Synthesis of Acetylation burdock fructooligosaccharide (BFO)

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Abstract. Burdock Fructooligosaccharide (BFO) were acetylated using acetic anhydride. The maximal degree of acetylation (DSAC) was 2.15, which was obtained by varying the reaction factors such molar ratio of acetic anhydride to fructofuranose unit (FU). The FT-IR, ¹H NMR and ¹³C NMR spectra showed the introduction of acetylate group, and the reaction occurred at C-6, C-4 and C-3 in the fructofuranose unit of BFO. The molecular weight estimated by HPGPC were 5527.9-6816 g/mol for A-BFO (DSAC=1.0).

1. Introduction

Burdock fructooligosaccharide (BFO) is a fructosan oligomer isolated from the root tissue of Arctium lappa, which is composed of a linear chain of twelve β-2, 1 linked fructofuranose residues with one terminal α-1, 2 linked glucopyranose (GF12) [1]. The BFO has the bioactivities of defense and protection both cucumbers and tomatoes against several diseases caused by pathogens [2-6], and the fructooligosaccharide (FOS) from burdock with bioactivities including antioxidant activity, antibacterial activity were also reported [7-11]. The resource of BFO is abundant: burdock is commonly planted as a popular vegetable and traditional medicine in China, Japan and many other Asian countries; the amount of BFO in the air-dried root tissue is about 17.0%, by the simple water extraction and ethanol precipitation, the distribution of BFO was over 90% and the yield was 85% [1]. The BFO is one of the most intensively studied fructooligosaccharide-inulin (FOSI) [12], synthesis of new BFO derivatives with improving bioactivity, good water-solubility, or other new properties is meaningful for widening its application range. But until now, synthesis of new derivatives of BFO and other fructooligosaccharide (FOS) from burdock has not been reported.

BFO is a of kind inulin with the degree of polymerization (DP) 13, whose structure is shown in Fig. 1. Then we proposed to synthesis the acetylated BFO (A-BFO) using acetic anhydride. The A-BFO was amphipathic, which greatly benefited to obtain high performance products and widen the potential applications of BFO in material and biological chemistry fields. This study addressed the acetylation of BFO, the characterizations of A-BFO were performed by FT-IR, ¹H NMR, ¹³C NMR and high-performance gel permeation chromatography (HPGPC).
2. Experimental

2.1. Material and Methods
Burdock root was obtained from the local market and stored at -20 °C until used. N, N-dimethylformamide (DMF), Acetic acid anhydride were bought from Shanghai Yue Ling Chemical Company. The DMF is a chromatographically pure grade and the other chemicals and reagents were of analytical grade.

Nuclear magnetic resonance spectra were obtained on Bruker Avance 300 MHz spectrometer, using TMS as internal standard substance. Infrared spectra were recorded on a Nicolet NEXUS 670 FT-IR spectrometer, and the samples were prepared as KBr pellets.

The degree of acetylation (DSAC) designates the average number of acetyl groups on each sugar-residue, which was determined from the relative intensities of signals of the acetyl groups at 2.05 ppm and those of all carbohydrate signals in $^1$H NMR spectra [13]. The DSAC was calculated using Eq. 1,

$$DSAC = \frac{[\text{Sum of integrals for acetyl groups at 2.05 ppm}]}{3} \div \frac{[\text{Sum of integrals for carbohydrate signals at 3.5-5.4 ppm}]}{7}$$

2.2. BFO preparation
BFO was obtained according to the previously described method [1]. Briefly, roots of *A. lappa* were sliced and placed in 95°C water for 2 hours. Next, the extracted solution was precipitated with ethanol, deproteinised with chloroform, decoloured using a resin column, and separated and purified by molecular exclusion chromatography. When analyzed by HPGPC, BFO has a symmetrical peak, indicating a homogenous fraction. The average molecular weight was 2134 g/mol.

2.3. The acetylation reaction of BFO
The BFO (0.326 g) was dissolved in DMF (10 mL) and stirred for 10 min at room temperature. Subsequently, a specific amount of acetic anhydride was added. The mixture was stirred for 3 hours at 40°C, then 10 mL of ice water was added to terminate the reaction. The mixture was cooled to room temperature and the pH was adjusted to 8-9 with 15% NaOH solution. The collecting liquid was concentrated, dialyzed for 72h and lyophilized to give the product of A-BFO.

2.4. Assessment of homogeneity and molecular weight
The homogeneity and molecular weight of the A-BFO were determined by high-performance gel-permeation chromatography (HPGPC) (Agilent technologies- 1100, USA) equipped with a TSK-gel G2000PW column (7.5 mm × 300 mm, column temperature 30 °C) and Refractive Index Detector (RID, detecting temperature 35 °C). A small amount of sample solution (15 μL) was performed in each run at a flow rate of 0.5 mL/min with water as their mobile phase. The column was calibrated
with T-series dextran (T-10, 40, 70, 500, 2000) as standards, and the molecular weight of A-BFO fractions was estimated by reference to the calibration curve.

3. Result and Discussion

3.1. Synthesis
In the acetylation reaction of BFO, hydrolysis or degradation of BFO is probably happened in the high reaction temperature, and the acetic anhydride is easy to hydrolyze in the presence of water. Then chromatographically pure grade of DMF were used to avoid the hydrolysis or degradation of BFO and the hydrolyze of acetic anhydride in these reaction. In the reaction temperature $40^\circ C$, reaction time 3 hours, a series of acetylated BFO with different DS$_{AC}$ were obtained by varying the acetic anhydride/FU molar ratio. As Fig. 2 shown, the maximal DS$_{AC}=2.15$ was obtained with the reaction condition: acetic anhydride/FU molar ratio: $8/1$, reaction time: 3 h and reaction temperature: $40^\circ C$. In the experimental, the yield of the A-BFO is in range of 85% to 95%.

![Figure 2](image)

**Figure 2.** Influence of Molar ratio: acetic anhydride/fructofuranose unit (FU) on the DS$_{AC}$ in acetylation reaction of BFO. Reaction time: 3h, reaction temperature: $40^\circ C$.

3.2. FT-IR spectra of A-BFO
Fig. 3 showed the FT-IR spectra of A-BFO with different DS respectively. In Fig. 3, by comparing IR spectra of A-BFO with IR spectra of BFO, the new absorption at 1739, 1237 and 1375 cm$^{-1}$ were assigned to the C=O stretching vibration, C–O–C stretching vibration and CH$_3$ symmetric deformation vibration of carbonyl groups. Moreover, the absorption band of acetyl group were enhanced accompanied with DS$_{AC}$ increasing in Fig 3. These results above indicate the acetylation reactivity of BFO.
Figure 3. FT-IR spectra of BFO and acetylated BFO with different DSAC: a: BFO, b: DSAC=0.7, c: DSAC=1.0, d: DSAC=2.1

3.3. $^1$H NMR and $^{13}$C NMR spectra of A-BFO

The $^1$H NMR spectra of BFO and A-BFO (DSAC=1.0) recorded on Bruker Avance 300 MHz in D$_2$O. Refering to the data on inulin and BFO reported early [1, 14-15], detailed assignments of the signals are listed in Table 1, and the C atom numbers on fructose residues were marked as Fig. 1. From table 1, we can conclude that the bands of protons of BFO were about 0.36 ppm downfield shift caused by introduction of acetyl groups. The $^{13}$C NMR data indicated that the reaction position of sulfation are at the C-6, C-4 and C-3 of the fructofuranose. As a whole, the $^1$H NMR and $^{13}$C NMR data confirmed the presence of O-acetyl groups.

Table 1. $^1$H NMR and $^{13}$C NMR chemical shifts (ppm) for residues of BFO and A-BFO (DSAC=1.0) in D$_2$O

| Compound     | Fructose: $\rightarrow$1)-β-D-Fruf-(2$\rightarrow$ | Glucose: α-D-Glop-(1$\rightarrow$) |
|--------------|-----------------------------------------------|---------------------------------|
|              | 1         | 2         | 3         | 4         | 5         | 6         | 3'        | 4'        | 6'        | C=O | Acetyl | G1 |
| BFO          | H         | 3.56      | 4.12      | 3.96      | 3.78      | 3.70      |           |           |           |      |        |    |
|              | C         | 60.3      | 103.0     | 76.8      | 74.1      | 81.0      | 62.0      |           |           |      |        |    |
| A-BFO (DS=1.0) | H       | 3.62      | 4.15      | 4.00      | 3.76      | 3.73      | 4.43      | 4.27      | 3.81      |      |        |    |
|              | C         | 60.7      | 103.1     | 76.8      | 74.1      | 80.9      | 62.0      | 78.2      | 74.4      | 65.3 | 173.4  | 20.2|

3.4. Molecular weights of A-BFO

Besides DS, the molecular weight of polysaccharide is another important parameter influencing its properties. Then the molecular weight of A-BFO (DSAC=1.0) were measured by HPGPCC method, and the HPGPC profiles of them are shown in Fig. 4. The molecular weight of A-BFO (DSAC=1.0) was obtained as 5527.9-6816 g/mol. The results indicated that no degraded occurred in the acetylated process of BFO under this mild reaction conditions.
Figure 4. High-performance gel permeation chromatograph (HPGPC) profiles of BFO and A-BFO (DS\textsubscript{AC}=1.0).

4. Conclusion
In summary, acetylated derivatives of BFO were synthesized using acetic anhydride, and the maximal degree of sulfonation (DS\textsubscript{AC}) were respectively 2.15. The FT-IR spectra, \textsuperscript{1}H NMR and \textsuperscript{13}C NMR of the products shown the characteristic of A-BFO, and the reaction position of acetylation are at the C-6, C-4 and C-3 of the fructofuranose. The molecular weight of the A-BFO (DS\textsubscript{AC}=2.15) determined by HPGPC are 5527.9-6816 g/mol, which indicated that no degradation of BFO occurred in the reaction process.

By introduction of acetyl groups, hydrophobic acetyl ester were formed and hydrophilic hydroxyl groups decreased for BFO, then the water-solubility of the A-BFO decrease with DS\textsubscript{AC} increased. In the experiment, we observed that A-BFO with low DS\textsubscript{AC} can dissolve in water, but the A-BFO with DS=2.15 was almost insolvable in water. Now we are investigating new applications of A-BFO in biological chemistry and material fields.

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