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Phytochemical profiles, nutritional constituents and antioxidant activity of black wolfberry (*Lycium ruthenicum* Murr.)

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ABSTRACT

Black wolfberry (*Lycium ruthenicum*, LR) is one of edible and medicinal fruits used widely in West China and contains many nutritional and health benefits of bioactive components. In this study, the phytochemical profiles and nutritional constituents in LR berries from seven production regions were systematically investigated and analyzed quantitatively. And the antioxidant activity of LR berry extracts was also measured by several methods, including DPPH, ABTS, FRAP and ORAC assays. Dietary fibers, soluble sugars, organic acids, minerals and vitamin C were rich in LR berries, and low variation of nutritional constituent contents were detected in these regions. Furthermore, the concentration of the functional components (such as polyphenols, phenolic acids, flavonoids and polysaccharides) varied significantly in LR berries from different regions, and there were no significant differences of these components in the wild and cultivated berries from the same region. Generally, these functional components had significantly positive correlations with antioxidant activity, with exception for total flavonoids and total carotenoids. Overall, LR berries from Qaidam Basin of Qinghai, not only represented abundant nutritional constituents and phenolics, polysaccharides and betaine, but possessed significantly higher antioxidant activity than those of the other production regions, indicating that they can conduct as excellent sources of antioxidants used in nutraceutical formulations and functional foods.

1. Introduction

Black wolfberry (*Lycium ruthenicum*, LR), is an edible and medicinal fruit tree, which widely distributes in the arid desert and saline-alkali region of Northwest China (Liu et al., 2012). Since LR berries have high concentration of nutritional and functional components such as soluble sugars, organic acids, minerals, vitamins, anthocyanins, polyphenols, flavonoids, polysaccharides and carotenoids (Liu et al., 2013; Wang et al., 2018a; Li et al., 2019; Liu et al., 2019), LR plant has been used as a traditional Chinese herb for treatment of abnormal eye disease, menopause, menstruation, and hypertension, as highlighted in the classical "Compendium of Materia Medica" and "The Four Medical Tantras". Of these constituents, anthocyanins and polysaccharides have a wide range of physiological and biological activities (Peng et al., 2014; Zhang et al., 2019; Chen et al., 2019). Therefore, the consumption of LR berry was great high recently and this plant has become a rising concern in the fields of medicines and food products (Wang et al., 2018a).

In recent years, the continuous expanding of LR planting area, especially in Qinghai and Xinjiang Province, China, has promoted the development of LR berry industry. Meanwhile, it was increasingly becoming prevalent and interested in the research of component and product development of LR fruits. The variation of secondary metabolites was high in different regions, resulting in uneven quality of LR berry (Wang et al., 2018b). Additionally, there were a few studies related with the analysis of nutritional and phytochemical composition in wild LR (Nzeuwa et al., 2017; Li et al., 2019), and they have only focused on single production region. However, systematically analysis and comparison of nutrients and functional components in LR berries located from different production areas were rarely reported, and there was no research for comparative evaluation of multiple functional components in cultivated and wild LR berries.

In this work, the determination and analysis of nutritional constituents and phytochemical profiles were reported firstly in mature LR berries from 27 localities consisting of seven big regions, including wild and cultivated fruits in Northwest China. The correlations between functional components (such as total polyphenols, flavonoids, phenolic acids, polysaccharides and carotenoids) and ecogeographic factors were also investigated, which would be beneficial for LR cultivation, wildlife

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tending and conservation. In addition, the efficiency of DPPH, DPPH, ABTS, FRAP and ORAC tests were compared to evaluate antioxidant activities and their correlations with these components in LR berry extracts. These results could be helpful for further evaluation and utilization of LR resources.

2. Materials and methods

2.1. Chemicals and reagents

Petunidin-3-*O*-rutinoside (*trans*-*p*-coumaroyl)-5-*O*-glucoside (PRG) was the main anthocyanin in the ripe LR fruits. The high purity of PRG has been prepared through the previous extraction and purification methods (Liu et al., 2020). Protocatechuic acid, caffeic acid, p-coumaric acid, vanillic acid, ferulic acid, chlorogenic acid, quercetin, myricetin, naringenin-7-*O*-glucoside, quercetin-rhamno-di-hexoside, kaempferol-3-*O*-rutinoside, rutin, gallic acid, betaine, zeaxanthin, β-carotene, neoxanthin, β-cryptoxanthin, lutein, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), glucose, fructose, sucrose, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and Folin-Ciocalteu phenol reagent were purchased from Macklin (Shanghai, China). Methanol, acetonitrile formic acid and so on, belonged to the HPLC grade, were mainly purchased from Xinlanjing Chemical Co., Ltd. (Yuxi, China). And other chemicals were purchased at the analytical grade, primarily from Solarbio (Beijing, China). The HPLC grade water was prepared from the Milli-Q system (Millipore, Billerica, MA, USA).

2.2. Plant materials

The samples of ripe black wolfberry (LR) were collected from the Northwest China, which primarily represented the geographical distribution of natural populations of species (LR) in China. There were seven big distribution areas included 27 locations (Fig. 1 and Table S1), and the LR samples contained wild and cultivated fruits. For every location, each replicate (about 1.5 kg fruits) sampled from several trees. Approximately 100 g fresh berries were used to measured and evaluated the firmness, total titratable acidity (TTA) and total soluble solid (TSS). And most of the fruit samples were dried at a constant temperature of 45℃ for 72 h and then ground and passed through a 45 mesh sieve. The dry sample powders were stored at -18℃ for later analysis, and the results were expressed as dry weight basis (DW). For every collection, three replicates were carried out in this research.

2.3. Measurement of fruit quality (firmness, TTA and TSS)

Fruit firmness, total titratable acidity (TTA) and total soluble solid (TSS) were determined and analyzed using the previous method (Khan et al., 2017). The fruit firmness was determined by a digital GY-2 penetrometer with a 2-mm diameter tip (Top Cloud-agri Technology Co. Ltd., Zhejiang, China), and the values were expressed as N. TSS of selected LR fruits juice were measured by ZG-WYA2S Abbe refractometer (Zhuoguang Instrument Technology Co. Ltd., Shanghai, China), and the results were calculated using %. The values of TAA were measured by titrating 15 g of a homogenized sample of LR fruits juice with 0.1 M NaOH solution at a pH of 8.20, and the result was expressed as g/L. Then, the average values were calculated for each parameter, respectively.

2.4. Nutritional assessment of LR berries

2.4.1. Proximate composition

The dry powdered samples of LR berries were measured and analyzed for proteins, fat, ash, dietary fiber, amino acid and carbohydrates through the Association of Official Analytical Chemists (AOAC) procedures (AOAC, 2016). The detailed methods for determination referred to AOAC 984.13 (crude protein), AOAC 920.39 (crude fat), AOAC 935.42 (ash), AOAC 985.29 (total dietary fiber) and AOAC 994.12 (amino acid), respectively. The total carbohydrate content was calculated by the percentage difference, following the equation: total carbohydrates (g/100 g) = 100 − (% crude protein + % ash + % crude fat + % total dietary fiber). And total energy was calculated by the following formula: Energy (kcal/ 100 g) = 9 × (% crude fat) + 4 × (% crude proteins + % total carbohydrates) + 2 × (% total dietary fiber).

2.4.2. Soluble sugars and organic acids

The contents of soluble sugars and organic acids in dry and powdered LR fruits were measured by the previously described method (Zhang et al., 2015). An Agilent 1200 HPLC (high performance liquid chromatography) system (Agilent Technology, USA) coupled to evaporative light-scattering detector (ELSD) was used to identify and quantify glucose, fructose and sucrose in each fruit sample using an Agilent ZORBAX Carbohydrate column (250 mm × 4.6 mm, 5 μm i.d.). Organic acids were analyzed using the HPLC system, and fitted with a diode array detector and an Agilent ZORBAX Eclipse XDB-C18 column (250 mm × 4.6 mm, 5 μm i.d.). The detection wavelength was selected at 214 nm. Organic acids and soluble sugars were identified according to their retention time by comparison with standards, respectively.

2.4.3. Fatty acids

A lipidic fraction was obtained using Soxhlet extraction by the AOAC (940.28) method, and a trans-esterification process was carried out by BF_3-methanol. Fatty acids were determined and analyzed by an Agilent 7890A gas chromatograph coupled with a dual flame ionization detector and an Agilent HP-88 column (60 m × 0.25 mm, 0.25 μm F.T.). Peak identification was carried out through comparation of their chromatographic retention time with those of authentic standards C4 ~ C24. Triplicated tests were applied in the experiments, and the data was expressed as the relative percentage of each fatty acid.

2.4.4. Mineral composition

Mineral preparation was based on diluting the ash obtained previously as described method, with HCl 17.5 % (p/v) and HNO_3 30 % (p/v) mixing 1:1 solution (2 mL), and completing to volume (50 mL) with distilled water (Guil-Guerrero and Rebolloso-Fuentes, 2009). The determination and analysis of minerals was carried out by an ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer), model iCAP 7400 (Thermo Fisher Scientific, Waltham USA) equipped with a double pass nebulization camera and a spray nebulizer.

2.4.5. Vitamins

The 2,4-dinitrophenylhydrazine method was carried out for determination of vitamin C, which was in conjunction with spectrophotometric measurement (Majidi and Qubury, 2016). The determination methods of vitamin B and E were carried out according to Xie et al. (2010) and Psomiadou and Tsimidou (1998) with some modifications, respectively. Vitamin B and tocopherols (vitamin E) were determined and analyzed using the HPLC system, and fitted with Waters 1525 HPLC and a Dikma Spursil C18-EP (250 mm × 4.6 mm, 5 μm i.d.), and Agilent 1260 HPLC and an Agilent Eclipse XDB-C18 (250 mm × 4.6 mm, 5 μm i.d.), respectively. The vitamins were identified and analyzed through their retention time by comparison with standards, respectively.

2.5. Determination of the contents of total phenolic, flavonoid, carotenoid and LRP

Total phenolic (TP) and total flavonoids (TF) were extracted by ultrasonic extraction method. An ultrasonic extractor (JP Ultrasonic 180ST, Skymen Cleaning Equipment Co., Ltd., Shenzhen, China) were applied for extraction of powdered LR berries (5.0 g) with 100 mL 85 %

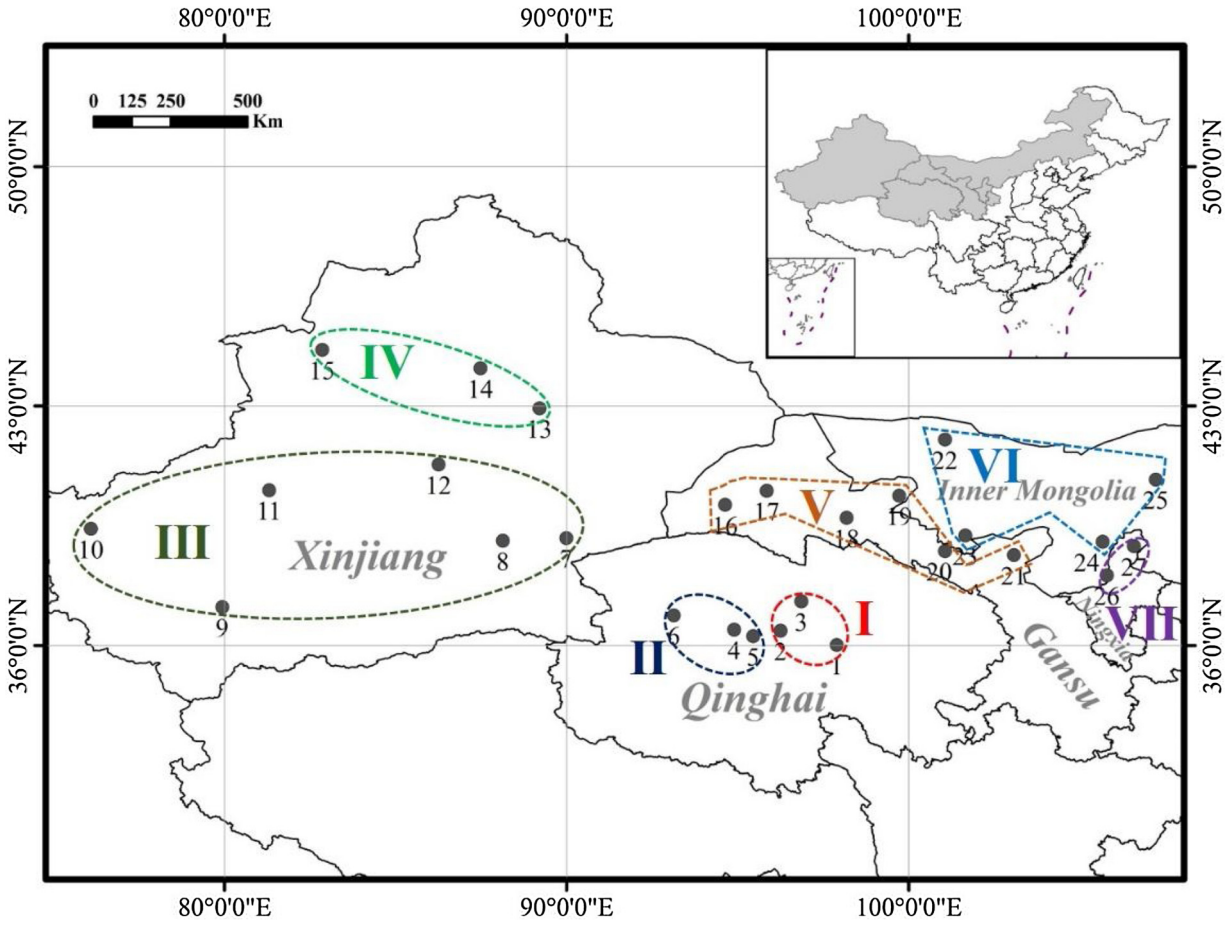

Fig. 1. Geographical locations of LR berry samples in Northwest China. For names of the numbered positions and ecogeographic information see list in Table S1.

ethanol (v/v) for 0.5 h. And 300 W and 40℃ were selected as the extraction power and temperature, respectively. The determinations of TP and TF were carried out by the Folin-Ciocalteu method and aluminum chloride colorimetric method (Zhang et al., 2016), respectively. They were expressed as mg/g DW of gallic acid equivalents (GAE) and mg/g DW of rutin equivalents (RE), respectively.

The determination and analysis of total carotenoid (TC) was carried out the previous method with some modifications (Lichtenthaler and Wellburn, 1983). The powdered LR berries (5.0 g) was homogenized with 100 mL of petroleum ether:acetone (1:0.5, v/v), and the residues were extracted repeatedly several times. After the petroleum ether layer filtration through sodium sulfate, the total volume of filtrates was made up to 100 mL using petroleum ether. The detection wavelength was 451 nm and 2500 was the extinction coefficient. The value of TC content was expressed as mg/g DW of β-carotene equivalents (βCE).

The preparation and decoloration of total *L. ruthenicum* polysaccharides (LRP) were carried out according to our previous method described by Liu et al. (2013). The content of total LRP was determined and analyzed by phenol-sulfuric acid method using $_D$-glucose as a standard. The results were expressed as mg/g DW.

2.6. Analysis of betaine, phenolic compounds and carotenoids composition

The betaine was determined and analyzed by HPLC method previously described by Lee et al. (2011) with slight modifications. The HPLC system was consisted of Waters 1525 HPLC and a Dikma Hilic column (250 mm × 4.6 mm, 5 μm i.d.), and the betaine was identified and analyzed through the retention time by comparison with standards.

The preparation and extraction of phenolic compounds were carried out by ultrasonic method. The powdered LR berries (2.0 g) was extracted in an ultrasonic extractor using 30 mL 85 % ethanol (v/v) for 30 min. And 300 W and 40℃ were selected as the working power and temperature, respectively. The residues were extracted repeatedly three times. Supernatants, collecting from all the extractions, were diluted to 100 mL using ethanol. After filtration with a 0.22 μm organic membrane, the solution (10 μL) was determined and analyzed through the SPD-M20A HPLC system equipped with a LC-20AD HPLC pump, a SIL-20AC autosampler, a CTO-20AC thermostated column compartment and a SPD-20AV photodiode array detector. The ZORABX SB-C18 column (250 mm × 4.6 mm, 5 μm i.d.) was used and the detection wavelengths (330 and 360 nm) were set to measure the contents of phenolic acids and flavonoids, respectively.

The carotenoid compounds were extracted and analyzed by the previous method (Zhang et al., 2016). HPLC analysis was carried out by the SPD-M20A system (Shimadzu, Japan) consisting of a LC-20AD HPLC pump and a SPD-20AV photodiode array detector, equipped with a YMC (Schermbeck, Germany) C30 analytical column (250 mm × 4.6 mm, 5 μm i.d.) including a C30 guard column (20 mm × 4.6 mm, 5 μm i.d.). The wavelength was set at 450 nm to detect carotenoid compounds which were quantified through their respective retention time by comparison with standards. The data was expressed in mg/g DW.

2.7. Evaluation of antioxidant capacity

The antioxidant activity of LR extracts was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP) and oxygen radical absorption capacity (ORAC) assays. The extract samples were obtained using ultrasonic extraction method, which were

consistent with extracts for analysis of phenolics. The procedures of these four assays were carried out by the method of Thaipong et al. (2006) with some modifications. For DPPH assay, the previously diluted extracts (150 μL) were allowed to react with DPPH solution (0.5 mM, 2850 μL) for 24 h in the dark. Then, the detection wavelength was selected as 515 nm. For ABTS assay, fresh ABTS solution was prepared for each test. The diluted extracts (150 μL) were allowed to react with ABTS solution (7.0 mM, 2850 μL) for 2 h in the dark condition. Then, 734 nm was selected as the detection wavelength using the spectrophotometer. The standard curves of assays were linear between 25 and 600 (ABTS) or 800 (DPPH) μM Trolox.

For FRAP assay, the fresh FRAP solution was prepared by mixing 25 mL acetate buffer (300 mM), 2.5 mL TPTZ solution (2,4,6-tripyridyl-s-triazine, 10 mM), and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution (20 mM) and then warmed at 37°C before using. The diluted extracts (150 μL) were allowed to react with 2850 μL of the FRAP solution for 0.5 h in the dark condition. Then the absorbance of the colored product was detected at 593 nm. The concentration range of Trolox (25 − 800 μM) was used to make the standard curve. If the FRAP (or DPPH, ABTS) value tested was over the linear range of the standard curve, additional dilution was needed to be done. For ORAC assay, phosphate buffer prepared at 37 °C (pH 7.4) was applied for analysis. And the peroxyl radicals were produced by 2,2′-azobis (2-amidino-propane) dihydrochloride, and this reagent should be prepared fresh for each test. The substrate was fluorescein, and the excitation at 485 nm and emission at 520 nm were set as fluorescence conditions. The linear range of Trolox (0 − 50 μM) was used to make the standard curve. All the values of above-mentioned assays were expressed in μM TE/g DW.

2.8. Statistical analysis

All the tests were performed in triplicate, and the data were presented as mean ± standard deviation (SD). Additionally, all the component content and antioxidant value were expressed as dry weight basis (DW). Statistical analysis was carried out using SPSS 16.0 (IBM, New York, USA) and GraphPad Prism 5.1 software (San Diego, California, USA). The evaluation of statistical data comparisons was carried out by one-way ANOVA (analysis of variance) test, and the determination of the relationship between experimental variables was performed accurately by Pearson's rank correlation coefficients. The two-tailed tests were used for estimation of the differences, which were significantly highlighted while $p < 0.05$.

3. Results and discussion

3.1. Assessment of LR berry quality

The qualities of LR fresh berries, including firmness, TAA and TSS, were measured and shown in Table 1. The highest firmness was located at WIM region, while EQBQ had the lowest firmness. All the LR berries firmness ranged from 4.9 to 5.6 N, with 5.2 N as an average value. There were no significances of firmness among the different production regions.

Table 1
Determination of fresh LR berries quality.

No.	Locality	Code	Firmness (N)	TTA[a] (g/L)	TSS[b] (%)
I	East Qaidam Basin of Qinghai	EQBQ	4.9 ± 0.1	2.1 ± 0.2	14.3 ± 0.3
II	West Qaidam Basin of Qinghai	WQBQ	5.0 ± 0.2	1.8 ± 0.1	14.5 ± 0.5
III	South of Xinjiang	SXJ	5.5 ± 0.3	1.6 ± 0.1	13.7 ± 0.3
IV	North of Xinjiang	NXJ	5.3 ± 0.3	1.9 ± 0.2	14.6 ± 0.2
V	Hexi Corridor of Gansu	HCG	5.1 ± 0.1	1.8 ± 0.3	13.5 ± 0.2
VI	West of Inner Mongolia	WIM	5.6 ± 0.2	1.6 ± 0.2	11.6 ± 0.4
VII	Yellow River basin of Ningxia	YRBN	5.3 ± 0.1	1.5 ± 0.1	12.5 ± 0.1

[a] TTA, total titratable acidity.
[b] TSS, total soluble solid.

The TAA range of all fruits was 1.5–2.1 %, and 1.8 % was the average value. The most acidic of the fruit juice was from EQBQ, while YRBN had the least acidic. The TTA values of SXJ, WIM and YRBN were characterized by low TTA (< 1.8 %), which indicated that the LR fruits located these regions had good taste than other production regions.

The TSS of the ripe fruits was presented in terms of °Brix. The value of TSS was highest in NXJ with a °Brix of 14.6 %, followed by the WQBQ (14.5 %) and EQBQ (14.3 %). And the TSS value of WIM was 11.6 %, which was significantly lower than above-mentioned regions. TSS contains sugars, acids, vitamins, minerals and secondary metabolites, which is the sum of these substances in berries. WIM and YRBN had low TSS levels, suggesting the fruits from these regions had lower metabolites than other production regions.

3.2. Nutrient composition

The Table 2 presented detailedly the proximate composition and energetic value of wild LR berries from different production regions. The most abundant macronutrient in LR berries was carbohydrates, with 66.2 % as an average value. Dietary fiber was the second major macronutrient (13.5 %, average), followed by proteins, ash and fat. However, the nutritional value and energy contribution of all fruits had no significant differences among the different regions. This result might be explained by the different ecogeographic factors of LR distributions that can rarely cause variable nutritional concentrations.

Soluble sugars, organic acids and amino acids of the wild LR berries were also exhibited in Table 2. Glucose, fructose and sucrose were the main soluble sugars measured in fruits. The result showed that glucose was the most abundant soluble sugar, followed by fructose and sucrose. The contents of glucose and fructose were high in LR fruits from Qaidam Basin of Qinghai (WQBQ and EQBQ) and YRBN, while SXJ had lowest content valued 32.78 and 5.24 g/100 g DW, respectively. Sucrose content was relatively low, and insignificant differences were detected in all the regions. Six organic acids were determined (Table 2), and malic acid and citric acid were the abundant organic acids in the LR fruits, followed by quinic acid and succinic acid. Oxalic acid and fumaric acid were the lowest. There were some variations of individual organic acid in LR fruits located at different regions, and the content of each organic acid was basically higher in LR fruits from Qaidam Basin of Qinghai than other regions. However, the sum of the organic acids had no statistically significant differences in all berries samples.

Regarding amino acid (Tables 2 and S2), the content in fruits displayed very different situations. Twenty amino acids were identified, glutamate, aspartate and arginine were the main nonessential amino acids (NEAA), while phenylalanine and tryptophan were the main essential amino acids (EAA) in fruits. NEAA content ranged from 4.21 (YRBN) to 6.56 g/100 g DW (WIM), with 5.22 g/100 g DW as an average value. And EAA content ranged from 1.67 (YRBN) to 3.19 g/100 g DW (EQBQ), with 2.48 g/100 g DW as an average value. There were statistically significant differences in the content of total amino acids between WIM (or EQBQ) and YRBN.

The determination and analysis of fatty acids in LR berries were presented in Tables 3 and S3. Thirteen fatty acids were captured and identified in the berries, and no significant difference was found in each fatty acid between berry samples from different regions. PUFA (Polyunsaturated fatty acids) were the major group, which was predominantly due to the presence of linoleic acid (C18:2) with an average value of 53.98 %, followed by oleic acid (C18:1, 21.12 %) and palmitic acid (C16:0, 12.33 %). Similar data were reported by Chi et al. (2016) in LR berries collected from Qinghai-Tibet Plateau, which presented linoleic acid (54.11 %) and oleic acid (20.70 %) as the main fatty acids.

As shown in Table 3, three kinds of vitamins were identified, being vitamin C predominant with 13.69 mg/100 g DW as an average value,

Table 2
Proximate composition, soluble sugars, organic acids and amino acids in wild LR berries.

	LR berries located different regions						
	EQBQ	WQBQ	SXJ	NXJ	HCG	WIM	YRBN
Nutritional value (g/100 g DW)							
Proteins	11.5 ± 0.3	11.0 ± 0.2	9.7 ± 0.2	9.9 ± 0.3	9.1 ± 0.2	13.2 ± 0.5	9.2 ± 0.1
Fat	3.0 ± 0.1	3.3 ± 0.1	3.0 ± 0.2	3.3 ± 0.1	3.9 ± 0.2	4.1 ± 0.4	3.5 ± 0.1
Ash	6.3 ± 0.1	5.6 ± 0.1	6.7 ± 0.2	5.9 ± 0.1	6.4 ± 0.21	7.3 ± 0.3	6.6 ± 0.1
Dietary fiber	12.1 ± 0.1	14.2 ± 0.3	13.2 ± 0.1	13.8 ± 0.2	14.2 ± 0.3	13.8 ± 0.2	13.1 ± 0.1
Total carbohydrates	67.0 ± 1.2	66.0 ± 1.7	67.3 ± 2.5	67.2 ± 0.8	66.5 ± 3.0	61.6 ± 4.2	67.5 ± 3.3
Energy contribution (kcal/100 g)	366 ± 2	366 ± 2	361 ± 3	366 ± 1	365 ± 2	363 ± 3	365 ± 2
Soluble sugars (g/100 g DW)							
Glucose	36.50 ± 0.85	36.03 ± 0.70	32.78 ± 1.24	34.49 ± 0.63	35.57 ± 1.40	33.85 ± 1.35	36.15 ± 0.66
Fructose	7.62 ± 0.26	7.72 ± 0.25	5.24 ± 0.31	5.98 ± 0.20	5.24 ± 0.42	6.12 ± 0.17	7.03 ± 0.22
Sucrose	0.58 ± 0.03	0.64 ± 0.02	0.47 ± 0.05	0.46 ± 0.01	0.51 ± 0.05	0.38 ± 0.02	0.50 ± 0.01
Sum	44.70 ± 1.13	44.39 ± 0.97	38.49 ± 1.59	40.93 ± 0.84	41.32 ± 1.88	40.35 ± 1.55	43.68 ± 0.89
Organic acids (g/100 g DW)							
Oxalic acid	0.016 ± 0.002	0.014 ± 0.001	0.011 ± 0.001	0.019 ± 0.002	0.017 ± 0.002	0.012 ± 0.001	0.011 ± 0.001
Fumaric acid	0.032 ± 0.002	0.030 ± 0.002	0.018 ± 0.003	0.021 ± 0.001	0.023 ± 0.003	0.015 ± 0.002	0.020 ± 0.001
Succinic acid	0.28 ± 0.03	0.24 ± 0.01	0.23 ± 0.04	0.14 ± 0.01	0.17 ± 0.03	0.21 ± 0.02	0.17 ± 0.01
Malic acid	1.84 ± 0.05	1.87 ± 0.04	1.62 ± 0.09	1.70 ± 0.02	1.59 ± 0.12	1.52 ± 0.05	1.66 ± 0.03
Citric acid	1.77 ± 0.03	1.80 ± 0.04	1.34 ± 0.06	1.67 ± 0.03	1.63 ± 0.09	1.42 ± 0.02	1.75 ± 0.04
Quinic acid	0.75 ± 0.01	0.67 ± 0.01	0.46 ± 0.05	0.64 ± 0.02	0.60 ± 0.07	0.49 ± 0.04	0.57 ± 0.01
Sum	4.69 ± 0.13	4.63 ± 0.10	3.68 ± 0.22	4.19 ± 0.09	4.03 ± 0.30	3.67 ± 0.13	4.18 ± 0.10
Amino acids (AA, g/100 g DW)							
Essential AA	3.19 ± 0.38	1.96 ± 0.24	2.76 ± 0.60	2.61 ± 0.21	2.08 ± 0.49	3.09 ± 0.25	1.67 ± 0.14
Nonessential AA	6.29 ± 1.28	4.63 ± 1.14	5.05 ± 1.61	4.92 ± 0.64	4.91 ± 1.45	6.56 ± 1.47	4.21 ± 0.57
Sum	9.48 ± 1.65	6.59 ± 1.38	7.81 ± 2.20	7.53 ± 0.85	6.99 ± 1.95	9.65 ± 1.72	5.88 ± 0.72

followed by vitamin B and tocopherol or vitamin E. Vitamin C concentration ranged from 12.93 (NXJ) to 14.92 mg/100 g DW (WQBQ), and the total vitamin B content ranged from 2.29 (NXJ) to 7.82 mg/100 g DW (WQBQ), with 4.00 mg/100 g DW as an average value. Vitamin B12 belonged to the predominant vitamin B in the fruits, followed by vitamin B2 and B1. The content of vitamin B12 was highest in WQBQ (5.88 mg/100 g DW), and WIM had the lowest content (1.22 mg/100 g DW). Regarding tocopherols, fruits presented very different profiles. The results showed that α-tocopherol was the predominant tocopherol in all LR berry samples. The region from WIM had

Table 3
Fatty acids, carotenoids, vitamins and minerals in wild LR berries.

	EQBQ	WQBQ	SXJ	NXJ	HCG	WIM	YRBN
Fatty acids (relative percentage, %)							
SFA[a]	18.99 ± 0.48	18.49 ± 0.43	19.12 ± 0.70	18.64 ± 0.28	19.26 ± 0.45	18.71 ± 0.35	18.93 ± 0.21
MUFA[b]	21.38 ± 0.60	22.04 ± 0.53	21.21 ± 0.73	22.34 ± 0.35	21.92 ± 0.73	21.72 ± 0.30	22.28 ± 0.24
PUFA[c]	59.63 ± 1.00	59.47 ± 1.15	59.67 ± 1.40	59.02 ± 0.83	58.82 ± 1.62	59.57 ± 0.85	58.79 ± 0.66
Carotenoids (μg/g DW)							
Lutein	52.4 ± 2.3	48.7 ± 2.6	44.5 ± 4.7	50.5 ± 1.8	55.6 ± 4.4	60.5 ± 2.7	60.2 ± 1.5
β-Cryptoxanthin	36.7 ± 1.5	32.1 ± 1.0	35.7 ± 3.2	46.3 ± 2.7	38.4 ± 3.0	47.7 ± 1.2	41.3 ± 0.6
Zeaxanthin	80.4 ± 1.2	78.6 ± 2.5	75.8 ± 5.3	79.2 ± 2.9	81.3 ± 4.8	86.5 ± 3.3	87.4 ± 2.7
Neoxanthin	1239.8 ± 49.5	1242.3 ± 27.2	1251.0 ± 89.7	1367.3 ± 57.8	1344.6 ± 91.8	1527.0 ± 57.1	1437.5 ± 32.0
β-carotene	110.0 ± 3.8	97.6 ± 3.5	98.4 ± 8.6	105.5 ± 2.4	109.7 ± 7.4	112.6 ± 5.0	113.7 ± 6.9
Vitamin (mg/100 g DW)							
α-Tocopherol	0.95 ± 0.07	1.25 ± 0.14	0.89 ± 0.10	0.52 ± 0.08	0.74 ± 0.15	1.56 ± 0.18	0.63 ± 0.03
γ-Tocopherol	0.38 ± 0.05	0.36 ± 0.06	0.15 ± 0.05	0.13 ± 0.01	0.18 ± 0.06	0.42 ± 0.05	0.12 ± 0.01
δ-Tocopherol	0.02 ± 0.00	0.04 ± 0.01	ND[d]	ND	0.02 ± 0.00	ND	ND
Vitamin C	14.36 ± 0.72	14.92 ± 0.65	14.05 ± 1.50	12.93 ± 0.49	13.37 ± 1.28	13.02 ± 0.79	13.20 ± 0.42
Vitamin B1	0.24 ± 0.01	0.19 ± 0.01	0.15 ± 0.02	0.14 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	0.14 ± 0.01
Vitamin B2	1.55 ± 0.04	1.75 ± 0.07	0.58 ± 0.05	0.71 ± 0.01	0.72 ± 0.04	1.06 ± 0.03	1.17 ± 0.05
Vitamin B12	3.92 ± 0.37	5.88 ± 0.49	2.75 ± 0.38	1.44 ± 0.03	1.64 ± 0.22	1.22 ± 0.20	2.59 ± 0.08
Macroelements (mg/g DW)							
K	15.6 ± 1.0	12.3 ± 1.4	17.6 ± 2.6	17.1 ± 0.8	16.6 ± 2.9	17.1 ± 1.5	14.8 ± 0.5
Na	3.4 ± 0.4	3.7 ± 0.6	3.4 ± 0.7	2.6 ± 0.2	3.3 ± 0.7	3.2 ± 0.5	2.9 ± 0.1
Ca	1.5 ± 0.1	1.0 ± 0.1	1.5 ± 0.3	1.6 ± 0.1	1.2 ± 0.2	1.3 ± 0.1	1.0 ± 0.1
Mg	1.5 ± 0.1	1.3 ± 0.1	1.4 ± 0.2	1.3 ± 0.1	1.2 ± 0.2	1.7 ± 0.2	1.0 ± 0.1
Microelements (μg/g DW)							
Zn	11.5 ± 0.6	9.9 ± 0.5	11.3 ± 1.2	14.9 ± 0.8	14.6 ± 1.9	23.6 ± 1.5	10.8 ± 0.6
Fe	103.1 ± 7.0	116.5 ± 8.3	24.3 ± 2.4	10.0 ± 1.0	29.6 ± 3.3	18.7 ± 1.6	13.7 ± 0.7
Sr	10.5 ± 0.9	25.7 ± 2.1	16.4 ± 1.8	15.0 ± 0.7	13.8 ± 2.0	15.4 ± 1.3	11.3 ± 0.5
Mn	8.2 ± 0.4	9.1 ± 0.7	8.9 ± 1.1	8.0 ± 0.2	7.3 ± 0.9	8.3 ± 0.6	6.8 ± 0.1
Cu	6.4 ± 0.2	3.8 ± 0.1	6.4 ± 0.5	8.6 ± 0.1	7.5 ± 0.6	11.5 ± 0.2	6.3 ± 0.1

[a] SFA, saturated fatty acids.
[b] MUFA, monounsaturated fatty acids.
[c] PUFA, polyunsaturated fatty acids.
[d] ND, not detected.

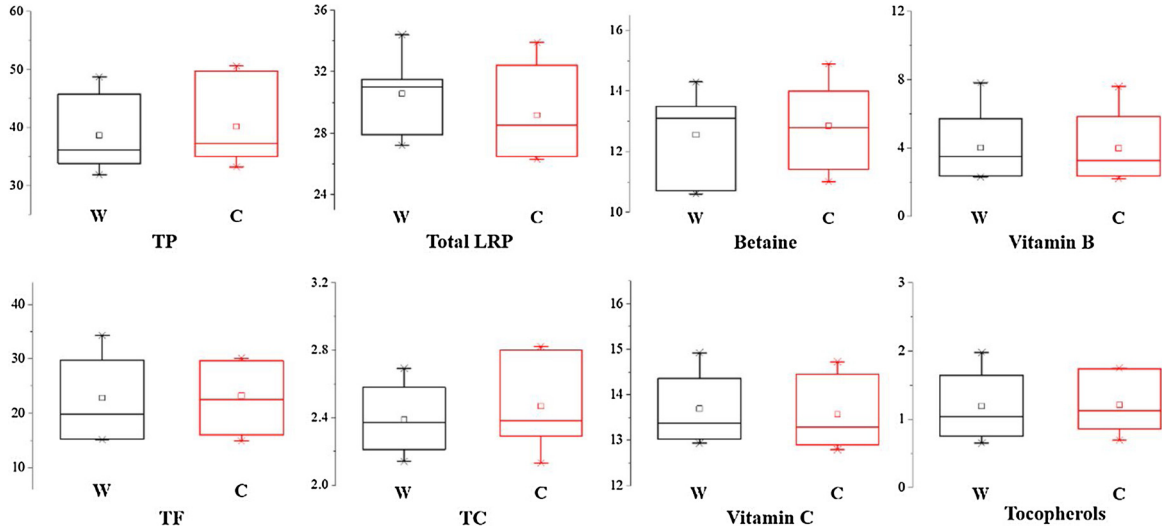

Fig. 2. Range and distribution of total phenolic (TP, mg GAE/g), total flavonoid (TF, mg RE/g), total LR polysaccharide (total LRP, mg/g), total carotenoid (TC, mg βCE/g), betaine (mg/g), vitamin C (mg/100 g), vitamin B (the sum of vitamin B1, B2 and B12, mg/100 g) and tocopherols (the sum of α-, γ-, δ-tocopherol, mg/100 g) in wild and cultivated berries of LR. The horizontal lines and the small boxes in the interior of the box are the median values and mean values, respectively. Approximately 99 % of the data fall inside the whiskers. The data outside these whiskers are indicated by crosses. W, wild fruits; C, cultivated fruits; the contents of these functional components were expressed as dry weight basis (DW).

the highest content of tocopherols (1.98 mg/100 g DW), consisting of α-tocopherol (1.56 mg/100 g DW) and γ-tocopherol (0.42 mg/100 g DW), and NXJ had the lowest tocopherols contents (0.65 mg/100 g DW). The tocopherol contents in NXJ, YRBN and HCG were below 1.00 mg/100 g DW, which were significant lower in the production regions from WIM and Qaidam Basin of Qinghai (WQBQ and EQBQ).

Minerals, including macroelements and microelements, were measured and analyzed in berries from different regions (Table 3). The results showed that four macroelements, K, Na, Ca and Mg, were rich in LR fruits, being K the most abundant one with 15.9 mg/g DW as an average value, followed by Na, Mg and Ca. There was statistically significant difference between microelements determined in different regions. The fruits in Qaidam Basin of Qinghai (WQBQ and EQBQ) contained much higher contents of Fe compared to other regions, while NXJ had the lowest content (10.0 μg/g DW). Notably, approximately 12-fold variation in Fe content was detected among the LR berry samples. Moreover, the fruits from WQBQ had high content of Sr and Mn, valued 25.7 and 9.1 μg/g DW, respectively, and the lowest contents of Cu and Zn were also found in WQBQ. Investigating some fruit berries, Pires et al. (2015) reported macroelements (K and Mg) and microelements (Fe, Sr and Zn) in grapes, mango pulp, strawberries and Barbados cherries, at contents lower than those studied here for LR ripe berries. Therefore, LR berries are excellent sources of minerals, since values were similar to or higher than those of berries consumed around the world.

3.3. Individual carotenoid profiles

Five carotenoids were identified and quantified systematically in this work (Table 3), including lutein, β-cryptoxanthin, zeaxanthin, neoxanthin and β-carotene. Neoxanthin was the predominant carotenoid, and it ranged from 1239.8 (EQBQ) to 1527.0 μg/g DW (WIM) in the different regions, with 1344.2 μg/g DW as an average value. And the second most abundant carotenoid was β-carotene, which ranged from 97.6 (WQBQ) to 113.7 μg/g DW (YRBN), and the average value was 106.8 μg/g DW. Zeaxanthin, as the third most abundant carotenoid, ranged from 75.8 (SXJ) to 87.4 μg/g DW (YRBN) in the berry samples, with 81.3 μg/g DW as an average value. Lutein and β-cryptoxanthin possessed low levels in all samples, with amount ranging from 44.5 (SXJ) to 60.5 μg/g DW (WIM) and 32.1 (WQBQ) to 47.7 μg/g DW (WIM), respectively.

As a summary of the sum of the carotenoid compounds, the contents of the carotenoids were generally higher in WIM and YRBN than in Qaidam Basin of Qinghai (WQBQ and EQBQ) and SXJ. However, statistically insignificant differences were detected in the total carotenoid compounds among LR berry samples from different regions. It was reported that zeaxanthin, β-cryptoxanthin and β-carotene were the main carotenoids in the ripe berries of *L. barbarum*, especially zeaxanthin and its esters as the predominant carotenoids (Peng et al., 2005; Zhang et al., 2016). These three carotenoid compounds were much lower in *L. ruthenicum* than *L. barbarum* fruits, and it was speculated basically that other varieties of pigments, such as anthocyanins and anthocyanidins, could contribute to color of ripe LR berries.

3.4. Analysis of functional components (LRP, TP, TF, TC, betaine and vitamins)

For all the wild fruits from different regions, the content of total LRP ranged from 27.2 (WIM) to 34.4 mg/g DW (WQBQ). Two regions contents, WIM and YRBN, were below the average content (30.6 mg/g DW), and Qaidam Basin of Qinghai (WQBQ and EQBQ) had significantly higher LRP content than these two regions. The TP content ranged from 31.9 (YRBN) to 48.7 mg GAE/g DW (EQBQ), with 38.7 mg GAE/g DW as an average value. Four regions, YRBN, HCG, SXJ and WIM, being below the average content, had significant lower TP content than the regions of Qaidam Basin of Qinghai. For the TF analysis, the highest content (34.3 mg RE/g DW) was from WIM, followed closely by WQBQ and EQBQ, and both YRBN and HCG had the lowest content (15.2 mg RE/g DW). Regarding TC, two regions, EQBQ and HCG, presented the average content (2.4 mg βCE/g DW). The TC content ranged from 2.1 (YRBN) to 2.7 mg βCE/g DW (WIM), and no significant differences were detected in all the regions. Betaine, one of the important alkaloids in LR fruits, was also determined and analyzed. The betaine content ranged from 10.6 (WQBQ) to 14.3 mg/g DW (NXJ). For the regions of Qaidam Basin of Qinghai (WQBQ and EQBQ), the betaine values were below the average content (12.6 mg/g DW), and the statistically significant differences of betaine contents were found between these regions and NXJ.

Comparison of the main functional components between cultivated and wild LR berries was also investigated (Fig. 2), and the differences in

the range and distribution were clearly visible. For the analysis of cultivated berries, the contents of TP, total LRP and TC were 33.2–50.6 mg GAE/g DW, 26.3–33.9 mg/g DW and 2.1–2.8 mg βCE/g DW, respectively, which had slightly greater variation in these three components contents than wild berries. The average values were similar in cultivated and wild fruits, and their differences were not statistically significant ($p > 0.05$). Moreover, the higher variation of TF content was detected in wild berries than the cultivated berries, but the average content of TF was slightly lower in wild samples (22.8 mg RE/g DW) than in cultivated berries (23.2 mg RE/g DW). The content of betaine in cultivated fruits was 11.0–14.9 mg/g DW, which had slightly higher variation than wild berry samples. Their averages of betaine were 12.9 and 12.6 mg/g DW, respectively, and no significance of average was found between cultivated and wild fruits.

The ripe LR berries contain much vitamins, especially vitamin C, vitamin B and tocopherols. The content of vitamin C ranged from 12.79 to 14.72 mg/100 g DW in cultivated fruits, with slightly higher variation than in wild fruits, and the average of vitamin C content had no significantly difference in the cultivated and wild berries. Notably, about 3.6-fold variation in total vitamin B content and 3.1-fold variation in content of tocopherols were found among all the fruits examined. The cultivated fruits had lower variation in both total vitamin B and tocopherols content than the wild fruits. However, both of tocopherol and vitamin B average levels were very close to, or even the same in cultivated and wild fruits, with values of 1.19 and 1.21 mg/100 g DW, and 4.00 and 3.99 mg/100 g DW, respectively.

3.5. Individual phenolic profiles and chemometric analysis

In this work, 12 phenolic compounds were systematically determined and analyzed in a collection of ripe LR berries from different production areas (Fig. 3 and Table S4), including six phenolic acids (caffeic acid, protocatechuic acid, vanillic acid, p-coumaric acid, ferulic acid and chlorogenic acid) and six flavonoids (quercetin, myricetin, naringenin-7-*O*-glucoside, rutin, kaempferol-3-*O*-rutinoside and quercetin-rhamno-di- hexoside). For the analysis of phenolic acids in the wild LR fruits, protocatechuic acid and p-coumaric acid were the major components, with 1.94 and 1.16 mg/g DW as average values, respectively, and their contents were significantly higher than in other *Lycium* fruits (Zhang et al., 2016). Protocatechuic acid content ranged from 1.40 to 2.35 mg/g DW, and WQBQ possessed the highest content, followed closely by EQBQ, and WIM had the lowest content. The content of p-coumaric acid ranged from 0.79 (WIM) to 1.73 mg/g DW (EQBQ), and three regions, WIM, HCG and YRBN, were below average value (1.16 mg/g DW). The contents of other four phenolic (caffeic acid, ferulic acid, chlorogenic acid and vanillic acid) differed slightly in different production regions, and their average values were 0.74, 0.53, 0.46 and 0.18 mg/g DW, respectively. Generally, the fruits located at Qaidam Basin of Qinghai (WQBQ and EQBQ) had higher content of total phenolic acids (TPA) than other production areas, and WIM had the lowest content.

Among the flavonoids in the wild fruits, the predominant flavonoid compounds were *naringenin-7-O-glucoside* and quercetin-rhamno-di-hexoside, which ranged from 4.74 to 6.23 mg/g DW and 4.30–5.86 mg/g DW, respectively. And their average contents were 5.36 and 5.13 mg/g DW, respectively, which were significantly higher than other flavonoids values. Moreover, there were statistically significant differences of these two flavonoids contents between HCG and Qaidam Basin of Qinghai (WQBQ and EQBQ). The contents of the second most abundant flavonoids were kaempferol-3-*O*-rutinoside and rutin, which ranged from 2.08 to 3.96 mg/g DW and 1.64–3.60 mg/g DW, with 2.97 and 2.25 mg/g DW as average values, respectively. WIM had the highest contents of these two flavonoids, and HCG and NXJ had the lowest contents. Furthermore, both quercetin and myricetin contents were lower than 1.50 mg/g DW. WQBQ had the highest quercetin content (1.36 mg/g DW), while the lowest content was detected in YRBN (0.79 mg/g DW), and quercetin was detected in SXJ, EQBQ, WIM and WQBQ more than the average value of 1.09 mg/g DW. The myricetin content varied from 0.42 to 0.58 mg/g DW, with 0.50 mg/g DW as an average value, and no significant difference was found among these different regions. Overall, the flavonoids contents were generally higher in Qinghai (WQBQ and EQBQ) and Inner Mongolia (WIM) than in Gansu (HCG), Ningxia (YRBN) and Xinjiang (SXJ and NXJ), and HCG had the lowest content.

Additionally, above phenolic compounds in LR cultivated fruits were also investigated and analyzed (Fig. 3 and Table S4). The results indicated that the range and distribution of these phenolics in cultivated and wild berries were similar at the same production regions. However, currently, the cultivated berries had slightly greater variation in phenolics contents than wild berries, but their differences of the average contents between cultivated and wild fruits were statistically insignificant. In addition, there were high variation of the phenolic compounds in both cultivated and wild berries, with exception of caffeic acid, vanillic acid and myricetin, the significant differences of the phenolics contents were detected in the fruit samples from different production regions.

Furthermore, hierarchical cluster analysis (HCA) and principal components analysis (PCA) were conducted statistically in this work. Based on the quantitative analysis of the functional components (TP, TPA, TF, PRG, LRP and TC), the data matrix was established through the statistical software. And then the relationships among the LR berry samples were revealed by the construction of distinctive dendrogram

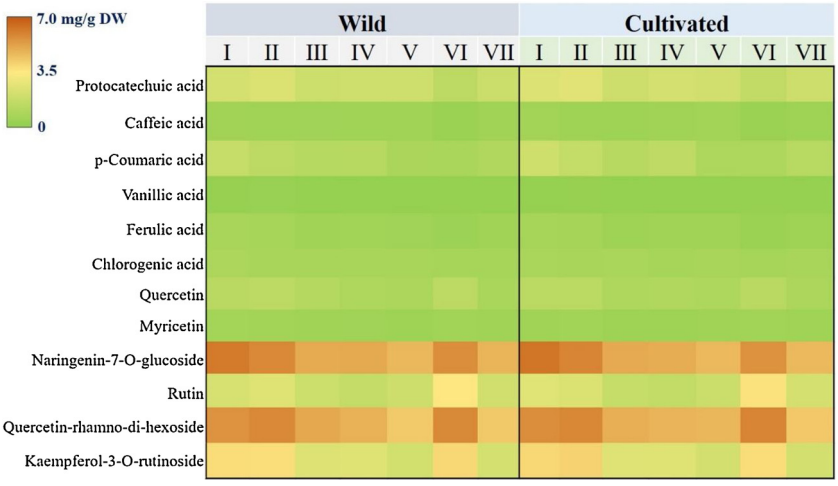

Fig. 3. Distribution of phenolic acid and flavonoid contents of wild and cultivated LR berries. I ~ II represented the different production regions of LR berry.

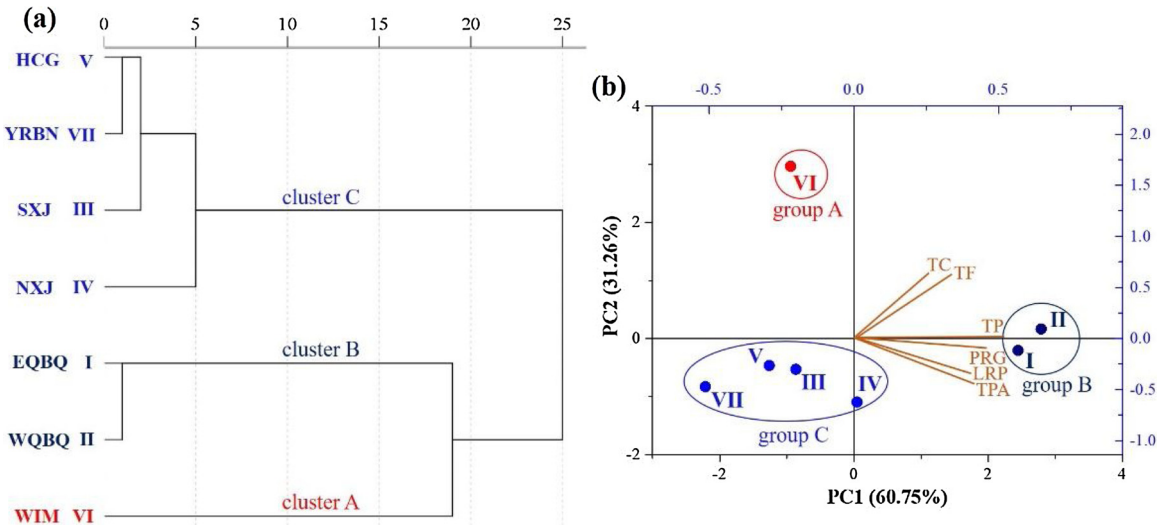

Fig. 4. Chemometric analysis of the functional components (TP, TPA, TF, PRG, LRP and TC) in 7 batches of LR. (a) Dendrogram of hierarchical cluster analysis of LR from different regions; (b) Two-dimensional plot of the functional component contents in LR berry samples investigated in principal components analysis.

(Fig. 4a). The HCA provided the sufficient evidence that seven batches of LR berries were clustered clearly into three groups, and they were cluster A (WIM), cluster B (EQBQ and WQBQ) and cluster C (the rest of berry samples). PCA was firstly carried out using the contents of these LR functional components, which could contribute to investigate further differentiation of the secondary metabolite compositions in LR berries with its geographical origin. The result of PCA presented the loading plots in Fig. 4b. The first two principal components (PC) were obtained on the basis of eigenvalues higher than 1, which approximately accounted for 92.01 % of the total variance. And the values of PC1 and PC2 were 60.75 % and 31.26 %, respectively. The scatter points were clearly exhibited through the loading of the variables, and three groups were distinctly classified in all the berry samples: sample code VI belonged to group A, group B contained sample codes I and II, and the rest of LR berry samples consisted of group C. Therefore, the result of PCA was consistent with HCA analysis, which could be applied to accurately navigate the classification and differentiation for LR geographical origin.

3.6. Correlation between functional components and ecogeographic factors

With the increasing awareness of high content of secondary metabolites (such as TP, TF and LRP) in berries, LR attracted recently a large number of researchers for systematic study of these resources. However, the exact reasons for the high metabolite contents were still not clear. It was conjectured that LR genomic difference and unique geographical factors (high altitude, drought and strong sunshine) could greatly contribute to the accumulation and biosynthesis of these secondary metabolites. Thus, the investigation of correlations between LR berry functional components and ecogeographic factors were also carried out in the present work (Table S5).

Generally, the contents of functional components were directly proportional to altitude and annual mean sunshine hours, and varied inversely with latitude. This could be emerged as a result of the changes in precipitation or temperature conditions. In order to persuasively clarify this phenomenon, the annual average humidity and temperature were also deliberately investigated in different LR production regions. It indicated that a close relationship between the components contents and these two factors was revealed through the statistical analysis. There was a significantly negative correlation between TP and annual mean temperature. And TF and TC also negatively correlated with annual mean humidity ($p < 0.05$). Additionally, the positively significant correlations were detected between altitude and TP, vitamin C and vitamin B, respectively.

The contents and variations of these components in LR were increased with higher altitude, which indicated that generation and accumulation of large numbers of secondary metabolites were favorable for LR plant adaptation of the high altitude, low temperature and strong ultraviolet habitat. The germination rate of LR was greatly affected by the factors of temperature and humidity. And this provided the evidence for explanation of the natural distribution of LR species and the adaptability of these plants to arid and alpine cold environments. At relatively high altitudes, germinations of LR seeds depend on relatively low temperatures which can also make plants more genetically diverse (Liu et al., 2012). Additionally, the contents of these functional components were also proportional to annual mean sunshine hours, especially the significant correlation between liposoluble components (TC and tocopherols) and sunshine hours. This showed that the accumulation of these metabolites was positively correlated with the duration of sunshine. Furthermore, abundant sunshine hours were conducive to the seedling survival and growth of LR plants, with performing great impact on genetic diversity of LR and accumulation and biosynthesis of secondary metabolites such as flavonoids, carotenoids, anthocyanins and vitamins (Liu et al., 2020, 2012; Wallis et al., 2011; Gairola et al., 2010).

In particular, significant relationships between some ecogeographic factors (latitude, annual mean temperature and humidity) and betaine were positively correlated, and negative correlation between betaine and altitude was detected ($p < 0.01$). LR widely distributes in salinized desert of West China, such as the region of Qinghai-Tibet Plateau with relatively high altitude. The content of betaine increased with temperature and humidity, and decreased with altitude, which could contribute to the adaptation of saline-alkali soil and dry wilderness (Cui et al., 2008). The special morphological and physiological characteristics of LR (e.g. drought- and salt-resistance), make it an ideal plant for preventing soil desertification and alleviating the degree of soil salinity-alkalinity, which are special important for the agroforestry and ecosystem at the remote region (Liu et al., 2012; Zhang et al., 2007).

3.7. Antioxidant activity of LR berry extracts

The antioxidant activities of wild LR berries were determined and evaluated by DPPH, ABTS, FRAP and ORAC methods (Table 4). The DPPH˙ scavenging activities of LR berry extracts from different regions ranged from 315.7–460.5 μM TE/g DW, with 393.9 μM TE/g DW as an average value. EQBQ had the highest DPPH value, followed WQBQ and

Table 4
The main phytochemicals contents and antioxidant activities of wild LR berries from different regions.

Samples	LRP[a]	TP	TF	TC	PRG	Betaine	DPPH[b]	ABTS[b]	FRAP[b]	ORAC[b]
EQBQ	31.3 ± 1.6	48.7 ± 3.1	27.3 ± 1.0	2.4 ± 0.0	25.1 ± 1.3	10.7 ± 0.6	460.5 ± 3.6	453.6 ± 3.9	539.4 ± 4.5	587.0 ± 4.1
WQBQ	34.4 ± 1.2	45.8 ± 3.7	29.7 ± 1.8	2.6 ± 0.1	24.3 ± 2.0	10.6 ± 0.4	437.8 ± 3.2	485.6 ± 4.2	528.7 ± 3.4	569.3 ± 4.6
SXJ	31.0 ± 2.3	35.5 ± 4.5	19.8 ± 2.7	2.3 ± 0.2	14.2 ± 2.3	12.4 ± 1.1	364.1 ± 4.3	382.2 ± 4.8	430.5 ± 6.1	508.6 ± 5.9
NXJ	31.5 ± 0.7	39.0 ± 2.8	18.3 ± 2.0	2.2 ± 0.0	20.3 ± 0.9	14.3 ± 0.5	370.3 ± 2.9	405.7 ± 2.4	471.5 ± 2.9	520.1 ± 3.8
HCG	30.7 ± 2.5	33.8 ± 4.1	15.2 ± 2.4	2.4 ± 0.3	14.4 ± 2.4	13.1 ± 1.4	410.7 ± 4.7	426.5 ± 5.7	451.2 ± 5.8	511.3 ± 5.5
WIM	27.2 ± 1.5	36.1 ± 2.9	34.3 ± 3.0	2.7 ± 0.1	15.5 ± 1.0	13.5 ± 0.5	315.7 ± 2.2	327.8 ± 4.0	412.0 ± 3.7	465.9 ± 4.2
YRBN	27.9 ± 1.0	31.9 ± 1.5	15.2 ± 1.5	2.1 ± 0.0	13.7 ± 0.7	13.3 ± 0.4	398.0 ± 2.6	375.1 ± 1.8	377.0 ± 3.0	490.5 ± 3.3

[a] Total LRP (LR polysaccharide), PRG (the main anthocyanin in LR berries) and betaine levels were expressed as mg/g DW, and TP (total phenolic), TF (total flavonoid) and TC (total carotenoid) levels were expressed as mg GAE/g DW, mg RE/g DW and mg βCE/g DW, respectively.
[b] The values were expressed as μM TE/g DW.

HCG, WIM had the lowest value. The ABTS values varied from 327.8 (WIM) to 485.6 μM TE/g DW (WQBQ), and the values of Qaidam Basin of Qinghai (WQBQ and EQBQ) and HCG were more than average value (408.1 μM TE/g DW), which were higher significantly than values from the rest of production regions. However, the FRAP values in YRBN and WIM regions, with 539.4 μM TE/g DW and 528.7 μM TE/g DW, respectively, were significantly higher than the values from other production regions. The FRAP values of YRBN and WIM, with 377.0 and 412.0 μM TE/g DW, respectively, were significantly lower than the average value (458.6 μM TE/g DW). Similarly, there were significantly higher ORAC values from Qaidam Basin of Qinghai (WQBQ and EQBQ) than other production regions. And the ORAC values ranged 465.9–587.0 μM TE/g DW, with 521.8 μM TE/g DW as an average value, and WIM had the lowest values.

Overall, the antioxidant abilities of LR berry extracts from the region of Qaidam Basin of Qinghai (WQBQ and EQBQ) were higher significantly than those of the other regions measured, with following HCG and NXJ, and WIM generally had lowest activity.

Additionally, the Pearson correlations between the functional component (TP, TPA, TF and so on) and antioxidant activity were investigated and the result was presented in Table 5. In general, the main functional components in LR fruits, TP, TPA, TF, PRG, total LRP and TC, were positively correlated with the values of antioxidant activities. There were highly significant correlations between TP and FRAP ($p = 0.002$, $r_s = 0.938$), TP and ORAC ($p = 0.007$, $r_s = 0.894$). Interestingly, TPA significantly correlated with all values of antioxidant tests, which could be inferred that TPA played an important role in antioxidant ability of LR berries. PRG, the predominant anthocyanin in LR berries, was correlated positively with ABTS ($p = 0.050$, $r_s = 0.755$), FRAP ($p = 0.002$, $r_s = 0.930$) and ORAC ($p = 0.008$, $r_s = 0.885$), which presented that their relationships were significant. And the similar correlation was occurred in total LRP. However, the components of TF and TC had no significant correlation with any antioxidant test. As the main functional components in LR berries, phenolics, flavonoids, polysaccharides and carotenoids differently contributed to the antioxidant activity. Moreover, the large contributors for antioxidant activity were phenolics and LRP, which further presented the evidence with phenolics (TP, TPA and PRG) and polysaccharides as the fundamental functional components in LR fruits.

Table 5
Pearson correlation (r_s) between functional components and antioxidant activities of LR berry extracts.

		DPPH	ABTS	FRAP	ORAC
TP	r_s	0.629	0.718	0.938[a]	0.894[a]
	p	0.130	0.069	0.002	0.007
TPA[c]	r_s	0.877[a]	0.894[a]	0.839[b]	0.971[a]
	p	0.010	0.007	0.018	0.000
TF	r_s	−0.117	0.006	0.374	0.177
	p	0.802	0.989	0.408	0.704
PRG	r_s	0.640	0.755[b]	0.930[a]	0.885[a]
	p	0.122	0.050	0.002	0.008
Total LRP	r_s	0.621	0.892[a]	0.815[b]	0.801[b]
	p	0.136	0.007	0.025	0.030
TC	r_s	−0.114	0.054	0.335	0.090
	p	0.807	0.909	0.462	0.848

[a] $p < 0.01$.
[b] $p < 0.05$.
[c] TPA, total phenolic acid (the sum of six phenolic acids).

4. Conclusion

In conclusion, LR berries had high contents of dietary fibers, soluble sugars, vitamins, minerals, anthocyanins, phenolic acids, flavonoids and polysaccharides. Generally, there were no significant differences of nutritional constituent contents in different regions, while the concentrations of the secondary metabolites (functional components) varied significantly in LR berries located at these regions. And the ecogeographic factors (such as altitude, temperature, humidity and so on) could play an important role in the high variation of these secondary metabolites. However, no significances of the functional components were found in the wild and cultivated fruit samples from the same production region, which suggested that it would be worth promoting for the development of cultivated LR. In addition, LR berries located from Qaidam Basin of Qinghai, not only possessed rich sources of potentially functional components (such as phenolics and polysaccharides), but also presented higher antioxidant ability than those of the rest of five regions. Therefore, these findings provide scientific evidence of the abundance of nutritional and functional components in black wolfberry (*L. ruthenicum*) and indicate that these Qinghai berries have considerable application in the industries of food, nutraceuticals and pharmaceuticals.

CRediT authorship contribution statement

Zenggen Liu: Writing - original draft, Methodology, Software, Conceptualization, Data curation. **Baolong Liu:** Software, Validation. **Huaixiu Wen:** Data curation. **Yanduo Tao:** Visualization, Investigation. **Yun Shao:** Writing - review & editing, Supervision.

Declaration of Competing Interest

In this research, the authors have no competition and no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2020.112692.

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