and condensation process to enhance the yield of extraction of functional constituents and reduce the discomfortable odor from Chunma.

Acknowledgements

This research was supported by Mu-Ju Health Food Association and Cooperation Center for Small & Medium Industry of Chonbuk National University.

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(Accepted June 10, 2005)