Solid-state NMR characterization of triacylglycerol and polysaccharides in coffee beans

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Abstract

It is important to understand the structural characteristics of triacylglycerol (TAG), polysaccharides and trace elements in coffee beans, so that residues can be reutilized in applications including biodiesel oils. Here, we performed ^{1}H and ^{13}C solid-state NMR measurements on Indonesian green beans, roasted beans, and spent coffee grounds (SCGs). In the NMR spectra, there were liquid-like TAG containing linoleic acids based on observed signals of –CH=CH–CH_2-CH=CH– group in an acyl chain, which play a role in decreasing TAG's melting point. We have found TAG was still abundant in the SCGs from NMR spectra. After lipids were removed from SCGs, the intensity of the TAG signal decreased considerably, with approximately 64% of the TAG was successfully extracted. We described the chemical structure of TAG in coffee beans and demonstrated that it is possible quantify the amount of extracted TAG using solid-state NMR.

Introduction

Coffee is one of the world's most popular beverages, and more than 400 billion cups of coffee are consumed each year [1]. Approximately 9.5 million tons of coffee beans were consumed in 2017, and this is increasing year by year [2]. Approximately 8 million tons of spent coffee grounds (SCGs) are generated annually [3]. They are now classified as food industry waste, and there is a responsibility to establish an efficient and comprehensive method for reusing or adding value to SCGs. Research on SCGs has sharply increased in the past ten years [1]. SCGs can be reused to produce fuel for industrial boilers, as a substrate for the cultivation of microorganisms, and as a raw material to produce ethanol, among other uses [4]. Biodiesel is a source of renewable energy, and the production of biodiesel has been dramatically increased in past ten years. According to some reports, oils from SCGs have the potential to be used in biodiesel oil [5-7]. Furthermore, coffee beans contain polysaccharides in their cell walls such as cellulose and hemicellulose. [3,6,8]. The extracts from coffee oils, including SCGs, have not been completely characterized at the molecular level, which is important SCGs can be used for green chemistry applications.

Coffee beans contain a complex mixture of hundreds of different compounds. Lipids are abundant in coffee beans and SCGs [3,8,9]. Lipids still represent about 10 to 20 % of the mass of SCGs [1,10,11]. The major lipid in coffee beans is triacylglycerol (TAG) [10,12], which is widely found in animal fat and vegetable oil. TAG consists of three acyl chains with saturated and unsaturated fatty acids. The unsaturated fatty acids are oleic (18:1(n-9)), linoleic (18:2(n-6)) and linolenic (18:3(n-3)) acids [10].

Nuclear magnetic resonance (NMR) is an effective and non-destructive analytical tool to identify the structure and dynamics of molecules [13–15]. Solution NMR analysis of the extracts of roasted coffee beans (RCBs) and green coffee beans (GCBs) has been used to analyze the structure of organic compounds and investigate their metabolomics to classify beans from different geographic regions [16,17]. Solid-state NMR characterization is a direct method for analyzing insoluble biomacromolecules, such as polysaccharides in plant cell walls, membrane proteins, and amyloid peptides [18–22]. Additionally, the use of solid-state NMR with magic angle spinning (MAS) does not require any complex sample treatment. If we could reveal detailed structural information about the lipids in coffee beans via solid-state NMR analysis, we could estimate their amounts and the degree of unsaturation in lipid molecules without needing to extract from

coffee beans. This would lead to a deeper understanding of how lipids extracted from SCGs can be used as raw materials to generate biodiesel oil. Therefore, we investigated the structures of TAG and polysaccharides from GCBs, RCBs and SCGs using ^{1}H and ^{13}C solid-state MAS NMR techniques.

Materials and Methods

Sample preparation

The GCBs and RCBs were commercially available *Robusta* coffee varieties in Indonesia that had been washed. GCBs and RCBs were ground using an electric coffee grinder (Kalita, EG-45) for 70 s and for 40 s respectively, to a uniform particle size. Then hot water was added to ground RCBs. Wet-ground RCBs were thinly spread on filter paper and completely dried at room temperature at least 1 day. Completely dried ground RCBs were called as SCGs. For the proton/deuterium exchange experiment, 450 mg of RCBs was suspended in 5 mL of 99.5% D_2O (CIL) and then the RCBs were dried at room temperature. TAG containing only oleic fatty acids in acyl chains ($\geqq$ 97%, Sigma Aldrich) was used as a reference sample.

SEM

The internal structures of the GCBs and RCBs were observed with field emission scanning electron microscope (FE-SEM) (HITACHI, SU8010). GCBs were rapidly freeze-dried and polished by embedding in a resin block to expose the section. RCBs were divided into a several mm-sized pieces using a cutter and then were lyophilized by a freeze-dryer (EYELA, FDU-2100).

Extracting lipids from SCGs

The equivalent of a cup of coffee SCGs, 10 g was gently stirred in 100 mL of *n*-hexane at room temperature for 1 day. The solvent was removed by reduced pressure evaporation at 80°C (EYELA, SB-1200). Approximately about 70 μl of lipids were extracted as a clear yellow brown oil in liquid form, and solid residues were filtered (Figure 1). The extraction rate of TAG was estimated by comparing integrated intensity ratio of the signal at 2.6~2.7 ppm in ^{1}H-MAS NMR spectra. (Figure 3 (b) and Figure 4 (b))

^{1}H and ^{13}C solid-state NMR experiments

The GCBs, RCBs, SCGs samples as well as the solid residue and liquid lipids extracted from the SCGs were directly packed into a 4.0 mm outer diameter zirconia NMR rotor. ^{1}H-MAS, ^{13}C cross polarization-magic angle spinning (CP-MAS) and ^{13}C dipolar decoupling-magic angle spinning (DD-MAS) solid-state NMR spectra were recorded at room temperature with a recycle delay time of 4 s on a 600 MHz spectrometer (Bruker Avance III) equipped with a 4.0 mm E-free MAS probe. The MAS frequencies were at 12.0 and 10.0 kHz for the ^{1}H and ^{13}C NMR experiments, respectively. The DD-MAS method primarily detects ^{13}C NMR signals from the mobile components based on their spin-lattice relaxation times compared with the recycle delay times [21, 22]. ^{1}H and ^{13}C chemical shifts were referenced to tetramethylsilane at 0.0 ppm, respectively (Figure 1).

Results and Discussion

SEM observations

The cell walls in the GCBs and RCBs had strong honeycomb structures with pore sizes of several tens of μm as shown in Figure 2. The honeycomb structure in the SEM image of GCB is partly distorted because of the removal of moisture during lyophilization. However, a similar honeycomb structure of the cell walls is evident in the GCB when observed with an optical microscope (Supporting Information Figure S1). The pores of the GCB and RCB are filled with liquid lipids, and the moisture, which was present in the GCB but not in the RCB, was believed to be absorbed into the cell walls (see NMR section below).

Solid-state NMR characterization of TAG

Firstly, we observed ^{1}H and ^{13}C solid-state NMR spectra of GCBs, RCBs and SCGs as shown in Figure 3. In general, ^{1}H MAS NMR signals of a solid-state sample are quite broad due to large huge ^{1}H-^{1}H homonuclear dipolar interactions [21]. However, all three ^{1}H MAS NMR spectra of GCBs, RCBs and SCGs had similar narrow spectral patterns, except there was a broad signal at around 4.5 ppm in the GCBs (Figure 3 (a) to (c)).

We confirmed that the three fatty acids in the TAG molecule were a mixture of oleic, and linoleic acids. The ^{1}H NMR peaks in all three spectra corresponded to A-J of TAG in Figure 3 and complete assignments of ^{1}H NMR signals corresponding to A-J were summarized in Supporting Information Table S2. The **F** signal (2.6 ppm) in all three spectra belongs to protons directly bonded with the carbon sandwiched between two –

C=C– bonds as -CH=CH-C**H**$_2$-CH=CH- in linoleic acid. Most –C=C– bonds in natural unsaturated fatty acids have a *cis*-conformation, which causes a bend in the alkyl chain and molecules that are not well stacked. TAG is likely a liquid in coffee beans at room temperature because of the –C=C– bonds in the unsaturated fatty acids. All the peaks assigned to TAG in the ^{1}H MAS NMR observations were remarkably sharp despite the use of natural products without any pretreatment. It appears that the amount of unsaturated fatty acids in coffee beans is higher than the amount of saturated fatty acids. The scale of the SCGs spectrum in Figure 3 (Left) was enlarged five times because the intensity was smaller than that of the GCBs and RCBs. This indicates that some TAG was extracted during coffee brewing, decreasing the amount of TAG in the SCGs compared to the same volume of RCBs. There was a broad signal at approximately 4.5 ppm in the GCBs due to fixed moisture, which accounts for approximately 5 to 10% of the dry weight [23,24]. The broadness of this signal is likely due to the slow mobility of water molecules absorbed into cell walls. The moisture content of coffee beans is sharply reduced during the roasting process; consequently, signals derived from water in cell walls were not appeared at around 4.5 ppm in the ^{1}H NMR of RCBs (Figure 3 (b)) or SCGs (Figure 3(c)).

The ^{13}C DD-MAS NMR spectra of the GCBs, RCBs and SCGs revealed similar patterns of chemical shifts (Figure 3 right). All the sharp peaks, except the broad peak of **K** (60~63 ppm) and **L** (70~77 ppm), could be assigned to TAG, with the -C=C- double bond of linoleic acids at around 130 ppm (Supporting information Table S2). **K** and **L** are derived from the polysaccharides that constitute cell walls in coffee beans. Components of the cell wall have restricted their molecular mobility due to high crystallization, so **K** and **L** are not as sharp as the peaks assigned to TAG.

After extracting the lipids from the SCGs, the liquid lipids and the solid residues were analyzed by ^{1}H MAS NMR and ^{13}C DD-MAS NMR (Figure 4). The sharpness of the peaks in Figure 4 (a) and (c) suggest a high purity and consistent quality in the extracted TAG from the SCGs. A single peak appeared at 172.3 ppm in (c) indicates the absence of free fatty acids and that is a great advantage for transesterification process of biodiesel production in general [25]. These results are also consistent with chemical shifts of commercial TAG, whose fatty acids are only oleic acids (Supporting Information Figure S2). The broad peak around 4 ppm in Figure 4 (b) may be due to water added to the brewing process or adsorbed water during the drying process. The peak at 110 ppm (Figure 4 (c)) is from the Teflon coating spacer used to avoid spillage of liquid lipids in

the zirconia NMR rotor during the acquisition. The signals marked with an asterisk (*) in Figure 4 (d) are likely the polysaccharides that constitute the cell walls of coffee beans, which indicates that the cell walls were not destroyed during the hexane treatment. On the other hand, the ^{13}C NMR signals of TAG in the dried residue (Figure 4 (d)) were relatively low compared to those of the RCBs and SCGs (Figure 3), suggesting that the TAGs were mostly extracted by the hexane treatments, but a small amount of TAG remained. The sharpness of the peaks in ^{1}H MAS NMR and ^{13}C DD-MAS NMR spectra (Figure 4 (a) and 4 (c)) indicated the uniformity of extracted lipids from SCGs, and a high level of similarity with the commercial triacylglycerol sample (Supporting Figure S3 and Table S2) demonstrated the high purity of the TAG. Different from soybean oil, palm oil and cottonseed oil which are typical vegetable oils generated biodiesel fuel [26], SCGs does not need newly cultivated lands nor completely compete with food. From our NMR results, SCGs are promising raw materials for generating biodiesel fuel. However, TAG seems to remain in the residues after delipidization process (Figure 4 (a) and 4 (c)), so an improvement extraction rate of TAG would be future issues. In addition, solid-state ^{1}H MAS NMR technology is also useful in the field of food science, as it is possible to evaluate the quality of TAG rapidly.

Solid-state NMR characterization of polysaccharides

The spectra from ^{13}C CP-MAS NMR show the main components of the cell walls (Figure 5). Cellulose and hemicellulose are polysaccharides that constitute the primary cell wall. There were high intensity peaks between 60 and 110 ppm representing carbons, constitute the five- or six-membered rings of polysaccharides. Galactomannan, arabinogalactan and cellulose are the most common polysaccharides in the coffee beans [8]. Using SPINASSIGN based on the RIKEN ^{1}H and ^{13}C chemical shift database of metabolites, the ^{13}C NMR signals at 81 and 102 ppm could be assigned to the galactose group of polysaccharides [27]. This indicates signals at 81 and 102 ppm are from polysaccharides included in hemicellulose. The broad signals of E, F and G are likely TAG and glycoprotein sidechains [28], lignin aromatic carbons [28, 29] and the carbonyl groups of lignin, hemicelluloses and the protein backbone [30]. The spectral similarities of the GCBs, RCBs, SCGs and solid residues indicates that the cell wall structure was maintained through the roasting, grinding, and delipidization processes. The presence of strong intramolecular and intermolecular hydrogen bonds in cellulose microfibrils and

hemicellulose may explain the stable structure of the cell walls [8, 31]. Deuterium secondary isotope shifts on ^{13}C NMR signals can be used to identify exchangeable hydroxyl protons [32]. A comparison of the ^{13}C CP-MAS NMR spectra of RCBs and D_2O-suspended RCBs revealed that the C6 signal of the suspended RCBs shifted partly toward a higher field shift of 1 ppm (at 61.8 ppm) (Supporting Information Figure S3). This suggests that water molecules can be reached easily at the cell walls and that they dynamically interact with polysaccharides via hydrogen bonds. Cellulose can become an attractive source of cellulose nanofibers oxidized by water-soluble 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), which is a promising new functional material [33]. The production of cellulose nanofibers from coffee bean will be our next focus.

Conclusions

Thick cell walls in the GCBs and RCBs were observed to form honeycomb structures even after grinding or brewing, and lipids were contained in pores. ^{1}H and ^{13}C solid-state NMR measurements have been performed to characterize the lipids and polysaccharides in coffee beans that had not been processed, those that had been roasted, those that were left over from coffee brewing, and those that had undergone lipid extraction. The structure of lipids in coffee beans was determined by NMR analysis. TAG have mixture of oleic and linoleic fatty acids, and they were successfully isolated in liquid form via delipidization process. Structure of major polysaccharides as galactomannan, arabinogalactan and cellulose in the coffee beans were characterized by ^{13}C solid-state NMR. These characterizations will help advance research on generating biodiesel fuel from the TAG.

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Disclosure statement

No potential conflict of interest is reported by the authors.

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Figure Captions

Figure 1. The experimental design of solid-state MAS NMR characterization in coffee beans [green coffee beans (GCBs); roasted coffee beans (RCBs); spent coffee grounds (SCGs)] and solid residue and liquid lipids extracted from SCGs.

Figure 2. SEM images of cell walls in (a) GCBs and (b) RCBs.

Figure 3. [Left] ^{1}H MAS NMR spectra of (a) GCBs, (b) RCBs and (c) SCGs (scale magnified five times), with letters from A to J indicating peaks that correspond to structures in two fatty acids (oleic, and linoleic acid); and [Right] ^{13}C DD-MAS NMR spectra of (d) GCBs, (e) RCBs, and (f) SCGs, with letters K and L indicating polysaccharides. For the ^{1}H MAS NMR, all three spectra were accumulated from 200 scans and each experimental time is 7 minutes. For the ^{13}C DD-MAS NMR, all three spectra were accumulated from 13,000 scans and each experimental time is around 11 hours.

Figure 4. [Left] Spectra from ^{1}H MAS NMR of (a) extracted liquid TAG and (b) solid coffee residues after hexane treatment (Number of scans = 200, total experimental time = 4 min 6 sec.), and [Right] spectra from ^{13}C DD-MAS NMR of (c) extracted liquid TAG (Number of scans = 600, total experimental time = 30 min 33 sec.) and (d) solid coffee residues after hexane treatment (Number of scans = 13000, total experimental time = 11 h 1 min 6 sec.).

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Figure 5 [Inset] Structure of cellulose; and spectra from ^{13}C CP-MAS NMR of (a) GCBs, (b) RCBs, (c) SCGs, and (d) solid coffee residues after hexane treatment (Number of scans = 5000, total experimental time = 4 h 14 min 43 sec.).

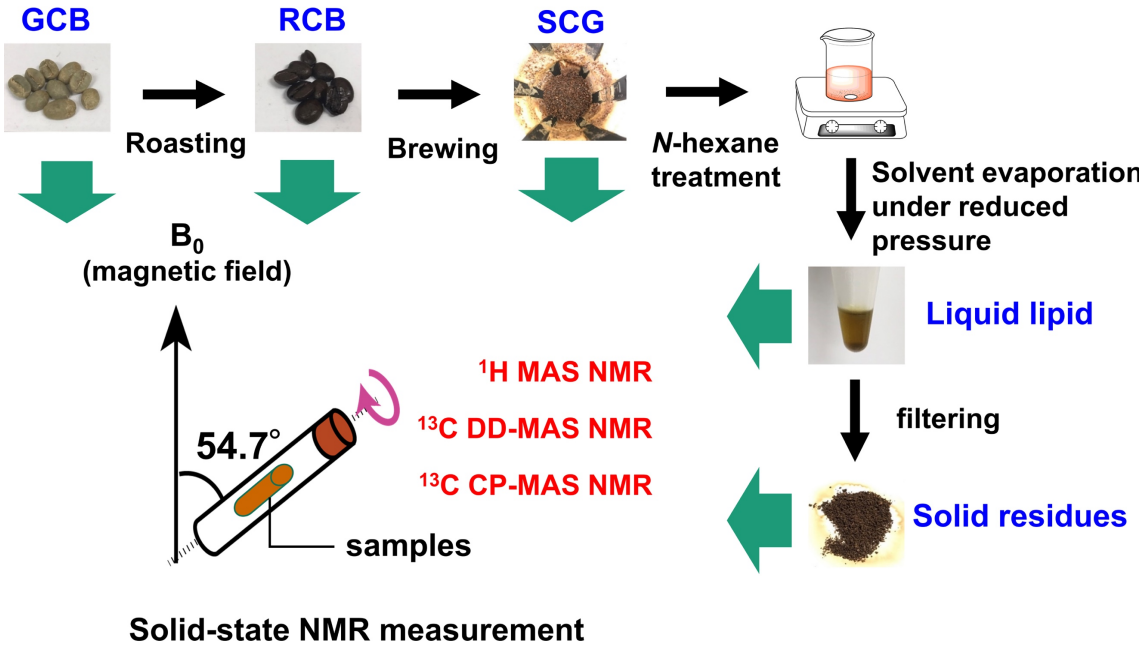

Figure 1
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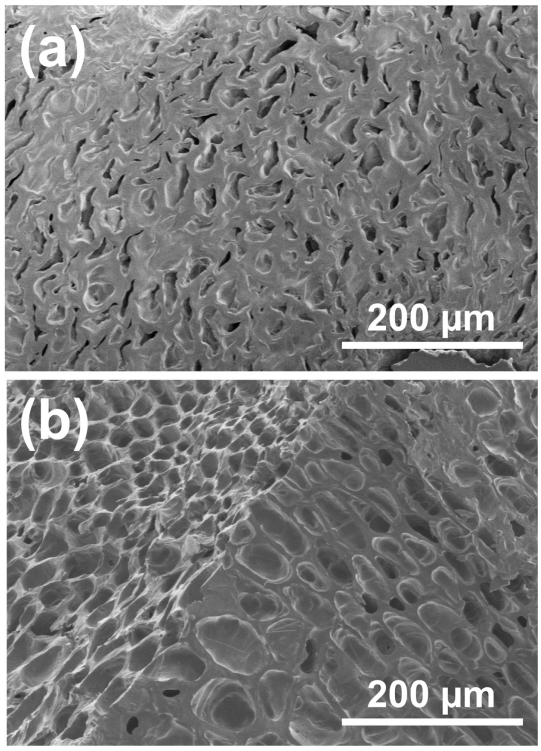

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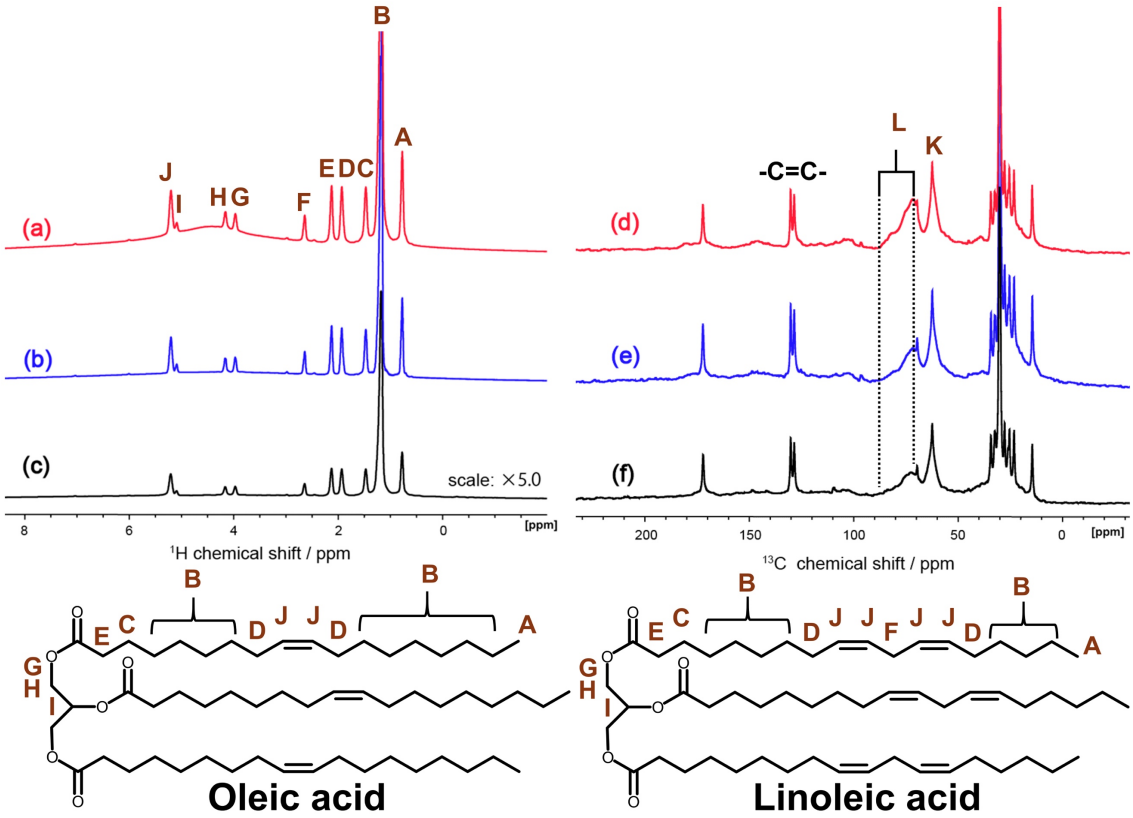

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Figure 3 N. Kanai et al. (2018)

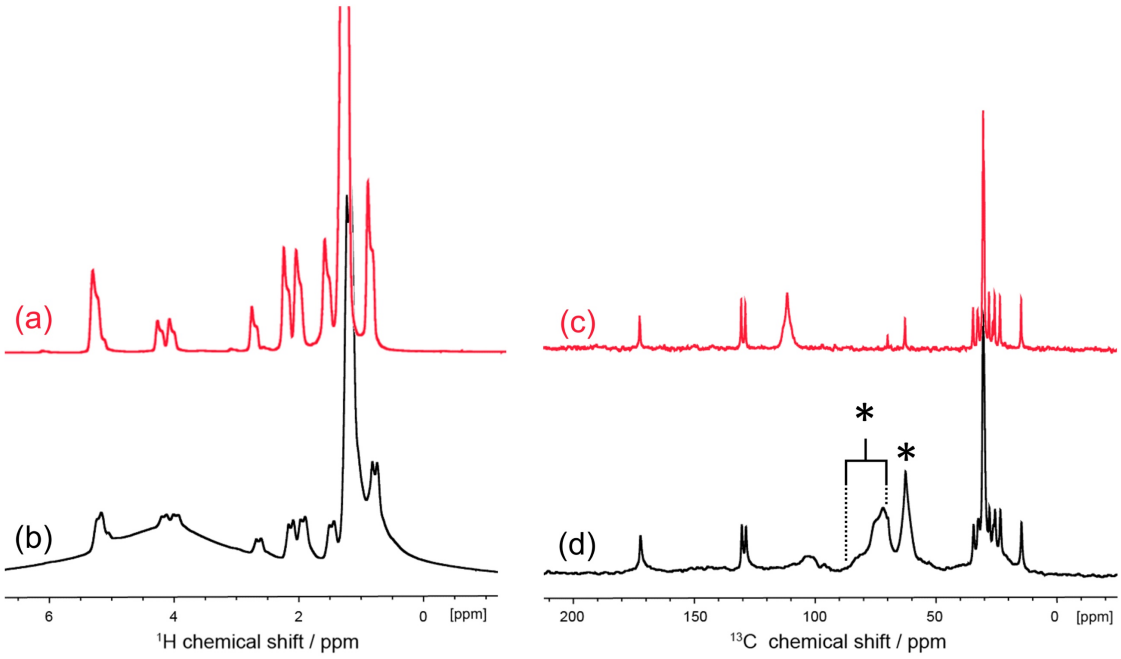

Figure 4
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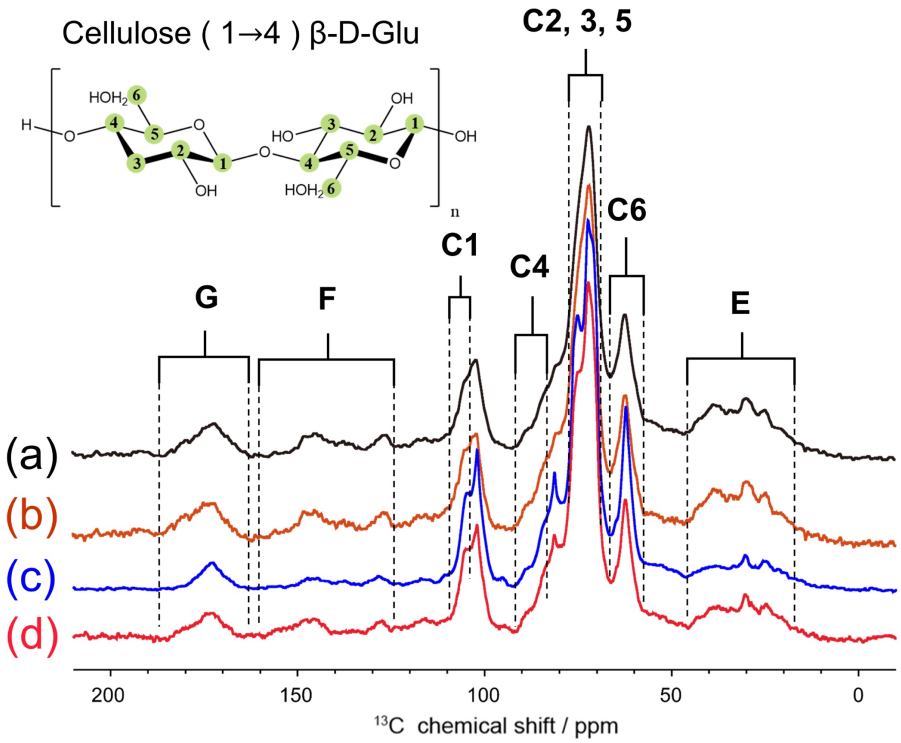

Figure 5
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