

Physicochemical Characterization of a Low-Molecular-Weight Fructooligosaccharide from Chinese Cangshan Garlic (*Allium sativum* L.)

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ABSTRACT: A novel low-molecular-weight fructooligosaccharide (LMWF) from garlic (*Allium sativum*) was isolated and identified. The structure and physicochemical properties of the LMWF were determined by chemical and spectroscopic methods, size-exclusion chromatography, atomic force microscopy (AFM), dynamic rheometry, and differential scanning calorimetry (DSC). The results showed that the LMWF was a neo-ketose with a molecular weight of 1770 Da. The LMWF had a (2,1)-linked β -D-Fruf backbone with (2,6)-linked β -D-Fruf side chains, and it was mainly composed of fructose. The branch degree was 18.1%, and the intrinsic viscosity was 3.06 mL/g. The spherical particles of the LMWF were observed by AFM, and their size was relatively uniform. With an increase in the water content, the peak temperature (T_p), onset temperature (T_o), and endset temperature (T_c) increased, while the gelatinization enthalpy (ΔH_{gel}) decreased. The LMWF was more stable at a water content of 10%.

KEYWORDS: Fructooligosaccharides, structure, physicochemical properties, NMR, AFM

■ INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the Liliaceae family, which has been used in folk medicine for centuries. Fructan is one of the main bioactive components of garlic. Fructans are classified into three families according to the type of linkage. Inulin-type fructans are composed of 2,1-linked β -D-fructofuranosyl units. Levan-type fructans are composed of 2,6-linked β -D-fructofuranosyl units. Graminan-type fructans are composed of both 2,1-linked and 2,6-linked β -D-fructofuranosyl units.¹ The molecular masses of most fructooligosaccharides and fructopolysaccharides are between 1000 and 4500 Da.¹⁸ An oligosaccharide of 1800 Da, isolated from garlic bulbs, was composed of 10 fructose units connected to a terminal glucose by β -2,1-linkage.² The molecular weight of a garlic fructan was reported to be about 9000–10 000 Da, with the monosaccharide units connected by β -2,1-linkages and traces of β -2,6-linkages for the branched chain.³ A polysaccharide with a molecular weight of 7100 Da and composed of fructose, glucose, and galactose in a ratio of 85:14:1 was isolated from garlic cultivated in China. The monosaccharide units of this polysaccharide were mainly connected by β -2,1-linkages.⁴ Another fructan isolated from garlic grown in China was composed of fructose and galactose in a ratio of 51:1 and had an average molecular weight of 7100 Da.⁵

Most of the studies on carbohydrates from garlic have focused on identification and structural characterization.^{2,3,6,7} Thus far, there are no reports on the conformation, viscosity, and thermal properties of carbohydrates from garlic.

The aim of this study was to characterize the structure of a low-molecular-weight fructooligosaccharide (LMWF) isolated from a Chinese garlic cultivar (Cangshan). The physicochemical properties of the fructooligosaccharide were also investigated.

■ MATERIALS AND METHODS

Materials. Cangshan garlic (*A. sativum* L.) was obtained from a local market in Tianjin, China. Trifluoroacetic acid (TFA), monosaccharide standards (D-rhamnose, L-arabinose, D-galactose, D-xylose, D-mannose, and D-glucose), and T-series dextran standards were obtained from Sigma-Aldrich (Shanghai, China). All other chemicals and reagents were purchased locally and were of analytical grade.

Isolation and Purification of the LMWF. The LMWF was purified using a polyamide column (60 × 3.0 cm inner diameter) with water as the mobile phase, applied at a flow rate of 0.4 mL/min. The carbohydrate fraction was collected. Then, a diethylaminoethyl (DEAE)-cellulose (OH⁻) column (45 × 2.5 cm inner diameter) was used to obtain the main carbohydrate fraction, using water as the mobile phase. A Sephadex G-25 column (50 × 2.5 cm inner diameter) was then employed to purify the carbohydrate fraction, using double-distilled water as the mobile phase. The purified fructooligosaccharide (LMWF) was obtained with a yield of about 6 g/100 g from raw garlic.

Molecular Distribution Analysis. The molecular-size distribution of the LMWF was determined on a Shimadzu LC-20AT HPLC system (Kyoto, Japan) equipped with a refractive index detector (Shimadzu RID-10A) and combined with a Shimadzu LC solution 1.26 SP1 system. Chromatographic separations were performed on a Shodex OHPak SB-802.5 HQ column (300 × 8.0 mm inner diameter; YMC Co., Ltd., Kyoto, Japan). The mobile phase was ultrapure water, applied at a flow rate of 0.8 mL/min. The column was kept at 30 °C, using a CTO-20A Prominence column oven (Shimadzu). The injection volume was 20 μ L. T-series dextran standards were used to obtain the standard curve.

Monosaccharide Composition Analysis. The neutral monosaccharide composition of the LMWF was determined by gas

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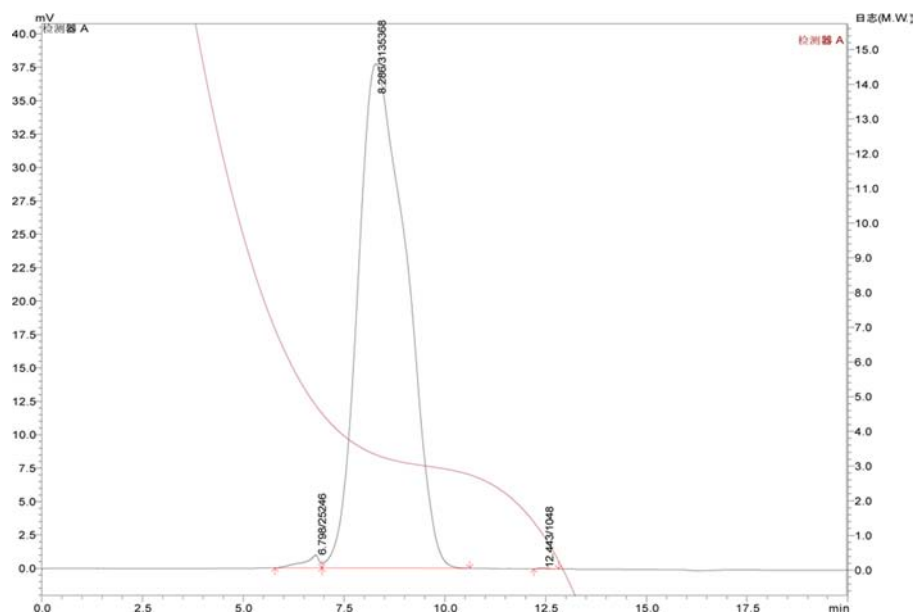


Figure 1. HPLC profile of LMWF from Cangshan garlic.

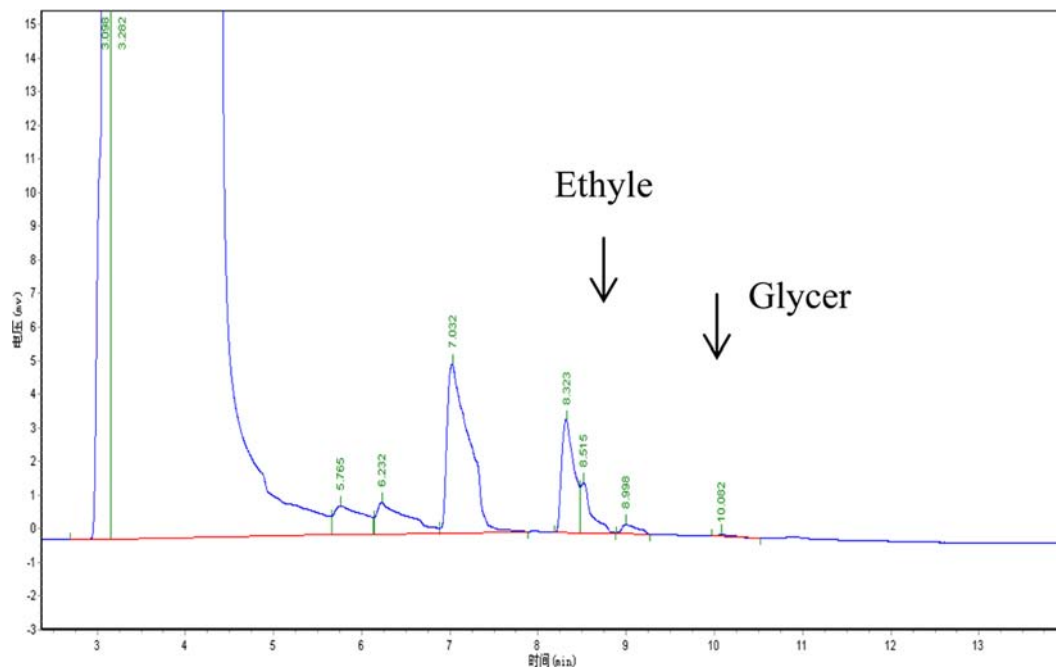


Figure 2. GC chromatogram of Smith degradation products of LMWF.

chromatography (GC) after converting the monosaccharides into acetylated derivatives, according to the method by Chen et al.⁸

Periodate Oxidation and Smith Degradation. Periodate oxidation and Smith degradation of the LMWF was carried out as described in our previous report.⁹

Infrared (IR) Spectroscopy. Fourier transform infrared (FTIR) spectra over the 4000–400 cm^{-1} range were recorded with a Vector 22 FTIR spectrometer (Bruker Optics, Ettlingen, Germany). The maximum measurement resolution was 4 cm^{-1} . Samples were prepared by grinding 1 mg of LMWF with 150 mg of KBr and pressing the mixture into very thin disks.

^1H and ^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy. ^1H and ^{13}C NMR spectra were recorded using a 400 MHz NMR instrument (Bruker Avans, Fallanden, Switzerland) equipped with a dual probe in the FT mode, at 20 °C. Samples were prepared in D_2O at a concentration of 20 mg/mL. Chemical shifts were expressed

in parts per million (ppm) relative to 2,2,3,3-tetradeuterio-3-(trimethylsilyl)-propanoic acid sodium salt, which was used as an internal chemical shift reference at 0 ppm.

Scanning Electron Microscopy (SEM). SEM observations of the LMWF were made at accelerating voltages of 5–30 kV, using a Philips XL-3 equipped with a tungsten filament. Samples were short-pulse-coated with gold to prevent damage caused by overheating and analyzed on their flesh side at increasing magnification folds (200–2000).

Atomic Force Microscopy (AFM). The LMWF was imaged with AFM according to the method by Chen et al.⁸ A stock solution (1.0 mg/mL) was prepared by dissolving LMWF with double-distilled water. The solution was diluted to a final concentration of 1.0 $\mu\text{g}/\text{mL}$. About 5 μL of the diluted LMWF solution was applied to the surface of a mica sample carrier, allowed to dry, and imaged in air at room temperature. The atomic force microscope was a JSPM-5200 AFM

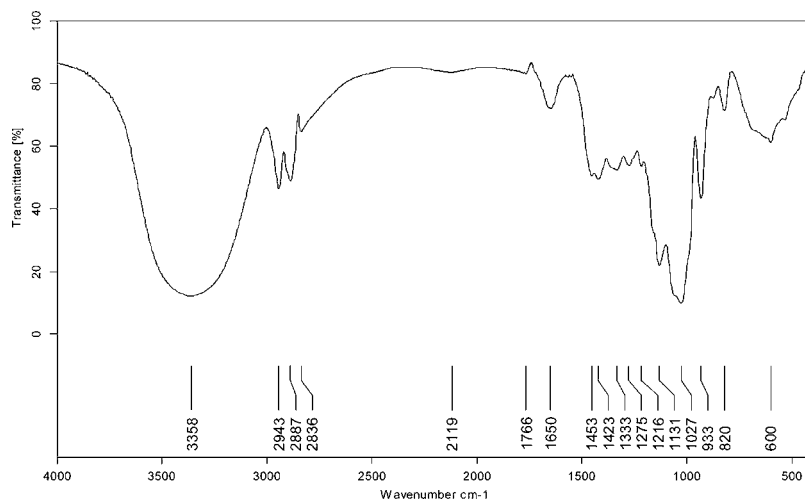


Figure 3. IR spectrum of LMWF.

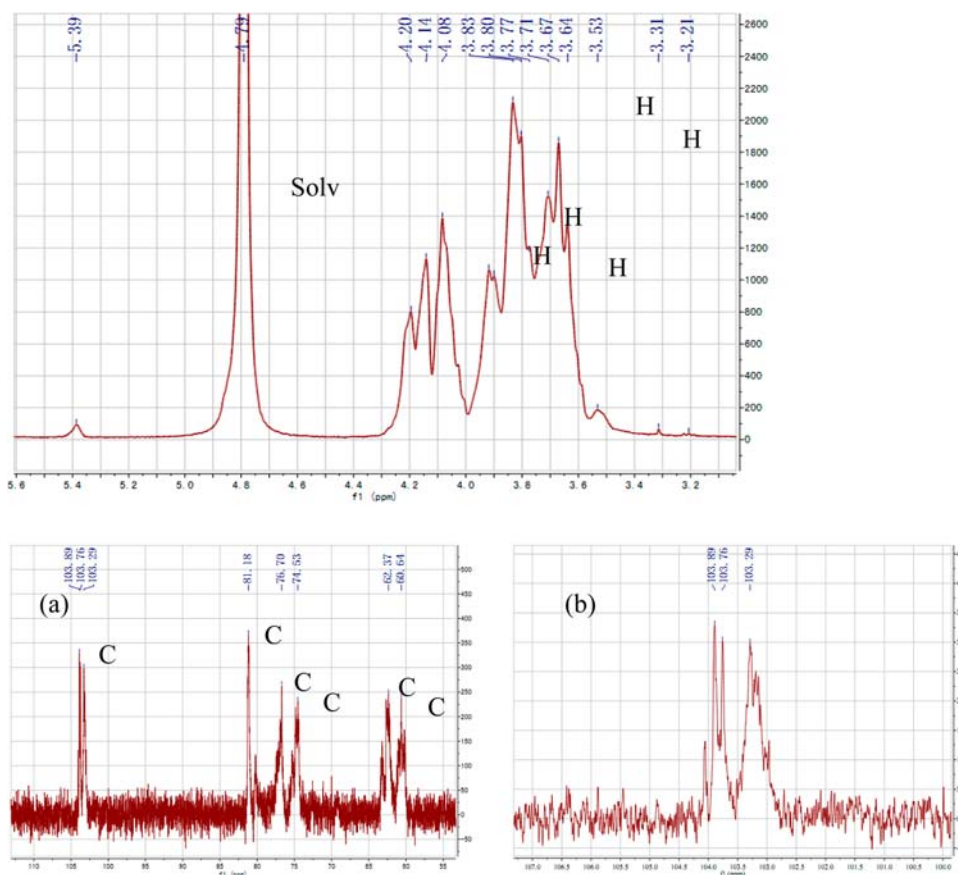


Figure 4. ^1H and ^{13}C NMR spectra of LMWF from Cangshan garlic (a, ^1H NMR; b, ^{13}C NMR).

(JEOL, Japan), operated in the tapping mode. The resulting imaging force was estimated to be around 0.05–3.0 nN, and the resonant frequency was about 2 kHz.

Intrinsic Viscosity Analysis. The intrinsic viscosity of the LMWF was determined using an Ubbelohde glass capillary viscometer (Lunjie, Shanghai, China) according to the method by Ma et al.¹⁰

Thermodynamic Analysis. The thermodynamic characteristics of the LMWF were determined using differential scanning calorimetry (DSC; Perkin-Elmer, Waltham, MA). The dried LMWF was placed in an aluminum pan. The pan was heated from 20 to 200 °C at a rate of 15 °C/min. The onset temperature (T_o), peak temperature (T_p),

conclusion temperature (T_c), and melting enthalpy (ΔH) were recorded.¹¹

RESULTS AND DISCUSSION

Homogeneity and Molecular-Weight Distribution.

The chromatogram (Figure 1) showed a relatively symmetric peak profile of the purified carbohydrate fraction, which suggested that the fraction was homogeneous. The molecular weight of the purified carbohydrate fraction was 1770 Da, with a M_w/M_n of 1.31. These results suggested that the carbohydrate

fraction was a LMWF different from those previously reported for garlic.^{3,4}

Monosaccharide Analysis. The monosaccharide composition of the LMWF was determined by TFA hydrolysis and derivatization followed by GC analysis. The purified LMWF was mainly composed of D-fructose with traces of D-glucose, which indicated that this was a novel LMWF from Cangshan garlic.

Periodate Oxidation and Smith Degradation. Periodate oxidation and Smith degradation are used for the structural characterization and sequence determination of carbohydrates. The LMWF showed HIO₄ uptake, but there was no production of formic acid after oxidation. The consumption of HIO₄ was equal to the amount of monosaccharide after 60 h of periodate treatment (ratio of 1:1), suggesting the presence of large amounts of fructose with 2 → 1, 1 → 4, and 2 → 6 linkages but no 1 → 6 linkages. The periodate-oxidized products were hydrolyzed and examined by GC (Figure 2). The absence of erythritol suggested that there was no (1 → 4)-linked backbone in the LMWF structure. The presence of glycerin suggested the presence of 2 → 1 and 2 → 6 linkages. These results agreed with previous reports on the structure of carbohydrates from garlic.^{1,12}

FTIR Analysis. FTIR spectroscopy can be used for the approximate identification of compounds when combined with data from chemical analyses. The FTIR spectrum (Figure 3) showed the typical absorption peaks of the fructofuranosyl group, as well as several signals in the anomeric region. The main band at 3358 cm⁻¹ was assigned to -OH. The bands around 2943 and 2887 cm⁻¹ were assigned to -CH₃ and -CH₂, respectively. The bands in the region of 1650 cm⁻¹ indicated the presence of a carbonyl. The appearance of characteristic peak bands at both 1131 and 1027 cm⁻¹ suggested the presence of a ketal group (C-O-C-O-C). The absorption band at 933 cm⁻¹ was assigned to fructofuranosyl. The absorption bands in the region of 820 cm⁻¹ indicated the presence of the -CH₂ of fructofuranosyl. The spectral data indicated that the oligosaccharide had the characteristic configuration of inulins.¹³

NMR Spectroscopy Analysis. The ¹H and ¹³C NMR spectra of the LMWF are shown in Figure 4. The proton and carbon chemical shifts were assigned according to the literature (Table 1).^{2,14}

Table 1. ¹H and ¹³C NMR Spectral Assignments (δ) for LMWF

assignments	¹ H NMR	assignments	¹³ C NMR
H-1	3.57–3.74	C-1	59.9–61.4
H-2		C-2	102.8–104.1
H-3	4.12–4.30	C-3	76.3–77.7
H-4	4.00–4.12	C-4	74.1–75.9
H-5	3.74–3.87	C-5	80.5–81.5
H-6	3.87–4.00	C-6	61.5–63.4

In the ¹H NMR spectrum, most of the structural reporter resonances appeared in the region between 4.3 and 5.9 ppm and two main regions were evident. The region between δ 4.4 and 5.3 was assigned to anomeric resonances. α-Glycosides usually resonate at a position lower by 0.3–0.5 ppm compared to β-glycosides. Thus, α-anomeric protons appear at 4.8–5.3 ppm, whereas β-anomeric protons appear as doublets between 4.4 and 4.8 ppm. The vast majority of the remaining ring

proton resonances are found in a second region width of 3.4–4.2 ppm, with subsequent overlapping problems.^{15–17} The D₂O signal appeared at δ 4.79. There was a small anomeric proton signal at δ 5.39, which probably belonged to glucose because the signal corresponding to the anomeric proton of ketose was absent. These results, together with those of GC analysis, suggested that there were traces of glucose in the LMWF.

The ¹³C NMR shielding data of oligosaccharides are closely related to the chemical shifts of each monosaccharide. The chemical shifts of the pyranoside and furanoside forms of the same monosaccharide are quite different and can be used for the determination of the ring size. In O-glycosides, anomeric carbon signals resonate in a distinctive region of 90–112 ppm and O-linked carbohydrates appear at 98–112 ppm. The anomeric resonances are of the methine type in aldoses and the quaternary type in ketoses. The anomeric region is useful for determining the number of O-linked monosaccharides and estimating their relative proportions.¹⁵ The rest of the methine and methylene resonances appear between 60 and 85 ppm. A hexose monosaccharide gives rise to five resonances. Generally, signals in the region of 80–85 ppm correspond to the C-5 of ketofuranose. Aldose has one methylene resonance, whereas ketoses have two resonances in the region of 60–70 ppm.¹⁵ According to the NMR data from the literature,^{13,15–18} the monosaccharide residues of LMWF were inferred to be β-D-fructofuranosyl residues.

As shown in Figure 4b, the ¹³C spectrum of LMWF contained four different signals for the C-2 of the β-fructofuranosyl residues. The signal at δ 103.3 was typical of the C-2 of a 2,1-linked residue. Chemical shifts at δ 103.9 and 103.8 were assigned to terminal and branched residues, respectively. The small signal at δ 104.1 is likely to correspond to a 2,6-linkage. The ratios indicated that the LMWF consisted of 65.0% of 2,1-linkages. The amount of 2-linked β-D-fructofuranosyl residues (18.7%) was a little higher than that of branched β-D-fructofuranosyl residues (18.1%). The results of NMR analysis were similar to those previously reported for garlic carbohydrates, although the molecular weights differed.

Morphological Observations. As observed in the SEM image (Figure 5), the LMWF surface showed an aggregative state of unconsolidated fragments. Particles with spherical shape were observed by AFM (Figure 6a; 1.0 × 10⁻³ mg/mL). Figure 6b, which corresponds to the three-dimensional AFM image of the LMWF, shows that the size of the spherical particles was relatively uniform. The spherical shape might be due to the compact aggregation and electric charge between the LMWF and mica slice. The diameter of the spheres was 0.03–0.05 nm, and their thickness was 0.05–0.08 nm.

Intrinsic Viscosity Properties. Intrinsic viscosity (η) is a distinctive property of polysaccharides. Infinite dilutions of polysaccharides can be considered as optimal for the evaluation of the contribution of individual molecules to the rheological properties of the whole solution, because dilute solutions can be viewed as systems in which individual polysaccharide coils are independent and free to move.¹⁹ The fitted linear equation was $y = 30.52x + 3.0561$ ($R = 0.9918$). The intrinsic viscosity of the LMWF was 3.06 mL/g, being lower than that of other polysaccharides, which can be explained by its low molecular weight.

Thermal Properties. The thermal properties of a broad range of fructooligosaccharides containing 10–85% (w/w) of water were studied by DSC. All thermal properties of the LMWF, including T_p , T_o , T_c and ΔH_{gel} , were determined

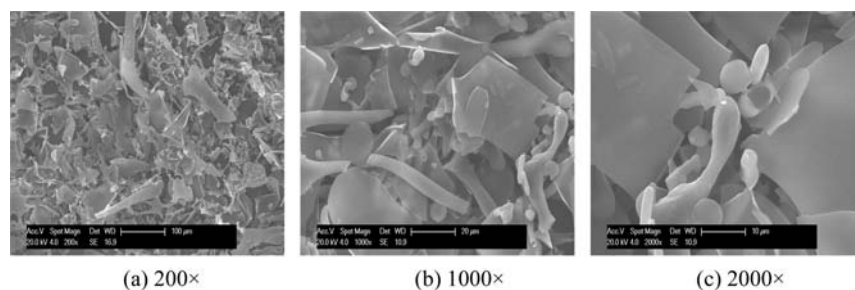


Figure 5. SEM image of LMWF.

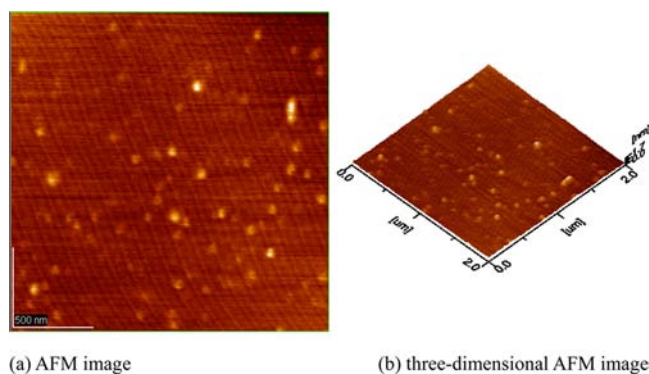


Figure 6. AFM image of LMWF.

(Table 2). Following room temperature conditioning, an endothermic event accompanied by material softening was

Table 2. Thermal Properties of LMWF at Different Moisture Contents^a

water content (% w/w)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)
10	214.0	214.4	221.8	113.9
25	201.6	202.4	207.6	198.5
40	201.8	203.8	209.3	206.7
55	179.8	185.8	193.7	640.9
70	149.62	180.2	186.4	1910.0
85	133.5	163.1	168.7	2510.0

^a T_o , onset temperature; T_p , peak temperature; T_c , endset temperature; and ΔH_{gel} , enthalpy of gelatinization.

observed at 45–65 °C for all samples, except those above their glass transition temperature. With the increase of the water content, the T_p , T_o , and T_c increased, while the ΔH_{gel} decreased. The highest T_p (214.4 °C), T_o (214.0 °C), and T_c (221.8 °C) were observed for LMWF containing 10% (w/w) of water, while the lowest T_p (163.1 °C), T_o (133.5 °C), and T_c (168.7 °C) were observed for LMWF containing 85% (w/w) of water. The highest ΔH_{gel} was 2510 J/g, for LMWF with a water content of 85% (w/w). The lowest ΔH_{gel} was only 113.9 J/g, for LMWF with a water content of 10% (w/w). This suggested that the LMWF was more stable at a low water content. These results were in agreement with those reported by Appelqvist et al.²⁰

In conclusion, a novel fructooligosaccharide from Chinese Cangshan garlic (*A. sativum* L.) was isolated and identified. The compound had a (2,1)-linked β -D-Fruf backbone with (2,6)-linked β -D-Fruf side chains, and the size of its spherical molecules was relatively uniform. The compound was more stable at a low water content.

It is expected that the results of this study will be helpful for future investigations on the structure–activity relationship of fructooligosaccharides and the processing of garlic-derived products in the food industry.

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Notes

The authors declare no competing financial interest.

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