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Isolation and characteristics of polysaccharide from *Amorphophallus* corrugatus in Vietnam

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ABSTRACT

A polysaccharide has been isolated from the tuber of *Amorphophallus* corrugatus (one of the most abundant *Amorphophallus* species in Vietnam forests). The chemical structure, molecular weight, moisture uptake and degree of crystallization of polysaccharide were investigated by nuclear magnetic resonance spectroscopy (NMR), viscosimetry, differential scanning calorimetry (DSC) and X-ray diffraction. The results shown that the polysaccharide was a glucomannan with a backbone of (1,4)-linked β-D-mannopyranosyl residues and β-D-glucopyranosyl residues, and was demonstrated to be composed of D-mannose and D-glucose in 2.25/1.0 molar ratio and having a relatively low degree of branching at C6 positions of the hexose residues. The degree of acetylation was about 7.57% and the acetyl group might attach to glucose and mannose residues at positions C2, C3, and C6. The molecular weight of glucomannan was $M_v = 1.57 \times 10^6$ Da. The polysaccharide was amorphous and the moisture uptake was about 8.37%.

1. Introduction

Glucomannan (GM) is a polysaccharide of the mannan family, very abundant in nature, specifically in softwoods (hemicelluloses), roots, tubers and many plants bulbs. Despite the variety of sources, the most commonly used type of GM is named konjac glucomannan (KGM) or konjac flour, which is extracted from tubers of *Amorphophallus* plants. Irrespective of its origin, GM is composed of β-1,4-linked D-mannose and D-glucose monomers. There may be certain short side branches at the C-3 position of the mannoses and acetyl groups randomly present at the C-6 position of a sugar unit (Kato & Matsuda, 1969; Smith & Srivastava, 1959). The acetyl groups frequently range from 1 per 9 sugar units to 1 per 20 sugar units (Kato & Matsuda, 1969; Koroskenyi & Mccarthy, 2001). In addition, there may be some differences in molecular structure of KGM from different species. However, the mannose/glucose monomer ratio may vary depending on the original source of GM. For example, it has been reported that konjac GM has a molar ratio of around 1.6:1, whereas GMs extracted from Scotch pine and orchid tubers have ratios of 2.1:1 and 3.6:1, respectively. These values should be regarded cautiously given the variability observed depending on the studies and, in particular, on the analytical procedures (Ishrud, Zahid, Viqar, & Pan, 2001).

Historically, konjac glucomannan has been used in traditional Asian foods such as noodles, tofu and other food products. konjac flour (made from the tubers) has been a healthy Japanese jelly called "konyaku" for over two centuries. By expanding in the stomach, KGM can be useful for people trying to lose weight. Fiber-containing foods are known to help reduce cholesterol, improve bowel functioning and assist in weight reduction by creating a feeling of fullness. Several small controlled studies have found KGM to be effective for reducing total cholesterol levels in otherwise healthy adults. KGM reduced bad cholesterol (LDL) and, according to some studies, increased good cholesterol (HDL) (Martino et al., 2005; Shimizu et al., 1991). In addition, KGM may improve other risk factors for heart disease, such as high triglyceride levels and high blood pressure (Vuksan et al., 1999). Several studies have suggested that KGM may also help the body to regulate blood sugar levels and, therefore, could be helpful in treating diabetes (Huang, Zhang, & Peng, 1990). Additionally, KGM might be helpful for individuals who experience episodes of low blood sugar (Chen, Sheu, Tai, Liaw, & Chen, 2003; Sood, Baker, & Coleman, 2008).

In the studying and application processes of glucomannan that was isolated from *Amorphophallus* species, the isolation step played an important role because the method used for collecting of GM might affect to scope of applications of this product. As shown in reported papers, GM was usually isolated from the dried state of *Amorphophallus* tubers (Ye, Kennedy, Li, & Xie, 2006), or by immersing the dried tubers in aqueous methanol (Li & Xie, 2006). Up to now, there are not methods for direct extraction of GM from initial tubers of *Amorphophallus* without drying to be reported.

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Being a member of the philodendron (arum) family, the *Amorphophallus* corrugatus was expected to contain polysaccharide that its structure was constituted from mannose and glucose unit (usually named glucomannan). Therefore, the aim of this work was isolation and characterization (using FTIR, NMR, X-ray, DCS analyses) of polysaccharide from *Amorphophallus* corrugatus. The polysaccharide obtained was a promising candidate for applications in food, film-forming for packaging or encapsulating, pharmaceutical and biomedical products, designing the new drug carriers, etc.

2. Experimental

2.1. Materials

The tubers of *Amorphophallus* corrugatus were collected from the mountain and hilly areas mainly in the North West of Vietnam. Ethanol, n-butanol, chloroform, acetone, sodium chloride, etc. were commercial products of Merck. Co., (Germany). All other chemicals and reagents used in experiments were of analytical grade.

2.2. Isolation and purification of polysaccharide from *Amorphophallus* tuber

Polysaccharide from the tubers of *Amorphophallus* corrugatus was extracted and purified as follows: *Amorphophallus* tubers (100 g) were sliced to about 8–10 mm in thickness, and then pulverized by a mill. The crude flour was dispersed in 200 ml of distilled water with stirring at 500 rpm for 5 h at room temperature. The extracted solution was filtered and then kept overnight at 5 °C. Then the solution was centrifuged at 16,000 rpm for 30 min at room temperature. Clear solution was separated and then concentrated under vacuum for obtaining 50 ml of residue. The residue was precipitated with 200 ml of 90% ethanol under stirring (500 rpm) at room temperature. This precipitated mixture was filtered and redissolved in 50 ml of distilled water and then precipitated with ethanol again. This process was repeated three times. Then the collected precipitate was washed with ethanol and acetone three times.

The cake material was dissolved in distilled water and deproteinization was carried out with 100 ml/time Sevag reagent ($CHCl_3$/n-BuOH, v/v = 4:1) (Navarini, Gilli, & Gombac, 1999). Then the aqueous solution was concentrated and a residue was obtained. The residue was precipitated with ethanol, and then kept at room temperature for 24 h. After that the precipitate was collected, washed with ethanol and acetone for three times and freeze-dried. In this way the polysaccharide was obtained. Lastly, the polysaccharide was dried at 50 °C for 3 h and used for further characterization. The polysaccharide content (PC) was calculated using the following formula:

$$\text{PC}\ \ \% = \left(\frac{m_1}{m_2}\right) \times 100\% \quad (1)$$

where m_1 and m_2 were weight of polysaccharide and original *Amorphophallus* tubers, respectively.

2.3. Molecular weight

The intrinsic viscosity of polysaccharide was measured by Ubbelohde viscometer according to method of Li, Xie, & Kennedy (2006) Briefly, various solutions of polysaccharide with different concentrations were prepared by dissolving polysaccharide in double distilled/deionized water. The solutions were heated to 80 °C and maintained at this temperature for 1 h and then cooled to the room temperature. Salt solution was prepared beforehand in water at room temperature at certain concentrations, and then, it was mixed with the polysaccharide aqueous solution, with gently stirring at room temperature for 10 min before the measurements. The intrinsic viscosity in 0.2 mol/l NaCl aqueous solution was measured by Ubbelohde viscometer (0.58 mm) at 25 °C (ultra thermostat, ±0.05 °C). Mark–Houwink parameters were fixed according to $\eta = 5.96 \times 10^{-2} \times M_v^{0.7317}$ (Li and Xie, 2006; Wanchun et al., 2008).

2.4. FTIR spectroscopy

FTIR spectrum of the polysaccharide was recorded on the FTIR-Impact 410 spectrometer under dry air at room temperature using KBr pellets in the range between 4000 and 400 cm^{-1}. The peaks were assigned by comparison with the data reported in the literature (Zhang et al., 2001).

2.5. NMR spectroscopy

NMR spectra of polysaccharide were recorded on the 500 MHz Bruker Avance spectrometer, the sample concentrations being about 5 g/l for ^1H NMR and 20 g/l for ^{13}C NMR, in D_2O at 353 K. Assignments of signals and identification of the sugar residues were assigned by combinations of ^1H–^{13}C HSQC NMR and comparison of the chemical shifts with published data on similarly substituted sugar residues (Hua, Zhang, Fu, Chen, & Chan, 2004).

2.6. Thermal analysis

Thermal analysis of polysaccharide was conducted with a Netzsch TG 209 (Germany) under air atmosphere with a flow capacity of 20 ml/min. The scan was carried out at a heating rate of 10.0 °C/min from 50 °C to 700 °C. The sample weight was about 6.0 mg. The weight loss of the sample at temperature from 50 °C to 180 °C due to the absorptive water or moisture uptake.

2.7. X-ray diffraction

X-ray diffraction pattern for the polysaccharide was analyzed using a Siemens D5000 (Japan) diffractometer equipped with a Cu Kα target at 40 kV and 30 mA with a scan rate of 4°/min. The diffraction angle ranged from $2\theta = 5°$ to $2\theta = 65°$.

3. Results and discussion

3.1. Isolation of polysaccharide from Amorphophallus tubers

In recent years, there were many studies concentrating on glucomannan and its applications. The isolation and purification of glucomannan from *Amorphophallus* plants were the important steps in the study process about this material. According to the traditional methods, the isolation and purification process were often carried out in the dried state of *Amorphophallus* tubers, that meant the tubers of *Amorphophallus* plant were dried before the konjac glucomannan was isolated (Ye et al., 2006). In our isolation process, because of the solubility of polysaccharide, which could be directly separated from the *Amorphophallus* tubers by grinding it with the presence of water. In this process, the polysaccharide, fat and protein were also dissolved by water. The protein was separated as mentioned in experimental part. The polysaccharide content resulted from *Amorphophallus* corrugatus calculated using the formula (1) was about 15% of original tuber. Thus, polysaccharide could be isolated directly from the original tuber of *Amorphophallus* corrugatus. The molecular weight of the polysaccharide measured according to method of Li et al. (2006) was about 1.57×10^6 Da.

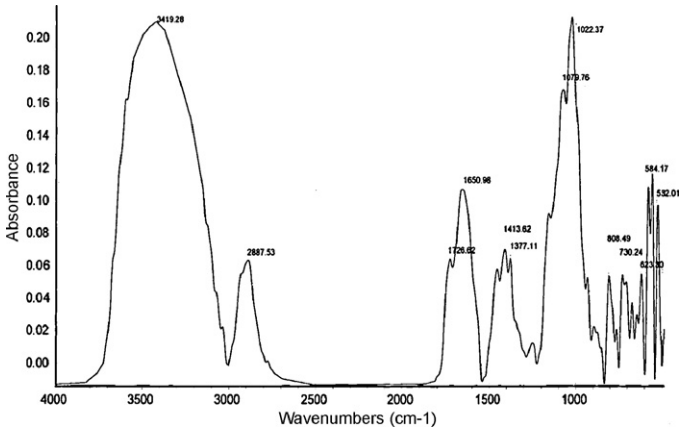


Fig. 1. The FTIR spectrum of polysaccharide isolated from *Amorphophallus* corrugatus.

3.2. FTIR analysis

The FTIR spectrum of the polysaccharide in the wavelength range of 4000–400 cm^{-1} was shown in Fig. 1. In the spectrum of polysaccharide, the wide band observed at 3000–3700 cm^{-1} could be attributed to the O–H stretching of the glucomannan. The band at 2887 cm^{-1} was attributed to the asymmetric stretching of C–H, while the band at 1650 cm^{-1} was ascribed to adsorbed water and the bands at 1413 and at 1377 cm^{-1} to the angular deformation of C–H. The C–O ether bond shown stretching at about 1150 cm^{-1} while the C–O alcohol bond shown stretching at 1079 and 1022 cm^{-1}. The characteristic peaks observed at 808–900 cm^{-1} were assigned to the β-pyranose between mannose and glucose unit. The peak at 1726 cm^{-1} was attributed to carbonyl of acetyl group. These results were in agreement with the data reported by Zhang et al. (2001).

3.3. NMR spectroscopy

The ^{1}H NMR spectrum of the polysaccharide was shown in Fig. 2. The peaks were assigned by comparison with chemical shift data reported in the literature (Capek, 2009; Ishrud et al., 2001). The ^{1}H NMR chemical shifts of polysaccharide signals were assigned as in Table 1. The results in Table 1 shown that polysaccharide were constituted from mannose and glucose units.

It could be clearly seen from Fig. 2 that the signals attributed to the hydrogen atoms linked to C2 to C6 of both glucose and mannose units were not well separated. This was due to the complex nature of the spectra of polysaccharides. Meanwhile, the signals

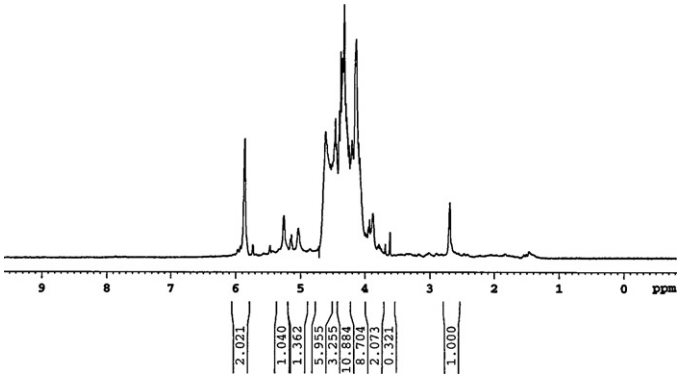
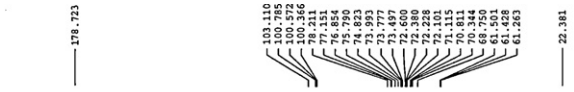


Fig. 2. The ^{1}H NMR spectrum of polysaccharide isolated from *Amorphophallus* corrugatus (5 g/l in D_2O at 80 °C).

Table 1
^{1}H NMR chemical shift data (δ ppm) of polysaccharide isolated from *Amorphophallus* corrugatus.

Signals	Mannose	Glucose
C1	100.36; 100.57; 100.78	103.11
C2	70.81; 70.4; 71.15	73.49
C3	72.10; 72.22; 72.38	73.99; 73.77
C4	77.15; 76.85	78.21
C5	75.79	74.82
C6	61.26; 61.42	61.50



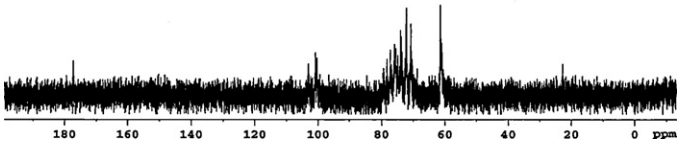


Fig. 3. The ^{13}C NMR spectrum of polysaccharide isolated from *Amorphophallus* corrugatus (20 g/l in D_2O at 80 °C).

attributed to the hydrogen-H1 linked to the carbon-C1 of both glucose unit (5.023 ppm) and mannose unit (5.875; 5.256 ppm) were well separated. Therefore, the mannose/glucose ratio in polysaccharide molecule could be calculated using the integrals of H1 in the ^{1}H NMR spectrum. According to this method, the mannose/glucose molar ratio in polysaccharide molecule was 2.25/1.0.

The ^{13}C NMR spectrum of polysaccharide was shown in Fig. 3. The ^{13}C NMR chemical shift data were assigned as in Table 2.

The ^{13}C NMR spectrum showed characteristic anomeric signals at δ 103.11 ppm due to C1 resonances of β-D-glucose residues, at δ 100.36; 100.57 and 100.78 ppm due to C1 resonances of D-mannose residues. Splitting of the anomeric signal of D-glucosyl unit and D-mannosyl one in the ^{13}C NMR spectrum of the polymer was due to different mutual arrangement of D-glucosyl and D-mannosyl units in the main chain (Capek, Alföldi, & Lišková, 2002). The C4 chemical shifts of the glucosyl and mannosyl units involved in glycosidic linkages appeared at δ 78.21 ppm and (77.15; 76.85 ppm), respectively. The signals at δ 75.79, (72.10, 72.22, 72.38 ppm) and (70.81, 70.4, 71.15 ppm) were assigned to C5, C3 and C2 of mannose residues, respectively. The characteristic resonances of C5, C3 and C2 of 1,4-linked glucose residues were observed at δ 74.82 ppm, (73.99; 73.77 ppm) and 73.49 ppm, respectively. The signals in the high magnetic field at δ 61.2–61.5 ppm were generated by the res-

Table 2
^{13}C NMR chemical shift data (δ ppm) of polysaccharide isolated from *Amorphophallus* corrugatus.

Signals	Mannose	Glucose
H1	5.857; 5.256	5.023
H2	4.613; 3.90–3.94	3.879
H3	4.399	4.508; 4.535
H4	4.375	4.179
H5	4.137	4.246
H6	4.187; 4.350; 4.600	4.187; 4.350; 4.600

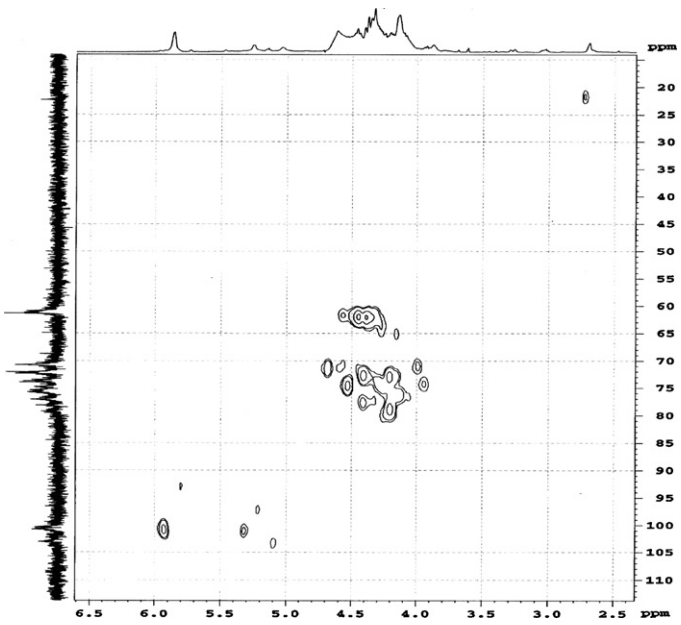


Fig. 4. The ^{1}H–^{13}C HSQC NMR spectrum of polysaccharide isolated from *Amorphophallus* corrugatus.

onances of nonsubstituted C-6 of glucosyl and mannosyl residues. Besides, a low intensity signal at δ 68.75 ppm could be assigned to substituted C6 of glucosyl or mannosyl residues.

The above assignments were consolidated by using the ^{1}H–^{13}C HSQC NMR spectrum of polysaccharide (Fig. 4). It was could be seen that the dominant H1/C1 cross peaks could be identified at δ 5.85/100.5–100.7 ppm and 5.25/100.3 due to β-1,4-linked mannose residues. The H1/C1 cross peaks of glucose units could be observed at δ 5.02/103.1 ppm. Cross-peaks at δ 4.17/78.2 and 4.37/77.1–76.8 were due to H4/C4 signals of substituted glucosyl and mannosyl residues, respectively. Cross peaks at δ 4.13/75.7 ppm, δ 4.39/72.1–72.3 ppm and (δ 4.61/70.4–70.8, δ 3.90–3.94/71.1 ppm) were due to the H5/C5, H3/C3 and H2/C2 cross peaks, respectively, of mannosyl residues involved in 1,4-linkage. The cross peaks of glucosyl residues were also observed at δ 4.24/74.8 (H5/C5), 4.50–4.53/73.7–73.9 (H3/C3) and 3.87/73.4 (H2/C2). The H6/C6 cross peaks of both substituted and nonsubstituted mannosyl and glucosyl residues appeared at δ 4.18–4.60/61.2–61.5 ppm.

Thus, the results confirmed a linear structure of polysaccharide composed of 1,4-linked D-mannosyl and D-glucosyl units in the molar ratio of 2.25/1.0 and the β configuration of glycosidic bond in the main chain and the presence of short side chains at C-6. Thus, polysaccharide isolated from *Amorphophallus* corrugatus could be called as a glucomannan.

In order to determine the position of the acetyl groups at the glycosyl and mannosyl units, the NMR spectrum of glucomannan was very useful. As seen in Fig. 2, beside the signals with the strong intensity assigned to the H1 of both glucose and mannose units, some of the low intensity signals were also observed at δ 5.14, 5.32, 5.46, 5.73 and 5.92 ppm. In the ^{13}C NMR spectrum (Fig. 3), the signals belonged to anomeric carbons were not separated well. These data indicated that the acetyl groups of glucomannan were distributed randomly along the backbone of polymer. The acetyl groups might attach to the glucose and mannose residues at positions C2, C3, and C6. These results were in agreement with those of Hua et al. (2004).

3.4. Degree of acetylation

The FTIR spectrum of polysaccharide (corrugatus glucomannan) has absorptions at 1250 cm^{-1} (C–O) and 1740 cm^{-1} (C=O), suggesting the presence of ester linkages. In the ^{1}H NMR spectrum of corrugatus glucomannan, the signals showed at δ 2.690 ppm corresponding to CH_3 of acetyl groups. Besides, in the ^{13}C NMR spectrum of corrugatus glucomannan, the signals attributed to CH_3 and C=O of acetyl groups showed at δ 22.381 ppm and δ 178.723 ppm, respectively. These features could be used as evidences for conclusion the presence of acetyl group. Degree of acetylation (DA) of the corrugatus glucomannan could be determined by using the

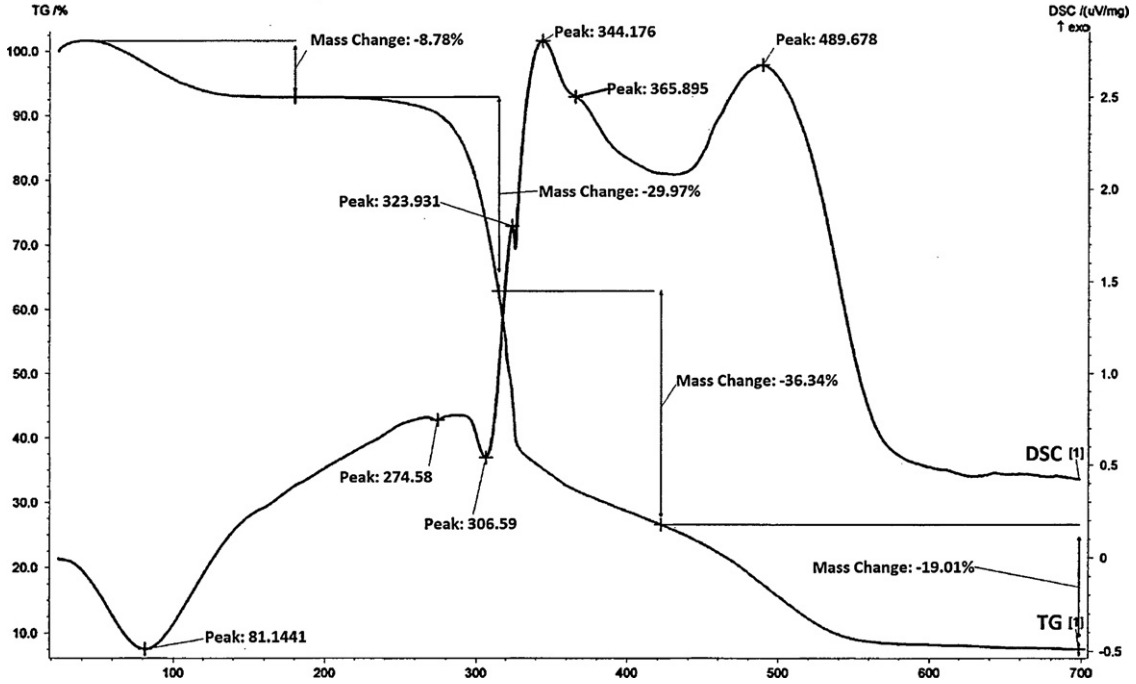


Fig. 5. The TG and DSC curves of polysaccharide isolated from *Amorphophallus* corrugatus.

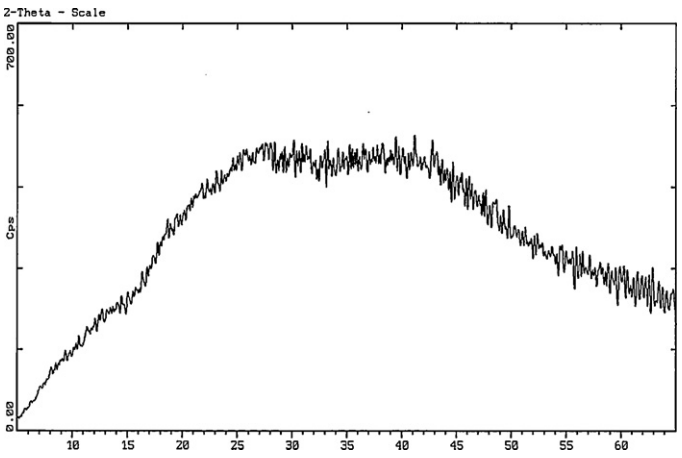


Fig. 6. XRD pattern of polysaccharide isolated from *Amorphophallus* corrugatus.

data of ^{1}H NMR spectroscopy. The DA value was estimated from the formula:

$$\mathrm{DA} = \left[\frac{(I_{\mathrm{CH_3}} \times 100\%)/3}{I_{\sum \mathrm{H1}}}\right] \quad (2)$$

where I_{CH3} was the integral of the hydrogen atom in $-COCH_3$ group and $I_{\Sigma H1}$ was total integral of the hydrogen atom of C1 in both glucose and mannose units. As shown in Fig. 4, the proton signal of acetyl group was separated well, so the value of acetyl content obtained could be quite accurate. The degree of acetylation of the corrugatus glucomannan was determined to be $\mathrm{DA} = [(1 \times 100\%)/3]/(2.02 + 1.04 + 1.36) \approx 7.57\%$.

3.5. Thermal analysis

The characteristic of TG and DSC curves of corrugatus glucomannan was presented in Fig. 5. The sample involved four steps of degradation: the initial weight loss at approximately 180 °C was due to the evaporation of water (moisture uptake), the loss weight was of 8.37%. While the weight loss in the second range (210–450 °C) corresponded to a complex process including the dehydration of the saccharide rings and depolymerization. As could be seen in Fig. 5, polysaccharide began to decompose at about 274 °C, rapidly lost about 66% of its weight up to 410 °C and then left about 19% up to 600 °C. The maximum rate of weight loss occurred at 306 °C. The result of TG analysis indicated that the weight loss of polysaccharide during heating could be attributed to a complex process including degradation of the saccharide rings and disintegration of macromolecule chains of polysaccharide. These results were in agreement with the results of Yu, Huang, Ying, and Xiao (2007).

3.6. X-ray diffraction

The X-ray curve of corrugatus glucomannan was shown in Fig. 6. As observed, the pattern of polysaccharide (Fig. 6), exhibited a non-crystalline state and only had a very broad peak around $2\theta = 20–45°$, which was consistent with the data reported by Xu, Li, Kennedy, Xie, and Huang (2007).

4. Conclusions

A polysaccharide with the weight content of 15% of original *Amorphophallus* tuber has been isolated from the tuber of *Amorphophallus* corrugatus. The structural investigations pointed out that the polysaccharide was a glucomannan with structural component consisted of β-1,4-linked D-mannosyl and D-glucosyl units in the molar ratio of 2.25/1.0. The presence of short side chains at C-6 was also observed. The degree of acetylation of the glucomannan was determined to be 7.57% and the acetyl groups might attach to the glucose and mannose residues at positions C2, C3, and C6. The molecular weight of glucomannan was about 1.57×10^6 Da. The glucomannan was amorphous and the moisture uptake was about 8.37%.

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