Identification and Determination of Fructooligosaccharides in Snow Chrysanthemum (*Coreopsis tinctoria* Nutt.)

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**Abstract**

**Objective:** Small molecules in snow chrysanthemum such as flavonoids, phenolic compounds and amino acids have been extensively investigated. No study to date has focused on water-soluble oligosaccharides. The objective of this study is identification and determination of water-soluble oligosaccharides in snow chrysanthemum. **Methods:** The oligosaccharides in snow chrysanthemum were identified by high performance thin layer chromatography (HPTLC), liquid chromatography-mass spectrometry (LC-MS) combined MS library and methylation analysis for the first time. Subsequently the oligosaccharides were determined by high performance liquid chromatography with a charged aerosol detector (HPLC-CAD). **Results:** The oligosaccharides in snow chrysanthemum were identified as inulin-type fructooligosaccharides (FOS). The yield of FOS (DP3–DP13) in the first extraction was over 97.6%. The RSDs of repeatability in three sample amount levels (0.08 g, 0.1 g, 0.12 g) are lower than 4.8% and the RSDs of stability are less than 3.5%. The recoveries of FOS (DP3–13) were ranging from 96.9% to 105.6%. The contents of FOS (DP3–DP13) in flowers of snow chrysanthemum from different regions of China were greatly variant. **Conclusion:** This is the first time to identify and quantify FOS in snow chrysanthemum which is helpful for its performance in the fields of biomedical, agriculture and functional food industry as well as development of the quality control methods. In addition, the identification approach developed in this work can also be used for screening potential natural sources containing FOS.

**Keywords:** *Coreopsis tinctoria* Nutt, fructooligosaccharides, qualitative/quantitative analysis

**INTRODUCTION**

*Coreopsis tinctoria* Nutt. (a member of the Compositae family) is native from North America but now distributed worldwide. In China, *C. tinctoria* has also named snow chrysanthemum (*Xueju* in Chinese) because it has been found around the snowline (above 3000 m) in Xinjiang of China. There is a long history for flowers of snow chrysanthemum used as tea-like beverage to prevent diabetes and cardiovascular diseases. In addition, further studies showed that the extracts from snow chrysanthemum have multiple biological functions such as antioxidant, antihyperglycemic activity, reducing blood lipid, antihypertensive effects, and so on. In recent years, due to the superb biological activity, snow chrysanthemum has become a hot topic in the market as well as in the scientific research. So far, small molecules in snow chrysanthemum such as flavonoids, phenolic compounds, and amino acids have been separated or determined. In addition, in our preliminary experiment (data not shown), high amount of oligosaccharides, similar with the functional fructooligosaccharides (FOS), has been detected in snow chrysanthemum. However, to our knowledge, no study to date has focused on water-soluble oligosaccharides in snow chrysanthemum. Therefore, qualitative and quantitative analysis of oligosaccharides in snow chrysanthemum is very necessary, which is helpful for their performance in the fields of functional food, agriculture, and biomedical industry.

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FOS are a class of simple carbohydrates, in which fructosyl units are bound to sucrose by a β-linkage.\cite{11} FOS are commonly used as natural food additives and officially classified as prebiotic dietary fiber in many countries.\cite{12} Further studies showed that FOS possess many biological functions, such as reducing the levels of cholesterol and blood glucose, improvement of calcium and magnesium adsorption, inhibiting putrefactive pathogens, lowering of blood pressure, prebiotic effects, and suppressing production of the reductase enzymes which can contribute to cancer.\cite{13-16} In addition, in recent research, FOS have been proved to be promising elicitors in postharvest disease control in many kinds of fruit.\cite{17} Actually, the content of FOS is one of the main indicators for quality control of many natural sources containing FOS.

Conventional methods for identification of FOS in natural sources require multiple-step column separation to obtain the pure compounds which are tedious and time consuming. High-performance thin-layer chromatography (HPTLC) combined detection reagents such as diphenylamine-aniline-phosphoric acid (DPA) is simple, rapid, and effective for preliminary identification of oligosaccharides in complex mixtures.\cite{18} For further identification, liquid chromatography–mass spectrometry (LC-MS) is very useful for investigation of the molecule mass of oligosaccharides and identification of their structures by comparison of the MS/MS data with that of reference standards or searching in the MS library. In addition, the glycosidic linkage information obtained from methylation analysis can further confirm the identified results from LC-MS. For the quantification of FOS, the high-performance LC with a charged aerosol detector (HPLC-CAD) method established in our previous work has been successfully used for the determination of FOS in edible plants.\cite{19} Therefore, in this work, the FOS in snow chrysanthemum have been identified by HPTLC, LC-MS, and methylation analysis. Subsequently, the contents of FOS with degree of polymerization (DP) 3–13 in different snow chrysanthemum and Chrysanthemum morifolium samples were determined using HPLC-CAD and microwave-assisted extraction method. This is the first time to identify and quantify FOS in snow chrysanthemum. In addition, this study is helpful for quality control of snow chrysanthemum.

**Materials and Methods**

**Materials and chemicals**

The snow chrysanthemum samples contain five parts (flowers, buds, seeds, leaves, and stems), four locations (from different places of Xinjiang province, China), and two *C. morifolium* (from Zhejiang and Anhui province). In addition, five parts of snow chrysanthemum (S1 and S3–S6) were from the same plants. The flower of snow chrysanthemum is the medical parts of this plant. Sample S1 was selected as the representative sample for qualitative analysis. The detailed characteristics are presented in Table 1. Voucher specimens of these samples were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China.

Inulin-type FOS (DP3–DP13) were separated and purified in our laboratory,\cite{20} and the purity was above 95% (determined by HPLC-DAD-ELSD). The structures [Figure 1a] were confirmed by using LC-MS, NMR, and methylation analysis according to our previous studies.\cite{21,22} Deionized water was prepared on a Millipore Milli-Q Plus system (Millipore, Bedford, MA). HPLC grade acetonitrile was purchased from Merck (Darmstadt, Germany). All other chemicals and reagents used were of analytical grade.

**Preparation of standard solutions**

The stock standard solution of inulin-type FOS (DP3–DP13) was prepared in ethanol–water (60:40 v/v) at final concentrations of 2.0 mg/mL. Working standard solutions were prepared by appropriate dilution with ethanol–water (60:40 v/v). All of the solutions were stored in the 4°C fridge.

**Sample preparation**

The extraction experiment was carried out with microwave-assisted extraction (Multiwave 3000, Anton Paar GmbH, Graz, Austria). The dried samples were powdered by a mill and sieved through a No. 40 mesh. Then, 2 mL of ethanol–water (60:40 v/v) and 0.1 g of the obtained powder were added into 5 mL extraction vessels. The vessels were weighted and then placed into the microwave instrument and extracted at the power of 400 W for 5 min at temperature of 80°C. After extraction, all vessels were cooled down to ambient temperature. Then, the vessels were weighted, and appropriate amount of extraction solvent was added to compensate for the weight loss. All extracts were centrifuged (5000×g, 5 min) and the supernatant was filtered through a 0.45 μm membrane (Millipore) and diluted to an appropriate concentration for further analysis.
Table 1: Characteristics of analyzed samples

| Codes | Varieties         | Parts      | Locations     |
|-------|-------------------|------------|---------------|
| S1    | *C. tinctoria* Nutt. (Yellow) | Flowers    | Dabancheng, Xinjiang |
| S2    | *C. tinctoria* Nutt. (Red)     | Flowers    | Dabancheng, Xinjiang |
| S3    | *C. tinctoria* Nutt. (Yellow) | Buds       | Dabancheng, Xinjiang |
| S4    | *C. tinctoria* Nutt. (Yellow) | Seeds      | Dabancheng, Xinjiang |
| S5    | *C. tinctoria* Nutt. (Yellow) | Leaves     | Dabancheng, Xinjiang |
| S6    | *C. tinctoria* Nutt. (Yellow) | Stems      | Dabancheng, Xinjiang |
| S7    | *C. tinctoria* Nutt. (Yellow) | Flowers    | Ke Liyang, Pishan, Hotan, Xinjiang |
| S8    | *C. tinctoria* Nutt. (Yellow) | Flowers    | Ke Liyang, Pishan, Hotan, Xinjiang |
| S9    | *C. tinctoria* Nutt. (Yellow) | Flowers    | Ke Liyang, Pishan, Hotan, Xinjiang |
| S10   | *C. tinctoria* Nutt. (Yellow) | Flowers    | Ke Liyang, Pishan, Hotan, Xinjiang |
| S11   | *C. tinctoria* Nutt. (Yellow) | Flowers    | A Ye, Minfeng Hotan, Xinjiang |
| S12   | *C. morifolium*        | Flowers    | Zhejiang      |
| S13   | *C. morifolium*        | Flowers    | Anhui         |

*C. morifolium: Chrysanthemum morifolium, C. tinctoria: Coreopsis tinctoria*

### High-performance thin-layer chromatography analysis

An HPTLC system (Desaga GmbH, Germany) with an AS30 HPTLC Applicator was used for HPTLC analysis. Sample S1 and *Morinda officinalis* How. extract solution (5–10 μL) were applied on a silica gel plate (Merck, Darmstadt, Germany) as bands 6 mm wide, 11 mm apart, and 10 mm from the bottom edge. The plate was firstly developed to a distance of 90 mm with the mobile phase 1-butanol/isopropanol/acetic acid/water, 7:5:2:1 (v/v/v/v). Then, the plate was dried in cool air and placed in the same chamber to develop a distance of 95 mm with the same mobile phase. Finally, the developed plates were colorized with DPA solution and heated at 105°C for 5 min on a YOKO-XR plate heater (Wuhan YOKO technology Ltd., China) and covered with transparent glass and photographed.

### High-performance liquid chromatography with a charged aerosol detector analysis

FOS (DP3–DP13) in snow chrysanthemum were determined by a Dionex Ultimate 3000 UHPLC system (Germering, Germany) that composed of RS autosampler, Ultimate 3000 degasser, pump, and RS column compartment. Detection was carried out with a Corona CAD instrument (ESA, Chelmsford, MA, USA). Chromatographic separation was achieved by using a Waters XBridge Amide column (4.6 × 250 mm id, 3.5 μm). The mobile phase consists of water (A) and acetonitrile (B) with gradient elution: 0–30 min, 75%–45% B; 30–32 min, 45%–75% B; and then equilibrated with 75% B for 10 min. The flow rate was 1.0 mL/min, injection volume was 5 μL, column temperature was 30°C, and the N\textsubscript{2} pressure of the CAD was 35 psi.

### Electrospray ionization-QTOF-mass spectrometry analysis

A high-resolution impact HD quadrupole time-of-flight (QTOF) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with an electrospray ionization (ESI) source was operated in both positive and negative ion modes. The capillary voltage was set at 4000 V in positive ion mode and 3500 V in negative ion mode. The mass range was set at m/z 100–2000 in the full scan mode. The source temperature was set at 250°C. Drying gas (N\textsubscript{2}) flow rate was set at 12 L/min.

MS\textsuperscript{2} data analysis of the three highest intensive ion fragments was intelligently performed in real time.

The MS library containing forty common oligosaccharides such as inulin-type FOS, raffinose family oligosaccharides, and xyloooligosaccharides was established in our library using Compass Library Editor (Version 4.2, Bruker Daltonik GmbH, Bremen, Germany). The match parameters containing Purity, Fit and RFit, and purity score was used to evaluate match levels of the ions masses and intensities between the library and the acquired targets. The Fit score indicates how well the masses and intensities of the library spectrum agree with those found in the acquired spectrum. Other masses contained in the acquired spectrum are ignored. The RFit score indicates how well the masses and intensities of the acquired spectrum agree with those found in the library spectrum. Other masses contained in the library spectrum are ignored.

### Methylation analysis

The extract solution of sample S1 was evaporated to dryness with a nitrogen evaporator and then 5 mg was dissolved in dry dimethyl sulfoxide (1 mL) and methylated by the method of Ciucanu and Kerek.\textsuperscript{[21]} The product was hydrolyzed in 2 mol/L trifluoroacetic acid (1 mL) for 3 h at 90°C and evaporated to dryness using a nitrogen evaporator. The methylated oligosaccharides were converted into their corresponding alditol by reduction with NaBH\textsubscript{4}. The resultant was neutralized with acetic acid, then dried, and acetylated using acetic anhydride. The partially O-methylated alditol acetates were analyzed by gas chromatography–mass spectrometer (GC-MS). The GC-MS conditions were the same as our previous work.\textsuperscript{[24]}

### Method validation

The HPLC-CAD method developed in our previous work was employed for quantitative analysis of FOS (DP3–DP13) in snow chrysanthemum. This method is accurate and specific for determination of FOS in natural sources. The calibration curves, precision, limits of detection, and quantification have been studied in previous work.\textsuperscript{[19]} In this work, we evaluated...
the extraction recovery, repeatability, stability, and accuracy to make sure this method is suitable for the quantitative analysis of FOS in snow chrysanthemum. For evaluation of the extraction recovery, sample S1 was extracted three times. After each extraction, the content of FOS (DP3–DP13) in the extract was determined and the extraction recovery was calculated by the equation: extraction recovery (%) = C_r/C_s × 100%, where C_r is the content of FOS DP3–13 in the first extract and C_s is content of FOS DP3–13 in total three extract. The repeatability was evaluated at three levels (0.08 g, 0.10 g, and 1.2 g) of the representative sample (S1). Each level was extracted and analyzed in triplicate. For measurement of stability, sample S1 was analyzed at 0, 1, 2, 4, 8, 12, and 24 h. Recovery was used to evaluate the accuracy of the method. Known amounts of individual standards were spiked into a certain amount (0.05 g) of sample S1. The spiked samples were analyzed in triplicate using the developed method. One-point external standard method was used for fast quantitative analysis of FOS in investigated samples by the equation: C_s = C_r × A_s/A_p, where C_s is the concentration of analyte in the sample extract solution, C_r is the concentration of analyte in the standard solution, A_s is the peak area of analyte in the standard extract solution, and A_p is the peak area of analyte in the standard solution (A_p is close to A_s).

Statistical data analysis
Hierarchical clustering analysis was performed by SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). A method named as between-group linkage was applied, and Euclidean distance mode, which is a pattern similarity measure, was selected as the measurement.

RESULTS AND DISCUSSION
Identification of oligosaccharides in snow chrysanthemum
The HPTLC results [Figure 1b] of crude oligosaccharides from snow chrysanthemum showed that the oligosaccharides in snow chrysanthemum were similar to that of the reference plant (Morinda officinalis How.), which has been proven to be rich in inulin-type FOS.[19,21] In this step, we used a reference plant instead of pure standards because the matrix effects in HPTLC analysis are weaker than that in many other methods such as HPLC. In addition, the reference plants are easy to obtain and inexpensive. The crude oligosaccharides from snow chrysanthemum were further analyzed by the HPLC-CAD. The results [Figure 1c] showed that the retention time of oligosaccharides in snow chrysanthemum was in accordance with that of reference compounds of inulin-type FOS (DP3–DP13). For further identification, LC-MS was employed. The high-resolution ESI-QTOF-MS was operated in positive and negative ion modes and the [M-H] of peaks 1–7 [Figure 1c] in snow chrysanthemum were detected at m/z 503.1618, m/z 665.2146, m/z 827.2665, m/z 989.3172, m/z 1151.3688, m/z 1313.4188, and m/z 1475.4695. The corresponding [M + Na]+ of peaks 1–7 were detected at m/z 527.1571, m/z 689.2105, m/z 851.2641, m/z 1013.3155, m/z 1175.3685, m/z 1337.4210, and m/z 1499.4722, but the response of these peaks is much lower than that in negative ion mode. The results were in accordance with that of hexose DP3–DP9. In negative ion mode, the MS/MS data showed that the fragment ions of oligosaccharides in snow chrysanthemum were generated by the glycosidic cleavages between two sugar residues and the cross-ring cleavages in the sugar ring. In addition, the glycosidic cleavages between two sugar residues were the predominant pathways, which were in accordance with that of inulin-type FOS.[22] For example, as shown in Figure 2a, the snow chrysanthemum oligosaccharides DP5 possessed the prominent fragment ions with m/z 665.2061 (C_4 or Z_4), m/z 647.2042 (B_4 or Y_4), m/z 503.1566 (C_4 or Z_4), m/z 485.1519 (B_4 or Y_4), m/z 341.1064 (C_2 or Z_2), and m/z 323.0991 (B_2 or Y_2), which were obtained by the cleavages of the glycosidic linkage. Figure 2b shows the FOS-DP5 fragmentation according to the nomenclature of Domon and Costello. Subsequently, we matched the MS/MS data of snow chrysanthemum oligosaccharides in the MS library developed by Compass Library Editor (Version 4.2, Bruker Daltonik GmbH, Bremen, Germany). The results showed that the values of match parameters (Purity, Fit, RFit) were all above 95% when matching the MS/MS data of snow chrysanthemum oligosaccharides (DP3–DP9) with that of inulin-type FOS standards (DP3–DP9) [Table 2]. The match parameters were all below 70% when matching the MS/MS data with other oligosaccharides in this library. The oligosaccharides with DP higher than 9 were difficult to ionize for LC-MS analysis because of the high molecule weight and low concentration, so the data were not shown. The MS/MS data of snow chrysanthemum oligosaccharides were also searched in the commercial glycan database of ProteinScape (Version 3.1.2450, Bruker Daltonik GmbH, Bremen, Germany) and the match results (match score more than 75%) are shown in Table 2 (based on the HPTLC results, the matched structures without fructose were excluded). The match results of oligosaccharides with DP higher than 5 were not shown because the match scores were lower than 70% in this library (might be derived from the different instrument, MS conditions, and sample matrix effects). Actually, there are many structure analogs in the match results. For example, DP3–2 and DP3–6 have the same monosaccharide composition but different glycosidic linkage. DP3–4 and DP3–7 have the same linkage but different monosaccharide composition. It is hard for us to select the accurate results. For further identification, methylation analysis was employed. The results [Figure 2c] showed that the presence of three derivatives for crude oligosaccharides of snow chrysanthemum, t-Fru (2,5-Ac2-1,3,4,6-Me4-mannitol and 2,5-Ac2-1,3,4,6-Me4-gluitol), t-Glc (1,5-Ac2-2,3,4,6-Me4-gluitol), and 2,1-Fru (1,2,5-Ac3-3,4,6-Me3-mannitol and 1,2,5-Ac3-3,4,6-Me3-gluitol) in a molar ratio was about 1:0.1:1:7.6. These results indicated that the main oligosaccharides of snow chrysanthemum had a backbone of 2,1-Fru and no branch. Based on these results, many match results in Table 3 were easy to exclude. However,
| Peak number | Molecular formula | [M-H] Experimental mass (m/z) | Mass error (ppm) | Fragments ion (m/z) | Identification | Purity (%) | Fit (%) | Rfit (%) | Compound |
|-------------|-------------------|-------------------------------|-----------------|---------------------|---------------|------------|--------|----------|----------|
| 1           | C$_{18}$H$_{31}$O$_{16}$ | 503.1618                     | 2.1             | 341.1094 (C$_2$ or Z$_2$), 323.0981 (B$_2$ or Y$_2$) | 97.2          | 97.6      | 98.1    | FOS-DP3 |
| 2           | C$_{24}$H$_{41}$O$_{21}$ | 665.2146                     | 1.0             | 485.1514 (B$_3$ or Y$_3$), 395.1199, 341.1090 (C$_2$ or Z$_2$), 323.0989 (B$_2$ or Y$_2$) | 99.6          | 99.6      | 99.6    | FOS-DP4 |
| 3           | C$_{30}$H$_{51}$O$_{26}$ | 827.2665                     | 1.1             | 665.2139 (C$_2$ or Z$_2$), 647.2042 (B$_2$ or Y$_2$), 503.1566 (C$_3$ or Z$_3$), 485.1519 (B$_3$ or Y$_3$), 341.1064 (C$_4$ or Z$_4$), 322.0989 (B$_2$ or Y$_2$) | 99.2          | 99.6      | 99.4    | FOS-DP5 |
| 4           | C$_{36}$H$_{61}$O$_{31}$ | 989.3172                     | 2.3             | 827.2651 (C$_2$ or Z$_2$), 809.2555 (B$_2$ or Y$_2$), 665.2139 (C$_3$ or Z$_3$), 647.2038 (B$_3$ or Y$_3$), 503.1616 (C$_4$ or Z$_4$), 322.0988 (B$_2$ or Y$_2$) | 97.9          | 97.9      | 98.1    | FOS-DP6 |
| 5           | C$_{42}$H$_{71}$O$_{36}$ | 1151.3688                    | 3.7             | 989.3155 (C$_2$ or Z$_2$), 971.3060 (B$_3$ or Y$_3$), 827.2650 (C$_3$ or Z$_3$), 809.2549 (B$_3$ or Y$_3$), 665.2137 (C$_4$ or Z$_4$), 647.2034 (B$_4$ or Y$_4$), 503.1618 (C$_4$ or Z$_4$), 322.0984 (B$_2$ or Y$_2$) | 95.6          | 95.6      | 96.3    | FOS-DP7 |
| 6           | C$_{48}$H$_{81}$O$_{41}$ | 1313.4188                    | 5.4             | 1151.3664 (C$_2$ or Z$_2$), 1133.3563 (B$_3$ or Y$_3$), 989.3165 (C$_2$ or Z$_2$), 971.3041 (B$_3$ or Y$_3$), 827.2650 (C$_3$ or Z$_3$), 809.2549 (B$_3$ or Y$_3$), 665.2138 (C$_4$ or Z$_4$), 647.2025 (B$_4$ or Y$_4$) | 95.7          | 95.1      | 97.2    | FOS-DP8 |
| 7           | C$_{54}$H$_{91}$O$_{46}$ | 1475.4695                    | 5.8             | 1314.4142 (C$_2$ or Z$_2$), 1295.4008 (B$_3$ or Y$_3$), 1151.3670 (C$_2$ or Z$_2$), 1133.3561 (B$_3$ or Y$_3$), 989.3160 (C$_2$ or Z$_2$), 971.3046 (B$_3$ or Y$_3$), 827.2652 (C$_3$ or Z$_3$), 809.2541 (B$_3$ or Y$_3$), 665.2139 (C$_4$ or Z$_4$), 647.2022 (B$_4$ or Y$_4$), 503.1618 (C$_4$ or Z$_4$) | 95.7          | 97.9      | 97.9    | FOS-DP9 |

FOS: Fructooligosaccharides

**Figure 2:** The liquid chromatography–mass spectrometry/mass spectrometry spectra (a), Domon and Costello nomenclature (b) for degree of polymerization 5 and methylation analysis of snow chrysanthemum oligosaccharides (c)
oligosaccharides such as DP3-2, DP3-5, DP4-1, DP4-6, DP5-4, and DP5-5 all contain backbone of 2,1-Fru, t-Fru, and t-GlcP. The molar ratio of t-GlcP and t-Fru in sucrose [detected in snow chrysanthemum by using HPTLC analysis, Figure 1b] was 1:1. If the structures were DP3-5, DP4-1, and DP5-6, the t-Fru was much higher than t-GlcP. Actually, the molar ratio of t-GlcP and t-Fru in snow chrysanthemum was about 1:1. Therefore, the oligosaccharides in snow chrysanthemum were unambiguously identified as inulin-type FOS.

**Method validation**

The FOS extraction conditions have been optimized in our previous work. In this work, we evaluate the extraction recovery to ensure the complete extraction of FOS. The results showed that the yield of FOS (DP3~DP13) in the first extraction was over 97.6%. Therefore, the extraction method was suitable for the extraction of FOS from snow chrysanthemum. The RSDs of repeatability in three sample amount levels (0.08 g, 0.1 g, and 0.12 g) are lower than 4.8% and the RSDs of stability are <3.5%. The results indicated that this method is suitable for FOS determination in snow chrysanthemum and the sample is stable during the tested period. The recoveries of FOS (DP3~DP13) were ranging from 96.9% to 105.6%. Therefore, the HPLC-CAD method developed in our previous study was suitable for quantitative evaluation of FOS (DP3–13) in snow chrysanthemum.

**Quantitative determination of fructooligosaccharides in snow chrysanthemum**

The inulin-type FOS (DP3~DP13) in different parts of snow chrysanthemum from different regions of China and two C. morifolium samples were determined by HPLC-CAD method. Indeed, as shown in Figure 3, many oligosaccharides with high DP (>13) were detected in the tested samples. These oligosaccharides were not quantified in this work because of the low content in snow chrysanthemum and the absence of reference compounds. The contents of the 11 investigated FOS in different samples are summarized in Table 4. It was noticed that the content of FOS in snow chrysanthemum, sample S1 from Dabancheng (Xinjiang Uygur Autonomous Region, China) was much higher than those from other areas. Sample S2 from Dabancheng (red flowers) was viewed as a variety of yellow snow chrysanthemum and the content of FOS in red flower was much lower than sample S1. Hierarchical clustering analysis of flowers of snow chrysanthemum from different origin and two flowers of C. morifolium was performed based on the content of FOS (DP3~DP13). The results [Figure 4] showed that most of flower samples were well grouped according to their origin. In previous studies, the contents of main flavonoids and phenolic acid in these samples were quantified and compared by HPLC and capillary zone electrophoresis. The difference between these samples was not so obvious. This indicated that the content of FOS in snow chrysanthemum might be more affected by the growing environment than flavonoids and phenolic compounds. However, for further investigate, much more samples are needed to investigate the most suitable growth environment, cultivating mode, and collection time for snow chrysanthemum with high quality.

For comprehensive utilization of snow chrysanthemum, FOS in other parts including buds, seeds, leaves, and stems from the
same area were also determined. The results showed that the content of FOS (DP3 ~ DP13) in stems (sample S6) is higher than other parts. Actually, the stems are usually disposed as waste or supplementary fuel in locals. This causes serious damage to the local ecological environment. Given the high content of FOS, the stems might be promising materials for biomedical and health food industry.

Chrysanthemum morifolium is a well-known functional tea beverage. FOS is one of the health components in this plant. In this work, the content of FOS in flowers of snow chrysanthemum and two C. morifolium was also compared. The content of FOS in C. morifolium was higher than that in snow chrysanthemum. As shown in Figure 4, C. morifolium and snow chrysanthemum samples (S2, S7 ~ S11) were divided in two groups. Sample S1 was in Group I because the content of FOS in this sample was much higher than that in other snow chrysanthemum samples.

### Table 4: Contents of the 11 analytes in tested samples

| FOS      | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10 | S11 | S12 | S13 |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| DP3      | 3.69| 2.02| 1.20| 1.10| 0.79| 2.98| 2.26| 1.76| 1.58| 1.20| 1.02| 4.97| 4.19|
| DP4      | 2.63| 1.45| 1.25| 0.25| 0.62| 2.13| 0.63| 0.90| 0.39| 0.24| 0.27| 2.98| 2.37|
| DP5      | 2.36| 1.09| 1.05| 0.11| 0.45| 1.88| 0.29| 0.67| 0.27| 0.16| 0.22| 2.56| 2.11|
| DP6      | 1.95| 0.78| 1.01| 0.09| 0.64| 2.50| 0.26| 0.62| 0.31| 0.14| 0.19| 1.68| 2.64|
| DP7      | 1.73| 0.65| 0.85| 0.08| 0.55| 2.21| 0.20| 0.61| 0.30| 0.11| 0.15| 1.57| 2.69|
| DP8      | 1.35| 0.45| 0.63| 0.06| 0.41| 1.71| 0.16| 0.44| 0.24| 0.08| 0.10| 1.29| 2.29|
| DP9      | 1.32| 0.50| 0.60| 0.04| 0.43| 1.61| 0.12| 0.46| 0.20| 0.06| 0.07| 1.15| 2.48|
| DP10     | 1.13| 0.35| 0.51| 0.08| 0.33| 1.30| 0.12| 0.37| 0.23| 0.07| 0.08| 0.95| 2.15|
| DP11     | 1.16| 0.33| 0.55| 0.07| 0.34| 1.40| 0.14| 0.36| 0.25| 0.07| 0.10| 0.97| 2.34|
| DP12     | 1.01| 0.26| 0.38| 0.06| 0.31| 1.29| 0.12| 0.35| 0.22| 0.07| 0.06| 0.78| 2.11|
| DP13     | 0.88| 0.26| 0.32| 0.06| 0.28| 1.14| 0.11| 0.25| 0.20| 0.07| 0.05| 0.69| 1.94|

FOS: Fructooligosaccharides

### Conclusions

The inulin-type FOS in snow chrysanthemum has been identified by HPTLC, LC-MS, and methylation analysis. The HPLC-CAD method was successfully employed for quantitative analysis of inulin-type FOS (DP3–13) in different parts, origins of snow chrysanthemum, and two flowers of C. morifolium. The results showed that FOS (DP3–DP13) in different parts of snow chrysanthemum from different regions of China were significantly different. This is the first time to identify and quantify FOS in snow chrysanthemum which is helpful for its performance in the fields of biomedical, agriculture, and functional food industry as well as development of the quality control methods. In addition, the identification approach developed in this work can also be used for screening potential natural sources containing FOS.

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### Conflicts of interest

There are no conflicts of interest.

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