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Functional constituents and antioxidant activities of eight Chinese native goji genotypes

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ABSTRACT

We quantified the levels of polyphenols, carotenoids and polysaccharides in fruits of the eight Chinese native goji genotypes, antioxidant activities of these fruit extracts were also evaluated by DPPH, ABTS and FRAP methods. Quercetin-rhamno-di-hexoside (435–1065 μg/g) and quercetin-3-O-rutinoside (159–629 μg/g) were found to be the predominant flavonoids. Chlorogenic acid was the most abundant phenolic acid (113–526 μg/g), while zeaxanthin (17–9306 μg/g) was the major carotenoid. The total antioxidant activities (TAA) of the berry extracts were significantly correlated with the total polysaccharide and phenolic contents, but not with total carotenoid (TC) levels. Overall, fruits of the Ningxia goji (*Lycium barbarum* L.) genotypes, DM (Damaye), NJ1 (Ningji No.1), BH (Baihua) and NH (Ningxiahuangguo) were not only rich in polyphenols, carotenoids and polysaccharides, but had significantly higher TAA than those of the other genotypes, suggesting that they represent an excellent source of antioxidants for human nutrition.

1. Introduction

Lycium plants are Solanaceous defoliated shrubs that primarily grow in northwest China and other parts of Asia. Their fruits (goji berries) are reported to have various biological activities and health-promoting properties and have been used in Asian countries as a traditional herbal medicine and functional food (Amagase & Farnsworth, 2011; Amagase, Sun, & Borek, 2009). Epidemiological studies have suggested that goji berries have antioxidant, anticarcinogenic and immune enhancing properties (Akbulut & Özcan, 2009; Karathanos & Belessiotis, 1997; Koyuncu, 2004; Pangavhane & Sawhney, 2002), and thus the consumption of goji berries may help prevent many chronic diseases such as diabetes and cancers (Chen, Zhu, & Han, 2011; Oki et al., 2006). These various nutritional and health promoting activities are provided by a structurally varied range of components, including phenolics, carotenoids and polysaccharides (Le, Chiu, & Ng, 2007; Luo, Cai, Yan, Sun, & Corke, 2004; Wang, Chang, Inbaraj, & Chen, 2010).

Phenolics are the most abundant secondary metabolites of plants and are beneficial components of a daily human diet (Ignat, Volf, & Popa, 2011). These phytochemicals exhibit a broad range of biological activities and are potential agents for prevention and treatment of many diseases (Szajdek & Borowska, 2008). In goji berries, the main phenolic compounds are phenolic acids and flavonoids (Szajdek & Borowska, 2008). Goji berries are also an excellent source of carotenoids, not only because of their relatively high content in fruits, but also due to the specific profile of carotenoid species. Carotenoids are important natural pigments responsible for the yellow, orange, and red colors of many fruits and vegetables (Amagase & Farnsworth, 2011; Amagase et al., 2009). In addition to their use as natural, nontoxic colorants, drinks, and cosmetics, carotenoids possess various biological activities, such as the antioxidant activity of pro-vitamin A. In the context of the health benefits of goji berries, *Lycium barbarum* polysaccharides (LBP) are one of the most valuable functional constituents and active compounds (Amagase & Farnsworth, 2011;

Abbreviations: TAA, total antioxidant activities; FCs, functional components; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzthiozoline-6)-sulfonic acid; FRAP, ferric reducing/antioxidant power; TSS, total soluble solid; TA, titratable acid; TP, total phenolic; TF, total flavonoid; TC, total carotenoid; LBP, *Lycium barbarum* polysaccharides; AAPH, 2,2′-azobis(2-(2-amidinopropane)dihy drochloride; TPTZ, 2,4,6-tris(2-pyridyl)-s-triazine; FW, fresh weight; GAE, gallic acid equivalents; RE, rutin equivalents; βCE, β-carotene equivalents; TE, trolox equivalents; TPA, total phenolic acid.

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Amagase et al., 2009). Indeed, LBP have been reported to have antioxidant, antiaging, antitumor, antidiabetic, cytoprotective, neuroprotective, and immunomodulatory effects (Chang & So, 2008).

China is the most important center of origin for the genus *Lycium*, and some important species or variety originated from the country (Yao, Peng, Zhou, Xiao, & Sun, 2010). Of the dozens of *Lycium* species around the world, approximately 90% of commercially available goji berries are produced by *L. barbarum*, which originated from the north central regions of China. Many other Chinese native wild species closely related to *L. barbarum*, such as *Lycium chinense*, *Lycium yunnanense and Lycium ruthenicum* are valuable germplasm resources for breeding and cultivation owe to their unique quality traits. Over the past few decades, only a few *Lycium* genotypes that are native to China have been described. Moreover, studies of *L. barbarum* carotenoids, phenolics, LBP composition and LBP function have only focused on one genotype (He, Yang, Jiao, Tian, & Zhao, 2012; Inbaraj, Lu, Kao, & Chen, 2010; Li, Li, & Zhou, 2007; Li, Zhou, & Li, 2007; Ming, Guanhua, Zhanhai, Guang, & Xuan, 2009; Peng et al., 2005; Wang, Chang, & Chen, 2009). There is therefore very little information regarding the main functional components (FCs) and antioxidant activities of native Chinese *Lycium* genotypes.

The objective of the present study is to determine the content and composition of main FCs (including polyphenols, carotenoids and LBP) in eight native Chinese goji genotypes and evaluate their antioxidant activities. The results obtained were compared with those of the four *L. barbarum* L. (Ningxia goji) genotypes. In addition, the contribution of each group of functional compounds to the total antioxidant activities (TAA) of these fruit extracts was also analyzed. These findings will provide useful information for future utilization of *Lycium* germplasm.

2. Material and methods

2.1. Chemicals

Folin–Ciocalteu phenol reagent, trolox (6-hydrox-2,5,7,8-tetra methylchromane-2-carboxylic acid), AAPH (2,2′-azobis(2-(2-amidi nopropane)dihydrochloride), TPTZ (2,4,6-tris(2-pyridyl)-s-tria zine), quercetin, myricetin, kaempferol, rutin, quercetin-rhamno-di-hexoside, quercetin-3-O-rutinoside, caffeic acid, *p*-coumaric acid, ferulic acid, vanillic acid, chlorogenic acid, lutein, β-cryptoxanthin, neoxanthin, β-carotene and zeaxanthin were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals of analytical grade used in this study were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Plant materials

Eight native Chinese goji cultivars were grown at the National Goji Germplasm Repository, Ningxia Academy of Agricultural Sciences, Ningxia, China (Table 1). Fruits were harvested at the commercial maturity stage based on external color and size uniformity (Fig. 1). For every cultivar, each replicate included 200 fruits sampled from ten trees. Among them, 50 fruits were used to determine the firmness, TA (titratable acid) and total soluble solid (TSS),

Table 1
Chinese goji genotypes used in the study.[a,b,c]

No.	Chinese name	Scientific name	Repository number	Abbreviation	Firmness (N)	TSS (%)	TA (%)
1	Damaye	*Lycium barbarum* L.	LYBAR64011050001	DM	6.5	15.5	1.3
4	Zhongguo	*Lycium chinense* Mill.	LYCHI 64011050001	ZG	5.4	15.5	1.6
3	Ningji No.1	*Lycium barbarum* L.	LYBAR64011050021	NJ1	5.8	19.3	0.8
2	Baihua	*Lycium barbarum* L.	LYBAR64011050004	BH	6.3	16.5	1.4
5	NingxiaHuangguo	*Lycium barbarum* L. var. auranticarpum K. F. Ching	LYBAR64011050018	NH	5.5	16.8	1.2
6	Yunnan	*Lycium yunnanense* Kuang et A. M. Lu	LYYUN64011050001	YN	5.6	16.2	2.1
7	Beifang	*Lycium chinense* Mill. var. potaninii (Pojark.) A. M. Lu	LYCHI 64011050012	BF	6.4	15.4	1.9
8	Heiguo	*Lycium ruthenicum* Murr.	LYRUT64011050001	HG	5.7	14.7	2.7

[a] TSS, total soluble solid.
[b] TA, titratable acid.
[c] Data are expressed as means ± standard deviation of triplicate samples.

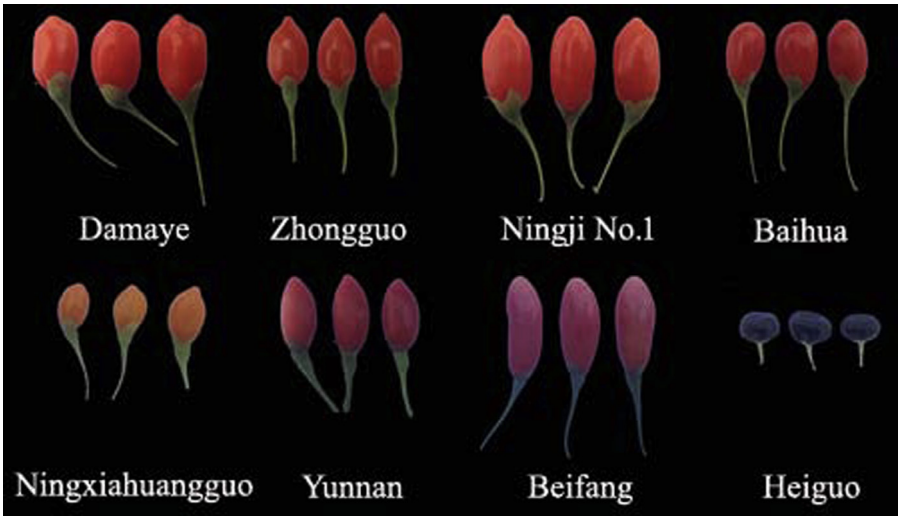

Fig. 1. Fruit of different goji genotypes analyzed in the study.

other 150 fruits were ground into a fine powder with liquid nitrogen in a freezer-mill (6750) apparatus (Glen Creston), and then passed through a 40 mesh sieve. Three replicates were carried out for every genotype. The resulting powders were stored at −80 °C until further use.

2.3. Determination of fruit quality

Fruit firmness was measured at the equator of the fruit using a penetrometer (Model: HL-300, Xianlin Non Detection Device Co. Ltd., Nanjing, China) with an 2 mm diameter head. Ten fruits were used as one replicate. Triplicates were used for each genotype samples.

TSS and TA measurements were conducted on juice samples collected from 40 fruits for one replicate, and three replicates were used. TSS value of fruit were measured with a hand-held refractometer (Model: B32T Brix Meter, Guangzhou Ruiqi Trade Co. Ltd., Guangdong, China). TA measurements were performed by titrating 6 mL of berry juice with 0.2 M NaOH until reaching a pH of 8.2. The results were expressed as mM/L $H^{\pm}$.

2.4. Determination of total levels of phenolic compounds, carotenoids and LBPs

Polyphenolic compounds were extracted by the method previously described by Ramful, Tarnus, Aruoma, Bourdon, and Bahorun (2011) with some modifications. Three grams of fruit powder was completely homogenized in 20 mL 80% methanol, and then centrifuged at 3000*g* for 10 min at 4 °C. Total phenolic (TP) content was determined according to the Folin–Ciocalteu method (He et al., 2012). Folin–Ciocalteu phenol reagent (0.5 mL) was added to the extract and the samples were vigorously shaken before 5 mL of 5% Na_2CO_3 solution was added. After the samples had been adjusted to a total volume of 25 mL with distilled water and mixed thoroughly, the solution was left to stand for 60 min at room temperature. The absorbance of the solution at a wavelength of 750 nm was measured using a Microplate spectrophotometer (Benchmark Plus, Biorad, Hercules, CA). The TP content of the samples was expressed as mg/g fresh weight (FW) of gallic acid equivalents (GAE).

The total flavonoid (TF) content was determined using the previous method (Jia, Tang, & Wu, 1999; Wang, Chuang, & Hsu, 2008). Briefly, 2.5 g of fruit powder was placed in a Soxhlet extractor and refluxed with methanol for more than 12 h at 85 °C. The extract was evaporated to dryness in a rotary vacuum evaporator at less than 40 °C and then dissolved with methanol. Exactly 0.3 mL of 5% $NaNO_2$ was added to a 1 mL extract in a 10 mL volumetric flask and kept for 6 min at room temperature. Addition of 0.3 mL of 10% $Al(NO_3)_3$ to the mixture, which was incubated for 6 min again, was followed by addition of 4 mL of 1 N NaOH and of methanol, up to volume. After incubating for 15 min at room temperature for color development, absorbance at 510 nm was measured. TF content was expressed as mg/g FW of rutin equivalents (RE).

The total carotenoid (TC) contents were determined according to the method described by Rodriguez-Amaya (1999) with some modifications. In brief, five grams of fruit powder was homogenized with 100 mL of methanol: petroleum ether (1:9, v/v) and the mixture were transferred to a separating funnel. Petroleum ether layer was filtered through sodium sulfate, transferred to a volumetric flask and total volume was made up to 100 mL with petroleum ether. Finally, the TC content was measured spectrophotometrically at wave length of 450 nm using an extinction coefficient of 2500 and the results were expressed as mg/g FW of β-carotene equivalents (βCE) (Gross, 1987).

Total LBP levels were determined according to the method described by Zhao et al. (2015) with slight modifications. Fruit (2.5 g) powder was added to 75 mL of 80% ethanol solution and was reflux extracted for 1 h. After filtering with gauze, the residues were washed with 80% hot ethanol solution and placed in a flask, heating in water bath extracted for 1 h, then washed with 75 mL of distilled water. The solution was set to 50 mL. A 0.3 mL aliquot of the sample solution was added to 2 mL of distilled water and 1 mL of phenol solution, and 5 mL of concentrated sulfuric acid was quickly added drop-wise with mixing, and the sample was left at room temperature for 5 min. The mixture was held in a boiling water bath for 15 min, and cooled to room temperature, and the absorbance of the solution was then measured at 490 nm. The glucose content was calculated using a glucose standard curve. The results were expressed as mg/g FW.

2.5. Analysis of phenolic composition by HPLC

Determination of the polyphenolic content was conducted by high pressure liquid chromatography (HPLC) as previously described (Zhang et al., 2014). Approximately 0.5 g of fruit powder was extracted with 12 mL 80% methanol and dimethyl sulfoxide (1:1, v/v). The homogenate was shaken for 12 h and centrifuged at 3000*g* for 10 min at 4 °C. The extraction solution was added to the residue and the same procedure was repeated. Supernatants from both extractions were collected and diluted to 50 mL with methanol. After filtration through a Millipore membrane (0.22 μm), 10 μL of the solution was injected into the HPLC system.

Chromatographic separation was performed using a reverse phase column (ZORABX SB-C18, 250 mm × 4.6 mm internal diameter). The mobile phase was composed of A (0.1% formic acid, aqueous) and B (methanol). Gradient elution was performed as follows: 0–20 min, 37–50% B; 20–35 min, 50–80% B; 35–40 min, 80–100% B; 40–50 min, 100% B; 50–60 min, 37–50% B. The column temperature was maintained at 25 °C and the flow rate was 0.7 mL/min. The detection wavelengths were set at 367 nm and 320 nm to detect flavonoids and phenolic acids, respectively. The results were expressed as μg/g FW.

2.6. Analysis of carotenoids composition by HPLC

Individual carotenoids were analyzed as previously described by Xu, Fraser, Wang, and Bramley (2006). Chloroform/methanol/Tris buffer (50 mM, pH 7.5) was added to three grams of fruit powder for extraction, and then centrifuged at 10,000*g* for 10 min. The chloroform phase was collected and the aqueous phase was extracted with chloroform. The two chloroform phases were combined and dried under nitrogen gas. Subsequently, the extract was saponified using diethyl ether and 6% (w/v) KOH in methanol to remove the chlorophyll compounds. Water and chloroform was then added, and the samples were finally dried under nitrogen gas.

HPLC analysis was carried using a Waters Alliance 2695 system (Waters Corporation, USA) consisting of a 2695 module and a 2996 PDA detector, equipped with a 250 × 4.6 mm i.d., 5 μm, YMC reverse-phase C_{30} column and a 20 × 4.6 mm i.d., YMC C_{30} guard. Chromatography program were as follows: 95% A (methanol), 5% B (80% methanol containing 0.2% ammonium acetate) for 6 min, a linear gradient to 80% A, 5% B, and 15% C (tertbutyl methyl ether) by 7 min, held until 12 min, gradient changed to 30% A, 5% B, and 65% C by 32 min, held until 48 min, then changed to 95% A and 5% B by 50 min, and then held to the end of analysis (60 min). Compounds were detected at 450 nm (lutein, β-cryptoxanthin, zeaxanthin and β-carotene) and 440 nm (neoxanthin) and quantified according to their respective standard curves. The results were expressed as μg/g FW.

2.7. Determination of antioxidant capacity

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) were used to evaluate the antioxidant capacity of the samples.

The DPPH free radical-scavenging activity was determined according to Brand-Williams, Cuvelier, and Berset (1995). A 50 μL aliquot of the previously diluted extract were allowed to react with 250 μL of DPPH (0.5 mM) for 24 h in the dark. Then the absorbance was detected at a wavelength of 515 nm. The standard curve was linear between 25 and 800 μM Trolox. Results were expressed in μM/g FW of trolox equivalents (TE).

The ABTS assay was performed according to the method of Almeida et al. (2011). A total of 5 mL of aqueous ABTS solution (7 mM) was added to 88 μL of a 140 mM potassium per sulfate solution. The mixture was kept in the dark at 29 °C for 14 h and the decrease of absorbance was measured for 6 min at a wavelength of 734 nm. The standard curve was linear between 25 and 600 μM Trolox. Results were expressed in μM TE/g FW.

The FRAP assay was carried out according to the method described by Benzie and Strain (1996). A 150 μL volume of reagent solution (0.1 M acetate buffer [pH 3.6], 10 mM TPTZ, and 20 mM ferric chloride [10:01:01, v/v/v]) was added to 20 μL of previously diluted extract and the absorbance was detected at a wavelength of 593 nm. The standard curve was linear between 25 and 800 μM Trolox. Results were expressed in μM TE/g FW.

2.8. Statistical analysis

All data were expressed as the means ± standard deviations of three biological replicates. Statistical analysis was performed using the SPSS v19.0 software (SPSS for Windows, Release 19.0, SPSS Inc.). Significant differences among all genotypes tested were calculated using a one-way ANOVA test, followed by Duncan's multiple-range test at a 5% level ($p \leqslant 0.05$).

3. Results and discussion

3.1. Characterization of fruit quality of goji genotypes

The firmness, TA and TSS of goji berry genotypes were shown in Table 1. The DM (Damaye) had the highest firmness, ZG (Zhongguo) had the lowest firmness. Even the firmness of all fruits tested ranged 5.4–6.4 N, no significances of firmness were found in all goji berries.

The TSS of the goji genotypes was expressed in terms of °Brix. The TSS of mature fruits ranged 14.7–19.3%. NJ1 (Ningji No.1) was characterized by the highest TSS value (19.3%), followed by the DM (Damaye) (18.5%). DM (Damaye), NJ1 (Ningji No.1), BH (Baihua) and NH (Ningxiahuangguo) had °Brix greater than 16.5%. The lowest TSS value was found in HG (Heiguo) with a °Brix of 9.7%. TSS is the sum of sugars, acids and secondary metabolites in fruits (Beckles, 2012). DM (Damaye), NJ1 (Ningji No.1), BH (Baihua) and NH (Ningxiahuangguo) had significantly higher TSS levels than the other genotypes tested, which suggested that *L. barbarum* genotypes had higher metabolites than other genotypes tested.

The TA of all fruits ranged 0.8–2.7%. The juice of HG (Heiguo) was the most acidic with a TA value of 2.7% while the juice of NJ1 (Ningji No.1) was the least acidic with a TA value of 0.8%. The TA values of DM (Damaye), NJ1 (Ningji No.1), BH (Baihua) and NH (Ningxiahuangguo) were characterized by low TA (<1.5%), which showed that *L. barbarum* L. genotypes (Ningxia goji) had good taste than other genotypes tested.

3.2. Total phenolic, total carotenoid and total LBP levels

For all goji genotypes, the TP levels ranged from 26.9 to 73.4 mg GAE/g FW, with the BH (Baihua) having the highest content, and HG (Heiguo) with the lowest content. DM (Damaye), ZG (Zhongguo), NJ1 (Ningji No.1), BH (Baihua) and BF (Beifang) had significantly higher TP levels than the other genotypes tested, with more than 50 mg GAE/g FW (Table 2). The TF levels ranged from 37.2 to 54.7 mg RE/g FW, and NJ1 (Ningji No.1) had the highest levels, while NH (Ningxiahuangguo) had the lowest. DM (Damaye), ZG (Zhongguo), NJ1 (Ningji No.1), BH (Baihua) and YN (Yunnan) had significantly higher TF contents than the other genotypes. TC levels ranged from 0.40 to 11.33 mg βCE/g FW among the genotypes, and BH (Baihua) had the highest, while HG (Heiguo) had the lowest TC content. Again, DM (Damaye), NJ1 (Ningji No.1), BH (Baihua), YN (Yunnan) and BF (Beifang) had significantly higher TC levels than the other genotypes. Finally, the total LBP contents ranged from 25.0 to 56.9 mg/g FW, with BH (Baihua) having the highest and ZG (Zhongguo) had the lowest values. The four *L. barbarum* L. genotypes all had more than 50 mg/g FW total LBP, which was significantly higher than those of the other genotypes.

3.3. Contents of individual phenolic compounds

A total of 11 phenolic compounds were identified from the Goji fruits tested, including six flavonoids (quercetin, myricetin, kaempferol, rutin, quercetin-rhamno-di-hexoside and quercetin-3-O-rutinoside) and five phenolic acids (caffeic acid, *p*-coumaric acid, ferulic acid, vanillic acid and chlorogenic acid) (Table 3). Among the flavonoids, quercetin-rhamno-di-hexoside and quercetin-3-O-rutinoside were the predominant flavonoid components, with quercetin-rhamno-di-hexoside contents ranging from 434.7 to 1065 μg/g FW. DM (Damaye) had the highest and BH (Baihua) had the lowest levels; however, DM (Damaye), NJ1 (Ningji No.1),

Table 2
Total phenolic, total flavonoid, total carotenoid, and total *L. barbarum* polysaccharide contents, and antioxidant activities of the different goji genotypes.[a,b,c,d]

No.	Genotypes	TP	TF	TC	Total LBP	DPPH	ABTS	FRAP
1	DM	58.4 ± 5.6 c	42.6 ± 4.3 bc	7.97 ± 0.56 d	55.9 ± 3.8 b	76.61 ± 4.3 b	78.2 ± 4.8 b	78.1 ± 1.7 c
2	ZG	68.8 ± 3.7 ab	45.3 ± 2.6 b	5.94 ± 0.80 e	25.0 ± 4.2 d	35.88 ± 2.2 f	59.3 ± 3.5 cd	57.7 ± 3.5 f
3	NJ1	73.4 ± 6.8 a	54.7 ± 3.2 a	11.33 ± 0.79 a	56.9 ± 2.1 b	85.46 ± 1.9 a	95.6 ± 4.9 a	92.5 ± 2.8 a
4	BH	64.3 ± 1.4 b	48.2 ± 5.3 b	7.83 ± 0.58 d	62.7 ± 1.7 a	77.47 ± 5.3 b	77.7 ± 5.8 b	87.1 ± 4.6 b
5	NH	30.3 ± 3.2 e	38.5 ± 3.8 e	3.64 ± 0.29 f	54.5 ± 1.2 b	62.28 ± 1.8 c	67.4 ± 6.5 c	72.7 ± 5.7 d
6	YN	40.0 ± 4.5 d	43.9 ± 2.9 bc	8.53 ± 0.94 c	48.4 ± 3.3 c	56.86 ± 3.9 d	64.3 ± 1.3 c	68.1 ± 3.8 e
7	BF	61.4 ± 2.9 b	37.2 ± 3.5 e	9.85 ± 0.79 b	49.6 ± 4.2 c	53.49 ± 4.6 d	67.3 ± 5.4 c	73.2 ± 5.1 d
8	HG	26.9 ± 4.5 e	36.1 ± 2.8 e	0.40 ± 0.05 g	56.1 ± 3.1 b	49.65 ± 3.1 e	47.8 ± 6.6 e	56.3 ± 6.9 f

[a] Data are expressed as means ± standard deviation of triplicate samples.
[b] Different lowercase letters between columns represent significant differences between samples ($p < 0.05$).
[c] TP, TF, TC and total LBP levels were expressed as mg GAE/g FW, mg RE/g FW), mg βCE/g FW and mg/g FW, respectively.
[d] Antioxidant activities of fruit extract were expressed as μM TE/g FW.

Table 3
Phenolics composition and content (μg/g FW) of different goji genotypes.[a,b]

No.	Cultivars	Flavonoid					Phenolic acid				
		Quercetin	Myricetin	Kaempferol	Rutin	Quercetin-rhamno-di-hexoside	Quercetin-3-O-rutinoside	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Vanillic acid	Chlorogenic acid
1	DM	269 ± 12.3 b	53.2 ± 8.9 f	69.3 ± 7.4 d	52.3 ± 6.8 d	1065 ± 65.3 a	522.5 ± 31.2 b	96 ± 17.5 d	189.6 ± 15.6 e	133.1 ± 15.4 c	52.2 ± 4.9 c	342.6 ± 8.1 d
2	ZG	123.6 ± 9.4 e	117.3 ± 4.9 a	59.6 ± 6.8 e	73.6 ± 5.3 a	823 ± 94.3 c	623.8 ± 51.3 a	133.5 ± 9.6 b	211.8 ± 25.8 d	98.9 ± 6.4 d	33.6 ± 3.2 e	411.6 ± 3.2 b
3	NJ1	89.6 ± 3.2 f	108.6 ± 3.2 b	25.8 ± 5.6 f	67.9 ± 8.8 b	1024 ± 23.9 a	628.9 ± 21.5 a	56.8 ± 6.4 f	176.4 ± 15.8 f	152.2 ± 9.8 b	17.8 ± 5.1 f	525.5 ± 6.5 a
4	BH	369.8 ± 44.5 a	16.89 ± 35.7 g	93.6 ± 6.7 a	43.5 ± 5.6 e	434.7 ± 6.4 f	233.2 ± 9.6 d	155.3 ± 18.8 a	309.8 ± 6.5 a	78.9 ± 6.3 f	33.6 ± 4.8 e	318.3 ± 8.7 e
5	NH	65.33 ± 9.5 f	78.9 ± 5.6 e	15.3 ± 2.8 g	55.8 ± 9.6 d	723.6 ± 56.9 d	366.4 ± 21.3 c	145.3 ± 5.2 a	252.5 ± 18.3 c	103.6 ± 5.5 d	68.4 ± 3.6 b	179.3 ± 6.3 f
6	YN	289.6 ± 14.3 b	96.3 ± 8.5 d	83.6 ± 9.4 b	43.2 ± 8.3 e	966.2 ± 69.3 a	356.6 ± 22.4 c	109.4 ± 8.5 c	273.6 ± 7.2 b	124.3 ± 6.9 c	43.2 ± 1.3 d	408.9 ± 11.8 b
7	BF	163.9 ± 4.2 d	102.8 ± 3.3 c	75.3 ± 5.5 c	61.7 ± 5.9 c	543.7 ± 66.2 e	158.9 ± 4.3 e	86.3 ± 5.4 d	193.4 ± 6.1 e	86.8 ± 3.3 e	73.5 ± 4.6 a	376.6 ± 34.3 c
8	HG	211.6 ± 11.8 c	112.6 ± 9.6 a	65.5 ± 5.7 d	76.1 ± 8.3 a	934.3 ± 87.7 b	536.9 ± 11.5 b	78.6 ± 8.7 e	283.9 ± 9.4 b	176.9 ± 13.6 a	46.9 ± 3.7 d	112.5 ± 8.4 g

[a] Data are expressed as means ± standard deviation of triplicate samples.
[b] Different lowercase letters between columns represent significant differences between samples ($p < 0.05$).

ZG (Zhongguo), YN (Yunnan) and HG (Heiguo) all contained over 800 μg/g FW quercetin-rhamno-di-hexoside. A substantial variation in quercetin-3-O-rutinoside levels was observed, although it was detected in all the genotypes, at levels ranging from 158.9 to 628.9 μg/g FW. NJ1 (Ningji No.1) had the highest and BF (Beifang) the lowest level. DM (Damaye), ZG (Zhongguo), NJ1 (Ningji No.1) and HG (Heiguo) were all relatively rich in quercetin-rhamno-di-hexoside, with levels more than 500 μg/g FW. Quercetin was the third major flavonoid, varying from 89.6 to 369.8 μg/g FW, with BH (Baihua) having the highest content, and NH (Ningxiahuangguo) with the lowest, while HG (Heiguo), YN (Yunnan), BH (Baihua) and DM (Damaye) all had more than 200 μg/g FW quercetin. Myricetin levels varied from 16.89 to 117.3 μg/g FW, with ZG (Zhongguo) having the highest value and BH (Baihua) had the lowest value. ZG (Zhongguo), NJ1 (Ningji No.1), BF (Beifang) and HG (Heiguo) had significantly higher myricetin content than the other genotypes. The levels of kaempferol ranged from 15.3 to 93.6 μg/g FW, with BH (Baihua) having the highest and NH (Ningxiahuangguo) the lowest content. DM (Damaye), BH (Baihua), YN (Yunnan), BF (Beifang) and HG (Heiguo) had significantly a higher kaempferol content than the other genotypes. Rutin was the least abundant flavonoid, with concentrations ranging from 43.2 to 76.1 μg/g FW. HG (Heiguo) had the highest rutin content. While Wang et al. (2010) previously reported that the neutral fraction quercetin-diglycoside was the predominant flavonoid in *L. barbarum* fruits, we found that quercetin-rhamno-di-hexoside was the dominant flavonoid in Chinese native *L. barbarum* fruits, as was also observed by Inbaraj et al. (2010).

Chlorogenic acid was the predominant phenolic acid in the goji berries tested, with levels ranging from 112.5 to 525.5 μg/g FW. NJ1 (Ningji No.1) had the highest chlorogenic acid content, HG (Heiguo) had the lowest, and DM (Damaye), ZG (Zhongguo), NJ1 (Ningji No.1), BH (Baihua), YN (Yunnan) and BF (Beifang) had more than 300 μg/g FW. *p*-Coumaric acid was the second most abundant phenolic acid, with levels ranging from 193.4 to 309.8 μg/g FW. BH (Baihua) had the highest levels of *p*-coumaric acid, BF (Beifang) had the lowest. BH (Baihua), NH (Ningxiahuangguo), YN (Yunnan) and HG (Heiguo) berries contained more than 250 μg/g FW *p*-coumaric acid. Ferulic acid and caffeic acid were the third most abundant phenolic acids and showed substantial variation in abundance between the goji genotypes, with ferulic acid ranging from 78.9 to 176.9 μg/g FW and caffeic acid from 56.8 to 155.3 μg/g FW, respectively. HG (Heiguo) had the highest ferulic acid content, while BH (Baihua) had the lowest. BH (Baihua) had the highest caffeic acid content and NJ1 (Ningji No.1) had the lowest. DM (Damaye), NJ1 (Ningji No.1), NH (Ningxiahuangguo) and YN (Yunnan) had significant higher ferulic acid levels than the other genotypes, and considerably higher caffeic acid levels were found in ZG (Zhongguo), BH (Baihua), HG (Ningxiahuangguo) and YN (Yunnan) compared to the other genotypes tested. Vanillic acid was a relatively minor phenolic acid in these genotypes tested, ranging from 17.8 to 68.4 μg/g FW, with NH (Ningxiahuangguo) having the highest level. Only DM (Damaye), NH (Ningxiahuangguo) and BF (Beifang) contained more than 50 μg/g FW vanillic acid. Again there is some discrepancy between these values and those reported in previous studies, since Wang et al. (2010) reported that chlorogenic acid was the dominant phenolic acid, as was observed here, while Inbaraj et al. (2010) found that dicaffeoylquinic acid and chlorogenic acid were the dominant phenolic acids.

3.4. Individual carotenoid content

Five carotenoids, including lutein, neoxanthin, β-cryptoxanthin, β-carotene and zeaxanthin were identified and quantified in the

Table 4
Carotenoid composition and content (μg/g FW) of different goji genotypes.[a,b]

No.	Genotypes	Lutein	β-Cryptoxanthin	Zeaxanthin	Neoxanthin	β-Carotene
1	DM	28.3 ± 5.7 f	499.6 ± 11.9 b	6474 ± 65.18 d	22.4 ± 4.8 e	260.9 ± 10.4 d
2	ZG	48.4 ± 0.4 d	337.4 ± 3.6 e	4791.5 ± 12.8 e	58.7 ± 0.2 d	79.8 ± 0.4 f
3	NJ1	52.7 ± 0.2 d	739.8 ± 9.6 a	9306 ± 111.2 a	15.6 ± 1.1 ef	413.9 ± 3.5 a
4	BH	39.9 ± 0.1 e	436.8 ± 9.4 c	6934 ± 82.1 c	9.6 ± 0.7 f	110.9 ± 6.3 e
5	NH	59.2 ± 2.9 c	55.3 ± 1.7 f	3076 ± 64.7 f	3.3 ± 0.1 g	233.5 ± 14.0 d
6	YN	65.5 ± 2.3 b	356.4 ± 2.7 e	4699 ± 103.8 e	156.9 ± 5.3 c	298.1 ± 7.7 c
7	BF	79.6 ± 0.2 a	388.3 ± 1.8 d	8566 ± 83.2 b	146.3 ± 3.3 b	342.9 ± 10.9 b
8	HG	10.5 ± 0.1 g	8.6 ± 0.1 g	17.01 ± 0.2 g	265.5 ± 2.6 a	18.8 ± 0.9 g

[a] Data are expressed as means ± standard deviation of triplicate samples.
[b] Different lowercase letters between columns represent significant differences between samples ($p < 0.05$).

current study (Table 4). Zeaxanthin was the predominant carotenoid, with levels ranging from 17.1 to 9306 μg/g FW in the genotypes. NJ1 (Ningji No.1) had the highest zeaxanthin content, with the exception of NH (Ningxiahuangguo), the zeaxanthin contents of other genotypes were more than 3000 μg/g FW. The concentration of the second most abundant carotenoid, β-cryptoxanthin, ranged from 8.6 to 739.8 μg/g FW and NJ1 (Ningji No.1) had the highest β-cryptoxanthin content, while HG (Heiguo) had the lowest content. Other than NH (Ningxiahuangguo) and HG (Heiguo), the β-cryptoxanthin levels in the other genotypes tested were more than 300 μg/g FW. β-Carotene was the third most abundant carotenoid, and ranged in concentration from 18.8 to 342.9 μg/g FW among the genotypes. BF (Beifang) had the highest β-carotene content, while HG (Heiguo) had the lowest content, and β-carotene was detected in DM (Damaye), NJ1 (Ningji No.1), NH (Ningxiahuangguo), YN (Yunnan) and BF (Beifang) more than 230 μg/g FW. Levels of neoxanthin varied from 3.3 to 265.5 μg/g FW and HG (Heiguo) had the highest neoxanthin content. YN (Yunnan), BF (Beifang) and HG (Heiguo) had high neoxanthin levels, with more than 140 μg/g FW. Low lutein levels were detected in all the goji genotypes, with amount ranging from 10.5 to 79.6 μg/g FW. BF (Beifang) had the highest lutein content, NJ1 (Ningji No.1), HG (Heiguo), YN (Yunnan) and BF (Beifang) contained more than 50 μg/g FW lutein.

A previous study reported that zeaxanthin and its esters were the major carotenoids in *L. barbarum* fruits. The content of zeaxanthin esters in ripening goji fruits can exceed 77.5% of TC, and zeaxanthin palmitate (phasalien) is especially abundant, comprising 31–56% of the TC (Peng et al., 2005; Weller & Breithaupt, 2003). In the present study, except for HG (Heiguo), the zeaxanthin content in other goji berry tested was over 84% of the TC. We propose that other pigment classes, such as anthocyanidins, may contribute to HG (Heiguo) color.

3.5. Antioxidant activities of fruit extracts from eight Lycium genotypes

The DPPH free radical-scavenging activities of extracts from fruits of the goji genotypes ranged from 36 to 85 μM TE/g FW. NJ1 (Ningji No.1) had the highest DPPH value, while ZG (Zhongguo) had the lowest DPPH value, and the DPPH values of DM (Damaye), NJ1 (Ningji No.1), BH (Baihua) and NH (Ningxiahuangguo) extracts were more than 60 μM TE/g FW, which was significant higher than values for other genotypes. The ABTS values ranged from 47.8 to 95.6 μM TE/g FW, NJ1 (Ningji No.1) had the highest ABTS value, while HG (Heiguo) had the lowest. DM (Damaye), NJ1 (Ningji No.1), BH (Baihua), NH (Ningxiahuangguo) and BF (Beifang) had significantly higher ABTS value than the other genotypes, with values more than 67 μM TE/g FW. The FRAP values ranged from 56.3 to 92.5 μM TE/g FW and NJ1 (Ningji No.1) had the highest value, while HG (Heiguo) had the lowest. DM (Damaye), NJ1 (Ningji No.1), BH (Baihua), NH (Ningxiahuangguo) and BF (Beifang) had FRAP values more than 73 μM TE/g FW, which was significantly higher than those of the other genotypes tested.

Table 5
Correlation coefficients between functional compounds and antioxidant activities of goji berry extracts.

	DPPH	ABTS	FRAP
TP	0.637*	0.716**	0.691*
TF	0.473*	0.313	0.338
TPA	0.667*	0.622*	0.585*
TC	0.347	0.312	0.260
Total LBP	0.943**	0.907**	0.867**

* Significant different ($P < 0.05$).
** Highly significant different ($P < 0.01$).

3.6. Correlation analysis between functional compounds and antioxidant activities

The correlations between functional compounds and antioxidant activities were shown in Table 5. TP was significantly correlated with DPPH ($p < 0.05$, $r = 0.637$), ABTS ($p < 0.01$, $r = 0.716$) and FRAP ($p < 0.05$, $r = 0.691$). While TF was not significantly correlated with ABTS and FRAP. There were significant correlations between TF and DPPH ($p < 0.05$, $r = 0.473$). It is interesting that total phenolic acid (TPA) correlated significantly with DPPH ($p < 0.05$, $r = 0.667$), ABTS ($p < 0.05$, $r = 0.622$), and FRAP ($p < 0.05$, $r = 0.585$), which suggested that TPA have important role in antioxidant activity of goji berry. No significant correlation was found between TC and any antioxidant measurement. We observed that LBP levels were significantly correlated with DPPH ($p < 0.01$, $r = 0.943$), ABTS ($p < 0.01$, 0.867) and FRAP ($p < 0.01$, $r = 0.907$). As the main FCs, phenolic compounds, carotenoids and LBPs contribute differently to the TAA of goji berries. In the present study, LBPs were the largest contributor to TAA, and the TP was the second contributor, which further provided the evidence that LBP as the main FCs in goji berries.

4. Conclusion

In conclusion, fruit of Chinese native goji genotypes generally had high levels of phenolic compounds, carotenoids and LBPs, although the abundance of these compounds varied significantly among these genotypes. Quercetin-rhamno-di-hexoside and quercetin-3-O-rutinoside were the predominant flavonoids of genotypes tested, while chlorogenic acid was the major phenolic acids and zeaxanthin was the most abundant carotenoid. Overall, berries of *L. barbarum* L. (Ningxia goji), including DM (Damaye), NJ1 (Ningji No.1), BH (Baihua) and NH (Ningxiahuangguo), not only represent a rich source of potentially FCs, but also exhibit a higher antioxidant activity than those of other *Lycium* genotypes. Thus,

Ningxia goji have considerable value for both consumption as food and nutraceutical purposes.

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