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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

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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 physicochemical 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 physalien 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 cerebrosides 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.

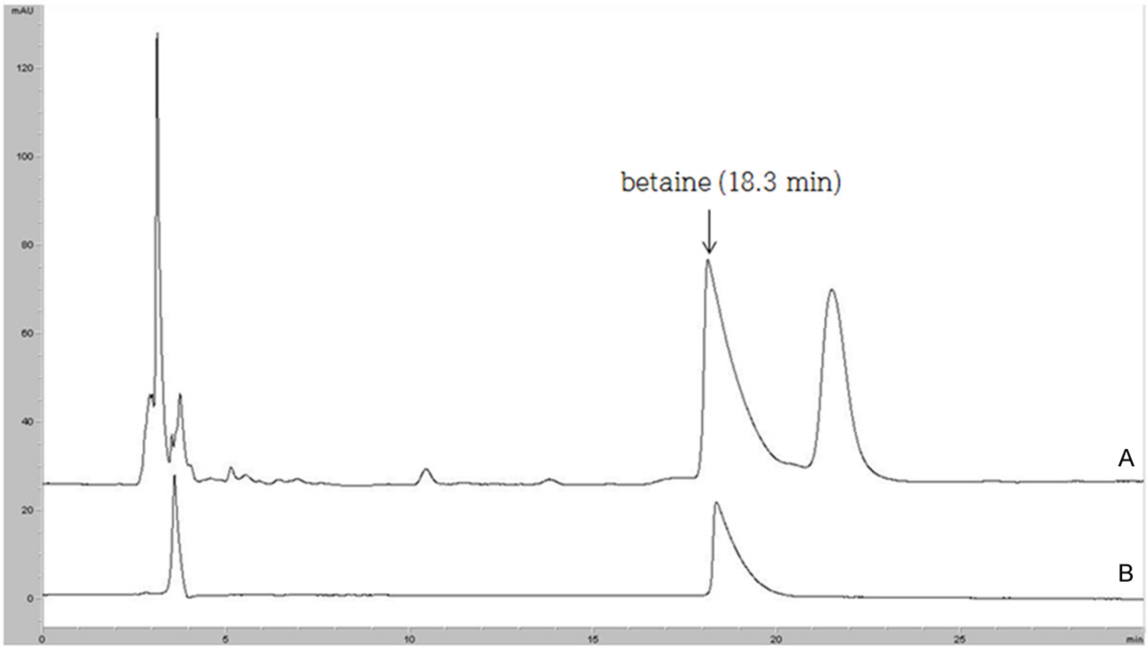

Figure 1. Analytical HPLC chromatogram of Lycii Fructus sample (A) and betaine standard (B).

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 Phenosphere 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 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%.

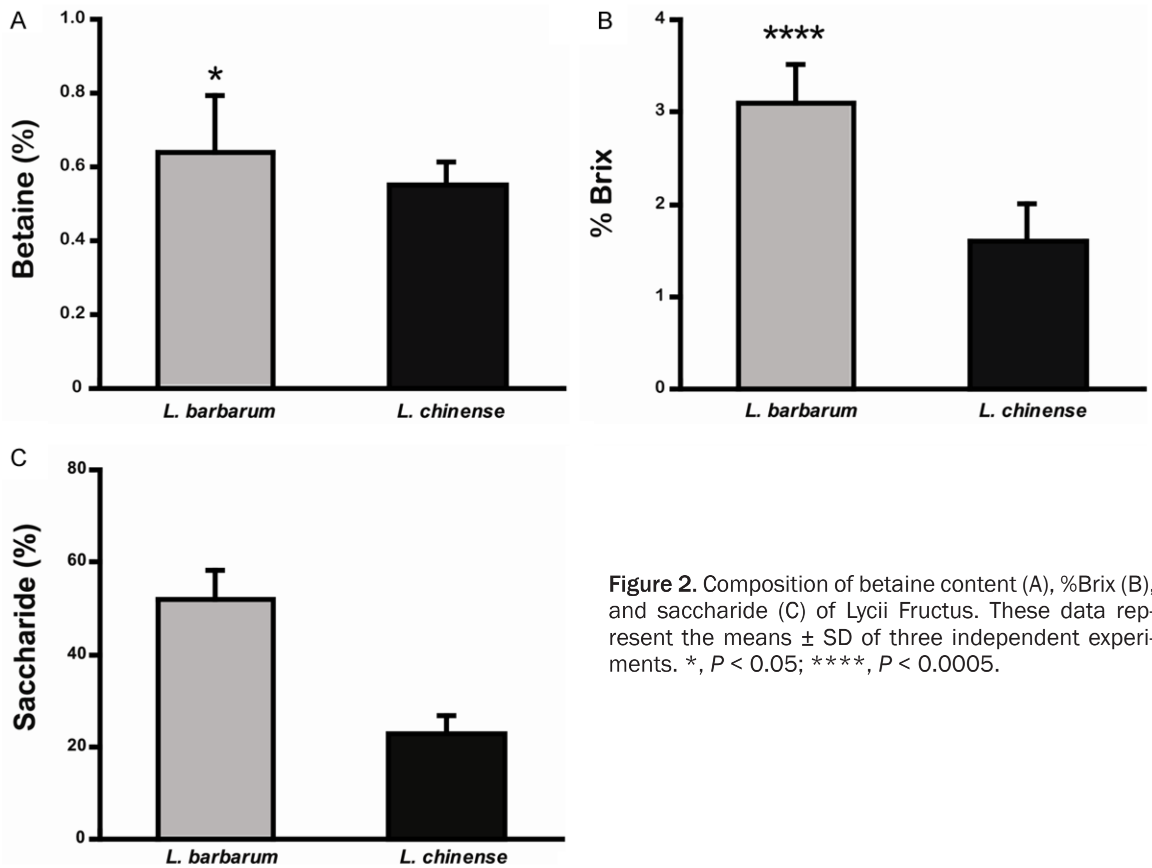

Figure 2. Composition of betaine content (A), %Brix (B), and saccharide (C) of Lycii Fructus. These data represent the means ± SD of three independent experiments. *, $P < 0.05$; ****, $P < 0.0005$.

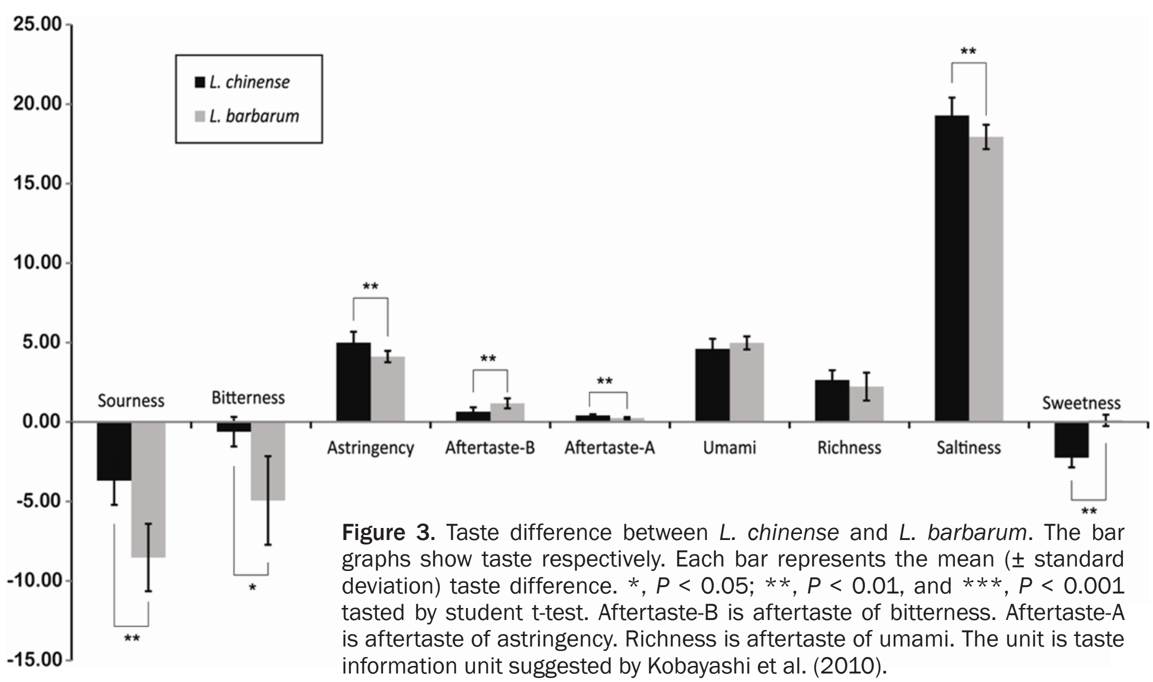

Figure 3. Taste difference between *L. chinense* and *L. barbarum*. The bar graphs show taste respectively. Each bar represents the mean (± standard deviation) taste difference. *, $P < 0.05$; **, $P < 0.01$, and ***, $P < 0.001$ tasted by student t-test. Aftertaste-B is aftertaste of bitterness. Aftertaste-A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

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

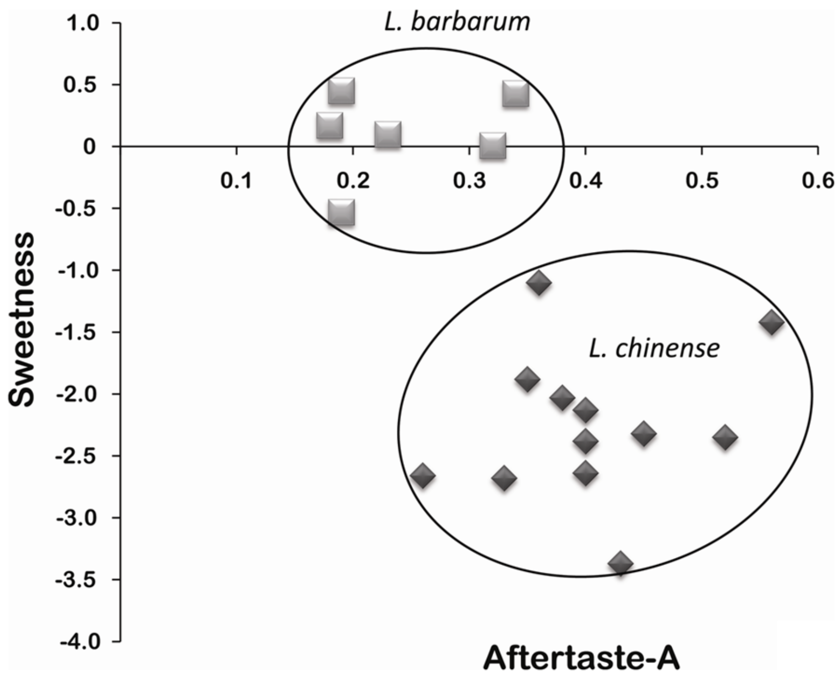

Figure 4. Taste clustering of *L. chinense* and *L. barbarum*. The unit is taste information unit suggested by Kobayashi et al. (2010).

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 ($C_5H_{11}NO_2$) 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 has been constantly discussed in the field of herbal medicine [20].

The taste sensors have been reported to be capable of comparison with up to 100 times the precision compared to human gustation [21], and they have been used in the taste assessment studies of domestic/international medicinal herbs [11, 21] as well as in the studies for distinguishing origins and species [13, 14]. As such, this study attempted to provide an objective index for Lycii Fructus by confirming the gustatory patterns and major ingredients.

When betaine content in 18 samples of 2 types of commercially available Lycii Fructus was analyzed, *L. barbarum* showed statistically higher content of betaine when 12 samples of *L. chinense* were compared with 6 samples of *L. barbarum*. This replicates the results of Moon [22] who reported that *L. barbarum* showed higher betaine content. However, it may be difficult to use the results as an accurate differentiation criteria as some samples of *L. chinense* showed a higher betaine content compared to *L. barbarum*.

When Lycii Fructus was measured with taste sensors after 15 hours of leaching at low temperatures (4-6°C) to prevent the corruption and transformation of the sample during the extraction process, significant difference was shown in most of the gustatory patterns between *L. barbarum* and *L. chinense*. Among the mean values of taste units showing significant difference, *L. barbarum* showed lower results than *L. chinense* for almost all tastes excluding aftertaste-B and sweetness. The Korean Pharmacopoeia lists the taste of Lycii Fructus to be sweet in the appearance criteria. In the actual gustatory patterns measured with taste sensors, *L. barbarum* showed a higher taste unit of sweetness compared to *L. chinense* matching the Brix (%) results measured with refraction saccharometer. In addition, when clustered based on the aftertaste-A of sweetness and astringency, *L. chinense* was clearly distinguishable from *L. barbarum*, confirming that sweetness can be used as a representative gustatory criteria for species comparison for the 2 types of Lycii Fructus (*L. barbarum* and *L. chinense*).

When the sweetness of Lycii Fructus was measured using refraction saccharometer, *L. barbarum* showed significantly higher results than *L. chinense* corresponding to the results of 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.

The differences in origin and species could be confirmed by analyzing the gustatory patterns and ingredients of 2 types of Lycii Fructus (*L. barbarum* and *L. chinense*), which could be used as the information for identifying the species of Lycii Fructus in the future.

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.

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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: anywon-1975@gmail.com

References

[1] Korea Food and Drug Administration. The 9th Korean Herbal Pharmacopoeia. Seoul, Korea: Korea Food and Drug Administration; 2007.
[2] Hwang EY, Hong JH, Choi JH, Lee EJ and Lee IS. Study on anti-obesity and hypoglycemic effects of Lycium Chinense Mill extracts. J Korean Soc Food Sci Nutr 2009; 38: 1528-1534.
[3] Kim DH, Lee SY, Kim NK, Youn BK, Jung DS, Choi EY, Hong SR, Yoon JY, Kang MH and Lee JY. Moderating effects of skin hyperpigmentation from Lycii Fructus and Lycii folium extracts. J Appl Biol Chem 2011; 54: 270-278.
[4] An TH, Lee SY and Cho J. Comparison on antioxidant activity of ethanolic extracts of Chinese matrimony vine. Korea J Crop Sci 2012; 57: 22-28.

[5] Han B, Park J, Park M and Han Y. Studies on the alkaloidal components of the fruits ofLycium chinense. Archives of Pharmacal Research 1985; 8: 249-252.

[6] Wettstein M, Weik C, Holneicher C and Haussinger D. Betaine as an osmolyte in rat liver: metabolism and cell-to-cell interactions. Hepatology 1998; 27: 787-793.

[7] Chin YW, Lim SW, Kim SH, Shin DY, Suh YG, Kim YB, Kim YC and Kim J. Hepatoprotective pyrrole derivatives of Lycium chinense fruits. Bioorg Med Chem Lett 2003; 13: 79-81.

[8] Kim KS, Shin SH, Jeong GH, Cheong CS, Ko KH, Park JH, Huh H, Lee BJ and Kim BK. Antidiabetic activity of constituents of Lycii Fructus. J Appl Pharmacol 1998; 6: 378-382.

[9] Kim SY, Choi YH, Huh H, Kim J, Kim YC and Lee HS. New antihepatotoxic cerebroside from Lycium chinense fruits. J Nat Prod 1997; 60: 274-276.

[10] Kim SY, Lee EJ, Kim HP, Lee HS and Kim YC. LCC, a cerebroside from Lycium chinense, protects primary cultured rat hepatocytes exposed to galactosamine. Phytother Res 2000; 14: 448-451.

[11] Anjiki N, Hosoe J, Fuchino H, Kiuchi F, Sekita S, Ikezaki H, Mikage M, Kawahara N and Goda Y. Evaluation of the taste of crude drug and Kampo formula by a taste-sensing system (4): taste of Processed Aconite Root. J Nat Med 2011; 65: 293-300.

[12] Hayashi N, Ujihara T, Chen R, Irie K and Ikezaki H. Objective evaluation methods for the bitter and astringent taste intensities of black and oolong teas by a taste sensor. Food Research International 2013; 53: 816-821.

[13] Choi GY, Kim YH, Chae SW, Lee HW, Ko BS and Lee MY. Discrimination of Chinese Glycyrrhiza uralensis and Uzbek Glycyrrhiza glabra using taste sensor. Kor J of Herbol 2011; 26: 35-39.

[14] Kim YH, Choi GY, Lee HW, Lee GH, Chae SW, Kim YH and Lee MY. Comparison of angelica species roots using taste sensor and DNA seuencing aalysis. Kor J of Herbol 2012; 27: 37-42.

[15] Toyota K, Cui H, Abe K, Habara M, Toko K and Ikezaki H. Sweetness sensor with lipid/polymer membranes : sweet-responsive substances. Sensor Mater 2011; 23: 465-474.

[16] Toyota K, Cui H, Abe K, Habara M, Toko K and Ikezaki H. Sweetness sensor with lipid/polymer membranes: response to various sugars. Sensor Mater 2011; 23: 475-482.

[17] Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. Beijing: China Medical Science and Technology Press, 2005.

[18] Park MY, Han HS and Lee YJ. A study on a morphological identification of Lycii Fructus. J Oriental Med Lab Kyongwon Univ 2006; 10: 139-154.

[19] Jeon WK, Yoo BK, Kim YE, Park SO, Parl SM and Ko BS. Effect of extracts for herbal medicines on the inhibition of whole blood aggregation. J Korean Soc Appl Biol Chem 2007; 50: 352-357.

[20] Choi MI, Lee HJ, Yun JB, Kim JY, Kang KS, Shin CG and Ju YS. The approach of properties-flavours theory and the study of morphological standard in Corni Fructus. Kor J of Herbology 2006; 21: 109-119.

[21] Kobayashi Y, Habara M, Ikezazki H, Chen R, Naito Y and Toko K. Advanced taste sensors based on artificial lipids with global selectivity to basic taste qualities and high correlation to sensory scores. Sensors (Basel) 2010; 10: 3411-3443.

[22] Lee BC, Park JS, Kwak TS and Moon CS. Variation of chemical properties in collected Boxthorn varieties. Korean J Breed Sci 1998; 30: 267-272.