

Variation of Nutritional and Antioxidant Characteristics of Extract of *Lycium barbarum* produced by using Different Extraction Processes

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Abstract: *Lycium barbarum* extract has a high potential to be developed as a health functional food due to the various health-promoting effects of *Lycium barbarum*. This study analyzed changes in nutritional and functional components depending on the extraction solvent (purified water and a mixture of purified water and alcohol) and the condition of the sample. The nutritional components (carbohydrates, protein, fat, ash), organic acids, amino acids, total phenolic compounds, and total flavonoids of the extract produced during the extraction process were analyzed. The nutritional composition and functional substances of the extracts showed some differences depending on the type of solvent and the condition of the sample. The amounts of crude protein (7.61%), crude fat (1.63%), carbohydrate (90.22%), and ash (0.54%) of dried *Lycium barbarum* extract using purified water as a solvent were similar to those of the powder sample extract. The highest content of citric acid was 4.31 mg/mL, similar to the case of acetic acid, when the powder sample used a mixture of purified water and alcohol as a solvent. The highest amino acid content was 357.39 mg/mL when the powder sample was mixed with purified water and alcohol as a solvent. The total amount of phenolic compounds was 686.16 g/L when the powder sample was extracted with a mixture of purified water and alcohol as a solvent. The highest total flavonoid content was 111.32 g/L when the powder sample was extracted with a mixture of purified water and alcohol as a solvent.

Keywords : Functional food, Antioxidant, Phenol compounds, Flavonoids, *Lycium barbarum*

1. Introduction

Since ancient times, our ancestors have enjoyed making and drinking beverages such as hwachae, sujeonggwa, and sikhye at home.

In addition, there are a variety of traditional drinks using green tea, grains, plant leaves, fruits, and flowering roots[1]. Recently, as consumer interest in health has rapidly increased, many studies have been conducted

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on natural functional food ingredients that can promote and prevent health. *Lycium barbarum* is a wolfberry tree (*Lycium chinensis* Miller) belonging to the Solanaceae family. It is a herbal medicine that grows or is cultivated in Korea, China, Taiwan, and Japan. In oriental medicine, it is treated as a non-toxic group of 120 types of medicinal herbs along with ginseng [2–3]. In Donguibogam, *Lycium barbarum* is described as being effective in nourishing, tonic, blood-replenishing, stimulating, and correcting organs[4].

Lycium barbarum contains fructose and small amounts of protein, fat, and fiber, and are also rich in minerals and vitamins. *Lycium barbarum* contains a large amount of functional ingredients such as betaine, rutin, kukoamine A, and β -sitosterol, and have anti-cancer effects[5], immunity-boosting effects [6], antimicrobial effects[7–8], anti-diabetic effects[9–10], and antioxidant effects[11–12]. It is also rich in essential amino acids such as lysine, threonine, and methionine, as well as tannin[13]. The composition of bioactive substances in plants is influenced by maturity, geographical location and climatic conditions, and physiological, biochemical and molecular changes during fruit growth[14–15]. *Lycium barbarum* has been shown to contain surprisingly high levels of antioxidants, fats, dietary fiber, essential amino acids, trace minerals and vitamins[16–18].

Lycium barbarum is traditionally grown in China, Tibet and other regions. China is Suppliers around the world supply approximately 25,000 to 30,000 tonnes of primary fruit annually. The largest amount of *Lycium barbarum* is produced in Asia (71.2%), followed by Africa (15.8%), the United States (8.3%), Oceania (3.4%), and Europe (1.3%) [19–20].

This study performed extraction processes for producing *Lycium barbarum* extracts based on the shape of dried *Lycium barbarum* and the extraction solvent used. Subsequently, a comparative analysis was conducted on the

nutritional composition, organic acid content, amino acid content, total phenolic content, and total flavonoid content of the extracts.

2. Materials and Methods

2.1. Experiment material

The dried *Lycium barbarum* used in the experiment were purchased at the Cheongyang-gun herb market. Folin-Ciocalteu reagent(FCR), aluminium chloride, gallic acid, and hesperidine were purchased from Sigma Aldrich(Seoul, Korea).

2.2. Preparation of *Lycium barbarum* extract

Lycium barbarum used to prepare hot water extract were divided into dried goji berries and powdered dried goji berries. *Lycium barbarum* used to prepare extracts using water and alcohol as solvents were also divided into dried *Lycium barbarum* and powdered dried *Lycium barbarum*. The DGWE, DGWAE, PGWE, and PGWAE represent the dried *Lycium barbarum* water extract, dried *Lycium barbarum* water plus ethanol extract, powdered *Lycium barbarum* water extract, powdered *Lycium barbarum* water plus ethanol extract, respectively. The preparation of *Lycium barbarum* extract was performed in a 5,000 mL extraction reactor by adding 300 g of solute to 3000 mL of solvent. Each *Lycium barbarum* sample was placed in a non-woven fabric and sufficiently submerged in the extraction solvent. A reflux cooling extraction device was connected to the top of the extractor, and then cooling water (4°C) was circulated to prevent water vapor from escaping to the outside. The extraction temperature was fixed at 80°C, and extraction was performed for 4 hr. After extraction was completed, the nonwoven fabric was taken out and gently squeezed by hand to recover the extract. The entire extract was filtered through non-woven fabric. Next, the extract was placed in a concentrator and concentrated to

30° Brix. *Lycium barbarum* concentrate was stored at -20°C until the experiment was performed. During the comparative laboratory test, an extract in the form of a beverage was prepared by correcting the solid content of *Lycium barbarum* beverage to 12° Brix.

2.3. Titratable acidity and pH measurement

The acidity of the extract was measured at the time of completion of extraction. A 0.1 N NaOH solution was used as the titration solution, and the amount of 0.1 N NaOH solution consumed up to the end point of pH 8.3 during titration was measured and converted to malic acid content. pH was measured using a pH meter.

2.4. Nutritional compounds analysis of extract

To analyze general ingredients, moisture content was measured using the 105°C normal pressure heating and drying method. Crude fat was analyzed using the Soxhlet extraction method, crude protein was analyzed using the Kjeldahi method, and crude ash was analyzed using the 550°C direct incineration method. The carbohydrate content was determined by subtracting the total content (%) of moisture, crude protein, crude fat, and crude ash based on 100% of the sample.

2.5. Composition of amino acid analysis of extract

0.5 g of the sample was weighed in a 18 mL test tube, sealed under reduced pressure by adding 3 mL of 6 N HCl, and hydrolyzed for 24 hours in a heating block set to 120°C . After hydrolysis was completed, the acid was removed with a rotary evaporator at 50°C , and then 10 mL of sodium loading buffer was added to the sample. Then, 1 mL was taken, filtered through a $0.2\ \mu\text{m}$ membrane filter, and quantitatively analyzed with an amino acid automatic analyzer (S433-H). As analysis conditions, a Cation separation column (LCA K06/Na) was used, column size was 4.6×150 mm, column temperature was $57 \sim 74^{\circ}\text{C}$, flow

rate was Buffer 0.45 mL/min, reagent 0.25 mL/min, Buffer pH. The range was 3.45~10.85, and the wavelength was 440 nm and 570 nm.

2.6. Organic acid analysis of extract

For organic acid analysis, 20 mL of distilled water was added to 0.2 g of the sample, stirred and extracted in a constant temperature water bath at 80°C for 4 hr, then dissolved in 20 mL of ethanol and analyzed by HPLC. Analytical HPLC (Shimadzu Co., JAPAN) was used, two shim-pack SCR-102H (300×8.0 mm) columns were used, and the guard column was shim-pack guard column SCR-102H (50×6.0 mm). was used. The mobile phase was 4 mM p-toluenesulfonic acid, the analysis temperature was 40°C , the flow rate was 0.7 mL/min, and the inject volume was 20 μL .

2.7. Total phenolic compound content

Quantification of phenolic compounds was analyzed using the Folin-Ciocalteu reagent (FCR) color development method using gallic acid (Sigma Aldrich Co., USA) as a standard [13]. The standard material was prepared by making a gallic acid (20, 40, 60, 80, 100 mg/L) solution and mixing 1 mL with 9 mL DIW (deionized water). For the analysis sample, mix 1.5 mL Na_2CO_3 (20 g/100 mL), 500 μL FCR, 6 mL DIW, 100 μL sample solution, and 100 μL standard gallic acid, react at room temperature for 2 hours, and measure absorbance at 765 nm. did. The control of the analysis sample was acetone: water (1:1), replacing the gallic acid solution. The total phenol compound content was evaluated as the amount of gallic acid (GAE) per g of dry sample.

2.8. Total flavonoid content

Quantification of total flavonoids was measured using aluminum chloride colorimetric method[14], and the standard material was prepared by making a solution of hesperidin

(Sigma Aldrich Co., USA) (20, 40, 60, 80, 100 mg/L) and making 1 mL. was prepared by mixing in 9 mL DIW. The analysis sample was prepared by mixing 0.5 mL sample solution, 1.5 mL 95% methanol, 0.1 mL 10% aluminum chloride (Sigma Aldrich Co., USA), 0.1 mL 1 M NaOH, and 2.8 mL DIW, and reacting at room temperature for 30 min. Absorbance was measured at 415 nm. The control of the analysis sample was methanol: water (1:1), replacing the hesperidin solution. The total flavonoid content was evaluated as the amount of hesperidin per g of dried sample.

2.9. Statistical analysis

Data are expressed as the mean \pm SD of five individual experiments. Analysis results were analyzed using ANOVA test and Tukey test ($P < 0.05$) for differences between data.

3. Results and Discussion

3.1. Contents of soluble solid of the extract

The method of producing extract from dried *Lycium barbarum* involves extraction and concentration processes, which relatively adds processing costs, but the product can be safely stored for a long time. The solvent used to manufacture extracts for manufacturing health functional foods and general foods is purified water or a mixture of purified water and alcohol. In general, the production of extracts from natural products such as *Lycium barbarum* uses hot water extraction. In this study, dried *Lycium barbarum* were used as raw material or powdered. Therefore, depending on the extraction process, it was classified into 4 categories. In the first process, dried goji berries were extracted using purified water as a solvent, in the second process, dried goji berries were extracted using a mixture

of purified water and alcohol, in the third process, powdered goji berries were extracted using purified water as a solvent, and in the fourth process, powdered *Lycium barbarum* were extracted using a mixture of purified water and alcohol. Fig. 1 shows the solid content of *Lycium barbarum* extract in purified water and a mixture of purified water and alcohol. The average solid content of the extracts was 7.9 to 9.7%. Comparatively, when extracted with a mixed solvent of purified water and alcohol, the solid content of the extract was higher than when a single solvent of purified water was used.

3.2. pH and titratable acidity of extract *Lycium barbarum* derived from different extraction processes

The pH and titratable acidity of *Lycium barbarum* extract are shown in Table 1. In general, the pH and acidity of fruits decrease and acidity increases due to organic acids produced through biosynthesis[16]. The pH and acidity of the extracts showed differences depending on the type of solvent and the condition of the sample. The pH and acidity of dried *Lycium barbarum* extract using purified water as a solvent were 5.03 and 0.75%, respectively. On the other hand, the pH and acidity of the extract using a mixture of purified water and alcohol as a solvent were 4.85 and 0.83%, respectively. On the other hand, when powdered *Lycium barbarum* was used, the pH was lowered and the acidity increased by about 20–25%.

The DGWE, DGWAE, PGWE, and PGWAE represent dried *Lycium barbarum* water extract, dried *Lycium barbarum* water plus ethanol extract, powdered *Lycium barbarum* water extract, powdered *Lycium barbarum* water plus ethanol extract. The data were expressed as mean (\pm SD) (n=5). The different superscripts on graphs represented different values ($p < 0.05$).

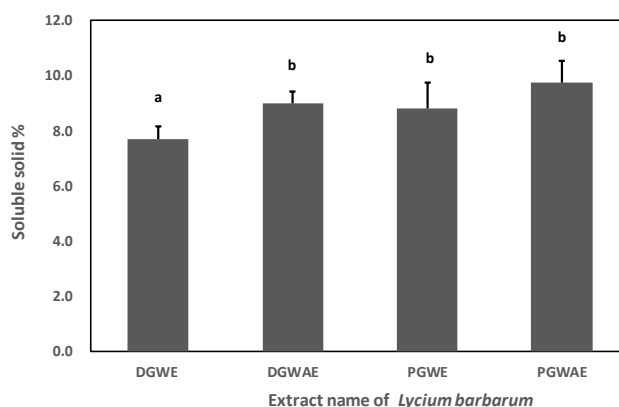


Fig. 1. The amount of soluble solid of extract of *Lycium barbarum* using different extraction process. The DGWE, DGWAE, PGWE, and PGWAE represent dried *Lycium barbarum* water extract, dried *Lycium barbarum* water plus ethanol extract, powdered *Lycium barbarum* water extract, powdered *Lycium barbarum* water plus ethanol extract. The data were expressed as mean (\pm SD) (n=5). The different superscripts on graphs represented different values ($p < 0.05$).

Table 1. pH and Acidity of *Lycium barbarum* extracts

	pH	Acidity (%)
DGWE	5.03 \pm 0.015 ^a	0.75 \pm 0.021 ^a
DGWAE	4.85 \pm 0.010 ^b	0.83 \pm 0.025 ^b
PGWE	5.22 \pm 0.010 ^c	0.67 \pm 0.015 ^c
PGWAE	4.79 \pm 0.010 ^b	0.83 \pm 0.015 ^b

Table 2. General nutritional composition of extract of *Lycium barbarum*

	DGWE	DGWAE	PGWE	PGWAE
Crude protein	7.61	5.65	8.71	7.25
Crude lipid	1.63	1.76	1.29	1.32
Carbohydrate	90.22	91.76	89.41	90.11
Crude ash	0.54	0.82	0.59	1.32

3.3. Nutrient distribution of extracts

Table 2 shows the composition of crude protein, crude fat, carbohydrates, and ash, which are important nutrients of *Lycium barbarum* extract. The nutritional composition of the extracts showed some differences depending on the type of solvent and the condition of the sample. The amounts of crude

protein (7.61%), crude fat (1.63%), carbohydrate (90.22%), and ash (0.54%) of dried *Lycium barbarum* extract using purified water as a solvent were similar to those of the powder sample extract. This trend was similar to when a mixture of purified water and alcohol was used as the solvent. Compared with the results of other studies, Pires et al.

reported the presence of carbohydrates (87 g/100 g), protein (5.3 g/100 g), fat (4.1 g/100 g), and ash (3.21 g/100 g) in dried goji berries[7]. In another study Goji berries contain moisture (75.32 g/100 g), carbohydrates (16.93g / 100 g), and dietary fiber (3.63 g/100 g), protein (1.98 g/100 g), fat (1.15 g/100 g), and ash (0.84 g/100 g) were reported[21].

The DGWE, DGWAE, PGWE, and PGWAE represent dried *Lycium barbarum* water extract, dried *Lycium barbarum* water plus ethanol extract, powdered *Lycium barbarum* water extract, powdered *Lycium barbarum* water plus ethanol extract. The data were expressed as mean (\pm SD) (n=5). The different superscripts on graphs represented different values(p<0.05).

3.4. Organic acids composition of extract

The organic acid content of goji berry extract is shown in Table 3. Regardless of the type of extraction solvent, the organic acids contained in the extract, in order of highest content, were acetic acid, citric acid, malic acid, oxalic acid, and tartaric acid. The composition and content of organic acids in goji berry extract also showed some differences depending on the type and state of the sample. There was no significant difference in the content of acetic acid, a representative organic acid involved in flavor, regardless of the solvent used in the powder sample, and in the case of the dry sample, the content was high when a mixture of purified water and alcohol

was used as the solvent. The highest content was 8.02 ± 0.48 mg/mL when the powder sample used a mixture of purified water and alcohol as a solvent. On the other hand, in the case of citric acid, significant differences in content were shown depending on the state of the sample and the extraction solvent. The highest content was 4.31 mg/mL, similar to the case of acetic acid, when the powder sample used a mixture of purified water and alcohol as a solvent. Pires et al.[7] reported the presence of organic acids and tocopherols in their study. Specifically, citric acid (1.29 g/100 g dw), succinic acid (0.77 g/100 g dw), and oxalic acid (0.010 g/100 g dw) were detected. Additionally, tocopherols such as α -tocopherol (0.23 mg/100 g dw) and δ -tocopherol (0.09 mg/100 g dw) were also identified.

The DGWE, DGWAE, PGWE, and PGWAE represent dried *Lycium barbarum* water extract, dried *Lycium barbarum* water plus ethanol extract, powdered *Lycium barbarum* water extract, powdered *Lycium barbarum* water plus ethanol extract. The data were expressed as mean (\pm SD) (n=5). The different superscripts on graphs represented different values(p<0.05).

3.5. Amino acid analysis of extracts

The amino acid analysis results of the extracts are shown in Table 4. Amino acids are taste-producing substances; sweet tastes include glycine, alanine, threonine, proline, and serine; bitter tastes include leucine, isoleucine,

Table 3. The composition of organic acids of extracts of *Lycium barbarum*

	Organic acids(g/L)				
	Acetic acid	Malic acid	Tartaric acid	Oxalic acid	Citric acid
DGWE	5.77 \pm 0.625 ^a	1.87 \pm 0.598 ^a	0.07 \pm 0.012 ^a	0.61 \pm 0.061 ^a	3.38 \pm 0.035 ^a
DGWAE	7.18 \pm 0.225 ^b	1.54 \pm 0.227 ^a	0.03 \pm 0.010 ^b	0.33 \pm 0.032 ^b	3.64 \pm 0.116 ^d
PGWE	7.62 \pm 0.513 ^b	4.72 \pm 0.183 ^b	0.47 \pm 0.030 ^c	0.84 \pm 0.040 ^c	3.97 \pm 0.061 ^c
PGWAE	8.02 \pm 0.482 ^b	4.76 \pm 0.075 ^b	0.59 \pm 0.075 ^c	0.86 \pm 0.031 ^c	4.31 \pm 0.074 ^b

Table 4. The composition of amino acids of extracts of *Lycium barbarum*.

	mg /100 g			
	DGWE	DGWAE	PGWE	PGWAE
Threonine	6.08	6.22	11.21	11.39
Serine	13.53	14.28	26.51	27.66
Asparagine	123.92	124.35	95.51	97.92
Glutamic acid	2.75	2.88	2.07	2.48
Proline	64.48	66.34	138.45	140.45
Glycine	1.75	1.93	0.64	0.79
Alanine	29.30	29.85	31.98	33.90
Valine	6.44	6.78	6.99	6.34
Cystine	9.22	9.31	N.D.	N.D.
Methionine	13.46	13.35	7.51	5.93
Isoleucine	5.92	5.98	2.01	1.84
Leucine	10.46	10.77	6.03	5.63
Tyrosine	4.77	4.92	0.25	0.45
phenylalanine	10.51	10.33	8.04	6.69
Histidine	3.32	4.28	2.97	2.91
Tryptopan	0.25	0.56	0.17	0.27
Lysine	1.91	2.12	0.47	0.55
Arginine	5.70	6.23	10.29	12.20
Total	313.8	320.48	351.1	357.39

methionine, phenylalanine, lysine, valine, histidine, and arginine; sour tastes include aspartic acid; and savory tastes include aspartic acid. The composition and content of amino acids in *Lycium barbarum* extract also showed some differences depending on the type and condition of the sample. In the case of powder samples, the total amino acid content of the extract was higher than that of the dry sample, regardless of the type of solvent. The highest amino acid content was 357.39 mg/mL when the powder sample was mixed with purified water and alcohol as a solvent. On the other hand, the lowest amino acid content of the extract was 313.8 mg/mL when the dried extract was extracted with purified water. The most abundant amino acids in goji berries are proline and serine, while the essential amino acids represent up to 30% of total free amino acids[22]. In addition,

goji berries have characterized non-protein amino acids, such as γ -aminobutyric acid, hydroxyproline, and citrulline, with specific metabolic functions[23].

The DGWE, DGWAE, PGWE, and PGWAE represent dried *Lycium barbarum* water extract, dried *Lycium barbarum* water plus ethanol extract, powdered *Lycium barbarum* water extract, powdered *Lycium barbarum* water plus ethanol extract.

3.6. Total phenolic and flavonoid compounds content

The total phenolic compounds and flavonoid contents with antioxidant properties of goji berry extract are shown in Fig. 2. The antioxidant content of goji berry extract was found to be high in dried and powdered samples when a mixture of purified water and alcohol was used as a solvent. When the powder

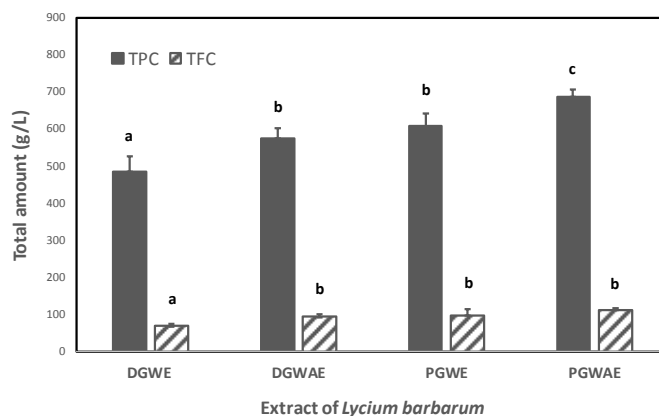


Fig. 2. Total phenolic (TPC) and total flavonoid (TFC) compounds of the extract of *Lycium barbarum*. Total phenolic and flavonoid contents were expressed as gallic acid equivalents(GAE) and hesperidine(HES) equivalents mg/g dried extract, respectively. The DGWE, DGWAE, PGWE, and PGWAE represent dried *Lycium barbarum* water extract, dried *Lycium barbarum* water plus ethanol extract, powdered *Lycium barbarum* water extract, powdered *Lycium barbarum* water plus ethanol extract. The data were expressed as mean (\pm SD) (n=5). The different superscripts on graphs represented different values(p<0.05).

sample was extracted with a mixture of purified water and alcohol as a solvent, it was 686.16 g/L. This trend was similar to the total flavonoid content of the extract. The highest total flavonoid content was 111.32 g/L when the powder sample was extracted with a mixture of purified water and alcohol as a solvent. It was reported that of 52 compounds, 15 phenolic acids and flavonoids of *Lycium barbarum* were positively identified based on both absorption and mass spectra, with the remaining 37 tentatively identified by comparison of absorption spectra with reported values in the literature. Internal standards 3-hydroxybenzoic acid and hesperidin were used for quantification of phenolic acids and flavonoids, respectively. Among the 15 positively identified compounds, quercetin-rhamno-di-hexoside was present in largest mass fraction (438.6 mg/g), followed by quercetin-3-O-rutinoside (281.3 mg/g), dicaffeoylquinic acid isomers (250.1 mg/g), chlorogenic acid (237.0 mg/g), quercetin-di-

(rhamnohexoside) (117.5 mg/g), quercetin-di-(rhamno)-hexoside (116.8 mg/g), kaempferol-3-O-rutinoside (97.7 mg/g), isorhamnetin-3-O-rutinoside (72.1 mg/g), p-coumaric acid (64.0 mg/g), caffeic acid (23.7 mg/g) and vanillic acid (22.8 mg/g). In this study, it was reported that the powdering process when producing extracts can increase the content of total phenols and flavonoids. Phenols and bioactive substances bound to cell walls and cell constituents are liberated through the powdering process or are exposed to solvent contact frequency[24]. Pires et al.[7] extracted reported the presence of nine-teen phenolic compounds (71 mg/g dw): eight flavonols (27.6 mg/g dw), seven phenolic acid derivatives (32.7 mg/g dw), one flavan-3-ol (10.4 mg/g dw), and three chlorogenic acids (25.07 mg/g dw). According to the authors, the principal phenolic compounds quantified were quercetin-3-O-rutinoside (16.6 mg/g dw) and p-coumaric acid (12.3 mg/g dw).

4. Conclusion

This study was conducted to produce raw materials for extracting functional tea and beverages using domestically cultivated dried *Lycium barbarum*. It demonstrated that the nutritional composition, organic acids, amino acids, total phenolic compounds, and total flavonoids of the extracts vary depending on the extraction method. It was found that the highest extraction efficiency was achieved when dried *Lycium barbarum* was extracted as a powder using a mixture of purified water and alcohol as the solvent. Therefore, it is believed that supplying *Lycium barbarum* extract suitable for use in tea and beverages can be applied to the field of health functionality.

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