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# Determination of Fructooligosaccharides in Burdock Using HPLC and Microwave-Assisted Extraction

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**ABSTRACT:** The root of burdock (*Arctium lappa* L.) is a commonly used vegetable in Asia. Fructooligosaccharides (FOS) are usually considered as its main bioactive components. Thus, quantitative analysis of these components is very important for the quality control of burdock. In this study, an HPLC-ELSD and microwave-assisted extraction method was developed for the simultaneous determination of seven FOS with degrees of polymerization (DP) between 3 and 9, as well as fructose, glucose, and sucrose in burdock from different regions. The separation was performed on a Waters XBridge Amide column (4.6 × 250 mm i.d., 3.5 μm) with gradient elution. All calibration curves for investigated analytes showed good linear regression ($r$ > 0.9990). Their LODs and LOQs were lower than 3.63 and 24.82 μg/mL, respectively. The recoveries ranged from 99.2 to 102.6%. The developed method was successfully applied to determination of ten sugars in burdock from different locations of Asia. The results showed that the contents of FOS in different samples of burdock collected at appropriate times were similar, and the developed HPLC-ELSD with microwave-assisted extraction method is helpful to control the quality of burdock.

**KEYWORDS:** *burdock, HPLC-ELSD, microwave-assisted extraction, carbohydrates, fructooligosaccharide*

## INTRODUCTION

Burdock (*Arctium lappa* L.) root, called "Niubang" in Chinese and "Gobo" in Japanese, has been known as a traditional Chinese medicinal and an edible perennial plant in China.[1] It has also been used therapeutically in Europe, North America, and Asia for hundreds of years. The plant has been cultivated as a vegetable in Asia, especially in China, Japan, and Korea for many years,[2] and the root is traditionally used in herbal remedies to treat tonsillitis, throat pain, arthritis, rashes, and various skin problems and as a diuretic, diaphoretic, and blood purifier.[3] Burdock usually contains abundant amounts of fructooligosaccharides (FOS), which are indigestible and cannot be absorbed in the human small intestine, but they do promote the proliferation of beneficial bacteria, particularly bifidobacteria, in the large intestine.[4,5] In addition, FOS has the characteristics of suppressing putrefactive pathogens and reducing the levels of blood glucose, serum cholesterol, phospholipids, and triglycerides.[6,7] Recently, cognitive-improving and cerebral protective effects of FOS have also been reported[8−10] Therefore, the content of FOS is the main indicator for the quality of burdock.

A series of methods, including colorization (phenol−sulfuric acid, dinitrosalicylic acid) method,[4] anion exchange chromatography (AEX),[11−13] and hydrophilic interaction chromatography coupled with refractive index detection[2] have been developed for the determination of sugars in burdock. However, the colorization method can only determine the content of total sugars, and the specificity is poor. AEX needs specific instruments and columns. The HPLC-RID method usually suffers from poor resolution because gradient elution is not available for RID, leading to poorly sensitive detection. Actually, evaporative light scattering detector (ELSD) is a universal detector, which has been widely applied in detecting analytes without UV absorption.[14,15]

In this study, an HPLC-ELSD and microwave-assisted extraction method was developed for the simultaneous determination of seven FOS with degrees of polymerization (DP) between 3 and 9, as well as glucose, fructose, and sucrose, in burdock from different regions.

## MATERIALS AND METHODS

**Chemicals, Reagents, and Materials.** Burdock was collected, respectively, from Jiangsu (A), Shandong (B), Heilongjiang (C), Henan (D and F), Jilin (E), and Gansu (G), China, as well as Korea (H). Among them, samples A, B, G, and H were cultivated, and the others (C, D, E, and F) were wild. Especially, sample C was obtained after seed collection. The materials were dried below 50 °C and then ground into fine powder. Fructose, glucose, and sucrose were purchased from Sigma (Steinheim, Germany). FOS (DP3−DP9) with DP between 3 and 9 (all purities determined by HPLC-DAD-ELSD were >95%) were separated and purified in our laboratory (Figure 1). The structures were confirmed by comparing their methylation, MS, and NMR data with the literature.[16,17] Acetonitrile for HPLC was purchased from Merck (Darmstadt, Germany). Purified water for HPLC was prepared using a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA). All other chemicals and reagents were of analytical grade.

**Preparation of Standard Solutions.** Mixed standard stock solution containing fructose, glucose, sucrose, fructooligosaccharides (DP3−DP9) was prepared in 60% ethanol. The concentrations of fructose, glucose, sucrose, and DP3−DP9 were about 10 mg/mL. The standard stock solution was stored in a refrigerator at 4 °C before use.

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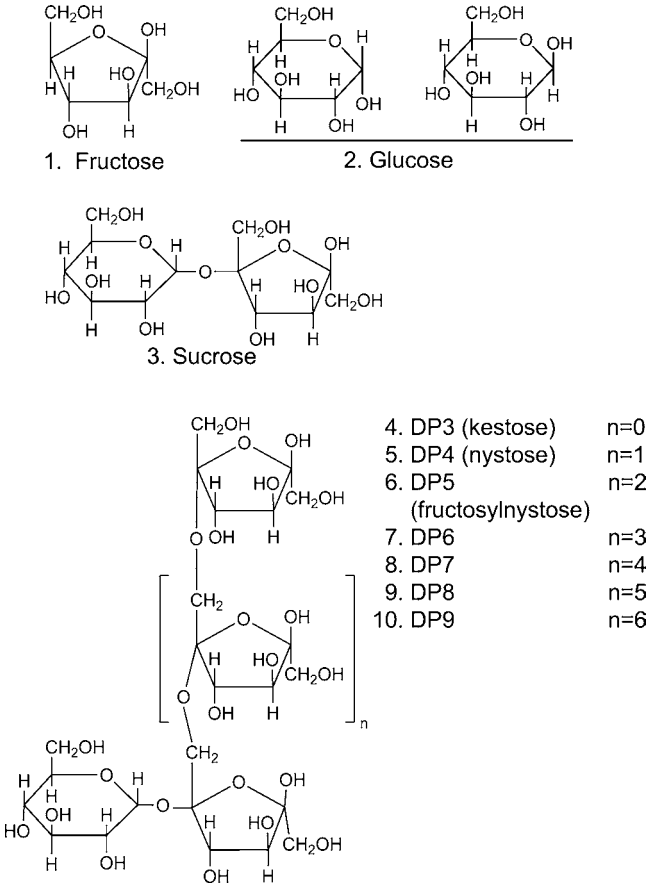


**Figure 1.** Structures of the 10 investigated sugars.

Working standard solutions were prepared from the stock solution by dilution with the appropriate volume of 60% ethanol.

**Sample Preparation.** Burdock powder (0.5 g, 40 mesh) was extracted by microwave-assisted extraction (Sineo Microwave Chemistry Technology Co. Ltd., Shanghai, China) under the optimized conditions. In brief, the extraction was performed at a power of 800 W with 10 mL of ethanol/water (60:40, v/v) for 10 min at the temperature of 80 °C. After centrifugation, 1 mL of supernatant was transferred into a 10 mL volumetric flask and made up to the volume with extraction solvent. Then the solution was filtered through a 0.45 $\mu$m filter before injection into the HPLC system for analysis.

**HPLC-ELSD Analysis.** All of the analyses were performed on an Agilent 1200 liquid chromatography system (Agilent Technologies, Palo Alto, CA, USA), equipped with a double pump, an online degasser, an autosampler, and a column temperature controller and with an analytical workstation (Chemstation for LC 3D Systems A10.02). The separations were carried out on a Waters XBridge Amide column (4.6 × 250 mm i.d., 3.5 $\mu$m). The column temperature was set at 30 °C. The mobile phase consisted of water (A) and acetonitrile (B) with gradient elution: 80−65% B at 0−20 min, 65−45% B at 20−35 min, 45−80% B at 35−40 min. The flow rate was 1.0 mL/min, and the injection volume was 10 $\mu$L. ELSD detector was from the Shimadzu (ELSD LT-II, Shimadzu Corp., Japan). The drift tube temperature was set at 50 °C, and the value of the gain was 8.

**Calibration Curves, Limit of Detection (LOD), and Limit of Quantification (LOQ).** Standard stock solutions containing reference compounds were prepared and diluted to appropriate concentrations for the construction of calibration curves. Six concentrations of 10 analyte solutions were injected in triplicate, and then the calibration curves were constructed by plotting the peak areas against the concentrations of each analyte.

Stock solutions were diluted to a series of appropriate concentration with 60% ethanol, and an aliquot of the diluted solutions was injected into the HPLC for determining the LOD and LOQ. The LOD and LOQ under the present chromatographic conditions were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively.

**Precision, Repeatability, Accuracy, and Stability.** Intraday variation was chosen to determine the precision of the method, and the mixed standards solutions were analyzed for six replicates within one day. The repeatability of the developed method was confirmed with six parallel samples (sample B) preparation and analysis. Stability was tested and analyzed at 0, 1, 2, 4, 8, 12, and 24 h. To check the accuracy of the developed method, the recovery experiments were carried out as follows: three different quantities (low, medium, and high) of the standards were spiked into the samples in the form of solution. The spiked samples were extracted, processed, and quantified in accordance with methods mentioned above.

## RESULTS AND DISCUSSION

**Optimization of Sample Preparation.** For optimizing the sample preparation, different extraction solvents (water and 20, 60, and 100% absolute ethanol) were investigated using universal design. The results showed that the extraction efficiencies of FOS using $H_2O$ or 20 and 60% ethanol as solvent were similar, and obviously higher than that of ethanol (Figure 2A). To reduce the extraction of water-soluble impurities such as polysaccharides and proteins, 60% ethanol was selected for sample preparation. Furthermore, different methods, including ultrasonic extraction, microwave-assisted extraction, and refluxing, were compared for the extraction of FOS in burdock. The results revealed that the extraction efficiency of microwave-assisted extraction was the highest (Figure 2B).

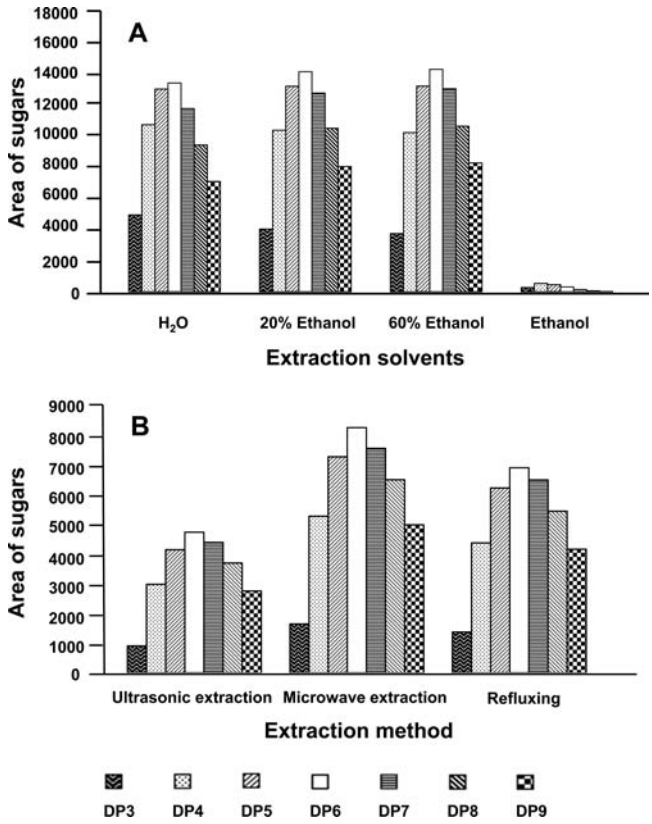


**Figure 2.** Effect of extraction solvent (A) and method (B) on the extraction efficiency of fructooligosaccharides with different degrees of polymerization (DP) in burdock.

Especially, the other parameters, including solvent volume (ratio of solid to liquid, 1:20, 1:30, and 1:40), extraction


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**Table 1. Design Matrix Based on L9($3^4$) Orthogonal Array and Measured Responses**

| run | ratio of solid to liquid | extraction no. | extraction time (min) | exaction temperature (°C) | total sugars (mg/g) |
|---|---|---|---|---|---|
| 1 | 1:20 | 1 | 10 | 80 | 422.0 |
| 2 | 1:20 | 2 | 20 | 90 | 360.5 |
| 3 | 1:20 | 3 | 30 | 100 | 280.7 |
| 4 | 1:40 | 1 | 20 | 100 | 319.3 |
| 5 | 1:40 | 2 | 30 | 80 | 284.4 |
| 6 | 1:40 | 3 | 10 | 90 | 267.0 |
| 7 | 1:60 | 1 | 30 | 90 | 309.1 |
| 8 | 1:60 | 2 | 10 | 100 | 255.3 |
| 9 | 1:60 | 3 | 20 | 80 | 279.9 |
| $K_1$ | 354.4 | 350.1 | 314.7 | 328.8 | |
| $K_2$ | 290.2 | 300.1 | 319.9 | 312.2 | |
| $K_3$ | 281.4 | 275.9 | 291.4 | 285.1 | |
| R[a] | 73. 0 | 74.2 | 28.5 | 43.7 | |

[a]R, extreme difference among $K_1$, $K_2$, and $K_3$.

**Table 2. Linear Regression Data, LOD, and LOQ of the Investigated Compounds**

| analyte | regression eq | $R^2$ | linear range ($\mu$g/mL) | LOD ($\mu$g/mL) | LOQ ($\mu$g/mL) |
|---|---|---|---|---|---|
| fructose | $Y = 1.696X - 2.790$ | 0.9984 | 80.0−1500.0 | 3.6 | 23.3 |
| glucose | $Y = 1.755X - 2.725$ | 0.9989 | 40.0−1400.0 | 2.6 | 24.8 |
| sucrose | $Y = 1.664X - 2.365$ | 0.9985 | 26.0−1050.0 | 1.8 | 13.6 |
| DP3 | $Y = 1.666X - 2.559$ | 0.9994 | 40.0−1400.0 | 2.8 | 17.6 |
| DP4 | $Y = 1.704X - 2.484$ | 0.9980 | 20.0−900.0 | 2.3 | 17.4 |
| DP5 | $Y = 1.686X - 2.534$ | 0.9988 | 30.0−1150.0 | 2.4 | 17.8 |
| DP6 | $Y = 1.694X - 2.509$ | 0.9983 | 30.0−1000.0 | 2.5 | 16.6 |
| DP7 | $Y = 1.684X - 2.561$ | 0.9988 | 30.0−1150.0 | 2.5 | 18.7 |
| DP8 | $Y = 1.673X - 2.579$ | 0.9984 | 40.0−1500.0 | 1.3 | 9.5 |
| DP9 | $Y = 1.698X - 2.448$ | 0.9989 | 20.0−950.0 | 1.9 | 15.1 |

number (1, 2 and 3), extraction time (10, 20, and 30 min), and extraction temperature (80, 90, and 100 °C) at three levels, were studied using Taguchi L9($3^4$) orthogonal design.[18] The sequence of experiments carried out was randomized to avoid any kind of personal or subjective bias.

The data of the orthogonal test are summarized in Table 1. The result ($R$ values) showed that the effect of the four parameters on extraction efficiency of sugars decreased in the order B ≈ A > D > C. The optimum method should be at the parameters of A1B1C2D1, but statistical significance could not be found. Actually, the overall $F$ for all parameters was <6.9, which was much less than the critical value, $F = 19.0$ ($n = 2$). Considering the ideal sample preparation should be accurate, rapid, cheap, and convenient, the optimized microwave-assisted extraction method was solvent, 60% ethanol; solvent volume, 20-fold to sample material (v/v); extraction number, 1; extraction time, 10 min; temperature, 80 °C.

**Table 3. Precision, Repeatability, Stability, and Recovery of the 10 Analytes**

| analyte | precision RSD (%) | repeatability RSD (%) | stability RSD (%) | accuracy | |
|---|---|---|---|---|---|
| | | | | recovery[a] (%) | RSD (%) |
| fructose | 1.5 | 1.2 | 2.0 | 99.2 | 1.8 |
| glucose | 0.7 | 0.1 | 1.7 | 102.6 | 1.7 |
| sucrose | 1.4 | 1.4 | 1.7 | 101.7 | 1.5 |
| DP3 | 1.5 | 1.8 | 1.2 | 102.1 | 1.5 |
| DP4 | 1.7 | 1.5 | 0.9 | 102.2 | 1.1 |
| DP5 | 1.8 | 1.4 | 1.9 | 99.9 | 1.4 |
| DP6 | 1.5 | 1.6 | 1.4 | 101.9 | 1.7 |
| DP7 | 1.1 | 1.2 | 1.4 | 100.9 | 1.6 |
| DP8 | 1.8 | 1.2 | 1.6 | 101.7 | 1.8 |
| DP9 | 1.3 | 1.1 | 1.5 | 99.8 | 1.3 |

[a]Recovery (%) = (found − original) × 100/added.

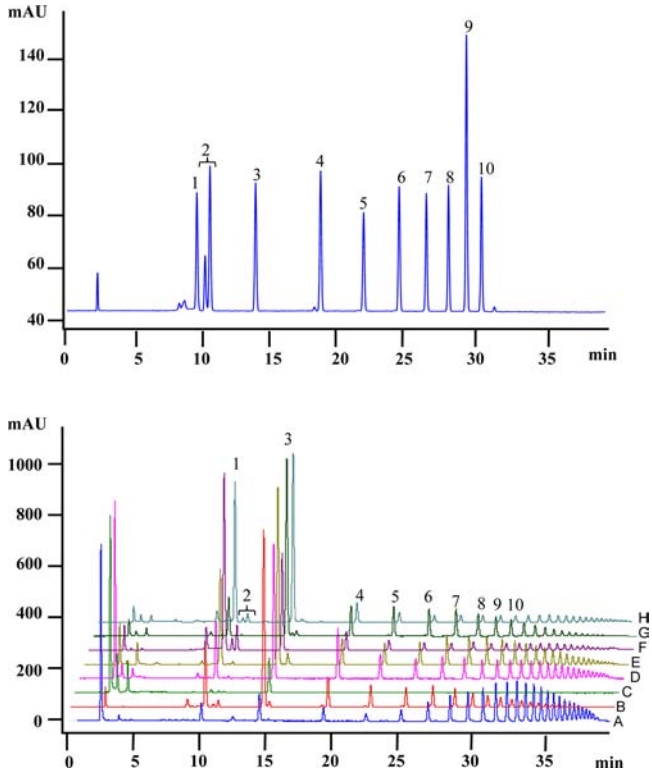


**Figure 3.** HPLC-ELSD chromatograms of (upper) the mixed standards and (lower) burdock extracts from (A) Jiangsu, (B) Shandong, (C) Heilongjiang, (D) Henan, (E) Jilin, (F) Henan, and (G) Gansu, China, and (H) Korea. 1−10, same as in Figure 1.

**Method Validation.** The linearity, regression, and linear ranges of the 10 analytes were determined using the developed HPLC method. The data indicated the calibration curves of the 10 analytes had good linearity ($r > 0.999$), and their LODs and LOQs were lower than 3.63 and 24.82 $\mu$g/mL, respectively (Table 2). The RSD of precision was <1.82%, and the recoveries ranged from 99.18 to 102.56%. The analytes were very stable in 60% ethanol solution during the tested period (Table 3).

**Quantitative Determination of FOS in Burdock.** The established HPLC-ELSD method was applied to the simultaneous determination of 10 sugars, including glucose, fructose, sucrose, and DP3−DP9, in burdock from different regions. The


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Table 4. Contents (Milligrams per Gram) of the 10 Analytes in the Tested Samples

| | samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| analyte | A | B | C | D | E | F | G | H |
| fructose | 20.89 | 51.93 | nd[a] | 23.29 | 46.72 | 74.44 | 58.68 | 110.10 |
| glucose | nd | 29.07 | nd | 26.46 | 26.93 | 32.38 | 28.37 | 28.46 |
| sucrose | 19.28 | 78.50 | 19.67 | 23.94 | 50.28 | 36.97 | 52.90 | 27.34 |
| DP3 | 25.25 | 36.90 | nd | 27.39 | 30.33 | 29.31 | 29.50 | 36.09 |
| DP4 | 13.18 | 23.17 | nd | 14.37 | 18.22 | 15.70 | 15.65 | 25.21 |
| DP5 | 16.75 | 25.61 | nd | 17.35 | 21.75 | 18.22 | 17.92 | 28.26 |
| DP6 | 22.34 | 31.26 | nd | 22.58 | 27.65 | 23.27 | 23.11 | 32.58 |
| DP7 | 17.71 | 26.61 | nd | 17.48 | 23.98 | 18.53 | 18.34 | 28.40 |
| DP8 | 11.95 | 17.22 | nd | 11.40 | 16.40 | 11.98 | 12.08 | 18.90 |
| DP9 | 14.88 | 18.59 | nd | 13.98 | 18.64 | 14.49 | 14.66 | 20.17 |
| sum (DP3–DP9) | 122.06 | 179.36 | nd | 124.55 | 156.97 | 131.50 | 131.26 | 189.61 |

[a]nd, not detected.

HPLC-ELSD chromatograms of the mixed standards and burdock samples are shown in Figure 3. The contents of 10 investigated sugars in burdock are summarized in Table 4. The results showed the contents of sugars in burdock including cultivated and wild samples from different regions were similar except sample C, which suggested the quality of burdock was stable, and the cultivation and environmental conditions might have less effect on the content of FOS, although their contents are higher in cultivated than in wild samples. Especially, there were almost no sugars except a small amount of sucrose detected in sample C, which was collected after flowering and fruiting. That is because FOS accumulate during root development and are then catabolized during regrowth and sprout development of the root. Therefore, the best collection time of burdock should be in the season before its blossoming. In addition, the results also indicated that FOS are the main nutrition in burdock, which are good markers for the quality of burdock. In this study, the total content of DP3–DP9 was 12.2–19.0%, which was higher than the previous report determined by HPTLC, which may have derived from the different samples and/or low sensitivity of HPTLC.[19] Total carbohydrates in burdock determined by colorimetry had poor specificity.[4] Actually, few previous studies have compared the contents of fructooligosaccharides, especially individual DP3–DP9, in burdock from different locations.

This is the first report of quantitative analysis of 10 sugars, especially DP3–DP9 fructooligosaccharides, in different samples of burdock, which is helpful to improve its quality. The developed HPLC-ELSD method, which is accurate and specific, can also be used for analysis of FOS in other plants.

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### Author Contributions

[‖]J.L. and X.L. contributed equally to this work.



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### Notes

The authors declare no competing financial interest.


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## ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; FOS, fructooligosaccharides; ELSD, evaporative light scattering detector; AEX, anion exchange chromatography; RID, refractive index detector; LOD, limit of detection; LOQ, limit of quantification; DP, degree of polymerization; GAP, Good Agricultural Practice

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