

The Utilization of Soybean Straw. I. Fiber Morphology and Chemical Characteristics

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To improve basic knowledge of the properties of soybean straw, its fiber properties, anatomical structure, and components were investigated in detail. Soybean straw contains less ash and silica than some non-woody biomass. Its stem and root have more lignin and holocellulose, but less nitrogen and protein contents than the pod. Additionally, it has much shorter and wider fibers, and the length-width ratio is also lower than other crop straws. Morphologically, there are three main tissues---the ground tissue, the vascular tissue, and the dermal tissue systems in the stem, and two different morphology portions – the intimal layer and the leathery layer – in the longitudinal-section of the pod. A variety of inorganic and metal elements are distributed across the whole stem or pod in different amounts. Lastly, the planetary ball-milled stem and pod are completely dissolved in 8% lithium chloride/dimethyl sulfoxide (LiCl/DMSO) solution. After regeneration, the lignin has the highest retention, followed by silica and sugars, but most of the ash can be removed in this process.

Keywords: Soybean straw; Morphology; Chemical composition; Elements distribution; Dissolution-regeneration

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INTRODUCTION

Soybean (*Glycine max*) straw is an abundant and renewable form of biomass with enormous potential as a low-cost, sustainable source of energy and chemicals (Ashori *et al.* 2014). Renewable biomass resources are increasingly regarded as important to the development of a sustainable industrial society and to the management of greenhouse gas emissions. Bio-fibers from agricultural residues such as soybean straw are widely distributed, inexpensive, recyclable, versatile, and are a biodegradable source of renewable lignocellulosic biomass (McKendry 2002; Liu *et al.* 2005). Most of the residues in common use are annual plants that develop their full fiber potential in one growing season (Ashori 2006), such as wheat straw, cotton stalks, rice straw, and reed. Even waste grass clippings function as an important fiber resource in countries such as China, where there is an extreme shortage of wood (Nemli *et al.* 2009). In fact, the rapid growth of wood-based manufacturing over the past decades combined with a concomitant global decline in forest resources has driven researchers worldwide to investigate a range of potential alternative raw materials such as non-wood biomass (Akgula and Camibel 2008). In addition to its role in wood-based industries, the lignocellulosic biomass comprised of agricultural residues is also a potential renewable feedstock for biofuels and biorefinery (Ragauskas *et al.* 2006; Lucia 2008).

Soybean is a species of annual legume herb native to East Asia that is widely grown for its edible bean, which has numerous uses (Michel 1995). It has hard pods, stout stems, and trifoliate leaves that are covered with fine brown or gray hairs. Before the soybean seeds are mature, all the leaves wilt and fall. In the axil of the leaves, there are the self-fertile flowers with the colors of white, pink, or purple. The plant is classed as an oilseed rather than a pulse by the UN Food and Agricultural Organization (FAO).

As reported by United States Department of Agriculture (USDA), soybean plantations in America covered 7.65 million acres in 2013, and would expand to 8.2 million acres in 2014. Global soybean production in 2013 was 267.1 million tons and would increase to 287.8 million tons in 2014. Given that the mass ratio of soybean straw to soybean fruit is 1.6 (Liu *et al.* 2006), there is a large amount of soybean straw that is grown annually as well.

At present, soybean straw is mainly used for animal feedstock, burned for rural energy, or disposed of arbitrarily in the field (Zhu *et al.* 2008; Terashima *et al.* 2009). Unfortunately, these current uses will inevitably result in environmental pollution as well as resources loss, particularly given the potential of soybean straw, which is rich in cellulose, hemicellulose, protein, and other organic matter. Even were it to be more methodically decomposed into the soil as humus, soybean straw could increase soil's organic carbon content, improve its fertility, and improve tillage performance.

According to recent research, soybean straw can be used to remove hazardous metal ions and dyes from aqueous solutions, such as Cu^{2+} (Zhu *et al.* 2008), black B, and acid orange 7 dyes (Ashori 2014). Some researchers also have suggested that it could be used to produce natural cellulose technical fibers with structure and properties similar to the cellulose fibers currently in use (Wang and Sain 2007; Reddy and Yang 2009). This would not only add value to the soybean crops but also provide a sustainable source for fibers (Castro *et al.* 1991).

In sum, more strategic utilization of soybean straw could yield enormous global economic, environmental, and social benefits. Yet more research needs to be conducted in two fundamental areas to enable scientists to move forward on these fronts. First, the basic understanding of soybean straw composition, microstructure, and properties must be extended and improved, and second, it is important to determine what would be the most advanced, environmentally sensitive utilization (Hames *et al.* 2003; Hamelinck *et al.* 2005).

In the context of these needs, the aim of this study was to determine and characterize the chemical components, inorganic element distribution, and microstructure of individual soybean straw fractions, which have not yet been comprehensively reported. This study also investigated the dissolution and regeneration of the soybean stem and pod in LiCl/DMSO.

EXPERIMENTAL

Materials

The harvested soybean straw used in this study was collected from the North of Jiangsu, China. The air-dried straw was first manually fractionated into stem, pod, and root, and then stored in plastic bags for further use.

Methods

Weight percentage of soybean straw fractions

About 5 kg of air-dried soybean straw was manually separated into stem, pod, and root. The separated fractions were weighed individually, and their dryness contents were determined according to Technical Association of Pulp and Paper Industry test method (TAPPI method) T258 om-02. Two batches of separation were conducted, and the weight percentage of each fraction was calculated on an oven-dry basis.

Fiber properties

The soybean stem was manually cut into strips 1 mm wide and 10 mm long, followed by treatment with a mixture of acetic acid and 30% hydrogen peroxide (1:1, v/v) at 60 °C for 72 h for cell dissociation. When the samples turned white, the macerated cells were filtered and thoroughly washed with distilled water (Li *et al.* 2012). The fibers were separated from each other using a disintegrator (GBJ-A, Experimental Instrument, Changchun, China), which did not introduce any mechanical damage to samples, and analyzed with the MorFiCompact FS-300 fiber quality analyzer (Techpap, France).

Morphological structure and elements distribution

Different morphological parts of soybean straw were removed and vacuum-dried for scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDXA). These samples of different parts were carefully cut to expose both the inner and outer surfaces, and then the specimens were coated with a thin gold-palladium film. The observation was carried out by using a Quanta 200 SEM-EDXA (FEI, USA).

Chemical characterization

For chemical analysis, air-dried soybean stem and pod were ground in a Wiley mill and passed through the 40- and 60-mesh screens. The extractives, ash and silica were analyzed according to TAPPI test methods T204 cm-97, T211 om-02, and T244cm-99, respectively. The holocellulose was obtained by treating the benzene-alcohol (2:1, v/v) extractive-free samples with sodium hypochlorite (NaClO₂) and acetic acid to remove lignin. The lignin content and sugar contents were determined according to NREL/TP-510-42618 (Sluiter *et al.* 2011). The carbon, hydrogen, and nitrogen determinations were carried out in an elemental analyzer (Vario EL III, Elementar, Germany), and the crude protein content was calculated from nitrogen content by multiplying by a coefficient of 6.25. The extractives contents were calculated based on oven-dried fractions, and other compositions were based on separate oven-dried benzene-alcohol (2:1, v/v) extractive-free fractions.

Inorganic components analysis

The 40- to 60-mesh oven-dried benzene-alcohol extractive-free fractions were digested in a microwave digester by adding 7 mL of HNO₃ and 1 mL of H₂O₂, to determine the contents of potassium (K), calcium (Ca), phosphorus (P), sodium (Na), magnesium (Mg), aluminum (Al), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu). The digested samples were then washed and diluted into a 50 mL volumetric flask with 18 MΩ ultrapure water. The elements were determined using a Thermo Scientific iCAP 6000 ICP-OES (Li *et al.* 2012).

Ball-milling, dissolving, and regenerating

The dried ground soybean stems or pods were milled in a planetary ball mill (Fristch GMBH, Pulverisette 7 premium line, Idar-Oberstein, Germany) for 4 h, and then dried under vacuum. The ball-milled stem or pod powder was suspended in DMSO with 8% LiCl (8% LiCl/DMSO), respectively, and stirred continuously at room temperature for 24 h (Wang *et al.* 2009). The dissolved ball-milled stem was regenerated from the clear solution with distilled water, then centrifuged and washed thoroughly. The regenerated sample was dried under vacuum at 40 °C for 24 h.

RESULTS AND DISCUSSION**Cell Morphology***Soybean stem*

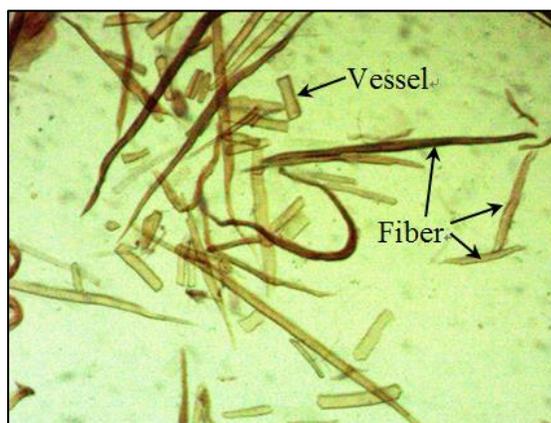
The harvested soybean straw always consists of stem and pod, which have different tissue structures, fiber properties, and chemical compositions. The length and width of the stem fibers are listed in Table 1.

Table 1. Fiber Dimensions of the Soybean Stem Compared with other Crops

Dimension	Soybean stem	Corn stover ^a	Wheat straw ^a	Sorghum straw ^a	Rice straw ^a	Reed straw ^a
Length (mm)	0.46	0.99	1.32	1.18	0.92	1.22
Width (μm)	24.2	13.2	12.9	12.1	8.1	8.5
Length-Width ratio	19	75	102	109	114	144

^a The fiber dimension of other crops were obtained from some references (Ogbonnaya *et al.* 1997; Ververis *et al.* 2004; Shatalov and Pereira 2006; Xu *et al.* 2006; Li 2012;)

The average length, width, and length-width ratio of soybean stem were 0.46 mm, 24.2 μm, and 19, respectively. The soybean stem had much shorter but wider fibers compared with other crops, such as corn stover, wheat straw, sorghum straw, rice straw, and reed straw. Thus, its length-width ratio was also lower than others (Ververis *et al.* 2004; Li 2012). As shown in Fig. 1, the short fibers are more than long fibers, and there are also some vessels present in the soybean straw.

**Fig. 1.** Optical microscope picture of the soybean straw fiber (Magnification: 8)

According to Page's theory (Page 1969), paper strength increases with the increment of fiber length which provides more fiber crossings on a typical fiber. If these short soybean straw fibers are used for paper making, they would be pulled out from each other without breaking. However, it is a potential lignocellulose material for biorefinery to produce bioenergy, chemicals and bio-materials.

Soybean is generally planted in the period from May to June. Because of the longer sunlight, higher temperature, and sufficient moisture at the time of planting, soybean plants grow rapidly (Hara 1995; Havel and Durzan 1996). As shown in Fig. 2, the stem is mainly constructed of a ground tissue system, a vascular tissue system, and a dermal tissue system. The ground tissue system in the stem is represented by the pith and cortex. The pith, which is composed of soft and light-colored spongy parenchyma cells, is located in the center part of the stem. These parenchyma cells have simple, thin primary walls, and they comprise the "filler" tissue in the stem. They are also responsible for storing and transporting nutrients throughout the plant. According to the high-resolution images of the parenchyma, there were a large number of pits on the cell wall. They work as the main channels for transporting water and nutrients between adjacent cells in the soybean stem. The cortex bounded on the outside by the epidermis is mainly composed of collenchyma cells that have irregularly thickened cell walls. Between the cortex and pith, there is the vascular tissue. It consists of two conducting tissues - the xylem and the phloem. The cells in this part have small diameters and thick cell walls. Some vessels that are distributed in this portion can transport water and nutrients from the roots throughout the plant. The dermal tissue system is represented by epidermis, which is the outer protective covering of the primary plant body. It serves as a boundary between the plant and the external environment, providing mechanical strength and protection to the plant against water loss, regulated gas exchange, secreted metabolic compounds, and absorbed water and mineral nutrients. Because the raw soybean straw used in this paper was collected after harvesting, all the cells were dead and dried. Therefore, cytoplasm cannot be found in these SEM photos.

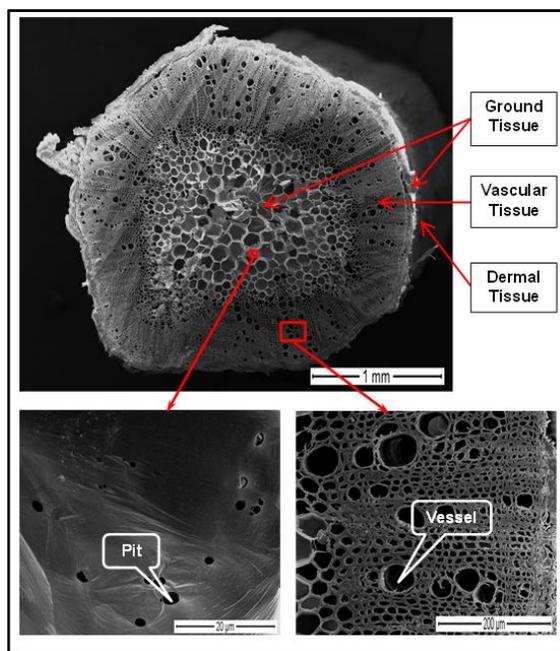


Fig. 2. SEM analysis of the soybean stem

Soybean pod

Although an organ homologous to soybean leaves, the pod shows no evidence of palisade tissue and spongy tissue (Cui *et al.* 2003; Fan *et al.* 2004). Figure 3 shows two different morphology portions – the intimal layer and the leathery layer – in the longitudinal-section of the pod. The intimal layer is seen to be composed of sclerenchyma with dense irregular shape and thick cells. The inner epidermis is very smooth and dense, consisting of interlaced reticulated fibers. With little, twisty lacuna, the leathery layer is much denser than the intimal layer. The outer epidermis consists of large amounts of pavement cells, trichomes, stomata, guard cells, and their subsidiary cells. The holes in the outer epidermis are the stomata of the pod. It is surrounded by two guard cells that control the opening and closing of the aperture. The guard cells are then surrounded in turn by subsidiary cells, which give support for the guard cells. In the root of trichomes, there were also some subsidiary cells that lead trichomes to grow in a certain direction. According to the available references (Raven *et al.* 2005; Szyndler *et al.* 2013), these cellular characteristics of soybean pod protect the soybean fruit, and control the transportation and distribution of water, air and nutrition within and between the pod and fruits. They also increase the soybean's tolerance of higher temperature, which is of great significance for photosynthesis and photosynthetic product output.

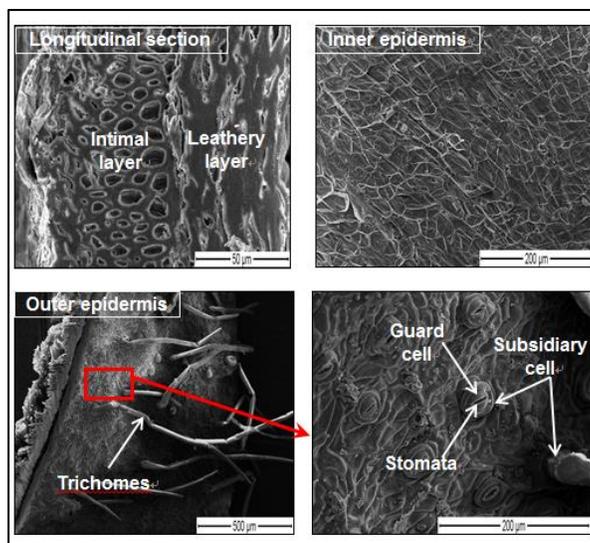


Fig. 3. SEM analysis of the soybean pod

Chemical Characteristics

The percentage of each fraction (stem, pod, and root) was determined based on the total dry weight of the sample. As shown in Table 2, the pod comprised nearly half of the weight of the whole straw, followed by the stem at 44.6%, with the root comprising the smallest amount.

Table 2. Weight Percentage of Soybean Straw Fractions on Dry Basis

Fractions	Weight percentage (%)
Stem	44.6
Pod	50.1
Root	5.3

These different fractions of soybean straw have different cell morphologies and play different functions. Thus, their chemical compositions can vary over a wide range. Some of them were measured, and results are listed in Tables 3 and 4.

Table 3. Soluble Components of the Soybean Straw Fractions

Composition ^a	Stem	Pod	Root
Benzene-alcohol extractives (%)	1.91	2.21	1.45
Hot water extractives (%)	9.22	18.63	9.01
1% NaOH extractives (%)	32.09	45.16	33.14
^a All these extractives were calculated based on the oven-dried fractions.			

According to the references, the extractives of biomass include all plant materials that are extracellular and not part of the three-dimensional cell wall structure, such as resin, fat, wax, pectin, starch, and inorganic pigment. They are present in different raw materials (Hames 2009). Total water and/or benzene-ethanol soluble materials are typically quantified gravimetrically and considered as extractives (Thammasouk *et al.* 1997; Chen *et al.* 2007). As indicated in Table 3, the amounts of extractives from the stem and root were similar, but much lower than the pod. Monosaccharides accounted for 30% to 46% of the total water extractives, which also included different kinds of aldol, aliphatic acid, inorganic ions, oligosaccharide, and some oligomers in phenolic glycoside. In the root and stem, the lignin contents were 25.28% and 24.07%, respectively. Both were higher than the pod (15.37%) by nearly 10%. The presence of high lignin content helps to resist outside mechanical injury as well as fungal invasion and explains why the stem and root are both very hard.

As shown in Table 4, the holocellulose content in the root (77.65%) was extremely similar to the stem (77.40%), but higher than the pod (69.62%). The pod had the highest nitrogen (0.86%), followed by the root (0.78%) and stem (0.52%), indicating that there were also more protein in the pod and root than in the stem. Additionally, compared with other lignocelluloses, soybean straw contained more ash and silica than most wood materials, but still less than some crop straws, such as wheat straw and corn stover (Liao *et al.* 2004).

Table 4. Chemical Composition of the Soybean Straw Fractions

Composition ^a	Stem (%)	Pod (%)	Root (%)
Total lignin	24.07	15.37	25.28
Klason lignin	22.45	12.64	23.86
Acid-soluble lignin	1.62	2.73	1.42
Holocellulose	77.40	69.62	77.65
Ash	2.64	5.86	2.27
Silica	0.76	0.51	0.85
Carbon	46.9	43.82	47.06
Hydrogen	5.95	5.85	5.94
Nitrogen as N	0.52	0.86	0.78
Crude protein	3.23	5.39	4.90
^a All these compositions were calculated based on the oven-dried benzene-alcohol (2:1, v/v) extractive-free fractions.			

The inorganic elements contents in the stem ranged from high to low as K>Ca>P>Na>Mg>Al>Fe>Zn>Mn>Cu. For the pod fraction, the sequence was Ca>K>Mg>P>Na>Fe>>Al >Mn>Zn>Cu, and for the root, the sequence was K>Ca>Na>P>Mg>Al>Fe>Zn>Cu>Mn. Clearly, K, Ca, P, Na, and Mg were the main inorganic components in soybean straw fractions. The contents of Al, Fe, Zn, Mn, and Cu in these three fractions were all lower than 100 ppm and could thus be considered trace elements.

No matter the main or the trace inorganic elements, they are mainly obtained from artificial fertilization, and are all the necessary nutrients for the growth of soybean plant. However, as reported in some references, they affect the utilization of this biomass in industry. When the soybean straws are used in pulping, these inorganic elements are the main constitution of ash. They present hazard to the continuous operation of the industrial black liquor recovery plant (Cardoso *et al.* 2009). In addition, the high lignin content in the stem is not desirable for chemical pulping, as it can lead to more chemical usage, longer digestion time, and higher energy consumption. If co-firing this biomass with coal directly in boilers for energy production, the high ash content would cause the operational problems (Szemmelveisz *et al.* 2009). It was also found that the common cations in ash, such as K⁺, Mg²⁺, Ca²⁺, Al³⁺, Mn²⁺, Fe³⁺, Cu²⁺, and Zn²⁺, showed negative effects on cellulase at different levels, except for the stimulative effects of Ca²⁺ and Mg²⁺ on β -glucosidase (Yu and Chen 2010). As for high potassium content in soybean straw fractions and its bad effects on many biomass-processing processes, it is economically necessary to separate and use them as chemicals.

Table 5. Inorganic Components in the Soybean Straw Fractions

Elements ^a	Stem (ppm)	Pod (ppm)	Root (ppm)
Potassium as K	3941.40	4356.10	2084.36
Calcium as Ca	3319.89	6700.25	1807.64
Phosphorus as P	719.30	981.27	672.59
Sodium as Na	690.78	146.74	1365.27
Magnesium as Mg	375.61	2870.42	387.36
Aluminum as Al	59.96	30.84	98.73
Iron as Fe	56.51	38.71	77.64
Zinc as Zn	12.05	12.80	8.82
Manganese as Mn	6.72	15.09	3.24
Copper as Cu	6.62	6.38	6.28

^a Element contents were all calculated based on the oven-dried benzene-alcohol (2:1, v/v) extractive-free fractions.

Inorganic Elements Distribution

As discussed above, Si, K, Ca, P, Na, and Mg were found to be the major inorganic elements in the soybean stem and pod. As shown in Fig. 4, these elements were distributed in the horizontal cross-section of the stem in different amounts. Obviously, the ground tissue had more and continuous K and Ca. O and P were distributed throughout the cross-section, especially in pith. S, Cl, C, B, Si, Al, Na, and Mg were almost evenly distributed within the stem. Additionally, scant amounts of Mn, Fe, Cu and Zn were distributed in the overall soybean stem with shallow brightness.

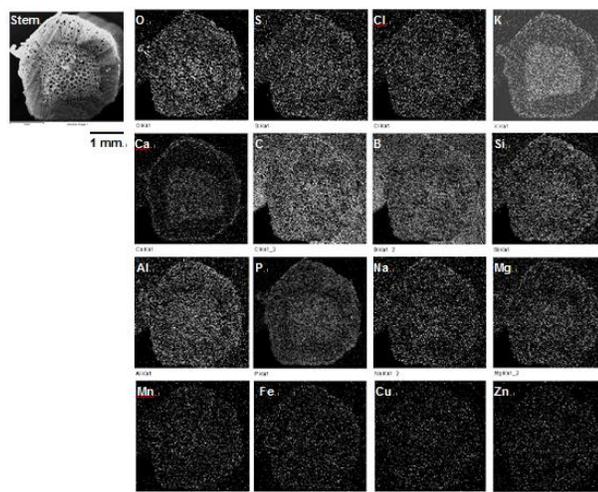


Fig. 4. Distribution of elements taken from the same area of the soybean stem, as indicated by SEM-EDXA

According to available information (Raven 2005), all these elements are essential for the growth of the soybean plant. From the branching stage, both the absorption and accumulation of N increased with the growth of the soybean plant, and reach peak during the seed filling period. The total amount of N needed in the soybean growth stage is four to five times more than other cereal with the same productivity. N deficiency can lead to dwarfs and little branches, with light-colored leaves that could turn yellow easily. The uptake and utilization of P have great impact on the overall growth of the soybean plant, and its maximum absorption appears from the branching to the seed filling period, although little in the seedling and flowering period. If there is not enough P, which is vitally important for soybean growth, the plant will develop sick leaves with brown spots, a small size seed, and even bad nodules will develop. The uptake of K mainly occurs at the early growth stage, and peaks in the podding period. K deficiency will also cause yellow leaves and decrease production. Other elements, such as Ca, Mg, Zn, B, and Mo, also affect the growth of leaves, nodule development, podding process, and the production of the soybean plant.

In Fig. 5 it is apparent that the elemental distributions in the intimal layer and leathery layer of the pod were similar to each other. In the intimal layer, the elements Mg, Cl, K, Zn, Na, and Al were present evenly. C was also abundant in most of the intimal layer, especially the position that did not have a large amount of O. There was just a bit of Ca in some parts of the epidermal surface. In the leathery layer, there were large amounts of Na, Cl, K, Mg, C and O. O content was a little bit lower in a certain portion where there was a higher content of K, Mg, and C.

Dissolution-Regeneration of Ball-milled Soybean

The ball-milled soybean stem/pod were dissolved in 8% LiCl/DMSO and then regenerated in distilled water. As shown in Fig. 6, both the ball-milled soybean stem and pod could dissolve in 8% LiCl/DMSO completely without any visible solid powder. As suggested by the available research literature, the solution in this system is brought about by undissociated ion pairs of LiCl molecules in DMSO, which interact with the oxygen atoms of hydroxyl groups of cellulose and disrupt irreversibly the hydrogen bonds between cellulose molecules (Petrus *et al.* 1995; Wang *et al.* 2013).

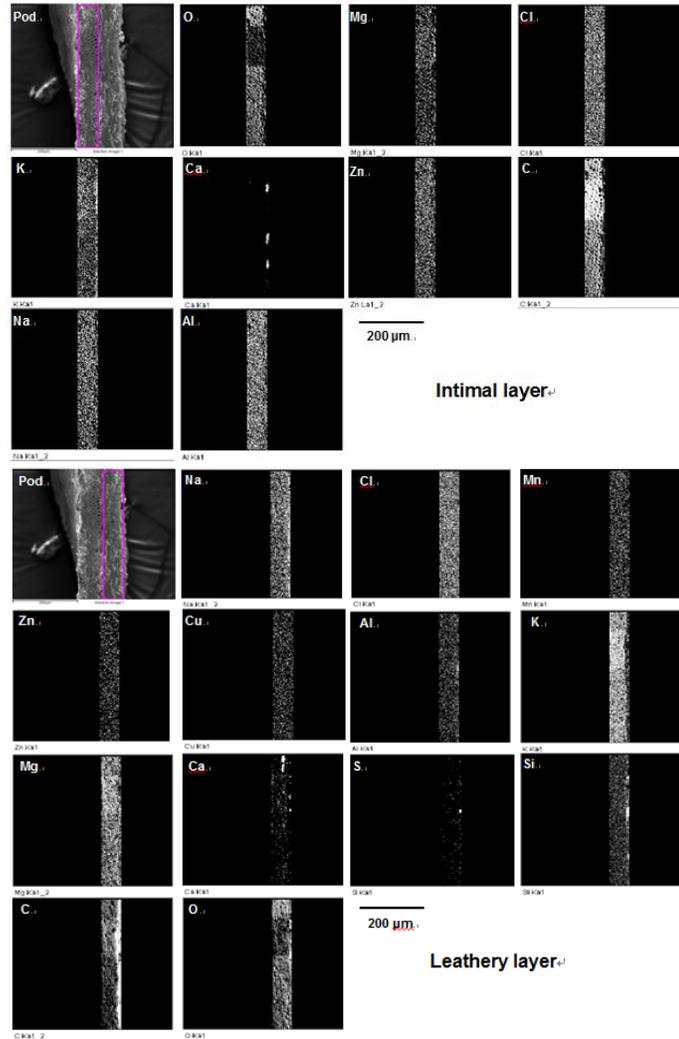


Fig. 5. Distribution of different elements taken from the same area of the soybean pod, as indicated by SEM-EDXA

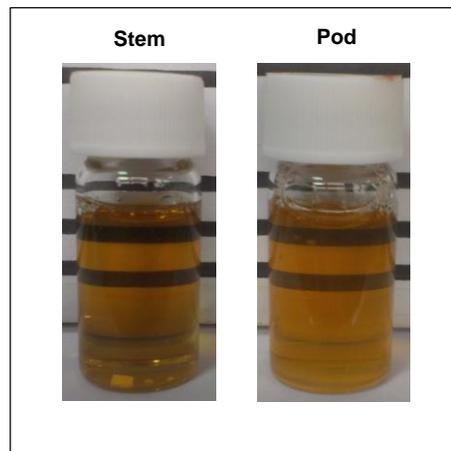


Fig. 6. Dissolution of the stem and pod with 4 h of ball milling in 8% LiCl/ DMSO with 1% (w/w) concentration

^a The white solid in the bottom is the stir bar.

Table 6. Chemical Composition of Regenerated 4 h Ball-milled Soybean Stem

Composition ^a	Raw stem (%)	Regeneration (%)	Retention (%) ^b
Yield	---	75.54	---
Total lignin	24.07	25.61	80.37
Klason lignin	22.45	24.16	81.29
Acid-soluble lignin	1.62	1.46	68.08
Total sugar	69.48	60.85	66.16
Glucan	43.97	44.11	75.78
Xylan	14.78	9.70	49.58
Rhamnosan + Galactan	4.11	2.56	47.05
Mannan + Araban	6.62	4.48	51.12
Ash	2.64	1.23	35.19
Silica	0.76	0.75	74.55
^a Both the composition of the raw stem and the yield of the regeneration were determined based on the extractive-free raw stem material. The compositions of regeneration were measured based on the oven-dried regeneration. The retention ratios of these compositions in the regeneration were calculated based on the contents of each composition in the extractive-free raw stem.			
^b Retention = [(The content of this composition in the regeneration × The yield of the regeneration) / (The content of this composition in the extractive-free raw soybean stem)] × 100%			

The characteristics of the regeneration are shown in Table 6. The total yield of regeneration was 75.54%. This also means that 24.46% of ball-milled soybean stem powder was lost in the dissolution-regeneration procedure. In the regeneration, the total lignin content was 25.61%, including 24.16% klason lignin and 1.46% acid-soluble lignin, which was higher than that of the raw stem. The retention of total lignin was 80.37%. Klason lignin and acid-soluble lignin retentions were 81.29% and 68.08%, respectively.

The content of sugars in this article is expressed in anhydro units, *i.e.*, corresponding to polymeric form as weight percentage of the total dry weight. The total sugar in the regeneration was 60.85%, which contained 44.11% glucan, 9.70% xylan, 2.56% of rhamnosan and galactan, and 4.48% of mannan and araban. The raw stem contained 69.48% total sugar, constituting of 43.97% glucan, 14.78% xylan, 4.11% of rhamnosan, and galactan, and 6.62% of mannan and araban. Most of the glucan was retained in the dissolving-regeneration process with a 75.78% retention ratio, which was higher than that of other sugars.

Additionally, there was 1.23% of ash and 0.75% silica in the regeneration. Thus 74.55% of the silica was retained, while the retention ratio of total ash was only 35.19%. This suggests that a large amount of ash was removed in the dissolving-regeneration process. These results lay the groundwork for further research on how different pretreating methods affect the dissolution, regeneration, and lignin isolation of the soybean stem and pod.

CONCLUSIONS

1. In soybean straw, the stem and root had higher lignin and holocellulose contents than the pod, which had more extractives, nitrogen, and protein.

2. Morphologically, the soybean stem had much shorter but wider fibers than other crops. There were three main tissues – the ground tissue, the vascular tissue, and the dermal tissue systems in the stem, and two different morphology portions – the intimal layer and leathery layer – in the longitudinal section of the pod.
3. K, Ca, P, Na, and Mg were the main inorganic components in soybean straw fractions. The trace elements contents of Al, Fe, Zn, Mn, and Cu were all lower than 100 ppm. All these inorganic and metal elements were distributed across the whole stem or pod in different amounts.
4. Additionally, the ball-milled stem and pod could be dissolved completely in 8% LiCl/DMSO. In the regeneration, the lignin had the best retention, which was followed by silica and sugars, but most of the ash could be removed in this process.

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