Original Article

Discrimination of *Lycium chinense* and *Lycium barbarum* by taste pattern and betaine analysis

Hye Won Lee, Young Hwa Kim, Yun Hee Kim, Gwan Ho Lee, Mi Young Lee

TKM-Based Herbal Drug Research Group, Korea Institute of Oriental Medicine, Daejeon, Republic of Korea

Received May 26, 2014; Accepted June 30, 2014; Epub August 15, 2014; Published August 30, 2014

**Abstract:** Lycii Fructus was used as natural products with therapeutic properties for a long time. Betaine is a natural amino acid and one of the major constituents of Lycii Fructus. It is reported that this fruit plays a role in reducing blood levels of homocysteine, a toxic byproduct of the amino acid metabolism. This study was used to establish infra based on oriental medicine through the analysis of correlation of taste, contents of betaine, %Brix and physico-chemical properties of Lycii Fructus. To investigate betaine, quantitative analysis was performed using HPLC separation system. In addition, %Brix and saccharide were estimated. Taste pattern analysis was measured using the taste sensing system, SA402B equipped with six taste sensors including newly developed sweetness sensor. Betaine quantitative analysis showed that *L. barbarum* 0.64 ± 0.15% (n = 6) was significantly higher than *L. chinense* 0.55 ± 0.1% (n = 12). And %Brix and saccharide composition of Lycii Fructus analysis showed that *L. barbarum* was significantly higher than *L. chinense*. The results of taste pattern analysis between *L. barbarum* and *L. chinense* showed a significant difference in almost every taste. In contrast, sweetness of *L. barbarum* was higher than *L. chinense*. When clustering with sweetness and bitterness, the two species are distinctly separated. In conclusion, these taste patterns, %Brix, betaine, and saccharide composition analysis could be applied to the establishment of herbal medicine marker for identification of different species in various regions.

**Keywords:** Lycii Fructus, *Lycium chinense*, *Lycium barbarum*, taste pattern analysis, betaine, %Brix

**Introduction**

Lycii Fructus, widely used in herbal medicine and the food industry, is defined in the Korean Pharmacopoeia, as *Lycium chinense* Miller, or *Lycium barbarum* Linné (Solanaceae) [1]. It grows naturally or is cultivated in the northeast and northwest of China, Taiwan, and Japan as well as in Korea.

Lycii Fructus is one of the medicinal herbs that is effective for diabetes accompanying inflammation and thirst, and in musculoskeletal strengthening. The anti-obesity and hypoglycemic effects of *L. chinense* extract [2] and hyperpigmentation moderating effect of the extract from *L. chinense* and dried leaves of *L. [folium]* have been reported [3]. *L. chinense* is known to be rich in betaine involved in the metabolism of methionine, zeaxanthin belonging to the same class as carotenoid, the derivatives of physaien and pyrrole, in addition to vitamins and amino acid [4, 5]. Known as an indicator of Lycii Fructus and to be effective in facilitating liver and stomach functions, enhancing musculoskeletal strength and the prevention of hypertension, atherosclerosis and anemia, betaine is involved in regulating the osmotic pressure in the liver cells and kidney cells in addition to being used as the treatment for homocystinuria [6]. Also, studies have been reported on the anti-diabetic effects of uracil, rutin and ascorbic acid isolated from *Lycii Fructus* as well as on the hepatocyte-protecting effects against hepatotoxicity caused by tetrachlorides of cerebroside and pyrrole derivative compounds or galactosamine [7-10]. Many case studies on the pharmacological effects such as hepatocyte protecting effects, hypotensive action and anti-diabetic effects suggest that these are related to the indicator component, betaine, which are also in line with the fact that it has mainly been used in herbal medicine for the purpose of strengthening, antipyretic agent, liver protection, and thirst relief.
Gustatory sensors can measure the aftertaste of bitterness, astringency and tastiness along with sourness, bitterness, astringency, tastiness, saltiness and astringency depending on the concentration of the taste substance through the artificial lipid membrane created to mimic human taste buds. It is widely used in gustatory comparison studies of drugs and food in Japan, including the gustatory comparison of crude drug and Kampo formula [11] and the comparison of bitterness and astringency between black tea and oolong tea [12]. Domestically, it has been used in the herbal medicine species-identifying studies, including differentiating Chinese licorice from Uzbekistani licorice [13] and the comparison of angelica roots [14]. In addition, its use is being expanded even further with the development of a sensor measuring sweetness [15, 16].

As such, this study compared betaine content in commercially available original species of L. barbarum and L. chinense listed in the Korean Pharmacopoeia [1] in order to explore an objective distinguishing method through quantitative assessment of these 2 species by analyzing the gustatory patterns, sweetness and sugar content, which could be objectively measured, unlike ambiguous physiognomic criteria.

Materials and methods

Materials

The Lycii Fructus used in the study was obtained from 15 herbal medicine distributors with known origin. These included 6 samples of L. barbarum from China and 12 samples of domestic L. chinense produced in Cheongyang, Jindo, Gongju and Yesan, which total 18 samples. Before being used in the study, these samples were checked against the appearance criteria for Lycii Fructus from the Korean Pharmacopoeia [1].

Reagents and device

The betaine used in the sample analysis was purchased from Wako Co. (Japan). The water used in the extraction was distilled 3 times. The acetonitrile used for qualitative and quantitative analyses was purchased as HPLC grade from J. T. Baker Inc. (Phillipsburg, NJ, USA). Other reagents used were special grade. The HPLC system used in betaine content analysis was Agilent (USA) 1100 series including Autosampler (G1313A), Column oven (G1316A), Binary pump (G1312), DAD detector (G1315B) and Degasser (G1379A). For software, Chemstation software (Agilent Technologies, USA) by Agilent Co. was used.

Gustatory sensor device, SA402B (Insent, Japan), was used after implementing 5 types of foodstuff sensor (CT0, C00, AAE, CA0 and AE1) in order to measure sourness, bitterness, astringency, tastiness and saltiness. Measurement was repeated 4 times in 2-step washing sample measurement mode. For sweet taste, GL1 sensor, the sweetness sensor was attached and measurement was repeated 5 times in GL1-test mode. Measurement results were calculated using analysis software (Taste analysis application, Insent, Japan) in Basic process mode. Taste information unit was used for the results calculated by the analysis software while the refraction saccharimeter PAL-1 (Atago, Tokyo, Japan) was used to measure sugar content in units of %Brix.

Preparation of samples

1) Preparation of samples for gustatory pattern and sugar level analysis 200 mL of water distilled 3 times was added to 20 g of each Lycii Fructus sample for 15 hours of leaching at cold temperature (4-6°C). It was filtered through hemp cloth and the same amount of water distilled 3 times was added for 2-fold dilution. This was used as the sample solution for gustatory sensor and sugar content measurement.

2) Preparation of sample and reference standard for betaine content analysis. The test was conducted based on the Korean Pharmacopoeia in order to measure betaine content. Sample preparation method is as below. 1.0 g of Lycii Fructus was accurately measured and extracted by reflux for 2 hours using 50% methanol 50 mL. It was then concentrated under reduced pressure and dissolved in deionized water. The pH of the solution was adjusted to 3.0 using diluted hydrochloric acid. The filtrate was put in the first column using strong acid cation exchange resin (form of H+); second column was done using weak acid cation exchange resin (form of H+) and strong base anion exchange resin (form of OH−), mixing in 1:2 ratio.
Discrimination of *Lycium chinense* and *Lycium barbarum*

Methods of Food Code, petroleum ether was used to remove lipids from the sample. 50% ethanol solution was used to extract sugars, and analysis was performed using HPLC-RI.

**Statistical processing**

The result values were indicated in mean ± SD. To test the significance of intergroup difference, one-way ANOVA and independent sample t-test were performed with P < 0.05.

**Results**

**Analysis of betaine content in Lycii Fructus**

Korean Pharmacopoeia\(^3\) lists chromatographic analysis methods of Lycii Fructus as NH\(_2\) column and betaine content greater than 0.5% when analyzing in a mobile phase of water and acetonitrile. When the content of betaine in 18 samples of commercially available Lycii Fructus was analyzed with HPLC-DAD, peaks were observed at retention time of 18.3 min for both the sample and reference standard of betaine (Figure 1).

As a result of betaine content analysis in a total of 18 samples of 2 types of Lycii Fructus, the mean of *L. barbarum* was 0.64 ± 0.15% and the mean of *L. chinense* was 0.55 ± 0.06%.

The filtrate was concentrated in reduced pressure. The residue was dissolved in 1 mL of distilled water and filtered through 0.45 μm PVDF membrane syringe filter (Pall, Port Washington, NY, USA) to be used in the study. The reference standard was prepared in betaine 10 mg/mL concentration using water distilled 3 times and was diluted in stepwise manner for use.

**HPLC analysis conditions**

The HPLC condition column used for betaine content analysis of the sample was Phensphere NH\(_2\) (250 × 4.6 mm, 5 μm, Phenomenex, USA). The ratio of water (A) and acetonitrile (B) used as a mobile phase was (A) : (B) = 15 : 85 (v/v) mixture in isocratic elution used for 30 minutes. Analysis wavelength was 210 nm, flow rate was 1.0 mL/min, column oven temperature was maintained at 30°C, and sample injection rate was 10 μL.

**Sugar content analysis in Lycii Fructus**

Sugar content measurement of each Lycii Fructus sample was carried out by SGS Korea (Ltd.) and the sample preparation method is as follows. According to the qualitative and quantitative test method of sugars based on the device analysis method from the General Test

---

Figure 1. Analytical HPLC chromatogram of Lycii Fructus sample (A) and betaine standard (B).
Discrimination of *Lycium chinense* and *Lycium barbarum*

Statistically, t-test results showed significant difference at $P < 0.05$, with *L. barbarum* showing a higher content compared to *L. chinense* (Figure 2).

Figure 2. Composition of betaine content (A), %Brix (B), and saccharide (C) of Lycii Fructus. These data represent the means ± SD of three independent experiments. *, $P < 0.05$; ****, $P < 0.0005$.

Figure 3. Taste difference between *L. chinense* and *L. barbarum*. The bar graphs show taste respectively. Each bar represents the mean (± standard deviation) taste difference. *, $P < 0.05$; **, $P < 0.01$, and ***, $P < 0.001$ tasted by student t-test. Aftertaste-B is aftertaste of bitterness. Aftertaste-A is aftertaste of astringency. Richness is aftertaste of umami. The unit is taste information unit suggested by Kobayashi et al. (2010).
Discrimination of Lycium chinense and Lycium barbarum

The analysis of sweetness and sugar content of Lycii Fructus

Results of sweetness and sugar content measurements were 3.1 ± 0.4 %Brix and 52 ± 6% for L. barbarum and 1.6 ± 0.4 %Brix and 22.5 ± 3.9% for L. chinense, respectively. L. barbarum showed higher values compared to L. chinense, with sweetness and sugar content both being approximately 2 times higher in L. barbarum compared to L. chinense (Figure 2).

Analysis of gustatory pattern of Lycii Fructus

As a result of analyzing the gustatory patterns of Lycii Fructus based on origin and species, the mean values of taste unit for L. barbarum were mostly lower than those of L. chinense. In particular, bitterness was -0.61 ± 0.93 for L. chinense and -4.95 ± 2.79 for L. barbarum; astringency was 4.99 ± 0.68 for L. chinense and 4.11 ± 0.36 for L. barbarum; saltiness was 19.29 ± 1.13 for L. chinense and 17.94 ± 0.76 for L. barbarum, with L. barbarum showing lower values. In contrast, sweetness was 0.11 ± 0.36 for L. barbarum and -2.25 ± 0.60 for L. chinense, with L. barbarum showing higher values compared to L. chinense. When the statistical significance of mean values was examined, significant difference was observed for almost all tastes at $P < 0.05$ and $P < 0.01$. In particular, a highly significant difference was observed for sweetness at $P < 0.001$ (Figure 3). In addition, when clustered based on the criteria of aftertaste-A for sweetness and astringency, a distinctive difference was seen between L. chinense and L. barbarum (Figure 4).

Discussion

Lycii Fructus is the medicinal herb listed first in the Divine Farmer’s Herb-Root Classic with bitter and cold taste, which is used for diabetes and systemic pain and is known to strengthen muscles and bones with long-term use. Korean Pharmacopoeia and Japanese Pharmacopoeia list L. chinense or L. barbarum as the original plants but the Chinese Pharmacopoeia only lists L. barbarum cultivated in the northwestern part of China since the origin. L. barbarum is listed as having excellent effects in the botanical list [17, 18].

The Korean Pharmacopoeia specifies the amount of indicator ingredient to be greater than 0.5% of betaine ($\text{C}_5\text{H}_{11}\text{NO}_2$) when quantified in a dried state. However, the criteria for appearance is somewhat abstract and subjective, as the Korean Pharmacopoeia and the Chinese Pharmacopoeia state that it has slight odor and sweet taste while the Japanese Pharmacopoeia states that it is sweet and has a slightly bitter aftertaste.

The odor theory, which is the index of the basic nature and efficacy of medicinal herbs, refers to four spirits and five tastes including the 5 tastes, i.e. bitter, hot, sour, sweet, and salty, and can be regarded as a direct experience of a series of specific chemical compounds through the human gustation [19]. The need to standardize the nature of medicine including five tastes into objective and quantifiable criteria...
Discrimination of Lycium chinense and Lycium barbarum

betaine content comparison, confirming that high betaine content was associated with high sweetness of Lycii Fructus. Also, when sugar content was analyzed, L. barbarum showed significantly higher content compared to L. chinense. This is believed to be due to the morphological difference as L. barbarum has meaty fruit while L. chinense has fibrous fruit.

In conclusion, these taste patterns, %Brix, betaine, and saccharide composition analysis could be applied to the establishment of herbal medicine marker for identification of different species in various regions.

Acknowledgements

This study was supported by Korea Institute of Oriental Medicine (K14103 and K12260).

Disclosure of conflict of interest

None.

Address correspondence to: Hye Won Lee, TKM-Based Herbal Drug Research Group, Korea Institute of Oriental Medicine, 1672, Yuseongdae-ro, Yuseong-gu, Daejeon, 305-811, Korea. Tel: +82-42-868-9506; Fax: +82-42-868-9301; E-mail: anywon1975@gmail.com

References

Discrimination of *Lycium chinense* and *Lycium barbarum*


