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Research Article

Comparative Metabolic Profiling of *Lycium* Fruits (*Lycium barbarum* and *Lycium chinense*) from Different Areas in China and from Nepal

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Lycium fruits (*Lycium barbarum, Lycium chinense*) are mainly cultivated and distributed in Northwest China. The fruits and root bark have been used in Chinese traditional medicine for centuries. In this study, *Lycium* dry fruit extracts from the main cultivation areas in China together with a sample from Nepal were subjected to a comparative metabolic profiling, including total carbohydrate content, total phenolic content, vitamin C content, carotenoid content, and mineral contents. Results showed that there was a slight difference in contents of nutrients and phytochemicals among samples from different areas. The total carbohydrate content was higher in the sample from Guazhou, Gansu province (69.47%), with an average value of total carbohydrate content of 61.59%, while the highest total phenolic content value was 14.13 mgGAE/g from Nepal. Data concerning vitamin C content ranged between 33.15 and 113.8 mg/100 g, with an average value of 55.29 mg/100 g. Zeaxanthin dipalmitate content in *Lycium* dry fruits ranged from 419.34 to 1008.90 μg/g among the different samples, with the highest content (1008.90 μg/g) observed in Tianjing. It appeared that we could not clearly differentiate *Lycium* samples in terms of their metabolic and mineral profile. The quantitative difference observed among samples might be linked to soil composition and environmental aspect of the harvest place. Our results were somehow in the same range as those reported in the literature. Therefore, *Lycium* fruits could be used as a dietary source of natural function foods and be worthy of development and utilization.

1. Introduction

Lycium fruits (*Lycium barbarum*, *Lycium chinense*), known as Chinese wolfberries or "gouqizi" in China, are solanaceous fruits that are largely cultivated in subtropical areas, mainly in Northwest China and other parts of Asia. *Lycium barbarum (L. barbarum)*, one plant of the genus *Lycium*, is known to have a variety of important biological functions, such as increasing longevity, enhancing immunity, antiaging, antitumour, antioxidant, improving eyesight, and enhancing kidney and liver functions [1–4]. The success related to the wide range of beneficial effects attributed to this plant has contributed to increase in its popularity, and thus, its recent use on the international functional food market [5]. Nowadays, out of the dozen of *Lycium* species around the world, approximately 90% of commercially available *Lycium* fruit (goji berries) preparations including dietary supplements, aromatized teas, juices, jams, snacks, soups, and other foodstuff are produced by *L. barbarum* originating from China. *Lycium barbarum* is, therefore, a typical example that might be used as nutraceuticals or directly eaten in the diet to maintain good health, and this is due to bioactive molecules like polysaccharides, phenolic compounds, and vitamins and minerals, which are mainly present in the fruits [6–8]. Recently, a wide range of phytochemical, analytical, and pharmacological studies focus on health benefits of

Lycium plants in general, specifically on *Lycium barbarum*, and therefore support its use as functional food [9–11].

Lycium chinense, a Chinese native wild species closely related to *L. barbarum*, is a valuable germplasm resource for breeding and cultivation owing to its unique quality traits. *Lycium* fruits and derived products represent a relevant source of nutrients, which contribute to the high nutritional quality of these fruits. Recently, much attention has been drawn to the fruits as a functional food. Polysaccharides were estimated to constitute 5–8% of dried fruits [12], and both their structure and activities were profoundly studied [1, 4, 5]. Potential health benefits of *L. barbarum* fruit polysaccharides have been recently reviewed [12, 13]. Compounds including phenolics, carotenoids, and ascorbic acid are antioxidants also present in *Lycium* fruits and are considered as important protective agents for human health [14]. These compounds were reported to help the human body to reduce oxidative deterioration or to protect food quality [15, 16].

Since product authenticity is an essential topic in the food sector and in the course of our continuing, quantify metabolites of biological importance from *Lycium* plants, in order to evaluate the use of the fruit extract as a food supplement, carbohydrate, phenolic, vitamin C, carotenoid, and mineral content of 13 samples of *Lycium barbarum* harvested in the three main production areas in China including semiarid (Ningxia, Gansu, and Inner Mongolia), plateau (Qinghai), and arid (Xinjiang) areas; a sample of *Lycium chinense* which is known to be exclusively cultivated in monsoon (Hebei) and a sample cultivated in Nepal were investigated and compared. The genotype, geographical origin, cultivation conditions, ripeness stage, storage time, and conditions affect the composition and biological properties of a plant [17, 18]. The importance of genetic origin for full characteristic of fruits was pointed out in numerous studies. Although Islam et al. [19] only reported a comparative study on phenolic profiles, antioxidant capacities, and carotenoid contents of red goji berry (*Lycium barbarum*) from Ningxia and black goji berry (*Lycium ruthenicum*), Zhang et al. [20] determined functional constituents and antioxidant activities of eight Chinese native goji genotypes grown at the National Goji Germplasm Repository, Ningxia Academy of Agricultural Sciences, Ningxia; to the best of our knowledge, this study is the first approach to compare the metabolic profile of *Lycium* fruits cultivated in the main cultivated areas of China and outside.

The present work is to assess a quality control analysis for goji berries that could be used in food industry as a reference on the fruits.

2. Materials and Sample Preparation

A total of 13 samples of dried fruits of *Lycium* (*L. barbarum,* goji berries) were collected from different areas in China including the Ningxia autonomous region, Xinjiang, Qinghai, and Gansu, along with a sample of *Lycium chinense* (goji berries), and a sample was purchased in the Nepalese market. All the 15 samples consisted of the entire dried fruits (Table 1), which were extracted with 30% methanol/water (*v*/*v*).

Table 1: Sample information of the goji berry collected.

Sample	Common name	Scientific name	Place of origin
JH	Red goji berry	*Lycium barbarum*	Jinghe county, Xinjiang province
ZT	Red goji berry	*Lycium barbarum*	Zhongning County, Zhouta, Ningxia Hui autonomous region
HWH	Red goji berry	*Lycium barbarum*	Zhongning County, Hong Wu Hill, Ningxia Hui autonomous region
TJH	Red goji berry	*Lycium barbarum*	Zhongning County, Tianjing Hill, Ningxia Hui autonomous region
SH	Red goji berry	*Lycium barbarum*	Shahai, Inner Mongolia autonomous region
TX	Red goji berry	*Lycium barbarum*	Tongxin, Ningxia Hui autonomous region
CH	Red goji berry	*Lycium barbarum*	Qinghai province
GEM	Red goji berry	*Lycium barbarum*	Qinghai province
NE	Red goji berry	*Lycium barbarum*	Nepal
DH	Red goji berry	*Lycium barbarum*	Delhi, Qinghai Province
YM	Red goji berry	*Lycium barbarum*	Gansu province
GZ	Red goji berry	*Lycium barbarum*	Guazhou County, Gansu province
LC	Red goji berry	*Lycium chinense*	Hebei Monsoon province
UFB	Red goji berry	*Lycium barbarum*	Inner Mongolia autonomous region
YNLF	Red goji berry	*Lycium barbarum*	Yichuan Nanliang farm, Ningxia Hui autonomous region

2.1. Chemicals and Reagents. All laboratory chemicals used for the extraction or content-determination protocol were of reagent grade. Distilled deionized water and ultrahigh purity commercial acids were used to prepare all reagents, standards, and samples. Hexane, methanol, nitric acid (HNO_3), sulfuric acid, MPA (metaphosphoric acid), glacial acetic acid, and sodium carbonate were from Sinopharm Chemical Reagent Co. Ltd. Acetone, phenol, ethanol absolute, and potassium dihydrogen phosphate were purchased from Shanghai Lingfeng Chemical Reagent Co. Ltd. Water was purified by using a Milli-Qplus system from Millipore (Bedford, MA, USA). Folin–Ciocalteu phenol reagent (FCR) was from Macklin Biochemical Co. Ltd (Shanghai); nylon filters (0.45 μm, 0.22 μm pore size) were from Nanjing Mabel Biotechnology Co., Ltd. All solvents of HPLC grade were from Dikma Technologies Inc., USA.

2.2. Standard Preparation and Calibration Curve. Standard curves were obtained using specific standards at different concentrations.

Zeaxanthin dipalmitate (purity >99%) was purchased from Aladdin Industrial Corporation (Shanghai). About 5 mg of zeaxanthin was accurately weighed, dissolved in

25 mL of hexane, and kept at −20°C as stock solution. Dilutions were prepared still in hexane each time immediately before analysis.

L-Ascorbic acid was from Macklin Biochemical Co. Ltd (Shanghai). Approximately 10 mg of L-ascorbic acid was weighed and dissolved in 100 mL methanol as a stock solution. For the calibration curve, a series of concentrations of L-ascorbic acid were prepared from the stock solution dissolved in methanol and injected in HPLC for analysis. Gallic acid was acquired from Aladdin Industrial Corporation (Shanghai), and D-glucose was from Sinopharm Chemical Reagent Co. Ltd. As mentioned above, for the two previous standards, a stock solution of gallic acid and D-glucose was prepared, respectively, and the calibration curve was determined according to the absorbance corresponding to series of concentrations measured at 750 nm and 490 nm, respectively, of the standards prepared.

2.3. Extraction of Lycium Dry Fruit Samples. *Lycium* fruit samples extraction procedure was as follows: three grams of fruit powder was completely homogenized in 20 mL of 30% methanol/water (*v*/*v*). Extracts were shaken for 3 h at 300 rpm using an orbital shaker; samples extracted were placed in the dark for 12 h. After 12 h, each extract was centrifuged at 3000 rpm for 10 min. The supernatant was stored at 4°C in the dark for further determination of total carbohydrate content (TCC) and total phenolic content (TPC).

2.3.1. Optimized Extraction of Carotenoids from the Dried Fruits. Carotenoids were extracted from *Lycium* dry fruits using the method described by Karioti et al. [2] with a slight modification. Briefly, around 500 mg of powdered fruits was weighed and before the extraction of carotenoids, the fruits were extracted by ultrasonication for 15 min with 50 mL of distilled water, and the supernatant was discarded. The residue was then washed with 20 mL of absolute ethanol and centrifuged, and the supernatant was again discarded. The residue was finally extracted with hexane/acetone mixture (50 : 50; *v* : *v*) in order to obtain carotenoids. The mixture was centrifuged at 4°C for 10 min. The extraction procedure was repeated until the residue remained colorless, and supernatants were combined. Samples were filtered and immediately injected. To ensure minimum exposure of samples to light and avoid high temperature during the experiment, the extraction process was fast. The carotenoid content from the dried fruit was expressed as microgram per gram (μg/g) edible dry weight (DW).

2.3.2. Optimized Extraction of Vitamin C from the Dried Fruits. Ascorbic acid was extracted by blending 5 g of fruit tissue with 25 mL of cold MPA/acetic acid solution (20 g MPA and 80 mL glacial acetic acid diluted to 1 L with distilled water) in a blender. The extract was centrifuged for 10 min at 10,000 rpm in a cold centrifuge (2–4°C), and the supernatant was collected. Samples were filtered through a 0.22 μm pore size filter, and 20 μL was injected in HPLC for analysis. All samples were kept at low temperature. Vitamin C content was expressed as milligram per 100 g (mg/100 g) edible dry weight (DW).

2.4. Instrumentation for Analysis. The ultrasonicator was a KQ5200 series operating at 70°C at a frequency of 50 kHz. Centrifuges used were Zonkia SC-2546 low-speed centrifuge (Anhui) and Eppendorf 5424R aerosol-tight operating at 4°C. The spectrophotometer was a MC visible spectrophotometer (722N) operating at 220 V and 50 Hz frequency manufactured by Shanghai Yi Electric Analytical Instrument Co., Ltd, China.

HPLC analysis for total carotenoid content was carried out using an Agilent 1100 binary pump liquid chromatograph equipped with a DAD detector. The column was a Zorbax SB-C_{18} (250 mm × 4.6 mm) maintained at 25°C. An isocratic elution system acetone/MeOH 55 : 45 (v/v) with a flow rate of 1.4 mL/min was used as the mobile phase and the UV-vis spectra were recorded at 350, 430, 450, 480, and 520 nm.

HPLC analysis for vitamin C content was carried out using an Agilent 1200 quaternary pump liquid chromatograph equipped with a DAD detector. The column was a Hypersil C_{18} (250 mm × 4.6 mm) maintained at 25°C. An isocratic elution system 0.2 M potassium hydrogen phosphate, pH 2.1~2.8, was used as the mobile phase, and the detector was set at 254 nm. A flow rate of 0.25 mL/min was used, and the runtime was 8 min. The injected volume of the samples was 20 μL.

2.4.1. Determination of TPC. The TPC was determined using the colorimetric method of Folin–Ciocalteu [21], with slight modifications. For the analysis, an aliquot (25 μL) of *Lycium* dry fruit extracts or standard solutions of gallic acid was pipetted into a 24-well microplate in triplicates containing 2 mL of distilled water, and then the Folin–Ciocalteu (250 μL) reagent was added. After 10 min, 750 μL of 7.5% sodium carbonate solution was added. The absorbance was measured at $\lambda = 750$ nm after 90 min of incubation at room temperature. Results were expressed as mg of gallic acid equivalent per gram (mg_{GAE}/g) of dry weight (DW) according to the calibration curve prepared using solutions of gallic acid. Methanol was used as a blank.

2.4.2. Determination of TCC. TCC was determined using the colorimetric method of phenol-sulfuric acid described in the People's Republic of China Exit Inspection and Quarantine Industry Standard SN/T 4260-2015. Briefly, 0.2 mL of different sample extracts of *Lycium* dry fruits was taken into boiling tubes containing 0.8 ml of distilled water. Then, 1 mL of 5% and 5 ml of 96% sulfuric acid were added one by one in each tube and shook well so that the phenol and sulfuric acid get mixed thoroughly with working samples. All the tubes were cooled down for 10 minutes and then placed in a water bath at 25–30°C for 15 minutes. Absorbance was detected at 490 nm with the help of a spectrophotometer. The blank was set with 1 ml of distilled water. Results were expressed as the percentage of D-glucose (%D-Glc) edible DW.

2.4.3. Determination of Mineral Content. To determine the mineral content in *Lycium* fruit samples, a standard protocol used by Kumari et al. [22] with slight modification was followed. Collected samples of *Lycium* fruits were first dried at 70°C in an oven until they reached a constant weight. After drying, about 0.5 g of the dried and ground sample was digested with 5 ml of 65% HNO_3 and 2 mL of 35% hydrogen peroxide (H_2O_2) and kept in a volumetric flask of 25 mL capacity. Completion of digestion was marked by the appearance of a colorless liquid. The volumes of the digested samples were completed to 25 mL with ultradeionized water, and mineral concentrations were determined. After digestion treatment, samples were filtrated through Whatman Ashless No 42 paper filter. The filtrates were collected in 50 mL flasks, and contents of various ions in the fruit were determined. Mineral content of the *Lycium* fruits extract samples was quantified against standard solutions of known concentrations, which were analyzed simultaneously. Results were expressed as mg/kg edible DW.

2.5. Statistical Analysis. Metabolic profile data executed on *Lycium* fruits extracts were analyzed via Microsoft Excel. Mean was calculated for each parameter, and data were expressed as the means of three replicates.

3. Results and Discussion

All the samples were analyzed for their macro- and micronutrients content. The nutrients chosen were reported to be responsible for the significant nutritive value and pharmacological activities of *Lycium* fruits (Liang, B. et al.). Moreover, *Lycium* fruits are eaten as both fresh and dry in daily life. All these constituents constitute a wide range of polarity and molecular weight, so they required analytical methods specific for each case.

3.1. Total Carbohydrate Content (TCC). From the data of TCC ranging from 61.76% to 69.47% as shown in Table 2, we could clearly observe that samples from Yichuan Nan Liang Farm (YNLF) had the lowest carbohydrate content of 61.76 compared to others, e.g., Zhouta 62.08%, followed by the sample from Inner Mongolia (UFB) 62.54%, Ge er mu 63.06%, Tianjing 63.28%, and Delhi 64.19%, while the highest value was recorded at 69.47% for the sample from Guazhou. These observations were in fair agreement with results reported by Niro et al. [23] in which nutritional evaluation of fresh and dried goji berries cultivated in Italy was performed. It appeared that the quantitative difference in the carbohydrate content among samples might be linked to varietal difference status of conservation and harvest time. From the results observed, the mean value of the total carbohydrate content in *Lycium* dry fruits was 61.59%.

3.2. Total Phenolic Content (TPC). The total phenolic content (expressed as mg_{GAE}/g) of all *Lycium* dry fruits samples is shown in Table 2. The sample from YNLF (11.82 mg_{GAE}/g) had a relatively higher total phenolic content than the other samples from the Ningxia Hui autonomous region. The highest value was 14.13 mg_{GAE}/g for the sample from Nepal, while the lowest was 8.36 mg_{GAE}/g for that from Zhouta. The average value of TPC of the 15 samples was recorded as 10.39 mg_{GAE}/g, which was 2 times higher than results reported by Kafkaletou et al. [24]. However, results reported by Zhang et al. [20], in which functional constituents and antioxidant activities of eight Chinese native goji genotypes was determined, were 3 times higher (ranging from 26.9 to 73.4 mg_{GAE}/g FW). It appeared that their samples had the highest moisture content compared to those analyzed in our study. It may also be due to experimental conditions and different solvents used. Environment aspect and harvest place may also be contributing factors.

Table 2: TCC, ZeC, Vit.C, and TPC of goji berries.

Samples	TCC	TPC	Vit.C	ZeC
JH	68.16	9.95	45.10	429.62
ZT	62.08	8.36	73.12	463.80
HWH	64.43	8.87	38.98	805.60
TJ	63.28	10.06	38.27	1008.90
SH	69.15	11.29	33.15	546.97
TX	66.61	9.21	41.94	638.70
CH	65.43	8.47	33.26	736.96
GEM	63.06	12.14	43.47	519.46
NE	69.43	14.13	99.70	635.39
DH	64.19	9.82	37.44	570.17
YM	66.33	12.15	49.01	677.57
GZ	69.47	11.84	56.30	499.26
LC	68.05	8.86	113.86	634.65
UFB	62.54	8.99	88.60	571.08
YNLF	61.76	11.82	37.66	419.34

Data are expressed as mean of three ($n = 3$) independent experiments. TCaC, total carotenoid content; Vit.C, vitamin C content; ZeC, zeaxanthin content.

3.3. Vitamin C Content (VitC). The data in this study concerning vitamin C content ranged between 33.15 and 113.86 mg/100 g, with an average value of 55.29 mg/100 g, as shown in Table 2. Our average value was somewhat close to results reported by Emine Kocyigit et al. and Donno et al. [14, 25]. However a much lower value of the ascorbic acid content was reported by Cheng et al. [26]. It might be due to the moisture content of samples analyzed. It may also be due to storage time and experimental conditions. Environment aspect and harvest place may also be contributing factors.

3.4. Carotenoid Content. The total carotenoid content of *Lycium* fruits is recorded in Table 2. Our current results in which zeaxanthin dipalmitate content ranged from 419.34 to 1008.90 μg/g among the different samples were somehow similar to those in the literature reported by Karioti et al. [2]. Differences observed among samples might be linked to the status of conservation and time of harvest. The lowest content was observed in YNLF, while Tianjing exhibited the highest zeaxanthin dipalmitate content.

3.5. Mineral Content. The content of mineral elements in *Lycium* fruits is reported in Table 3. Potassium (K) was

Table 3: Content of mineral elements in goji berries (mg/kg).

Samples	Mg	Ca	K	Cu	Fe	Mn	Zn
JH	691.6	568.5	12626.7	9.32	<0.25	6.85	18.8
ZT	836.7	634.9	13560.8	9.92	<0.25	7.91	18.72
HWH	728.4	548.5	13202.9	5.84	<0.25	7.23	20.44
TJ	996.1	699.4	14682.2	6.12	0.71	9.66	22.22
SH	762.4	391.2	12180.1	7.01	<0.25	7.08	13.93
TX	722.2	458.7	12540.1	6.06	1.85	5.95	11.54
CH	636.9	487.5	12957.5	11.52	13.69	6.90	18.14
GEM	687.4	616.6	13924.8	4.78	0.61	7.37	8.40
NE	775.2	637.7	14724.4	6.75	0.28	9.04	13.06
DH	794.1	585.8	14373.4	6.75	2.78	7.60	10.12
YM	779.5	593.1	12853.8	7.19	0.36	8.59	11.79
GZ	901.8	547.7	15149.2	7.07	<0.25	7.63	12.40
LC	732.1	645.8	14007.8	7.43	<0.25	8.69	9.26
UFB	797.8	650.5	12081.2	6.15	<0.25	8.94	12.93
YNLF	647.6	445	13376.8	6.07	<0.25	6.08	12.42

Data are expressed as mean of three ($n = 3$) independent experiments.

found to be the predominant element with an average value of 13482.78 mg/kg of dry weight (DW). The sample from Guazhou exhibited the highest amount (15149.2 mg/kg). The potassium content was followed by magnesium (Mg) content, with an average concentration of 765.98 mg/kg. Chaturvedi et al. [27] reported that magnesium prevents heart disease and growth retardation, while potassium was reported by Clausen and Poulsen [28] to play an important role together with sodium in regulating blood pressure and the body's acid-base balance. From the same table, we could also observe that Goji is a good source of calcium (Ca), with an average value of 567.3 mg/kg DW. The highest value of calcium content was found to be 699.4 mg/kg DW from TianJing, Ningxia province, and the lowest content was 391.2 mg/kg from Shahai. Table 3 also exhibits a discrete amount of copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn). Our results were somehow in the same range with those reported by Llorent-Martínez et al. [29], in which the aim was to characterize and compare the chemical composition of exotic superfoods in Spain, and Endes et al. [30], in which the aim was to determine physicochemical properties, fatty acid composition, and mineral contents of goji berry (*Lycium barbarum* L.), while Niro et al., [23] reported slightly different results. This difference might reflect the soil in which *Lycium* fruits were cultivated because essential and nonessential element concentration is dependent on the soil characteristics, the physiology of the plant, the water source composition, and fertilizers, insecticides, pesticides, and fungicides used in the plantations [31].

4. Conclusion

In conclusion, *Lycium* fruits had high levels of phenolic compounds, carotenoids, and carbohydrates. Quantitative results showed a variance among investigated samples, which may be linked to different sources of fruits, harvest time, manufacturing process, and storage conditions. Comparison of our results with those reported in the literature was somehow similar. Our results did not exhibit a global superiority of a specific growing area over others. Therefore, *Lycium* fruits could be used as a dietary source of natural function food and be worthy of development and utilization.

Data Availability

The results regarding the metabolic content and data used to support the findings of this study are included within the article (Tables 2 and 3).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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