Research Article

Tangwei Zhang[#], Jialin Ma[#], Xuelian Wu, Zhihua Hao, Ci Dun, Chen Chen^{*}

Qualitative and semi-quantitative assessment of anthocyanins in Tibetan hulless barley from different geographical locations by UPLC-QTOF-MS and their antioxidant capacities

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Abstract: Tibetan hulless barley (“qingke” in Chinese) is a valuable food in Tibet. Purple qingke (PQK) and black qingke (BQK), two special pigmented types of hulless barley, have traditionally been widely cultivated and consumed in Tibet for thousands of years. The composition and contents of anthocyanins of two cultivars are unknown. This study aimed to explore the composition and contents of anthocyanins of two cultivars and their antioxidant capacities. Six anthocyanins were identified by ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry from barley, most of the anthocyanins were acylated by malonyl group. The total anthocyanin contents ranged from 141 to 2,304 µg/g in PQK and from 248.7 to 2902.9 µg/g in BQK. Furthermore, qingke has strong antioxidant activity against DPPH, ABTS^{•+}, and FRAP. Qingke may be useful for treating or preventing diseases caused by the overproduction of radicals.

Keywords: Tibetan hulless barley, UPLC/Q-TOF-MS, anthocyanins

1 Introduction

Anthocyanins are the plant pigments that are responsible for spectacular red, blue, and purple colors of diverse plants. They are commonly found in foods, such as fruits, vegetables, and grains, and are important components of the human diet. Anthocyanins have been shown to have beneficial effects on human health as antioxidants and as anti-inflammatory, anti-diabetic, anti-obesity, and anti-cancer agents [1–6]. Therefore, the regular consumption of anthocyanin-rich food may help to prevent various chronic disorders and improve health. Based on daily food research in America, the daily consumption of anthocyanins was estimated to be 12.5 mg/person/day [7]. The major dietary anthocyanins were delphinidin, malvidin, and peonidin glycosides, which can be found in many plant foods, including purple sweet potatoes, grapes, berries, and wine [8–10].

Tibetan hulless barley, which is called “qingke” in Chinese and “ne” in Tibetan, is cultivated in the “Third Pole of the World,” the Tibetan Plateau. It has special characteristics of cold resistance and drought resistance, and it is very important for the ecosystem and agriculture in the remote area. It is the staple food for Tibetans and an important feed for livestock in the Tibetan Plateau. Purple qingke (PQK) and black qingke (BQK), two special pigmented types of hulless barley, have traditionally been widely cultivated and consumed in Tibet [11]. Tibetans have consumed qingke as daily food for thousands of years, because it is a rich source of protein, starch, sugar, and fiber [12].

Recently, much attention has been paid to qingke because of its anthocyanin content. Several previous studies have confirmed the high anthocyanin content of qingke and reported various methods to extract anthocyanins from qingke [13–16]. However, these studies focused on the development of methods for the extraction and the determination of total anthocyanin contents by spectrophotometric method. Only two studies determined the structures of anthocyanins in barley using high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) [17]. There is a lack of information on

[#] These authors contributed equally to this paper.

*** Corresponding author: Chen Chen**, Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, People's Republic of China, e-mail: chenchen1410@163.com, fax: +86-09716132750

Tangwei Zhang, Xuelian Wu, Zhihua Hao, Ci Dun: Institute of Agricultural Product Quality Standard and Testing Research, Tibet Academy of Agricultural and Animal Husbandry Sciences, Lhasa, People's Republic of China

Jialin Ma: Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, People's Republic of China

the types and amounts of anthocyanins in qingke grown in Tibet. Therefore, it is important to develop a method to characterize the anthocyanins in qingke.

In this study, a rapid and reliable method to characterize the anthocyanins in PQK and BQK based on ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC/Q-TOF-MS) was used. With this method, six anthocyanins were identified and the content of anthocyanins in barley was determined. Furthermore, the antioxidant activities of PQK and BQK were assessed.

2 Materials and methods

2.1 Chemicals and materials

Methanol and HPLC-grade acetonitrile (ACN) were obtained from Yuwang Chemical Reagents Co. (Shandong, China). Cyanidin-3-glucoside, 1,1-diphenyl-2-picrylhydrazyl (DPPH), trifluoroacetic acid (TFA), gallic acid (GA), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS⁺), 2,4,6-tripyridyl-S-triazine, and formic acid were obtained from Sigma (St Louis, USA).

Samples of PQK and BQK were collected from 15 different growing sites in Tibet during harvest and then air-dried until the moisture content decreased to approximately 10%. Details of the sample collection sites (altitude, longitude, and latitude) are shown in Table 1. Each sample was ground and sieved through an 80-mesh sieve before analysis.

2.2 Extraction procedure

Briefly, 1 g of samples was extracted with 10 mL of methanol (1% formic acid) using a sonicator (Advanced sonic processing systems, Oxford, CT, USA) at 16 kHz with 300 W power for 30 min at room temperature in darkness. The suspension was centrifuged at 10,000 g for 10 min in a Contifuge 28RS centrifuge (Heraeus, South Plainfield, NJ, USA), and then was filtered through a 0.22 µm membrane filter before HPLC analysis.

2.3 Identification of anthocyanins by UPLC/Q-TOF-MS

Anthocyanins in PQK and BQK were identified by UPLC/Q-TOF-MS analysis. A Waters ACQUITY UPLC TM system

Table 1: Geographical information of qingke

No.	Location	Longitude	Latitude	Altitude (m)
L1	Qushui	29.45°N	90.61°E	3,650
L2	Nimu	31.24°N	94.23°E	3,810
L3	Linzhou	30.14°N	91.31°E	3,760
L4	Bayi	29.74°N	94.46°E	3,100
L5	Milin	29.22°N	94.22°E	2,940
L6	Zuogong	29.58°N	97.68°E	3,810
L7	Mangkang	29.49°N	98.93°E	3,920
L8	Bianba	30.73°N	96.08°E	3,660
L9	Luolong	30.91°N	95.88°E	3,600
L10	Jiangzi	28.91°N	89.61°E	4,070
L11	Bailang	28.95°N	89.05°E	3,940
L12	Pulan	30.29°N	81.18°E	4,510
L13	Zhadu	31.47°N	79.79°E	3,700
L14	Gongga	29.19°N	90.95°E	3,570
L15	Nadong	29.30°N	91.76°E	3,560

(Waters Corporation, Milford, MA, USA), which was equipped with a binary solvent delivery manager, a thermostatically controlled column compartment, an auto-sampler, and a photodiode array detector, was used. The samples were separated on a ZORBAX Eclipse Plus column (100 mm × 4.6 mm i.d., 1.8 µm). The HPLC mobile phases consisted of H₂O with 0.1% TFA (solvent A) and ACN with 0.1% TFA (solvent B). They were eluted from 5% B to 5% B in 2 min, followed by a gradient to 15% B in 25 min. The flow rate was 0.8 mL/min, the column temperature was 25°C, and the detection wavelength was 525 nm. An AB Triple TOF 5600 plus (AB SCIEX, Framingham, MA, USA) mass spectrometer equipped with an electrospray ionization (ESI) source was used to perform the MS analysis. The major MS/MS parameters were as follows: source type, ESI; positive ionization mode; an *m/z* range of 100–1,500; ion Source Gases 1 and 2 (GS1 and GS2), 50 psi; a capillary voltage of 3.50 kV; a sampling cone voltage of 28 V; an extraction cone voltage of 4.0 V; a source temperature of 100°C; a cone gas flow of 50 L/h; and a collision energy ramp of 15–25 V.

2.4 Semi-quantification of anthocyanin content by HPLC

HPLC analysis was performed on an Agilent 1200 system (Agilent, USA). The Agilent 1200 system was equipped with an Agilent HPLC workstation, a pump, a DAD detector, and a column thermostat. Samples were separated on a ZORBAX Eclipse Plus column (250 mm × 2.1 mm

i.d., 5 μm). The HPLC mobile phases were H_2O with 0.1% TFA (solvent A) and ACN with 0.1% TFA (solvent B). The elution gradient was 12% B to 12% B in 10 min, followed by a gradient to 15% B in 20 min. The flow rate was 1.0 mL/min, the column temperature was 25°C, and the detection wavelength was 525 nm.

Cyanidin-3-glucoside was used as the standard to prepare the calibration curve used for semi-quantitative analyses of individual anthocyanins in PQQ and BQK. Each individual anthocyanin was expressed as micrograms of cyanidin-3-glucoside equivalents per gram dry sample weight.

2.5 Antioxidant activity

DPPH radical scavenging activity was measured by the method of previous report [18]. A volume of 5 μL of extraction sample was reacted with 995 μL of DPPH solution (0.4 mM) in a 96-well plate. The mixture was kept at room temperature in the dark for 30 min, and the absorbance (Abs) at 517 nm was measured by a microplate reader. GA was used as positive control. The calibration curve was established by plotting the DPPH scavenging ratio $(A_0 - A_t)/A_0$ (A_0 and A_t represent the absorbance at the initiation and termination of the reaction, respectively) against GA concentration. The results were expressed as milligrams of GA equivalents per gram of dry weight (DW).

The ABTS assay was performed according to the method of Wang et al. [19]. A volume of 5 μL of sample was added to 995 μL of diluted ABTS^{•+} and to reacted in the dark at room temperature for 10 min. The mixture was tested under 732 nm. GA was used as a standard compound, and the results were expressed as mg of GA equivalents per g of DW.

The FRAP assay was performed according to the method of Wang et al. [19]. A volume of 5 μL of sample was added to 995 μL of FRAP reagent and allowed to react at room temperature in the dark for 15 min. Absorbance at 593 nm was recorded. GA was introduced as a standard. The content of GA equivalents was used to express the results.

2.6 Data analysis

Mean values of each sample were obtained from three replications and used for further analysis. Data were analyzed with SPSS software (version 20) (IBM Corp., Armonk, NY, USA). Compound identification was performed using

the METLIN MS database and Food Composition and Method Development Laboratory database (U.S. Department of Agriculture).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 HPLC of anthocyanins

The HPLC profiles showing the anthocyanin peaks in PQQ and BQK samples are shown in Figure 1. The HPLC chromatograms were similar for the PQQ and BQK samples from different locations. Six peaks with large areas were detected in all samples and represented common characteristic peaks. The relative standard deviations of the retention times of peaks were less than 0.9%, confirming that the developed method was robust and suitable for analyses.

3.2 Anthocyanin identification

After HPLC separation, the anthocyanins were identified on the basis of the MS data and the molecular ion characteristics for each peak (Figure 2). The m/z ratios for intact anthocyanins and their daughter fragments are listed in Table 2. Peak 1 had an MS ion at m/z 449.1083, with a fragmentation pattern (m/z 287) consistent with the loss of a hexose molecule. The MS fragments with an MS ion at m/z 287 corresponded to cyanidin aglycone moiety. Thus, peak 1 was tentatively identified as cyanidin-3-glucoside [20]. Peaks 2 and 3 had the same molecular ion (m/z 535.1081) and fragmentation pattern, suggesting that they represented isomers of cyanidin (m/z 287) derivatives attached to malonylated glucopyranosyl residues. On the basis of the retention times of peaks 2 and 3, they were tentatively identified as cyanidin 3-*O*-(3''-*O*-malonyl-glucoside) and cyanidin 3-*O*-(6''-*O*-malonyl-glucoside), respectively [21]. Peak 4 had a characteristic cyanidin aglycone ion (m/z 287), suggesting that it represented cyanidin glycoside. The fragmentation pattern suggested that peak 4 had two malonylated β -glucopyranosyl residues. Thus, peak 4 was identified as cyanidin 3-*O*-dimalonylglucoside [22]. The intense ion peaks at m/z 605.1129 and 635.1236 detected for compounds 5 and 6 corresponded to $\text{C}_{27}\text{H}_{25}\text{O}_{16}$ and

Figure 1: HPLC analysis of qingke. (a) HPLC analysis of PQQ and (b) HPLC analysis of BQK.

$C_{28}H_{27}O_{17}$, respectively. The peak 5 at m/z 271 represented pelargonidin, peak 5 was identified as pelargonidin 3-*O*-dimalonylglucoside [23]. The peak 6 ion at m/z 301 indicated that it was peonidin, and peak 6 was identified as peonidin 3-*O*-dimalonylglucoside [23].

In this study, six anthocyanins were identified in both PQQ and BQK. Four of the six anthocyanin aglycones were derivatives of cyanidin, one of the most common naturally occurring anthocyanins. The main structural feature of anthocyanins in PQQ and BQK was a malonyl group.

Figure 2: MS chromatographic profiles of anthocyanins from qingke.

Table 2: Mass spectrometric data of anthocyanins of qingke

Peak	t_R (min)	$[M]^+$ (m/z)	Formula	Fragment (m/z)	Tentative identification
1	9.08	449.1083	$C_{21}H_{21}O_{11}$	287	Cyanidin-3-glucoside
2	10.92	535.1081	$C_{24}H_{22}O_{14}$	449, 287	Cyanidin 3- <i>O</i> -(3''- <i>O</i> -malonyl-glucoside)
3	11.7	535.1078	$C_{24}H_{22}O_{14}$	449, 287	Cyanidin 3- <i>O</i> -(6''- <i>O</i> -malonyl-glucoside)
4	13.35	621.1080	$C_{27}H_{24}O_{17}$	287	Cyanidin 3- <i>O</i> -dimalonylglucoside
5	14.62	605.1129	$C_{27}H_{24}O_{16}$	271, 249	Pelargonidin 3- <i>O</i> -dimalonylglucoside
6	14.99	635.1236	$C_{28}H_{26}O_{17}$	301, 286	Peonidin 3- <i>O</i> -dimalonylglucoside

Acylated anthocyanins are more stable than non-acylated anthocyanins. The malonyl structure also stabilizes anthocyanins. A previous study revealed that environmental factors can affect the anthocyanin profile by regulating the activities of enzymes that catalyze polyphenol biosynthesis [24]. The unique geography (high altitude) and harsh climatic conditions (cold, dry, and

strong ultraviolet radiation) in the qingke growing regions of Tibet would influence anthocyanin composition. Specifically, the relatively high concentrations of acylated anthocyanins in qingke may protect plants against ultraviolet damage. This trait is very important for the survival of plants exposed to high levels of ultraviolet radiation [25,26].

Table 3: Content of anthocyanins in qingke

Location	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
Content ($\mu\text{g/g}$) of PQQ						
L1	86.0 ± 0.2	107.3 ± 0.3	515.4 ± 0.9	522.3 ± 1.4	11.2 ± 0.1	4.8 ± 0.1
L2	100.7 ± 0.8	120.7 ± 0.3	460.3 ± 0.7	510.2 ± 0.9	80.9 ± 0.3	70.1 ± 0.3
L3	55.9 ± 0.4	86.4 ± 0.4	444.2 ± 0.7	508.7 ± 0.7	7.9 ± 0.1	4.4 ± 0.1
L4	9.7 ± 0.1	20.9 ± 0.2	58.7 ± 0.6	47.9 ± 0.5	2.4 ± 0.1	1.5 ± 0.1
L5	6.5 ± 0.1	11.1 ± 0.1	46.1 ± 0.4	41.1 ± 0.6	1.7 ± 0.1	2.0 ± 0.2
L6	33.6 ± 0.2	106.0 ± 0.2	209.2 ± 0.5	162.4 ± 0.7	16.2 ± 0.2	1.8 ± 0.1
L7	55.7 ± 0.2	70.0 ± 0.3	338.7 ± 0.9	354.1 ± 1.1	6.3 ± 0.1	2.8 ± 0.1
L8	19.2 ± 0.2	31.2 ± 0.2	144.2 ± 0.5	116.5 ± 0.6	2.4 ± 0.1	1.1 ± 0.1
L9	9.1 ± 0.2	23.7 ± 0.2	59.8 ± 0.6	71.8 ± 0.6	1.5 ± 0.2	1.3 ± 0.1
L10	186.2 ± 0.2	200.2 ± 0.7	850.9 ± 0.9	1049.6 ± 1.7	12.3 ± 0.2	4.3 ± 0.3
L11	112.2 ± 0.4	186.3 ± 0.8	825.6 ± 1.2	1096.21 ± 1.9	17.5 ± 0.2	4.0 ± 0.2
L12	13.1 ± 0.1	21.8 ± 0.3	105.0 ± 0.7	108.2 ± 2.2	1.6 ± 0.2	1.3 ± 0.1
L13	9.6 ± 0.2	19.2 ± 0.2	79.6 ± 0.7	80.1 ± 0.9	99.8 ± 0.7	3.0 ± 0.2
L14	66.2 ± 0.2	120.6 ± 0.4	538.9 ± 1.2	615.2 ± 1.1	14.2 ± 0.3	3.9 ± 0.2
L15	84.4 ± 0.3	136.6 ± 0.7	651.8 ± 1.4	901.9 ± 1.3	12.0 ± 0.3	9.5 ± 0.1
Content ($\mu\text{g/g}$) of BQK						
L1	230.3 ± 0.5	340.5 ± 0.8	760.7 ± 1.8	970.8 ± 1.6	190.4 ± 1.2	270.6 ± 0.5
L2	63.7 ± 0.4	99.8 ± 0.4	460.6 ± 0.8	531.1 ± 1.5	10.1 ± 0.2	$4.6 \pm 0.10.1$
L3	70.9 ± 0.6	109.2 ± 1.4	535.2 ± 0.8	521.5 ± 1.8	10.0 ± 0.1	5.3 ± 0.2
L4	40.3 ± 0.5	90.6 ± 1.2	140.7 ± 1.9	240.1 ± 1.4	60.6 ± 0.2	80.2 ± 0.2
L5	46.6 ± 0.7	86.1 ± 1.5	393.1 ± 2.0	416.6 ± 1.7	9.2 ± 0.1	6.3 ± 0.2
L6	33.3 ± 0.4	67.5 ± 0.2	304.8 ± 2.1	357.9 ± 1.7	6.3 ± 0.2	5.3 ± 0.2
L7	70.2 ± 0.8	100.8 ± 0.3	250.3 ± 1.7	340.5 ± 1.5	30.4 ± 0.2	70.3 ± 0.4
L8	59.5 ± 0.8	80.6 ± 1.6	371.4 ± 1.8	354.9 ± 1.1	6.2 ± 0.2	2.2 ± 0.2
L9	36.6 ± 0.8	71.8 ± 1.6	326.7 ± 1.3	380.6 ± 0.6	7.1 ± 0.2	6.2 ± 0.2
L10	134.0 ± 1.0	206.7 ± 1.8	851.4 ± 2.6	1178.2 ± 1.1	17.5 ± 0.3	5.0 ± 0.1
L11	150.7 ± 1.1	252.6 ± 1.9	989.9 ± 2.9	1489.1 ± 1.3	18.7 ± 0.3	1.8 ± 0.1
L12	14.7 ± 0.3	36.8 ± 0.9	108.6 ± 2.8	92.1 ± 0.7	2.3 ± 0.1	1.1 ± 0.1
L13	14.5 ± 0.2	24.9 ± 0.5	103.6 ± 1.3	101.9 ± 0.9	2.3 ± 0.1	1.6 ± 0.1
L14	56.6 ± 0.7	106.2 ± 0.9	444.0 ± 1.8	593.1 ± 1.4	8.3 ± 0.1	3.1 ± 0.3
L15	60.3 ± 0.8	120.4 ± 1.1	680.5 ± 1.5	770.7 ± 2.0	140.8 ± 0.8	90.7 ± 0.9

3.3 Anthocyanin quantification

Table 3 lists the contents of individual anthocyanins detected in PQK and BQK. The composition and total contents varied considerably among samples from different locations (Table 4). The total anthocyanin contents ranged from 141 to 2,304 $\mu\text{g/g}$ in PQK and from 248.7 to 2902.9 $\mu\text{g/g}$ in BQK. Lin *et al.* reported that the total anthocyanin contents were 2.69 mg/100 g in blue qingke and 9.55 mg/100 g in BQK [27]. The different contents were on the basis of different analysis methods. This study focused on the individual anthocyanin content. The predominant anthocyanins were cyanidin 3-*O*-(6''-*O*-malonyl- β -glucopyranoside) **3** and cyanidin 3-*O*-(3'',6''-di-*O*-malonyl- β -glucopyranoside) **4**, which accounted for nearly 80% of the total anthocyanin content in PQK and BQK. In another study, the total anthocyanin content in colored barley varieties ranged from 3.2 to 1037.8 $\mu\text{g/g}$ [28,29]. The higher range of concentrations detected in this study may be related to differences in the analyzed cultivars and growing conditions.

For PQK, the lowest and highest anthocyanin contents were detected in samples from locations L5 and L10, respectively, and the mean anthocyanin content was 995.1 $\mu\text{g/g}$. For BQK, the lowest and highest anthocyanin contents were detected in samples from locations L13 and L11, respectively, and the mean anthocyanin content was 1267.4 $\mu\text{g/g}$. Additionally, the total anthocyanin content was consistently higher in BQK than in PQK collected from the same locations.

Table 4: Total content of anthocyanins from same locations

Location	Total content of anthocyanins	
	PQK	BQK
L1	1247.1	2763.3
L2	1342.8	1169.9
L3	1107.5	1252.1
L4	141.0	652.5
L5	108.5	957.9
L6	529.3	775.0
L7	827.7	862.5
L8	314.5	874.8
L9	167.2	828.9
L10	2303.5	2392.8
L11	2241.9	2902.9
L12	251.0	255.6
L13	291.2	248.7
L14	1359.0	1211.3
L15	1796.2	1863.4

3.4 Correlation analysis

In order to analyze the variations in anthocyanin contents of PQK and BQK among different growing locations (longitude, latitude, and altitude) in Tibet, the correlations between anthocyanins and geographical details (longitude, latitude, and altitude) of the sample collection locations were analyzed (Table 5). The correlation coefficient of PQK was -0.52 , 0.07 , and 0.32 , and correlation coefficient of BQK was -0.60 , 0.04 , and 0.08 , respectively. For both PQK and BQK, anthocyanin content was negatively correlated with latitude ($P < 0.05$) and tended to decrease at increasingly southern latitudes.

3.5 Antioxidant activity

In this study, the antioxidant activity of qingke was examined, and the results are shown in Table 6. The DPPH result showed that qingke in L3 has the highest antioxidant activity among locations. The ABTS⁺ result showed that PQK in L6 and BQK in L10 have highest antioxidant activity, respectively. The FRAP⁺ result showed PQK in L3 and BQK in L11 have highest antioxidant activity, respectively. The anthocyanins of qingke showed that high antioxidant activity may be related to their structures. As we know, an increase in the number of hydroxyl substitutions on the B ring appeared to be associated with enhanced antioxidant activity. These results on antioxidant activity of anthocyanins imply that qingke may be useful for treating or preventing diseases caused by the overproduction of radicals. The cultivation of qingke rich in acetylated anthocyanins with strong antioxidant activities may be beneficial for Tibetans who are exposed to high ultraviolet radiation.

4 Conclusions

In this study, we applied UPLC/Q-TOF-MS to identify and quantify the anthocyanins in PQK and BQK samples collected from 15 different geographical locations in Tibet. To

Table 5: Correlation coefficients of anthocyanins and geographical details

Content	Longitude	Latitude	Altitude
PQK	-0.52	0.07	0.32
BQK	-0.60	0.04	0.08

Table 6: Antioxidant activity determined by DPPH, ABTS⁺⁺, and FRAP assays (mg/g DW)

Location	Cultivars	DPPH	ABTS ⁺⁺	FRAP
L1	PQK	35.21	121.25	65.82
L2		34.63	98.53	57.44
L3		37.94	102.85	71.84
L4		25.23	43.25	50.21
L5		22.38	38.25	44.52
L6		30.78	165.84	48.67
L7		31.59	94.85	58.94
L8		29.53	66.58	47.25
L9		23.56	51.46	30.54
L10		36.54	127.42	35.48
L11		34.99	75.94	60.33
L12		21.06	64.23	58.61
L13		21.65	41.97	28.52
L14		29.54	55.81	32.54
L15		27.38	56.94	36.59
L1	BQK	40.21	105.63	52.62
L2		45.84	77.64	50.14
L3		46.39	84.25	60.19
L4		23.13	49.55	35.51
L5		20.68	43.15	56.22
L6		35.19	125.97	60.27
L7		23.55	92.37	62.14
L8		36.13	59.02	38.85
L9		26.34	65.26	42.24
L10		32.81	132.82	46.78
L11		41.12	66.74	70.80
L12		18.94	62.13	66.16
L13		26.43	40.54	42.34
L14		27.83	62.52	39.14
L15		29.55	50.54	39.19

the best of our knowledge, this is the first report on the compositions and contents of anthocyanins in qingke cultivated in Tibet. Six anthocyanins were identified, with cyanidin 3-*O*-(6''-*O*-malonyl- β -glucopyranoside) and cyanidin 3-*O*-(3'',6''-di-*O*-malonyl- β -glucopyranoside) being the predominant anthocyanins in PQK and BQK. The extraction of qingke exhibited antioxidant properties. The results indicated that specific geographical factors should be used to identify qingke crops with outstanding anthocyanin contents. As well as being a healthy food, the anthocyanin-rich qingke varieties cultivated in Tibet have the potential for development into nutritional supplements and health-care products.

Abbreviations

ABTS ⁺⁺	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ACN	acetonitrile

BQK	black qingke
DPPH	1,1-diphenyl-2-picrylhydrazyl
DW	dry weight
ESI	electrospray ionization
GA	gallic acid
HPLC	high performance liquid chromatography
HPLC-MS/MS	high-performance liquid chromatography with tandem mass spectrometry
PQK	purple qingke
TFA	trifluoroacetic acid
UPLC/Q-TOF-MS	ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry

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Author contributions: Jilin Ma performed the experiments. Xuelian Wu, Zhihua Hao, and Ci Dun collected the samples. Tangwei Zhang carried out the data analysis. Chen Chen conceived and designed the experiment. All authors have read and agreed to the published version of the manuscript.

Competing interests: There are no competing interests.

Data availability statement: All data generated or analyzed during this study are included in this published article.

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