

## Research Article

# A Simple HPLC-ELSD Method for Sugar Analysis in Goji Berry

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Received 12 November 2015; Accepted 10 January 2016

Academic Editor: Patricia Valentao

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Fructose, glucose, and sucrose were identified and quantified in commercial samples of *Lycium barbarum* L. fruits (goji berries) by high performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD) method. This study described a rapid, simple, sensitive, selective, and reliable HPLC method suitable for the profiling of major sugars in berries, the evaluation of the nutritional/energetic properties, and assessment of the maturation stage. The proposed analytical method was validated and the results showed good precision, accuracy, and linearity. In all analyzed goji fruits, glucose and fructose were the predominant sugars, while sucrose content was about ten times lower than each monose. It was observed that glucose and fructose were detected in comparable quantities in all considered samples. Quantitative analysis showed that fructose, glucose, and sucrose content ranged from 154.20 to 259.13 mg g<sup>-1</sup>, from 152.92 to 284.60 mg g<sup>-1</sup>, and from 13.75 to 36.43 mg g<sup>-1</sup>, respectively.

## 1. Introduction

Recently, the interest in the composition of berry fruits has grown because of increased awareness of their possible health benefits, as they are rich sources of sugars, micronutrients, and other important substances such as phytochemicals. In fact, recent studies have revealed that berry species have essential positive effects on the human health which could be mainly ascribed to the presence of several taste and health related compounds such as sugars, phenolics, and organic acids [1, 2]. *Lycium barbarum* L. is a shrub from the family Solanaceae coming from Southeast Asia and is mostly grown in China [3]. Its fruit, goji berry, has become more popular for the last few years due to its public acceptance as a “super food” with highly advantageous nutritive; in fact this fruit is a rich source of sugars with interesting nutritional value [4]. Moreover, sugars, the main soluble constituents of goji berry, have a major effect on taste and represent an index of consumer acceptability. Modern consumers are increasingly interested in their personal health and expect the foods to be not only tasty and attractive but also safe and healthy. The fruit of *L. barbarum* has a variety of important biological functions, such as enhancing immunity, antiaging, antitumor, and antioxidant. *L. barbarum* polysaccharides and total sugars in the fruit constitute the major aspect of the

medicinal quality of goji fruit; for this reason the evaluation of its sugar composition is important [5].

The sucrose metabolism-related sugars in fruits mainly include glucose, fructose, and sucrose [6]. There are some scientific evidences that sucrose is the primary form of transported sugar in tomatoes [7, 8], but it only accounts for a small percentage, while glucose and fructose, in approximately equal contents, reach over 50% of the total water-soluble sugars in fruits [9].

In relation to the analytical aspects, amino-bonded phase HPLC with refractive index (RI) detectors has been extensively used for sugar analysis [10]. However, evaporative light scattering detector (ELSD) presents numerous advantages compared to RI detector, such as the sensitivity, the stability, and the compatibility with gradient elution [11]. In the present study, a simple, rapid, sensitive, selective, and reliable HPLC method using ELSD was developed and validated. Then it was successfully used for the simultaneous determination of glucose, fructose, and sucrose in commercial samples of goji berries.

## 2. Materials and Methods

**2.1. Goji Samples.** Sixteen commercial samples of goji berries (*L. barbarum*) were collected in 2015 from different sellers

TABLE I: Goji berry samples.

Sample	Purchasing	Origin	Selling size	Species
I	Herbalist's shop	Ningxia-RPC	By weight (100 g)	Unspecified
II	Herbalist's shop	Ningxia-RPC	By weight (100 g)	<i>L. barbarum</i>
III	Drugstore	Tibet	By weight (100 g)	<i>L. barbarum</i>
IV	Herbalist's shop	Ningxia-RPC	By weight (100 g)	<i>L. barbarum</i>
V	Drugstore	Agriculture no EU	Plastic bag (100 g)	Unspecified
VI	Herbalist's shop	Tibet	By weight (100 g)	<i>L. barbarum</i>
VII	Herbalist's shop	Ningxia-RPC	By weight (100 g)	<i>L. barbarum</i>
VIII	Herbalist's shop	Tibet	By weight (100 g)	Unspecified
IX	Herbalist's shop	Tibet	By weight (100 g)	<i>L. barbarum</i>
X	Supermarket	Agriculture no EU	Plastic bag (80 g)	<i>L. barbarum</i>
XI	Supermarket	China	Plastic bag (80 g)	<i>L. barbarum</i>
XII	Supermarket	Not reported	Plastic bag (35 g)	Unspecified
XIII	Herbalist's shop	Ningxia-RPC	By weight (100 g)	<i>L. barbarum</i>
XIV	Drugstore	Mongolia	Bag with pressure lock (100 g)	<i>L. barbarum</i>
XV	Supermarket	China	Plastic container (150 g)	<i>L. barbarum</i>
XVI	Supermarket	Qinghai-Tibetan Plateau	Plastic bag (100 g)	Unspecified

(herbalist's shops, drugstores, and supermarkets). Some information about the samples investigated in this work has been described in Table 1. The samples were stored in a dry place in the dark at room temperature until analyses. The samples were tested shortly after opening of the package and then immediately closed and left at room temperature in a dry place in the dark.

**2.2. Reagents.** HPLC grade acetonitrile (ACN), chloroform ( $\text{CHCl}_3$ ), and analytical grade reference compounds, D(-)-fructose ( $\geq 99\%$  GC), D-(+)-glucose ( $\geq 99.5\%$  GC), and D-(+)-sucrose ( $\geq 99.5\%$  GC), were purchased from Sigma-Aldrich (Milano, Italy), whereas deionized water ( $>18$  MX cm resistivity) was obtained from Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA).

**2.3. Preparation of Standard Solution.** Stock solutions of fructose, glucose, and sucrose were prepared in bidistilled water by weighing 2.5231, 2.5120, and 2.5363 g, respectively, and bringing the volume to 100 mL. The standard solution was diluted to five appropriate concentrations ranging from 2.50 to 0.31  $\text{mg mL}^{-1}$ . Stock solutions of the sugars were stable for 1 month at  $4^\circ\text{C}$  in the refrigerator. The working standard solutions were prepared as needed by appropriate dilutions of the concentrated stock solutions in the bidistilled water. Standard solutions were prepared fresh daily.

**2.4. HPLC Instrumentation and Chromatographic Condition.** The HPLC analyses were carried out using a LC-10ADvp pump (Shimadzu Corp., Kyoto, Japan) and ELSD (Sedex 55, S.E.D.E.R.E., France), operating at  $60^\circ\text{C}$  and nitrogen pressure of 230 kPa. Standard and sample solutions (20  $\mu\text{L}$ ) were directly injected on a Platinum Amino column (5  $\mu\text{m}$ , 250 mm  $\times$  4.6 mm i.d.; Grace, Lokeren, Belgium), maintained at a temperature of  $20^\circ\text{C}$  in a column oven (CTO-10ASvp, Shimadzu).

HPLC analysis was carried out by isocratic elution for 20 minutes using the mixture ACN :  $\text{H}_2\text{O}$  (80 : 20, v/v) at a

flow rate of  $1.2 \text{ mL min}^{-1}$ . The mobile phase was degassed by ultrasonic bath (Astrason Heat Systems, New Highway, Farmingdale, NY) prior to use. After each analysis, the column was washed with water. The chromatograms were acquired and the data handled using the Class-VP software (Shimadzu). All solutions of samples were filtered through 0.45 nylon membrane (Advanced Microdevices Pvt. Ltd., Ambala, India) before use.

**2.5. Calibration Curves and Limit of Detection and Quantification.** Twenty  $\mu\text{L}$  of each solution was injected in duplicate for the construction of calibration curves. The calibration curves were obtained by plotting peak area versus concentration of each sugar. The linearity was evaluated by linear regression analysis calculated by the least square regression method. The limits of detection (LOD) and limits of quantification (LOQ) were calculated according to the statistical method reported in Federal Register [12]. The LOD and LOQ values were experimentally verified by injections of standard solutions of the compounds at the LOD and LOQ concentrations.

**2.6. Accuracy.** The recovery test was used to evaluate the accuracy of the method. The recovery was evaluated after extraction of analytes from some selected goji berry samples spiked with a mixture of known amounts of fructose, glucose, and sucrose standards (1.0, 2.0, and 3.0  $\text{mg mL}^{-1}$ ). Then the spiked analytes were analyzed in triplicate as reported in Section 2.4 in order to assess the effectiveness and accuracy of the extraction step.

The percentage of recovery was calculated by using the following formula:

$$\text{Recovery (\%)} = \frac{[\text{analyte}]_{\text{spiked}} - [\text{analyte}]_{\text{unspiked}}}{[\text{analyte}]_{\text{added}}} \times 100, \quad (1)$$

where  $[\text{analyte}]_{\text{spiked}}$  is the recorded amount of sugar observed in the spiked sample,  $[\text{analyte}]_{\text{unspiked}}$  is the amount

TABLE 2: Regression parameters and LOD and LOQ for fructose, glucose, and sucrose.

Sugar	Retention time (min)	Regression equation	Linearity range (mg mL <sup>-1</sup> )	r <sup>2</sup>	LOD (μg)	LOQ (μg)
Fructose	7.25	y = 4100.34x - 642.06	0.31-2.50	0.9986	1.2	3.6
Glucose	8.37	y = 3699.65x - 603.23	0.31-2.50	0.9981	1.2	3.6
Sucrose	13.59	y = 3654.10x - 463.42	0.31-2.50	0.9998	1.0	3.0

of sugar observed in the unspiked sample, and [analyte]<sub>added</sub> is the amount of the sugar standards added in the spiked sample.

**2.7. Precision.** The intraday and interday precision of method were evaluated by six replicate injections of the standard solution (containing 0.62 mg mL<sup>-1</sup> of each analyte) for three consecutive days. The repeatability and reproducibility of the developed method were assessed by evaluating the intra- and interday percent relative standard deviation (% RSD) of retention times ( $t_R$ ), peak areas ( $P_A$ ), and sugar concentrations ( $C_S$ ).

**2.8. Sample Preparation for the Determination of Sugars.** The extraction of sugars from goji berry samples was adapted from a method described by Broeckling et al. [13]. For this, 5 g of goji berry tissue was homogenized in blender (Oster, model 869-50R, USA) for 2 minutes using 50 mL of bidistilled water; after that an equal volume of CHCl<sub>3</sub> (50 mL) was added. The obtained mixture was vortexed for 5 minutes and then incubated at 50°C for 30 minutes under magnetic stirring (F80 Model, FALC Instruments s.r.l., Treviglio, Bergamo, Italy). The extract was followed by centrifugation at 6500 rpm for 10 minutes; then the supernatant was separated and transferred to 250 mL flask and subjected to Rotavapor (B-480 Model, Büchi, Italy) for 5 minutes and successively to nitrogen flow for 10 minutes for removing CHCl<sub>3</sub> traces. All solutions of samples were filtered through 0.45 nylon membrane (Advanced Microdevices Pvt. Ltd., Ambala, India) and then analyzed in HPLC, as described in Section 2.4.

**2.9. Statistical Analysis.** The results of the analyses are expressed as the mean value and standard deviation (SD) based on three replicates. Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) was used for data analysis.

### 3. Results and Discussion

This study presents an analytical HPLC method for the simultaneous analysis and quantification of the major sugars present in goji berries. The representative chromatograms for the standard mixture and sample of goji berry, using Platinum column operating with ACN:H<sub>2</sub>O (80:20, v/v) as mobile phase at 20°C, are shown in Figures 1 and 2, respectively. The chromatographic peaks were identified by comparing their retention times with reference compounds and spiking of samples with the reference compounds. The chromatograms reported in Figures 1 and 2 showed that sugars were well

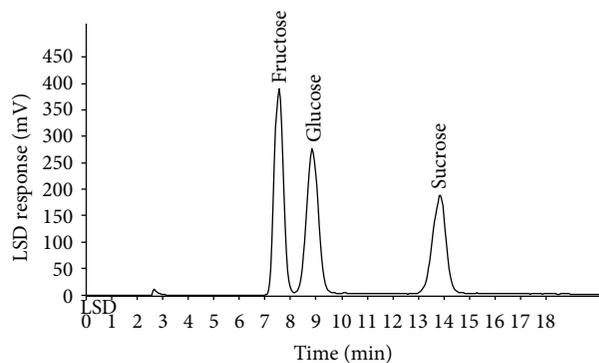


FIGURE 1: HPLC-ELSD profile of fructose, glucose, and sucrose standard compounds.

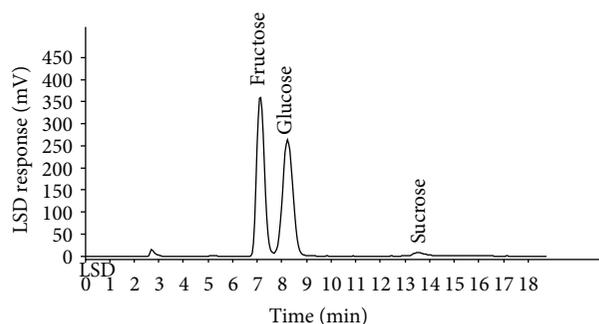


FIGURE 2: HPLC-ELSD profile of a sample of goji berries.

resolved and then their quantitative determination in goji berries was possible.

**3.1. Method Validation.** The reliability of the method in terms of linearity, precision, and accuracy was evaluated. In Table 2 the regression equations and LOD and LOQ for fructose, glucose, and sucrose are shown. The method allowed the separation of all analytes in short time (15 min). The linear regression performed for each compound gave a regression coefficient ( $r^2$ ) higher than 0.9980 (Table 2), which showed a linear relationship between the chromatographic response areas and concentrations for all compounds.

In Table 3, the results of precision and recovery for the considered compounds are reported. Day-to-day variations of retention times, peak areas, and analyte concentrations were determined on three different days using standard solution containing fructose, glucose, and sucrose. The results showed that intra- and interday % RSD of the retention times, peak areas, and concentrations had low values (less than

TABLE 3: Precision and recovery data for retention times, peak areas, and sugar concentrations.

Sugar	Intraday precision ( $n = 6$ , mean), % RSD									Interday precision ( $n = 18$ , mean), % RSD			Recovery (%)		
	Day 1			Day 2			Day 3			$t_R^a$	$P_A^b$	$C_S^c$	Spiked amount ( $\text{mg mL}^{-1}$ )	Mean	% RSD
$t_R^a$	$P_A^b$	$C_S^c$	$t_R^a$	$P_A^b$	$C_S^c$	$t_R^a$	$P_A^b$	$C_S^c$							
Fructose	0.10	0.41	0.24	0.11	0.52	0.28	0.14	0.65	0.34	0.13	0.41	0.37	1.0	95.4	1.12
													2.0	97.0	
														3.0	
Glucose	0.26	0.79	0.97	0.28	0.74	0.92	0.31	0.76	0.95	0.25	0.82	1.07	1.0	98.2	1.02
													2.0	99.0	
														3.0	
Sucrose	0.11	0.26	0.15	0.12	0.32	0.12	0.14	0.29	0.19	0.16	0.36	0.21	1.0	95.6	1.21
													2.0	97.0	
														3.0	

<sup>a</sup>% RSD of retention time.

<sup>b</sup>% RSD of peak area.

<sup>c</sup>% RSD of sugar concentration.

1.07%). Recovery studies were carried out to determine the accuracy of the method; a goji berry sample was analyzed before and after the addition of known amounts of fructose, glucose, and sucrose. The results indicated that the developed analytical method had good accuracy, with satisfactory recovery (95.4–99.0%) for the considered analytes. Therefore, the developed method had good linearity, precision, and accuracy for the simultaneous quantitative evaluation of fructose, glucose, and sucrose in fruit of *L. barbarum*.

**3.2. Optimization of Sample Extraction Method.** In order to optimize the extraction yield, a method was adapted from Broeckling et al. [13] and was modified to obtain the best conditions for HPLC analysis. Sample aliquot of 5 g was used in order to extract reproducibly sugars from commercial goji berries. This fruit is present on market as “sun-dried” product and in this condition any analysis with good reproducibility is very hard to perform. In fact, due to the gluey nature of the fruits, grinding is very difficult; therefore classical mill pulverization is not feasible, whereas anhydrication technique such as lyophilization does not resolve the problem [14]. The described method used the berries without pretreatments; in fact the fruits were directly homogenized with bidistilled water; in this way both homogenization and a first extraction step were carried out. The second step was performed using the same volume of chloroform with respect to water at 50°C for 30 minutes; this was an important clean-up step to complete the extraction procedure; in fact the goji fruit is a very complex matrix with a significant amount of pigments and lipids that are extracted with chloroform, the discarded phase. Then, at the end of clean-up step (after 30 min), the sugar content was totally extracted from goji berries and successively it was possible to perform the HPLC analysis.

**3.3. Optimization of HPLC-ELSD Conditions.** For the best results, the following detector parameters were optimized.

**3.3.1. Mobile Phase and Column Temperature.** Different ratios of acetonitrile and water were tested. In general, by increasing water percentage in the mobile phase, the retention times

were lower, but the resolution decreased. So the optimum composition of the mobile phase was selected as ACN:H<sub>2</sub>O (80:20, v/v).

Column temperature was regulated at 20°C. In this condition the background noise was eliminated considerably and an improved response value was observed.

**3.3.2. Drift Tube Temperature.** The HPLC analysis with light scattering technique offers the advantage to detect the organic molecules by their mass and hence it is useful in quantitative determination of non-UV sensitive compounds. An important parameter for optimizing ELSD conditions is the drift tube temperature [15]. Good peak symmetries and efficiencies were obtained by means of a drift tube temperature at 60°C.

**3.4. Application for the Determination of Sugars in Goji Berries.** The developed HPLC-ELSD method was applied to analyze fructose, glucose, and sucrose in sixteen samples of goji berries. In all samples fructose, glucose, and sucrose were successfully identified in 15 minutes (Figure 2). Table 4 reports the content of sugars in goji samples examined in this study. In all samples, fructose and glucose were recorded in large amount (154.20–259.13 mg g<sup>-1</sup> and 152.92–284.60 mg g<sup>-1</sup>, resp.), while sucrose content was about ten times lower (13.75–36.43 mg g<sup>-1</sup>) than each monose. Mikulic-Petkovsek and collaborators [16] reported 23.1 g kg<sup>-1</sup> for both fructose and glucose and 0.38 g kg<sup>-1</sup> for sucrose, but they considered fresh cultivated berries. Zheng and collaborators [17], instead, reported content similar to our results and underlined the different content of hexoses with respect to sucrose: the former accounted for 97.92% of the total content of the three sugars plus the starch (0.026%), while the latter accounted for only 1.82%. As the results obtained in this study are in agreement with those reported by the cited authors for ripe fruits, we can conclude that all the analyzed goji samples reached the ripening stage. This research helps expand knowledge on goji simple sugars, since very few data are available in literature. In fact most of the studies focused

TABLE 4: Fructose, glucose, and sucrose contents ( $\text{mg g}^{-1}$ , mean values  $\pm$  SD,  $n = 3$ ) in goji samples.

Sample	Fructose	Glucose	Sucrose
I	186.02 $\pm$ 1.84	183.42 $\pm$ 1.91	21.84 $\pm$ 0.92
II	196.53 $\pm$ 0.71	180.34 $\pm$ 1.84	21.12 $\pm$ 0.85
III	214.41 $\pm$ 9.83	183.52 $\pm$ 25.39	33.93 $\pm$ 2.83
IV	193.14 $\pm$ 3.82	172.11 $\pm$ 0.92	19.75 $\pm$ 0.99
V	178.60 $\pm$ 19.59	182.34 $\pm$ 19.59	28.70 $\pm$ 1.27
VI	194.02 $\pm$ 7.07	189.72 $\pm$ 3.11	23.32 $\pm$ 0.00
VII	208.34 $\pm$ 0.00	203.15 $\pm$ 3.75	24.91 $\pm$ 0.00
VIII	202.44 $\pm$ 20.22	179.80 $\pm$ 18.46	20.22 $\pm$ 1.70
IX	154.20 $\pm$ 1.20	152.92 $\pm$ 0.57	13.75 $\pm$ 0.64
X	243.43 $\pm$ 27.79	243.41 $\pm$ 17.82	20.60 $\pm$ 1.27
XI	239.54 $\pm$ 3.32	259.72 $\pm$ 1.70	34.61 $\pm$ 1.68
XII	233.21 $\pm$ 16.33	221.14 $\pm$ 8.84	19.34 $\pm$ 1.34
XIII	184.23 $\pm$ 2.76	171.63 $\pm$ 3.68	18.50 $\pm$ 1.84
XIV	236.20 $\pm$ 6.72	222.01 $\pm$ 0.00	22.81 $\pm$ 1.13
XV	190.81 $\pm$ 0.00	208.52 $\pm$ 6.29	36.43 $\pm$ 1.27
XVI	259.13 $\pm$ 2.76	284.60 $\pm$ 6.51	26.25 $\pm$ 1.84

on the polysaccharide fraction [18, 19], characterized by a variety of biological activities.

#### 4. Conclusions

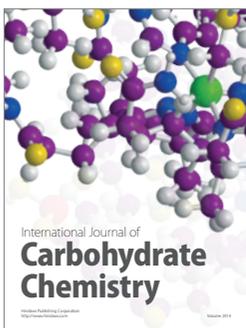
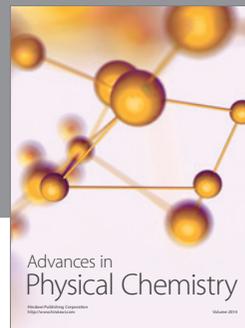
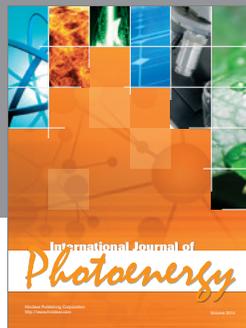
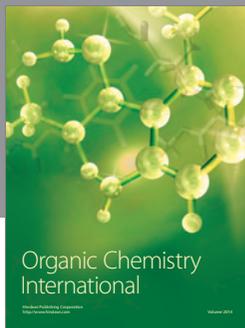
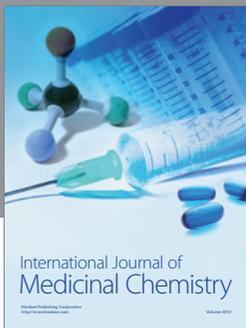
This study presents an analytical HPLC method for the simultaneous analysis and quantification of the major sugars in goji berries. By the obtained results we can conclude that this method is suitable to separate and quantify fructose, glucose, and sucrose without derivatization in less than 15 minutes. The regression equations revealed good linear relationships ( $r^2 = 0.9981\text{--}0.9998$ ) within test ranges. Moreover, LOD and LOQ for the considered analytes were in the range of 1.0–1.2  $\mu\text{g}$  and 3.0–3.6  $\mu\text{g}$ , respectively. The results of analysis carried out on sixteen goji samples were in good agreement with those previously obtained by other authors. Then, the proposed HPLC-ELSD method can be used to quantify simultaneously fructose, glucose, and sucrose in goji fruits.

#### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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