Analysis of Nutritional and Functional Components, Heavy Metals, and Pesticide Residues of Wolfberry (*Lycium barbarum* L.) Fruits From Minqin, China

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Abstract: To evaluate the nutrient content, bioactive constituents, and safety of wolfberries (*Lycium barbarum* L.) from Minqin county, China, high-performance liquid chromatography, gas chromatography, and other relevant approaches were employed to measure nutritional and functional components, heavy metals and pesticide residues, and the amino acid composition of wolfberries. The results indicated that for the basic nutritional composition of Minqin wolfberries (100 g dry weight), the content of proteins, crude fats, and carbohydrates were 13.71, 3.09, and 60.08 g, respectively; for analysis of functional components, the content of polysaccharides, carotenoids, betaine, ascorbic acid, total phenolics, and flavonoids were 2.11 g, 569.81 mg, 1.57 g, 45.08 mg, 81.95 mg, and 70.23 mg, respectively. The main basic nutritional composition of Minqin wolfberries included proteins and carbohydrates. Moreover, the wolfberry samples analyzed in this study contained abundant functional amino acids, such as aspartic acid, proline, and glutamic acid. High content of tryptophan, aromatic amino acids, and other essential amino acids were found in Minqin wolfberries. Aspartic acid was the most abundant, and lysine was the first limiting essential amino acid in wolfberries. Minqin wolfberries contained relatively high levels of fats, carotenoids, and ascorbic acid compared with wolfberries from other regions, and the levels of heavy metals and pesticide residues did not exceed the limits for wolfberries as a green food. The findings of this study provide a reference for the quality evaluation of wolfberries grown in Minqin county.

Key words: Minqin *Lycium barbarum*; nutritional composition; bioactive content; functional amino acid; heavy metal; pesticide residue

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民勤枸杞主要营养、功能成分、重金属及农药残留量分析

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摘要：本文为了探讨民勤枸杞的营养、活性成分和安全性评价，通过使用高效液相、气相色谱法和其他测定方法分析民勤枸杞的基本营养和功能成分、氨基酸组成、重金属和农药残留量。民勤枸杞的基本营养成分（以每 100 g 干重计）蛋白质、粗脂肪和碳水化合物含量分别为 13.71 g、3.09 g 和 60.80 g；功能成分多糖、类胡萝卜素、甜菜碱、抗坏血酸、总酚和总黄酮含量分别为 2.11 g、569.81 mg、1.57 g、45.08 mg、81.95 mg 和 70.23 mg。民勤枸杞主要基本营养成分为碳水化合物和蛋白质，并含有丰富的功能性氨基酸天冬氨酸，脯氨酸和谷氨酸。民勤枸杞中色氨酸和芳香氨基酸等必须氨基酸含量丰富；天冬氨酸含量最为丰富；赖氨酸是第一限制必须氨基酸。民勤枸杞含有相对较高的脂肪、类胡萝卜素和抗坏血酸含量；其重金属和农药残留量没有超绿色食品枸杞标准限制。研究结果可为民勤枸杞质量定位提供理论依据。

关键词：民勤枸杞；营养成分；活性成分；功能性氨基酸

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Wolfberry (*Lycium barbarum* L., also named goji, goji berry) is a species of boxthorn in the nightshade family that grows in China and other parts of Asia, and its ripe fruits has been used in Asian countries as a traditional herbal medicine and functional food in dried or fresh way[1]. Wolfberry fruits are rich for nutritional and bioactive contents such as polysaccharides, carotenoid, flavonoid, vitamins, amino acids and alkaloid[1~3]. It has been used to cure consumptive disease with syndrome of kidney essence insufficiency, aching lumbus and knees, dizziness and tinnitus, internal heat dispersion-thirst, blood deficiency chlorosis and blurred vision in the traditional Chinese medicine[2, 4]. Apart from that, pharmacology studies indicated the effects of wolfberry on anti-oxidant, immunomodulation, anti-aging, anti-tumor, invigorating liver, and lowing blood fat[2, 3, 5, 6]. The clinical research outcomes showed that wolfberry could significantly improve sleep quality, fatigue, intestinal function, sport performance and the general well-being of people[6].

The growing and using history of wolfberry is more than 2,000 years in China and some other Asian countries[6]. Wolfberry is distributed in the northwest and north China and is widely grown mainly in Ningxia, Gansu, Xinjiang and Qinghai province[3]. There exist some differences among properties such as the taste, nutritional content and bioactive content because the geographic and weather conditions of the wolfberry vary[7~9]. Therefore, analyzing the nutritional content and bioactive content and proceeding quality orientations of wolfberry in different places can establish an important basis for the further development of wolfberry related sectors.

Minqin county is located in Wuwei, Gansu province, which lies in the northeast Hexi Corridor and lower Shiyang River. Its east, west, and north sides are surrounded by Tengri Desert and Badain Jaran Desert. The weather in Minqin is temperate continental arid climate, giving the county some properties including cold in winter and hot in summer, lacking of rain, full of sunlight and large temperature difference day and night, which make Minqin an especially suitable place for agriculture[10]. Because of its geographical position, the cultivation area and industry scales of wolfberry have been enlarged recently. Now wolfberry has been one of the biggest three characteristic horticulture in Minqin. Therefore, this research analyzed and detected the nutritional, bioactive content, heavy metal and pesticide residue of Minqin wolfberry in order to provide some references for its future production and processing.

1 Materials and method

1.1 Materials and apparatus

1.1.1 Materials and reagents

Wolfberry (*Lycium barbarum* L., wolfberry which was mentioned in following text was *Lycium barbarum* L.) fruits (2014 and 2015 harvest season with different harvest time) from Minqin county (Gansu, China) were supplied by Forestry Bureau Minqin County (Gansu, China). The fruits was stored at -20 ℃ until all analysis were performed. All results represent averages of three time duplicate determinations.

Glucose (batch number: 150624) was purchased from Beijing Xin Ke Biological Technology Co., Ltd.Beta-carotene (batch number: MUST-15082508, purity>90%) and betaine (batch number: MUST-15042417, purity>98%) were purchased from Chengdu Biological Technology Co., Ltd. Gallic acid (batch number: MUST-15052813, purity>95%) and Folin-phenol reagent were purchased from the American Sigma Company. Ultrapure water was purchased from Hangzhou Wahaha Group Co., Ltd. Methanol, ethanol and other chemical reagents were purchased from Sinopharm Group Chemical Reagent Co., Ltd. ,which were all analytically pure.

1.2 Methods

1.2.1 Basic Composition analysis

Water content was determined by weight difference after drying sample, following the national standard[11]. Protein content was calculated from the nitrogen content (N×6.25) analyzed by Kjeldahl method[12]. Lipid content was determined by Soxhlet method[13,14]. Carbohydrate content was analyzed using phenol-sulphuric acid colorimetric method[15,16]. The linear equation of the glucose standard solution for Carbohydrate content was y=0.0014x + 0.00005, R^2=0.9992.

1.2.2 Polysaccharide content

Polysaccharide content was determined by using

phenol-sulphuric acid colorimetric method, following the national standard [16]. Sample (0.4 g, accurate to 0.0001 g) was refluxed with 80% ethanol and was filtered. The filtrate was matured with the procedure in the standard[16]. The linear equation of the glucose standard solution was y=0.0014x + 0.00005, R^2=0.9992.

1.2.3 Ascorbic acid analysis

Ascorbic acid content was determined by colorimetric method, following national standard[17]. The linear equation of the ascorbic acid standard solution was y=0.0307x + 0.0449, R^2=0.9977.

1.2.4 Carotenoid content analysis

The carotenoid content of the wolfberry sample was determined by spectrophometer (SpectraMax M2e, USA) at 448 nm using spectrophotometry method[18].

Beta-carotene used to make standard curve. 1 g wolfberry powder (accurate to 0.0001 g) was weighed and 15 mL petroleum ether and acetone (4: 1, *V/V*) was added to it according to a ratio of material to solvent 1: 15(*m/V*). Then ultrasonic extraction was taken at room temperature for 3 times, lasting for 20 min each time. After that, the solution was filtered and filtrate was then combined. The filter residue was washed until the filtrate turned transparent and was then diluted to 250 mL into a brown volumetric glass flask. The absorbance of the sample was measured at 448 nm. The carotenoid content was calculated with the standard curve equation. The linear equation of the beta-carotene standard solution was y=0.0358x+0.0015, R^2=0.9912.

1.2.5 Betaine content Analysis

Betaine content of the wolfberry analysed by HPLC (Shimadzu HPLC LC-10Atvp, Japan) via improving Zhang et al.'s method[19]. Betaine was extracted from wolfberry powder using methanol at 75 ℃ for 1 h. The filtrate was concentrated by vacuum concentration and was diluted with ultrapure water. The clarified extract was filtered through 0.22 μm Millipore filters and stored frozen until used. The analyses were performed by HPLC-SPD at 190 nm using a Inertsil ODS-SP column, 250 × 4.6 mm, particle size 5 μm. The chromatography was conducted with pure water at flow rate 500 μL/min at 30 ℃. Volumes of 5μL of standard solution or sample were injected. Betaine was identified on the basis of its retention time. The standard curve was made by using different betaine standard solutions and the betaine content of the wolfberry sample was calculated based on the standard curve. The linear equation of the betaine standard solution was y=(2E+06)x + 84955, R^2=0.9998.

1.2.6 Total phenolic

Total phenolic content was determined by using the Folin-Ciocalteu assay[20,21]. 1 g of sliced sample wax mixed by high speed dispersator in 25 mL of ethanol, and extracted twice at 45 ℃ in ultrasonic cleaner for 30 min. Sample was centrifuged at 6000 rmp for 10 min and sediment was extracted again. The supernatant was combined and measured the phenolic content. Sample (100 μL) was introduced into test tubes followed by 500 μL of Folin-Ciocalteau's reagent (10-time dilution) and 400 μL of sodium carbonate (7.5%, *m/V*). The mixture was vortexed and incubated in the dark for 60 min at room temperature. The absorbance was measured by spectrophometer (SpectraMax M2e, USA) at 765 nm. The result was expressed as garlic acid equivalent (mg GAE/100 g). The linear equation of the gallic acid standard solution was y=3.8567x+0.0439, R^2=0.9936.

1.2.7 Total flavonoids

The total flavonoid content was calculated by subtraction of the total phenolic content with non-flavonoid content. The phenolic content was extracted the method described above as 1.2.6. The total non-flavonoid content was measured according to Deetae et.al[20]. 500 μL extraction was mixed with HCl (500 μL, 20% *V/V*), followed by 37% formaldehyde solution (250 μL). The mixture was vortexed and left for 24 h at room temperature in the dark. The sample was then centrifuged at 12000 r/min for 10 min. The supernatant was measured by Folin-Ciocalteu method described above. The results were expressed as mg GAE/100g.

1.2.8 Amino Acids analysis

Amino acids composition analyzed by Hitachi L-8900 amino acid analyzer, following the national standard [22]. Essential amino acid scores were calculated according to reference amino acid requirements of adults (FAO/WHO/UNU, 2007) and child (FAO/WHO/UNU, 1985). The score was calculated using the following formula: amino acid score=amount of amino acid per test protein (mg/g)/amount of amino acid per protein in reference pattern (mg/g)×100.

1.2.9 Heavy metal analysis

Heavy metal including Pb, Cd, As, Hg and Cr

analysed by graphite furnace method, following the national standard[23~27].

1.2.10 Pesticide residue analysis

Pesticide residue including hexachlorobenzene (HCB), hexachlorocyclohexane isomers (HCHs), organochlorine pesticides (DDTs), dimethoate and deltamethyrin analysed by Agilent 7980A gas chromatograph, following the national standard[28].

1.2.11 Statistic analysis of data

All sample analysis were performed in triplicate and mean value repeated. The result are presented as means of three determinations ±SD.

2 Results and discussion

2.1 The nutritional content analysis of wolfberry from Minqin

The basic content analysis of wolfberry from Minqin was shown in Table 1. Carbohydrate and protein were the main basic nutrients of wolfberry. The carbohydrate content was 60.8% and the protein content was 13.7%. The carbohydrate content of wolfberry from Minqin was higher than that of other reported areas like Jinghe (Xinjiang, China), Zhongning (Ningxia, China) and Qaidam (Qinhai, China). The protein content of Minqin wolfberry was higher than that of Zhongning, but was almost the same as that of Qaidam (Table 1). In addition, the lipid content of the wolfberry from Minqin was 3.1%, which was higher than that of other reported places (Table 1).

The main bioactive content analysis of wolfberry from Minqin was shown in Table 1. The carotenoid content was 570 mg/100 g and the ascorbic acid content was 45 mg/100 g, both of which were higher than those of other reported places (Table 1). The betaine content, the total phenolic content and the flavonoid content were 1.57%, 81.95 mg/100 g and 70.23 mg/100 g respectively. The betaine content was higher than that of Jinghe and Zhongning. The flavonoid content was higher than that of Zhongning, but was lower than that of Qaidam. The polysaccharide content of Minqin wolfberry was 2.11%, which was lower than of the earlier reported result. The total phenolic content of wolfberry from Minqin was was 1.95 mg/100 g.

Table 1 Nutritional and functional composition of wolfberry (*Lycium barbarum* L.) from Minqin (100 g dry weight of basis)

Item	Unit	Content	Reported content from different area			
			Jinghe*	Zhongning*	Qaidam*	References
Protein	g	13.71±0.03	-	11.8~12.1	10.30~14.55	[7]
Lipid	g	3.09±0.01	-	0.9	0.33~0.75	[7, 29, 30]
Carbohydrate	g	60.80±0.85	52.11	42.33~50.38	37.88~46.02	[7, 8, 29~31]
Polysaccharide	g	2.11±0.13	9.06~11.96	5.82	3.01~8.33	[7, 8, 31~34]
Carotenoid	mg	569.81±13.27	106	202	66.54~210	[9]
Betaine	g	1.57±0.29	1.11	0.229~1.11	0.98~2.19	[8, 30, 31, 35]
Ascorbic acid	mg	45.08±3.02	-	18.4	-	[30]
Total phenolic	mg	81.95±1.03				
Flavonoid	mg	70.23±0.79		560	830-1110	[7, 31~33]

Note: *Jinghe (Xinjiang Uyghur Autonomous Region, China), Zhongning(Ningxia Hui Autonomous Region, China) and Qaidam (Qinghai, China) are the three main are for planting wolfberry (*Lycium barbarum* L.).

2.2 The analysis of amino acids composition of wolfberry from Minqin

The analysis of amino acids composition was shown in Table 2. The functional amino acids (FAA) were proved to have effects on metabolism regulation, anti-oxidant, anti-inflammation and promoting healing[36,37]. 72% amino acids of Minqin wolfberry were functional amino acids, which meant that Minqin wolfberry was a good resource of functional amino acids. Functional amino acids such as aspartic acid, proline and glutamic acid were important functional amino acids composition of Minqin wolfberry, the content of which were higher than those of other amino acids with a percentage of 46%. Aspartic acid was the most abundant

among those amino acids with 0.0171 g/ g.

Table 2 Amino acid composition analysis of Minqin wolfberry (dry weight of basis, $\times 10^{-2}$ g/ g)

Item		Content	Item		Content
aspartic acid[b]	ASP	1.71±0.50	lysine[a]	LYS	0.27±0.05
threonine[a]	THR	0.28±0.06	histidine[a]	HIS	0.17±0.03
serine	SER	0.44±0.10	arginine[b]	ARG	0.79±0.18
glutamate[b]	GLU	0.97±0.13	proline[b]	PRO	1.15±0.43
glycine[b]	GLY	0.27±0.03	tryptophan[a,b]	TRP	0.10±0.01
alanine	ALA	0.42±0.12	cysteine[b]	CYS	0.13±0.02
valine[a]	VAL	0.28±0.02	TAA		8.27
methionine[a,b]	MET	0.08±0.01	EAA		2.40
isoleucine[a]	ILE	0.21±0.03	FAA		5.97
leucine[a,b]	LEU	0.42±0.03	FAA/TAA(%)		72.12
tyrosine[a,b]	TYR	0.35±0.05	EAA/TAA (%)		28.97
phenylalanine[a]	PHE	0.24±0.05	(PRO,ASP,GLU)/TAA (%)		46.33

Note: Each value is expressed as means; amino acid abbreviations follows IUPAC standards; FAA is abbreviation for functional amino acids; EAA is abbreviation for total essential amino acids; TAA is abbreviation for total amino acids; a Essentiol amino acids; b Functional amino acids.

The amino acid in free state or binding form of plants were not only important resources of nitrogen, but also were resources of essential amino acids such as lysine, methionine and threonine[38]. As is shown in the Table 3, the content of Lysine was the lowest among amino acids, which was the first limiting amino acid for both children and adults. However, Minqin wolfberry had abundant tryptophan and aromatic amino acid (phenylalanine and tyrosine), which could be good resources of those amino acids for adults (Table 3).

Table 3 Essential amino acids scores for Minqin wolfberry

Amino acid	Adults		Children	
	Reference[a]	Score	Reference[b]	Score
HIS	15	83	19	65
ILE	30	52	28	55
LEU	59	52	66	47
LYS	45	43	58	34
MET+CYS	22	69	25	60
PHE+TYR	38	114	63	69
THR	23	87	34	59
TRP	6	124	11	68
VAL	39	52	35	58

Note: Abbreviations of amino acid are in Table 3. [a] Reference amino acid requirements of adults (FAO/WHO/UNU, 2007). [b] Reference amino acid requirements of child (FAO/WHO/UNU, 1985).

2.3 Heavy metal and pesticide residue analyses of wolfberry from Minqin

Heavy metal and pesticide residue contents of Minqin wolfberry were shown in Table 4. The Ph content of Minqin wolfberry was 0.0909 mg/kg; the Cd content of Minqin wolfberry was 0.0113 mg/kg; the Hg content of Minqin wolfberry was 0.00159 mg/kg; the As content of Minqin wolfberry was 0.0290 mg/kg; the Cr content of Minqin wolfberry was 0.1620 mg/kg (Table 4).

Pesticide residue including HCB, HCHs, DDTs, dimethoate and deltamethyrin of the wolfberry sample were not detected (Table 4). Those analysis outcomes were all within the limits of wolfberry green food standard[39].

Table 4 Heavy metal and pesticide residue analysis of Minqin wolfberry (mg/kg)

Item	Content	Item	Conten
Pb	0.0909	*o,p'*-DDT	ND
Cd	0.0113	*p,p'*-DDE	ND
As	0.0290	*p,p'*-DDD	ND
Hg	0.00159	*p,p'*-DDT	ND
Cr	0.1620	Dimethoate	ND
HCB	ND	Deltamethyrin	ND
HCHs	ND		

Note: ND is not detected.

3 Conclusion

3.1 The nutritional and bioactive contents, heavy metal and pesticide residue of wolfberry from Minqin were analyzed by this research. The result indicated that the carbohydrate content was 60.08%, which was the most abundant main basic nutrient. The fat content was higher than wolfberry of other reported places of origin with 3.09%. Minqin wolfberry was rich in functional amino acids, among which Aspartic acid was the most abundant amino acid and lysine was the first limited essential amino acid.

3.2 The analysis indicated that Minqin wolfberry was rich in bioactive content. The bioactive composition and content were as follows: the polysaccharide content was 2.11%; the betaine content was 1.57%; the total phenolic content was 81.95 mg/100 g and the flavonoid content was 70.23 mg/100 g. In addition, the carotenoid content was 570 mg/100 g and the ascorbic acid content was 45.1 mg/100 g, both of which were higher than wolfberry of other reported places of origin. Minqin wolfberry can be a good resource of carotenoid content.

3.3 Heavy metal and pesticide residue of Minqin wolfberry were within the limits of wolfberry green food standard, including heavy metal such as Pb, Cd, As etc. and pesticide residue such as HCB, DDTs, deltamethrin etc.

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References

[1] Amagase Harunobu, Farnsworth Norman R. A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of *Lycium barbarum* fruit (goji) [J]. Food Research International, 2011, 44(7): 1702-1717

[2] Potterat Olivier. Goji (*Lycium barbarum* and *L-chinense*): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity [J]. Planta Medica, 2010, 76(1): 7-19

[3] Rukeya Jiapaer, SUN Yu-jing, ZHONG Lie-zhou, et al. A review of phytochemical composition and bio-active of *Lycium barbarum* fruit (Goji) [J]. Journal of Chinese Institute of Food Science and Technology, 2013, 13(8): 161-172

[4] WANG Ya-jun, AN Wei, SHI Zhi-gang, et al. Research progress in wolfberry medicinal properties [J]. Journal of Anhui Agri. Sci., 2008, 36(30): 13213-13214, 13218

[5] Ulbricht Catherine, Bryan J Kathryn, Costa Dawn, et al. An evidence-based systematic review of goji (*Lycium* spp.) by the natural standard research collaboration [J]. Journal of Dietary Supplements, 2015, 12(2): 184-240

[6] Cheng Jiang, Zhou Zhi-Wei, Sheng Hui-Ping, et al. An evidence-based update on the pharmacological activities and possible molecular targets of *Lycium barbarum* polysaccharides [J]. Drug Design Development and Therapy, 2015, 9: 33-78

[7] YANG Ren-ming, JING Nian-hua, WANG Hong-lun, et al. Analysis and evaluation of nutritional composition and bioactive composition in chinese wolfberry (*Lycium barbarum* L.) Fruits from different regions of qinghai province [J]. Food Science, 2012, 33(20): 265-269

[8] ZHANG Lei, ZHENG Guo-qi, TENG Ying-feng, et al. Comparison research of fruit quality of *Lycium barbarum* L. in different regions [J]. Northwest Pharmaceutical Journal, 2012, 27(3): 195-197

[9] QU Yun-qing, ZHANG Tong-gang, LIU Dun-hua, et al. Cluster analysis on carotenoids contents in *Lycium barbarum* L. fruits from different origins [J]. Food and Machinery, 2015, 31(2): 76-79

[10] Economic information center of Minqin county. Minqin situation, in: natural conditions. government of Minqin county, Gansu. (2014)[05-15]. http://www.minqin.gov.cn/

[11] GB 5009.3-2010, National food safety standard: Determination of moisture in foods [S]

[12] GB 5009.5-2010, National food safety standard: Determination of protein in foods [S]

[13] GB/T 5009.6-2003, Deremination of fat in foods [S]

[14] SN/T 0878-2000, Rules of the inspection of fructus lycii for import and export [S]

[15] Mecozzi M. Estimation of total carbohydrate amount in environmental samples by the phenol-sulphuric acid method assisted by multivariate calibration [J]. Chemometrics and Intelligent Laboratory Systems, 2005, 79(1-2): 84-90

[16] GB/T 18672-2014, Wolfberry [S]

[17] GB/T 5009.86-2003, Determination of total ascorbic acid in

fruits, vegetables and derived products-Ftourometric method and colorimetric method [S]

[18] ZHANG Zhi-ning, ZHANG Wei. Determination of carotenoids in *Lycium barbarum* L. [J]. Nei Mongol Journal of Traditional Chinese Medicine, 2013, 32(34): 134

[19] ZHANG Zi-ping, GUO Rong, LIAO Guo-ling, et al. Comparative study on methods for determination of betaine from *Lycium barbarum* L. [J]. Acta Agriculturae Boreali-Occidentalis Sinica, 2007, 16(6): 292-295

[20] Deetae P, Parichanon P, Trakunleewatthana P, et al. Antioxidant and anti-glycation properties of Thai herbal teas in comparison with conventional teas [J]. Food Chemistry, 2012, 133(3): 953-959

[21] Ardestani A, Yazdanparast R. Inhibitory effects of ethyl acetate extract of *Teucrium* polium on *in vitro* protein glycoxidation [J]. Food and Chemical Toxicology, 2007, 45(12): 2402-2411

[22] GB/T 5009.124-2003, Determination of amino acids in foods [S]

[23] GB 5009.12-2010, National Standards of Food Safety: Determination of lead in foods [S]

[24] GB/T 5009.15-2003, Determination of cadmium in foods [S]

[25] GB/T 5009.11-2003, Determination of total arsenic and abio-arsenic in foods [S]

[26] GB/T 5009.17-2003, Determination of total mercury and organic-mercury in foods [S]

[27] GB/T 5009.123-2003, Determination of chromium in foods [S]

[28] NY/T 761-2008, Pesticide multiresidue screen methods for determination of organophosphorus pesticides, organo-chlorine pesticides, pyrethroid pesticides and carbamate pesticedes in vegetables and fruits [S]

[29] WANG Xiang-dong, WANG Li-peng, ZHU Jie, et al. Nutritional composition analysis of wolfberry fruit from Zhangye and Zhongning [J]. Forest By-Product and Speciality in China, 2012, 1: 40

[30] MA Jian-jun, ZHOU Tao, ZHU Li-xin. Comparison of chemical composition and nutritional components of genuine traditional *Lycium barbarum* L. produced form different habitats [J]. China Vegetables, 2009, 12: 11-14

[31] NIU Yan. Studies on main contents of *Lycium barbarum* L. and relation on environment factors [D]. Yinchuan: Ningxia University, 2005

[32] YANG Wen-jun. Study of reproductive characteristic of poa pratensis var. Anceps Gaud. Cv. Qinghai [D]. Xining: Qinghai University, 2005

[33] LI Guo-liang, LIU Yong-jun, SHI Jun-you, et al. Bioactive content anlyses of tsaidam wolfberry [J]. Chinese Journal of Analysis Laboratory, 2009, 28(S1): 286-288

[34] Ni Hui, He Aihua. Extraction and content determination of polysaccharide of Xinjiang *Lycium* barbarum L. [J]. Chinese Traditional Patent Medicine, 1993, 15(1): 39-40, 51

[35] LIU Zeng-gen, TAO Yan-duo, SHAO Yun, et al. Determination of betaine in *Lycium ruthenicum* Murr. and *Lycium barbarum* L. [J]. Chinese Journal of Spectroscopy Laboratory, 2012, 29(2): 694-697

[36] Wu G Y. Amino acids: metabolism, functions, and nutrition [J]. Amino Acids, 2009, 37(1): 1-17

[37] Wu G Y. Functional amino acids in growth, reproduction, and health [J]. Advances in Nutrition, 2010, 1(1): 31-37

[38] Choi Suk-Hyun, Ahn Jun-Bae, Kozukue Nobuyuki, et al. Distribution of free amino acids, flavonoids, total phenolics, and antioxidative activities of jujube (*Ziziphus jujuba*) fruits and seeds harvested from plants grown in Korea [J]. Journal of Agricultural and Food Chemistry, 2011, 59(12): 6594-6604

[39] NY/T 1051-2014, Green food-Wolfberry and its product [S]