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Full Length Research Paper

Analysis on the main active components of *Lycium barbarum* fruits and related environmental factors

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Fruits of *Lycium barbarum* L. (wolfberry or "Gouqi" in Chinese) are widely used as traditional Chinese medicine and functional food in China, South-east Asia, Europe and North America. Polyphenols, polysaccharides and carotenoids are the main active compounds in *L. barbarum* fruits. Based on a rapid sample preparation with ultrasonic-assisted extraction (UAE) established, contents of polyphenols, polysaccharides and carotenoids of *L. barbarum* fruits from 8 main producing areas of western China were analyzed and the related environmental factors were investigated. The results are: *L. barbarum* fruits from Zhongning showed the highest polyphenols contents (2.9749%) and the highest antioxidant activity (RSA = 56.82%), the highest content of polysaccharides (8.9041%) was from Jinghe, the highest content of carotenoids (0.1642%) was from Julu; the highest fruit weight and pulp weight were from Numhon. Contents of polysaccharides, polyphenols and carotenoids were significantly affected by environmental factors: the principle factor for polyphenols was soil organic matter (SOM) (r = 0.964), the principle factors for carotenoids were temperature-sunshine (r = 0.826), polysaccharides were mainly affected by soil available phosphorus (r = 0.75), fruit weight or pulp weight were negatively correlated with temperature (r = 0.953, 0.963). Each of the three active components has its own genuine producing area rather than Zhongning as the only genuine producing area authenticated.

Key words: *L. barbarum* fruits, polyphenols, antioxidant activity, polysaccharides, carotenoids, genuineness.

INTRODUCTION

Lycium barbarum L. is one of the important traditional Chinese medicinal plant species. It has been cultivated in Northwest China and used as daily functional food in China, Southeast Asia and many European countries. In the Chinese medicinal monographs "shennongbencaojing", "ben cao gang mu" and "ben cao hui yan", *L. barbarum* fruits were recorded as nourishing liver and kidney, enhancing eyesight, enriching blood, invigorating sex, reducing rheumatism" and so on. More functions were recently reported as immunity improvement (Lin et al., 2008), anti-oxidation (Lu et al., 2008), anti-radiation (Qian et al., 2004), anticancer (Chao et al., 2006), enhancing hemopoiesis (Hsu et al., 1999), anti-aging and enhancing sex (Yu et al., 2005).

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Polyphenols, polysaccharides and carotenoids are the important active compounds in *L. barbarum* fruits. Since *L. barbarum* fruits have become one of the most popular functional foods, *L. barbarum* has been cultivated in many areas of western China, which leads to the subsequent quality problems of *L. barbarum* fruits. Environmental factors of *L. barbarum* fruits are important for quality control on *L. barbarum* fruits. In order to make a systematic evaluation on the quality of *L. barbarum* fruits, the main active components of *L. barbarum* fruits from different areas were analyzed, the related ecological factors were investigated.

MATERIALS AND METHODS

Reagents and materials

Standard rutin were purchased from Chinese Authenticating Institute of Material Medica and Biological Products (Beijing, China).

β-carotene were purchased from Sigma Company, USA, 1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Wako Pure Chemical Industries, Ltd. (Japan), all the other chemicals were of analytical grade. Ripe fruits of the same cultivated variety of L. *barbarum* L. were collected from eight main producing areas in Northwest China. In every producing area, 5 locations (N1 - N5) were sampled as replicates. The fruits were dried with silica gel, and then frozenly milled with liquid nitrogen to quickly go through 60-mesh screen. Then the collected powders were dried with silica gel until constant weight.

Equipments

The following facilities were used: an ultrasonic cleaning bath (Desktop NC, Xi'an Taikang Biotechnology Co., Ltd., China, 40 kHz, 400 W) equipped with an automatic temperature regulation system, UV-VIS spectrophotometer Lambda 450 (PerkinElmer, Inc., USA), Agilent 1100 HPLC system consisted of Agilent 1100 ChemStation Rev.A.10.02, G1313A autosampler, G1311A quarternary pump, G1314A variable wavelength ultraviolet (UV) detector (VWD), and reverse phase ZORBAX SB-C8 column (5 µm, 4.6 × 250 mm, Agilent Technologies, USA).

Extraction and determination of polyphenols

0.2 g of the dried powders were placed in soxhlet extractor and refluxed with ether at 50°C water bath to remove oils for 2 h. After the ether was volatilized, the deoiled powders were put in an airproof box filled with silica gel for polyphenols analysis.

Ultrasonic-assisted extraction (UAE) is being used widely in analytical chemistry, facilitating different steps in the analytical process, particularly in sample preparation (Cabredo-Pinillos et al., 2006; Wang et al., 2006), expeditious, inexpensive and efficient alternative to traditional extraction techniques and microwave-assisted extraction (Jalbani et al., 2006). In order to make rapid and efficient extraction of polyphenols from *L. barbarum* fruits, UAE method was employed for sample preparation. Factors as extraction temperature, duration, concentration of ethanol and ratio of liquid to solid were optimized.

Methanol was regarded as not in compliance with good manufacturing practice (GMP) due to its high toxicity (Hemwimol et al., 2006), so in this study only ethanol was used for extraction of polyphenols. The deoiled powders were mixed with the appropriate extraction solvent in a 100 ml conical flask that was immersed in water of the ultrasonic cleaning bath. The bottom of the flask was approximately 2 cm above that of the bath and the liquid level in the flask was about 5 mm below the water surface in the bath. The extracts were filtered through 0.22 µm microporous membranes and the filtrates were collected for quantitation of polyphenols. Optimization on UAE parameters was conducted by orthogonal design and test (Table 1).The results were analyzed with SPSS 16.0.

Polyphenols in the extracts were determined according to the established method (Dong et al., 2009), namely, polyphenols of the extracts were directly determined at 258 nm with rutin as equivalents. Recovery was obtained by adding rutin into the extracts to investigate the accuracy of the determination on polyphenols of the samples prepared by the UAE method.

Polyphenols contents and radical-scavenging activity

The capacity of polyphenols of *L. barbarum* fruits to remove 1, 1-diphenyl-2-picrylhydrazyl radical was determined according to the method by Chon et al. (2009), namely, 1 ml of polyphenols extract and 5 ml of freshly prepared 0.1 mM DPPH methanol solution were thoroughly mixed and kept in the dark for 60 min. The absorbance of the reaction mixture at 520 nm was measured with the UV spectrophotometer. The blank was prepared by replacing the extract with methanol. The percentage of free radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = [1 - (A_{520\ nm,\ sample}/A_{520\ nm,\ blank})] \times 100$$

Analysis on carotenoids of *L. barbarum* fruits

Ultrasonic cannot only destroy the wall of plant cells but enhance mass transfer rates (Zhang et al., 2009; Dong et al., 2010), hence increase extraction rate. The optimized UAE process for *L. barbarum* fruit samples was also used for the extraction of polyphenols, carotenoids and polysaccharides from *L. barbarum* fruit samples. Six kinds of commonly used extraction solvents were used in carotenoids extraction to find out the proper extraction solvent. The same amount of dried fruit samples were weighted and extracted with the same amount of solvents under the extraction process modified from the UAE method established earlier, namely, extraction temperature of 40°C, extraction time of 30 min, solid to liquid of 3.0 mg/ml. The extraction was performed in three cycles. Then the extracts were scanned within 350 to 600 nm. In Figure 2, these extracts showed the special UV-visible absorption of β-carotene (Fraser and Bramley, 2004). Absolute ethanol extract showed the highest absorbance which indicated the highest extraction yield of carotenoids. So, absolute ethanol was used as the proper solvent for carotenoids extraction. The carotenoids extract by absolute ethanol was separated by HPLC method to detect possible interference (Figure 3). The HPLC conditions: acetonitrile (solvent A, 60%) and dichloromethane (solvent B, 40%), deaerated ultrasonically for 30 min, respectively, in advance were used as the mobile phase with a flow rate of 1.0 ml/min. The column temperature was set at 25°C and the sample volume injected was 10.0 µl. The detector was set at 453 nm.

β-carotene was used as standard for determination of carotenoids. Carotenoids concentrations were calculated according to the calibration, with β-carotene as standard. A good linear relationship was obtained within the range of 0.5 to 4.0 µg/ml, and the regression equation is: $y = 0.176 x + 0.0058$, $r = 0.9998$, where y is the absorbance at 453 nm, x is the concentration of β-carotene (µg/ml), r is coefficient correlation.

Determination of polysaccharides

The prepared dried fruit powders were weighed and packed with filter paper. The packed powders were placed in soxhlet extractor and refluxed with ether at 60°C water bath for 4 h to remove lipids. And the deoiled samples were further refluxed with 80% (v/v) ethanol at 80°C for 4 h to remove polyphenols and flavonoids. Finally, the samples were dried at 60°C for 4 h and then polysaccharides of the samples were extracted with UAE method established in this article. The extraction was performed in three cycles.

Phenol-sulfuric method (Masuko et al., 2005) was used for determination of polysaccharides. Polysaccharides concentrations were calculated according to the calibration, as glucose standard. A good linear relationship was obtained within the range of 8.5 to 38.5 µg/ml, and the regression equation is: $y = 0.0069x + 0.0035$, $r = 0.9998$, where y is the absorbance at 490 nm, x is the concentration of glucose (µg/ml), r is coefficient correlation.

Analysis on environmental factors related with the main active components and fruit weights

Potential influential environmental factors were collected and

Table 1. Orthogonal test and results (L_{16}, 4^4).

Test no.	Ethanol (%)	Extraction temperature(°C)	Liquid/ solid (ml/mg)	Extraction time (min)	Mean yield (%, n = 3)
1	50	30	1	30	2.65
2	50	50	1.5	40	3.05
3	50	60	2	50	2.51
4	50	70	2.5	60	2.54
5	60	30	1.5	50	3.08
6	60	50	1	60	3.25
7	60	60	2.5	30	3.15
8	60	70	2	40	2.82
9	70	30	2	60	2.46
10	70	50	2.5	50	3.38
11	70	60	1	40	2.91
12	70	70	1.5	30	2.65
13	80	30	2.5	40	2.28
14	80	50	2	30	2.24
15	80	60	1.5	60	2.12
16	80	70	1	50	1.93
K_1	2.69	2.62	2.69	2.67	
K_2	3.08	2.98	2.73	2.77	
K_3	2.85	2.67	2.51	2.73	
K_4	2.14	2.49	2.84	2.59	
R	0.94	0.49	0.33	0.18	
P value	0.002 **	0.011 *	0.037 *	0.170	

* Significant (P < 0.05); ** very significant (P < 0.01).

analyzed. These factors are: sunshine (kh/y), precipitation (mm/y), cumulative temperature (≥10°C/y), mean annual temperature (°C/y), frost-free period (d/y), and altitude (m) were collected from local weather records of producing areas, respectively. Soil factors as soluble salinity (%), SOM (soil organic matter, %), Soil available nitrogen, phosphorus and potassium were analyzed according to the method of Täumer et al. (2005). The factors as sunshine, precipitation, cumulative temperature, mean annual temperature, frost-free period, altitude, soluble salinity and SOM were analyzed by principle factors analysis with SPSS 16.0 to investigate correlations between these environmental factors and the main active components, because these are environmental factors deciding the genuineness of *L. barbarum* fruits. While soil available nitrogen, phosphorus and potassium can be easily changed by human farming, so the available nitrogen, phosphorus and potassium were analyzed by linear regression independently.

RESULTS

Preparation of the samples

A four-level OAD with an OA16 (4^4) matrix was chosen to optimize the UAE parameters (Table 1). Based on single-factor experiments (Figure 1), the levels are chosen as: ethanol concentration of 50/60/70/80%, extraction temperature of 30/50/60/70°C, ratio of liquid to solid of 1/1.5/2/2.5, extraction time of 30/40/50/60 min. The results of orthogonal test and extreme difference analysis are presented in Table 1. The P value analysis indicated that the influential order of the four factors on the extraction yield of polyphenols is ethanol concentration > extraction temperature > ratio of liquor to solid > ultrasonication time (Table 1). According to variance analysis, the contributions of ethanol concentration, extraction temperature and ratio of liquor to solid for the extraction yield of polyphenols are significant (P < 0.05), whereas extraction time was not significant factor which means UAE can significantly shorten extraction time. According to extreme difference analysis, the optimum extraction condition of polyphenols was deduced as: ethanol of 70%, liquid/solid of 1/2.5 (ml/mg), extraction temperature of 50°C, extraction time of 30 min.

Accuracy of determination on polyphenols from UAE extracts

The linearity range of standard rutin was determined as 4.0 to 15.0 µg/ml (R^2 = 0.9998). The equation was obtained by linear regression: y = 0.0419x - 0.1015 (data not shown), where y is absorbance, x is concentration of rutin (µg/ml). Recovery was obtained by adding standard rutin to extract solution to obtain total polyphenols. Final concentrations were 6.0, 11.0 and 15.0 µg/ml. The assay

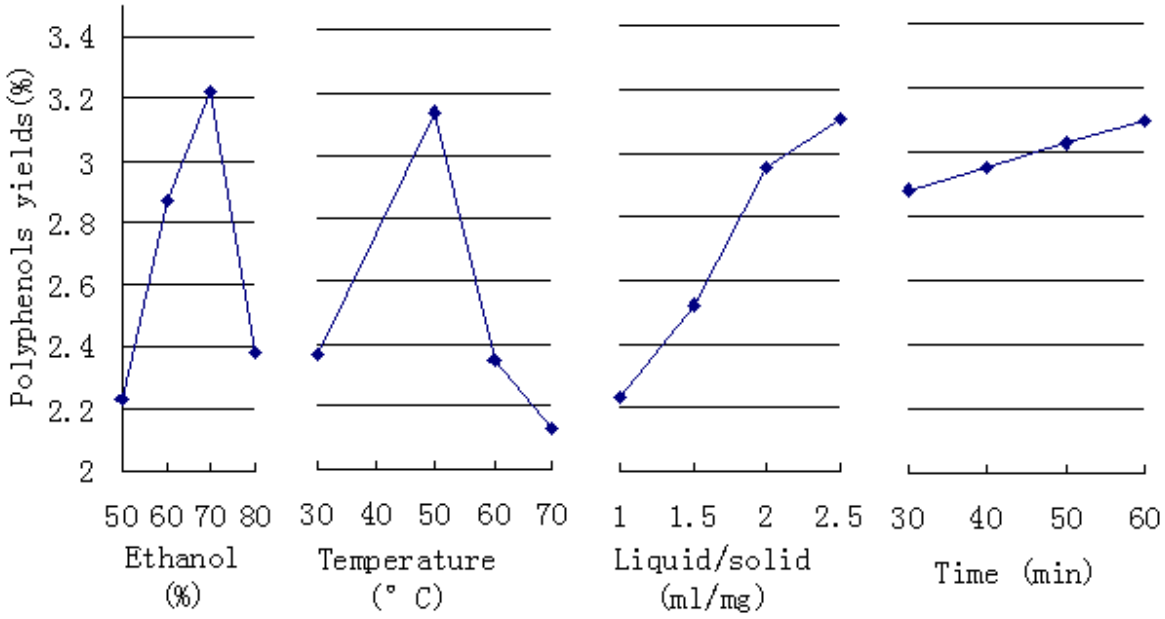

Figure 1. Effect of ethanol concentration, temperature, liquid/solid and extraction time on polyphenols yields.

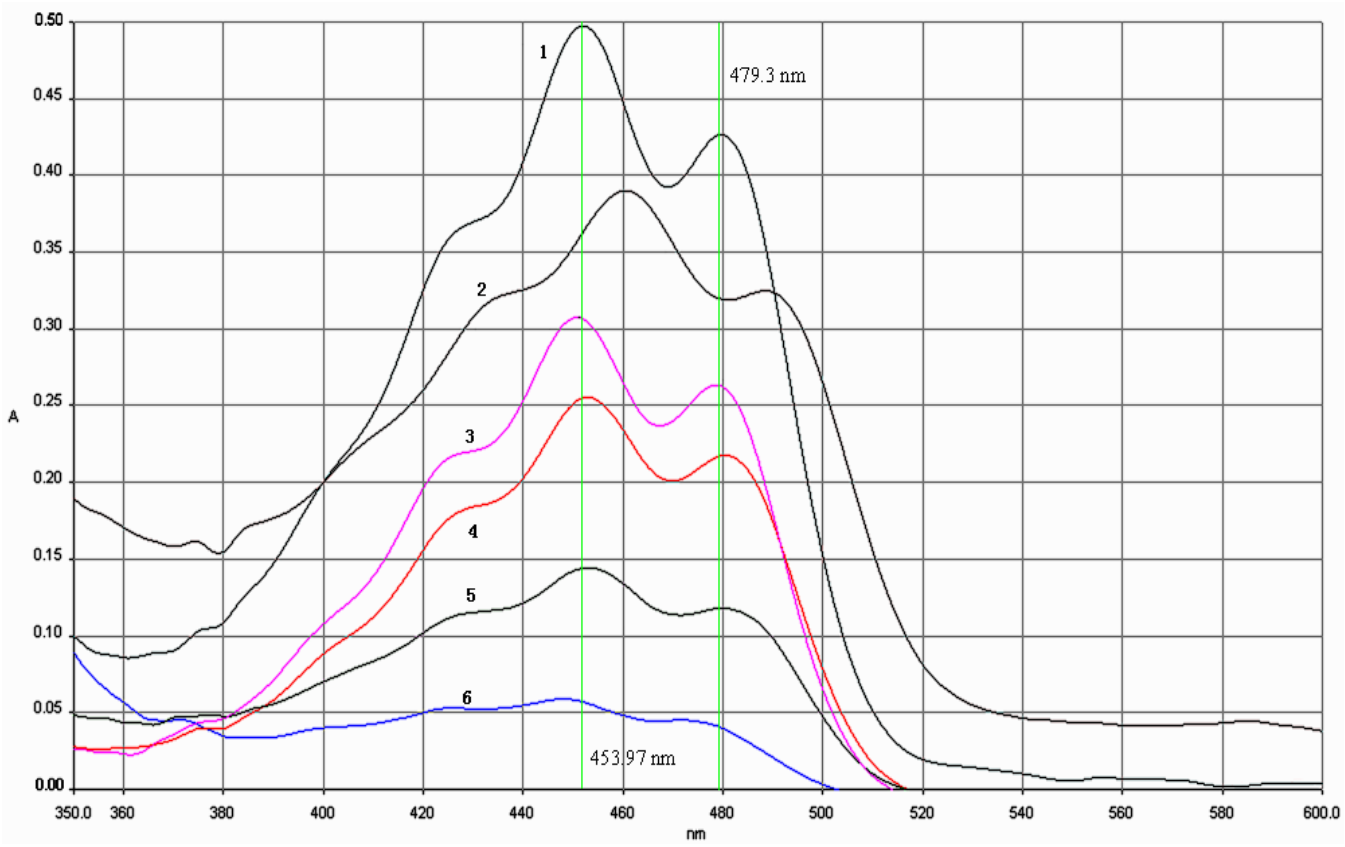

Figure 2. UV-Visible chromatography of carotenoids extracted by different solvents (1 = absolute ethanol, 2 = chloroform, 3 = aether, 4 = acetone/aether (1/1), 5 = acetone, 6 = absolute methanol).

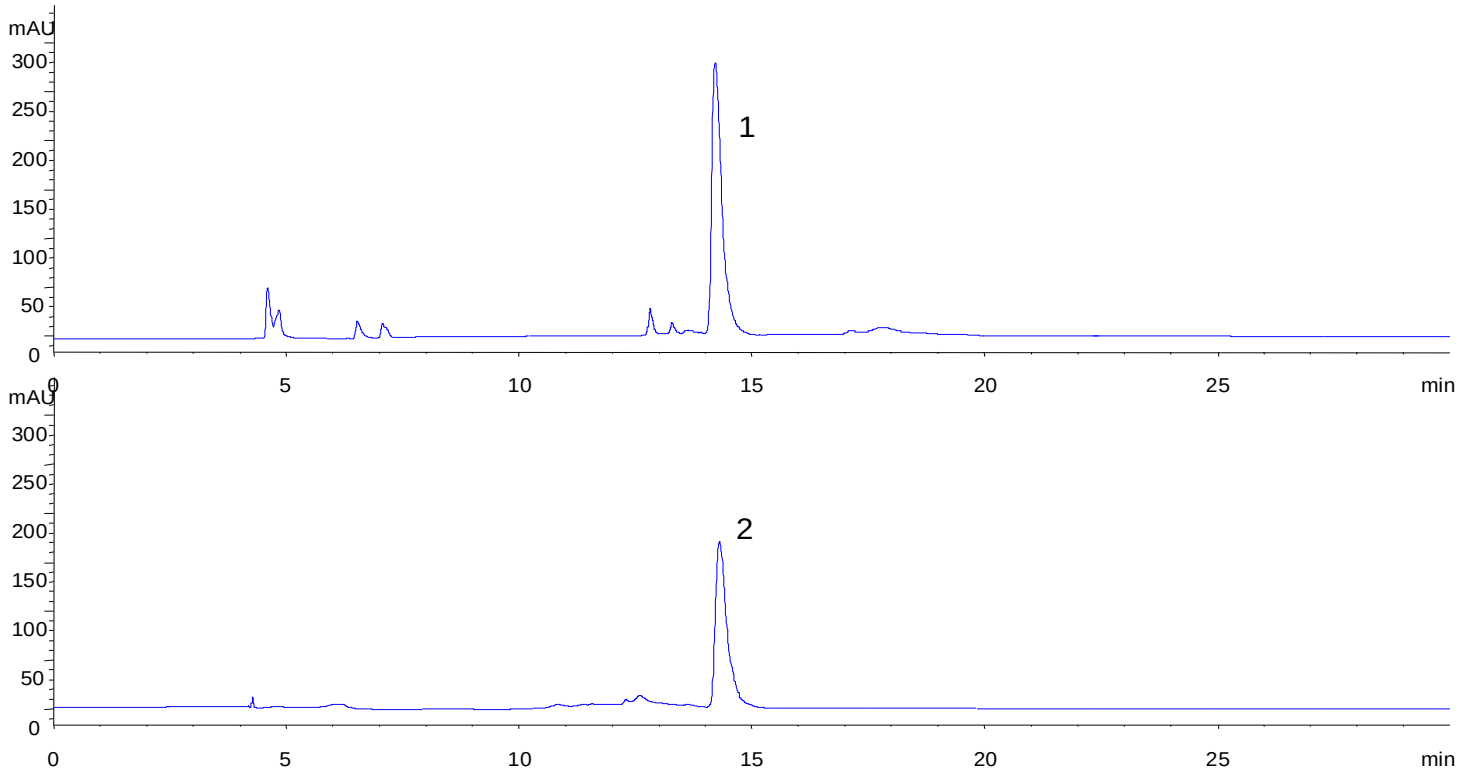

Figure 3. HPLC chromatography of caratenoids from *L. barbarum* fruit extract by absolute ethanol (1. carotenoids extract; 2.β-carotene).

Table 2. [a] Three active components levels of L. barabrum fruits from different producing areas and radical scavenging activity of polyphenols

Producing areas	PS (%)	C (%)	PP (%)	FW	PW	RSA(%)	R
ZN	5.3682^{C}	0.1005^{B}	2.9749^{A}	12.182^{C}	10.4231^{C}	56.82	0.968
SH	6.9639^{B}	0.1535^{A}	2.7081^{B}	13.738^{C}	11.3863^{C}	49.71	
NM	7.2293^{B}	0.0511^{D}	2.54056^{C}	19.713^{A}	16.9451^{A}	46.25	
GM	7.3586^{B}	0.0512^{D}	2.64098^{C}	18.531^{A}	16.3494^{A}	48.80	
DZ	7.4153^{B}	0.0570^{D}	2.63^{C}	17.98^{B}	15.4195^{B}	47.32	
QT	7.3190^{B}	0.0907^{B}	2.40148^{D}	7.984^{D}	7.0674^{D}	45.18	
JH	8.9041^{A}	0.0656^{C}	2.46116^{D}	13.313^{C}	11.8399^{C}	43.43	
JL	7.0099^{B}	0.1642^{E}	1.594^{E}	8.946^{D}	7.1368^{D}	30.82	

[a] : Each value is the mean of five replicates(RSD＜5%); ZN=Zhongning, SH=Shahai, NM=Numhon, GM=Goldmud, DZ=Dazi, QT=Qitai, JH=Jinghe, JL=Lulu; RSA=radical scavenging activity; R=correlation coefficient between polyphenols contents and radical scavenging activity; PS=polysaccharides; C=carotenoids; PP=polyphenols; FW= total weight/100fruits; PW=pulp weight/100fruits；R= correlation coefficient.

was conducted with 5 replicates. Recovery assay provided adequate range of 96.4 to 101.8% (data not shown).

Polyphenol levels and antioxidant activity

In Table 2, fruits of the highest polyphenols contents were from Zhongning (2.9749%), meaning Zhongning is the genuine area for polyphenols of *L. barbarum* fruits. The correlation coefficient between polyphenols contents and anti-oxidant activity of the 70% ethanol extracts was 96.83% which indicated that polyphenols may be the main potential antioxidants in the extracts and polyphenols extracts from Zhongning have highest antioxidant activity.

Table 3. Climatic and soil factors of 8 producing areas of *L. barbarum*.

*	S	Pr	C	M	F	A	D	SOM	N	P	K
ZN	2.972	250	3.349	9.2	153	1190	0.44	2.91	51.53	76.77	312.37
SH	3.200	171.6	3.447	8.5	157	1055	0.60	2.75	45.49	53.73	423.87
NH	3.090	38.9	1.921	4.4	112	2812	0.48	2.29	66.32	66.43	438.03
GM	3.150	45.8	2.014	4.2	140	3250	0.40	2.56	47.43	73.59	443.33
DZ	3.065	450	2.228	7.4	120	3667	0.37	1.90	53.29	65.37	535.75
QT	3.076	176	3.107	4.7	156	792	1.80	4.39	46.87	59.76	357.43
JH	2.800	111.6	3.582	7.2	175	366	0.03	0.52	25.36	18.92	121.10
JL	2.773	532	4.663	13.1	207	18	0.13	10.85	35.75	32.72	327.40

* Producing area codes are the same as those of Table 3. S = Sunshine (Kh/y); Pr = precipitation (mm/y); C = cumulative temperature (≥10°C/y); M = mean annual temperature(°C/y); F = frost-free period (d); A = altitude (m); D = dissoluble salinity (%); SOM = soil organic matter (%); N = available nitrogen (mg/kg); P = available phosphorus (mg/kg); K = available potassium (mg/kg).

Table 4. Linear correlations of *L. barbarum* fruits with climatic and soil factors producing areas.

Variable	PS (%)	C (%)	PP (%)	FW	PW
S*	+0.6402	-0.2260	-0.2323	+0.4738	+0.4685
Pr*	-0.5563	+0.5080	-0.2268	-0.3932	-0.4391
C*	-0.6166	+0.8300	-0.0650	-0.8309	-0.8545
M*	-0. 5641	+0.7931	-0.2919	-0.5150	-0.5684
F*	-0.6943	+0.7248	+0.0803	-0.7905	-0.7983
A*	+0.4678	-0.6950	-0.0294	+0.8699	+0.8717
D*	+0.1841	-0.0050	-0.1456	-0.3969	-0.3706
SOM*	-0.8454	+0.7022	-0.2667	-0.5866	-0.6273
N*	+0.4458	-0.3448	-0.5004	+0.5435	+0.5205
P*	+0.4644	-0.3338	-0.7500	+0.4180	+0.4159
K*	+0.2121	-0.1335	-0.3309	+0.5080	+0.4707

*: The codes are the same as those of Table 3 and 4.

Determination on contents of polysaccharides and carotenoids

Recovery was obtained by adding standard glucose to extracts to obtain polysaccharides. Final concentrations were 9.0, 25.0, and 38.0 µg/ml. The assay was conducted with 5 replicates. Recovery was within 98.7 to105.1% (data not shown). As shown in Table 2, the order of polysaccharides contents is Jinghe (8.9041%) > Qitai (7.3190%), Dazi (7.4153%), Goldmud (7.3586%), Numhon (7.2293%), Shahai (6.9639%), Julu (7.0099%) > Zhongning (5.3682%). So, Jinghe can be considered as the genuine area for polysaccharides of *L. barbarum* fruits.

β-carotene was added into the carotenoids extracts to obtain recovery, final concentration 1.0, 2.5, 4.0 µg/ml. The recovery was 97.45 to 109.3% (data not shown). As is shown in Table 2, the order of carotenoids contents is Julu (0.1642%) > Shahai (0.1535%) > Zhongning (0.1005%), Qitai (0.0907%) > Jinghe (0.0656%) > Numhon (0.0511%), Goldmud (0.0512%), Dazi (0.0570%). Julu proved to be the genuine area for carotenoids of *L. barbarum* fruits.

Correlation between environmental factors and the main active components of *L. barbarum* fruits

Environmental factors of eight producing areas were collected in Table 3. In Table 4, linear correlation analysis showed that polyphenols contents was strongly negatively correlated with SOM (r = 0.8454), which indicated that soil containing relatively low SOM is proper for polyphenols accumulation; carotenoids were positively correlated with effective cumulative temperature (r = 0.83), mean annual temperature (r = 0.7931) and frost-free period (r = 0.7248), meaning that areas of west China with relatively low temperature are not the best for carotenoids accumulation; fruit weight and pulp weight were strongly positively correlated with altitude (r =0.8699, 0.8717, respectively), strongly negatively correlated with cumulative temperature and frost-free

Table 5. Correlation of main active components and fruit weights with eigenvectors of principal factors by rotation and multiple regression.

Characters	P [a]	Extracted principle factors and equations [b]
Polyphenols (%)	0.024	$Y = +0.714 (X_1)^2$, r = 0.964
Carrotenoids (%)	0.000	$Y = +1.641 (X_2)^3$, r = 0.826
Weight/100 fruits (g)	0.000	$Y = -1.791(X_2)^3$, r = 0.953
Pulp/100 fruits (g)	0.000	$Y = -1.848 (X_2)^3$, r = -0.963
Polysaccharides (%)*		$Y = -0.819 (X_3)^3$, r =- 0.750

[a]: P value of the model by tests of KMO and Barteltt; [b]: X_1 = SOM factor (%); X_2 = accumulated temperature, frost-free days each year and altitude; X_3 = available phosphorus; *: analyzed by linear regression independently.

period (r = -0.8309, -0.7905, -0.8545, -0.7983, respectively), at the relatively high altitude, strong sunshine and high temperature variety between day and night are good for photosynthesis and accumulation of dried matters; polysaccharides was not significantly affected by other environmental factors but negatively correlated with soil available phosphorus (r = 0.75). Based on rotation and multiple regression analysis (Table 5), SOM was the principle factor for polyphenols contents (r = 0.964), effective cumulative temperature, frost-free period and altitude were the principle factors significantly affecting carotenoids contents, fruit weight and pulp weight (r = 0.826, 0.953 and 0.963, respectively). Altitude mainly leads to temperature-sunshine changes; so, effective cumulative temperature, frost-free period and altitude can be summed up as temperature-sunshine factor, namely, temperature-sunshine is the principle factor for carotenoids contents, fruit weight and pulp weight.

DISCUSSION

Genuineness of *L. barbarum* fruits and medicinal values

In ancient traditional Chinese medicine system, Zhongning had been authenticated as the genuine producing area where the *L. barbarum* fruits were of the highest medicinal quality. In pharmacopoeia of China (2005), polysaccharides were authenticated as the main active components of *L. barbarum* fruits. In this study, *L. barbarum* fruits from Zhongning showed the highest content of polyphenols (2.9749%) and the highest antioxidant activity (56.82%) with high correlation (r = 0.968), which indicated that content of polyphenols in *L. barbarum* fruits can accurately reflect the anti-oxidant ability and confirmed that Zhongning is the genuine producing area for polyphenols. It was reported that more than 100 human diseases are correlated with oxidation caused by free radicals in the body (Gutteridge, 1993). *L. barbarum* fruits from Zhongning presented the highest content of polyphenols and the highest antioxidant activity; it may be the main reason for the genuineness of *L. barbarum* of Zhongning.

L. barbarum fruits produced in different areas had different essential active components. Among the 8 producing areas, Zhongning proved to be the genuine area for polyphenols production, while polysaccharides genuine producing area was Jinghe, carotenoids genuine producing area was Julu, the highest fruit weight was from Numhon. Fruits from Zhongning contained the highest content of polyphenols, but the lowest content of polysaccharides in the 8 areas. So, polyphenols may be the really main active component rather than polysaccharides as for *L. barbarum* fruits from Zhongning.

Since *L. barbarum* fruits of different areas showed different contents of the main active components, their main medicinal use should be consequently different. Take for example, vitamin A deficiency was a worldwide serious health problem, especially in Africa, South America, Central America, and Southeast Asia (Sommer et al., 1995). *L. barbarum* fruits of Julu should be the best choice for vitamin A supplementation because *L. barbarum* fruits of Julu have the significantly highest content of β-carotene which was the precursor in vitamin A synthesis in human body. While *L. barbarum* fruits of Jinghe should be the best choice for polysaccharides supplementation and extraction (Dong et al., 2008).

Principle factors affecting the genuineness of *L. barbarum* fruits

The optimal environmental factors for each active component are not the same, meaning that each active component has its own genuine producing area. Relatively low SOM is good for polyphenols accumulation; relatively high temperature and low ultraviolet in the sunshine are proper for carotenoids synthesis and accumulation, while low temperature is good for dried matter accumulation hence leading to higher pulp weight. The climatic factors, SOM and dissoluble salinity are not related with polysaccharides. Only soil available phosphorus significantly affected the

content of polysaccharides. Phosphate is mainly stored in membrane system in plants; deficiency of phosphate may lead to the membrane phosphate transferred to growth center, which resulted in degradation of phospholipids, while galactose acts as substitute for phospholipids to maintain the normal structure and functions of membrane (Kochlan et al., 2004). Sun et al. (1997) reported that 69% of polysaccharides composition in *L. barbarum* fruits was galactose, which confirmed the mechanism that deficiency of phosphorus leads to polysaccharides accumulation.

Conclusion

Extraction with UAE proved to be the proper sample preparation for *L. barbarum* fruits. β-carotene is the main component in carotenoids of *L. barbarum* fruits, which can be a good source for VAD functional food. Absolute ethanol is the best solvent for carotenoids extraction concerning safety and efficiency. A direct UV determination of carotenoids from absolute ethanol extracts of *L. barbarum* fruits is convenient and accurate.

Contents of the main active components of *L. barbarum* fruits from the 8 areas were significantly different. So, medicinal values of *L. barbarum* fruits from different producing areas might not be the same.

Polyphenols of *L. barbarum* fruits from Zhongning were the main active components rather than polysaccharides. *L. barbarum* fruits of Julu should be the best selection for carotenoids supplementation because of highest β-carotene content, while *L. barbarum* fruits from Jinghe containing the highest content of polysaccharides should be the best source for polysaccharides supplementation or extraction.

Environmental factors have significant effects on genuineness and the main active components of *L. barbarum* fruits. The principle factors of polysaccharides, carotenoids and polyphenols are soil available phosphorus, temperature-sunshine and SOM, respectively. These principle factors provide a basis for scientific division of producing areas and quality control for *L. barbarum*.

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