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Research paper

Characterization of three tissue fractions in corn (*Zea mays*) cob

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ABSTRACT

Corn (*Zea mays*) cob is composed of three tissue fractions, chaff, woody ring, and pith, with dry weight percentages of 21.1%, 77.5%, and 1.4%, respectively. In this study, the cell wall components in these tissue fractions were characterized to examine their tissue morphology. The chemical compositions in the three fractions were relatively similar, and hemicellulose was the main component. Through sugar composition analysis, hemicellulose was mainly composed of xylan in all fractions, whereas the proportion of arabinose and galactose was different in the woody ring. From the alkaline nitrobenzene oxidation analysis, lignin in all fractions was composed of guaiacyl, syringyl, and *p*-hydroxyphenyl lignins, whereas their ratios varied in the three fractions. Furthermore, the amounts of cinnamic acids such as ferulic and *p*-coumaric acids, which are associated with corn lignin, were also different among the three fractions. With respect to the tissue morphology, the component cells in the three fractions were totally different each other. Furthermore, from the ultraviolet microspectrophotometry of each morphological region in the three tissue fractions, lignin concentration and distribution of cinnamic acids were different from one morphological region to another. The differences in chemical composition and lignin structures influence the decomposition behaviors in various treatments; thus, this information provides a clue to promote efficient utilization of corn cob into value-added chemicals.

1. Introduction

Corn (*Zea mays* L.) is one of the most produced foodstuff in the world along with sugarcane [1]. As a by-product of the corn production, corn cob is estimated to be globally produced with a yield of 164 Tg [2]. For its utilization, various studies have been conducted to produce valuable chemicals such as xylitol [3,4], ethanol [3,5–10], and cellulose nanofibers [11–13]. In current applications, corn cob is a resource for furfural production in China, and ethanol has been produced from corn cob since 2013 [14]. In order to utilize the lignocellulosics for biofuels or chemicals, it is essential to understand its chemical characteristics and structures.

The cell walls of the lignocellulosics are mainly composed of cellulose, hemicellulose, and lignin, and their components and compositions are different depending on the lignocellulosic species [15]. For the whole corn cob, several researchers have studied its chemical structures, especially for hemicellulose [9,16,17]. However, corn cob is composed of three tissue fractions: chaff, woody ring, and pith [18]. The shapes, densities, and physical structures are totally different among the three fractions, whereas their detailed chemical structures have not yet been characterized.

In this study, the chemical compositions and the characteristics of the main cell wall components such as cellulose, hemicellulose, and lignin, are examined for the separated three tissue fractions of the corn cob. Furthermore, their component cells and the distributions of lignin including cinnamic acids are examined using ultraviolet microspectrophotometry.

2. Materials and methods

2.1. Samples and chemicals

Corn used in this study was harvested in Langfang city, Hebei Province, China. The cultivar of corn is Chengyu. The sampling time and sample age were April 2015 and 0.4 years, respectively. The storage temperature and humidity before delivery to the laboratory were 10–20 °C and 60–70%, respectively. The sample condition during delivery was air-dried and the corn cob was separated from grains before packaging for delivery. This information was shown in accordance with the checklist for sample definition by Barton [19]. Upon arrival in the laboratory, three different tissue fractions (outer part, chaff; middle part, woody ring; inner part, pith) were separated using a knife as shown in Fig. 1, and the separated fractions were dried at 105 °C for 12 h to measure their oven-dried weight. The fractionated samples were then milled in a small grinder (Wonder Blender WB-1: Osaka Chemical Co., Ltd., Osaka, Japan), and used for various analyses. The analyses

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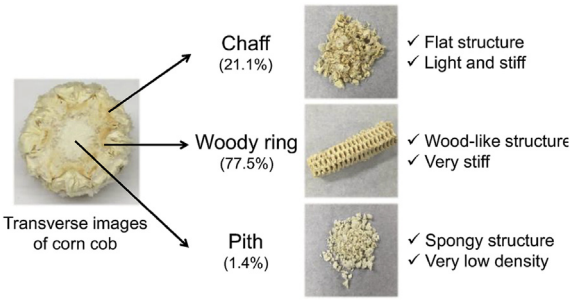

Fig. 1. Three tissue fractions of corn cob.

described below were conducted at least three times, and the average values were used. The chemicals used in this study were of reagent grade without any purification, purchased from Nacalai Tesque, Inc., Kyoto, Japan. The unit "%" used in this paper is based on weight of the sample.

2.2. Analytical methods

Chemical compositions in three tissue fractions were evaluated using the method of Rabemanolontsoa et al. [20]. The chemical components cellulose, hemicellulose, lignin, protein, extractives, starch, and ash were determined.

X-ray diffraction (XRD) was performed using a Rigaku RINT 2,200V (Rigaku Corp., Tokyo, Japan) with Ni-filtered Cu-Kα radiation (λ = 0.1542 nm) generated at 40 kV and 30 mA to evaluate the crystalline structure of cellulose and the standard method for holocellulose production [21]. In order to compare the cellulose crystallinity in each fraction, the crystallinity index was estimated using the calculation methods by Segal et al. shown below [22].

$$\text{Crystallinity index (CrI)} = \frac{I_{002} - I_{am}}{I_{002}} \times 100$$

I_{002} is the maximum intensity of the 002 lattice diffraction at 2θ = 22.5°, and I_{am} is the intensity of the diffraction at 2θ = 18.0°.

The composition of hemicellulosic saccharides was determined using the acid methanolysis method [23], and the obtained monosaccharides were quantified by gas chromatography–mass spectrometry (GC-MS) analysis using GCMS-QP 2010 Ultra (Shimadzu Co., Kyoto, Japan) after trimethylsilyl derivatization [2]. Furthermore, acetic acid content was analyzed using the acid hydrolysis method with 72% H_2SO_4 followed by 3% H_2SO_4 [24]. Subsequently, the obtained hydrolyzates were analyzed with high-performance liquid chromatography (HPLC; LC-10A, Shimadzu Co., Kyoto, Japan) [2].

For the analysis of the lignin structure, the alkaline nitrobenzene oxidation was performed in accord with standard methods, and the total yields of vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde were determined by gas chromatography (GC; GC 2014, Shimadzu Co., Kyoto, Japan) [25]. The vanillin and *p*-hydroxybenzaldehyde can be produced from ferulic acid and *p*-coumaric acid, respectively, because both acids are associated with corn lignin [26–30]. Thus, the yields of cinnamic-acid-derived vanillin and *p*-hydroxybenzaldehyde were subtracted from the original yield of the alkaline nitrobenzene oxidation products to obtain the actual yields of products from lignin. The yields of cinnamic-acid-derived vanillin and *p*-hydroxybenzaldehyde were estimated from the experiments with model compounds.

The corn lignin contained some cinnamic acids such as ferulic acid (4-hydroxy-3-methoxycinnamic acid) and *p*-coumaric acid (4-hydroxycinnamic acid). The fractionated flour was treated with 0.5 mol L^{-1} NaOH to extract the cinnamic acids [31]. The extracted portion was acidified with dilute HCl and then extracted with ethyl acetate. The ethyl acetate-soluble portion was then dehydrated and evaporated under vacuum. The obtained products were trimethylsilyl derivatized followed by GC-MS analysis [32].

2.3. Microscopic observations

The distribution of lignin including cinnamic acids were observed by UV microscopy [33]. Each tissue fraction was embedded in epoxy resin, and the samples were cut into 0.5-μm thick sections with a diamond knife mounted on a Leica Reichert Supernova Microtome (Buffalo Grove, IL, USA). The sections were placed on quartz slides, mounted with glycerin, and covered with a quartz coverslip before examination by an MSP-800 system (Carl Zeiss, Oberkochem, Germany) with a specified filter at 280 nm ± 5 nm. The morphological regions of each fraction were analyzed on a UV microspectrophotometry using photometric point-by-point measurements (spot size: 1 × 1 μm).

3. Results and discussion

3.1. Characteristics and chemical compositions of the three tissue fractions

The images of three tissue fractions from corn cob and their dried weight compositions are shown in Fig. 1. The corn cob is composed of three tissue fractions with physical structures different from one another. The outer part, chaff, is light and stiff, and its structure is wrinkled. The middle part, woody ring, is a lignified structure and very stiff like a woody xylem. The inner part, pith, is extremely light and its structure is spongy. The chaff, woody ring, and pith account for 21.1%, 77.5%, and 1.4% on an oven-dried weight basis, respectively.

The chemical compositions in three tissue fractions are presented in Table 1. Hemicellulose is the main component in all fractions, especially in the woody ring with 46.9%. Cellulose is the second largest component, after hemicellulose. The proportion of the holocellulose (cellulose + hemicellulose) is quite high in all regions, and these values are much higher compared with those of other lignocellulosics [15]. However, the lignin content is less than 20% in all fractions, smaller than that of woody biomass. Although the woody ring appears high in lignin content, its content is lower than that of the other two fractions. For the extractives, the pith contained 3.5%, which is the highest among three fractions. The ash content is the highest in the chaff and the lowest in the woody ring. Accordingly, there are some differences in the chemical composition between the three tissue fractions, whereas their overall compositions are relatively similar. Because each component was quantified independently, the total values are not necessarily equal to 100%. However, it was not adjusted.

For the further characterization, the detailed analyses of the main cell wall components such as cellulose, hemicellulose, and lignin were conducted for the three fractions.

Table 1
Chemical composition of three tissue fractions of corn cob.

Tissue fraction	(%)	Chemical composition (% on dried sample basis)							
		Cellulose	Hemicellulose	Lignin	Protein	Extractives	Starch	Ash	Total
Chaff	21.1	36.3	41.4	18.8	2.3	0.5	0.3	3.1	102.7
Woody ring	77.5	31.6	46.9	15.7	2.1	1.0	0.3	1.4	99.0
Pith	1.4	34.8	39.9	17.3	2.4	3.5	2.1	2.4	102.4