

Preparation of *Taraxacum kok-saghyz* Rubber and Biofuel Ethanol Simultaneously by the Yeast Fermentation Process

Shuai Zhao,¹ Xiang Jie,¹ Zhe Ma, Zheng Wang,* Jichuan Zhang,* Yushi Li, Qiuhai Nie, and Yong Ma

Cite This: *ACS Omega* 2023, 8, 24185–24197

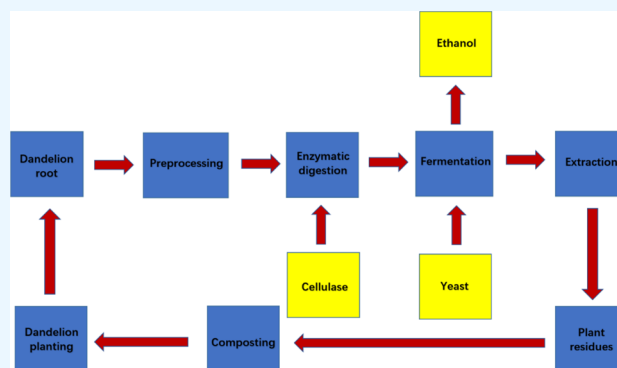
Read Online

ACCESS |

Metrics & More

Article Recommendations

ABSTRACT: *Taraxacum kok-saghyz* (TKS) rubber is considered the most ideal alternative source of natural rubber (NR). Extracting rubber from TKS with high quality, low cost, and low pollution is the basis of commercial development. The TKS roots were subjected to morphological observation and detailed compositional analysis. Scanning electron microscopy (SEM) images confirm that rubber filaments are physically entangled with plant tissues due to differences in molecular polarity. Compared with the traditional solvent TKS rubber extraction process, a new rubber extraction process developed in this study, namely, the microbial extraction ("ME") process, is less harmful to the environment and lower in cost. The "ME" process is divided into three steps: dilute acid pretreatment process, enzyme degradation process, and fermentation process. After each step is completed, the purity of TKS rubber will gradually increase from 84.8% to 93.8 to 95.5%. The TKS rubber finally obtained fully meets the requirements of the traditional rubber industry, especially the tire industry. Besides, the yield of biofuel ethanol, a by-product of cellulose fermentation, reaches 2.05 g/100 g of TKS roots (dry weight), which can effectively reduce the production cost of TKS rubber. In the rubber extraction process, microorganisms have little effect on the quality of TKS rubber. The results show that the molecular weight and chemical structure of TKS rubber is very close to NR, so the "ME" process can be used as a new method for large-scale extraction of TKS rubber.



1. INTRODUCTION

Natural rubber (NR) from *Hevea Brasiliense* is an important strategic material, widely used in aerospace, automobile industry, shipbuilding, rail transportation, and other fields.^{1,2} At present, the production and development of NR in the world are facing many challenges.^{3–5} The global NR-producing countries are mainly concentrated in the tropical rain forests of the Asia-Pacific region.^{6,7} The land suitable for planting *Hevea* trees in the world is limited, which will increase the difficulty of further increasing the output of natural rubber. Most of the *Hevea* trees in the Asia-Pacific region have narrow genetic genes,^{8,9} making their genetic transformation difficult. An *Hevea* tree capable of producing natural rubber latex requires a cultivation period of 5–7 years.^{10,11} *Hevea* trees also need to face the risks of natural disasters and insect pests during the vacuum period when no latex is produced. At the same time, rubber farmers in natural rubber-producing countries have not been able to obtain any benefits from rubber trees, causing many rubber farmers to abandon the cultivation of rubber trees. However, the tapping time and tapping direction of *Hevea* trees are different due to different trees,^{12–14} so it is difficult to mechanically operate the tapping work, and it can only be operated by manual work, which increases the

production investment and labor cost of natural rubber, thus restricting the development of natural rubber.

In response to the series of crises behind the natural rubber industry, scientists in the field of natural rubber have turned their attention to the second natural rubber. At present, there are more than 2500 kinds of plants that can biosynthesize natural rubber reported in the world, but most of them cannot be commercially used, and the appearance of TKS rubber has changed this situation. TKS rubber comes from a plant called *Taraxacum kok-saghyz* (TKS),¹⁵ which contains rubber mainly distributed in the roots;^{16,17} in the fresh TKS, the rubber is present in the lactiferous duct in the form of liquid latex,¹⁸ while in the dry TKS, the rubber is present in the form of solid rubber thread.¹⁹ TKS rubber has a similar chemical structure to natural rubber, and its main components are *cis*-1,4-polyisoprene. Therefore, TKS rubber is generally regarded as

Received: December 10, 2022

Accepted: April 28, 2023

Published: June 27, 2023

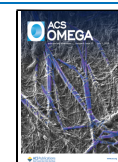




Figure 1. Pretreatment experiment operation steps.

the second natural rubber that can replace natural rubber in the world.

For the study of *TKS* rubber, the United States has launched the PENRA program in 2007, and the European Union (EU) launched the EU-PEARL program in 2008, but so far, no extraction technology can produce *TKS* rubber commercially. At present, the extraction technology of *TKS* rubber is the solvent method, which was established by Elmuradov²⁰ and others for the influence of the many nongum components of *TKS* in the rubber extraction on the purity of the extracted rubber.²¹ The principle of the solvent extraction method is to use different organic solvents to remove the nonadhesive components of *TKS* in sequence and use the good solvent of the rubber to extract the *TKS* rubber after the impurity removal is completed. However, this method consumes a large amount of organic solvent in the process of gum extraction, and the organic solvent used in the method not only has a high cost but also has certain physical harm to the gum extraction staff, so the method has not yet entered into industrial production and utilization.

Eskew²² developed a wet milling process for *TKS* rubber extraction due to the differences in the density and hydrophobicity of each tissue component in *TKS*. The principle of the wet milling method is that the density of rubber is lighter than that of water and will float on the water surface, while the nongum plant tissue sinks to the bottom due to water absorption in the water environment, which separates the rubber in the *TKS* from the nongum plant tissue. For the rubber extracted by this method, there are many impurities, and in addition to the high economic cost caused by the consumption of a large amount of water, it also takes a lot of time cost in the rubber extraction process, so the method is not yet mature for the production of high-purity rubber. Buranov and Elmuradov²⁰ proposed the dry-grinding method and applied for a patent for the shortcomings of wet-grinding in gum extraction. Compared with the wet grinding method, the dry grinding method has a similar principle to rubber extraction. Compared with the wet grinding method, the rubber purity of the method is improved, which can make the rubber purity reach 99% and reduce the energy consumption of water, but the extracted rubber still contains a large amount of nongum components, such as inulin, which cannot achieve

sufficient separation of rubber and nongum plant tissue. This technology has not developed a comprehensive extraction technology, and the cost of rubber extraction is too high to compete with clover rubber. Professor Cornish²³ of the United States discovered a new microbial fermentation method to extract rubber, namely, a new strain of nematode thermomyces lanuginosus STM, which produces hydrolases when grown on different lignocellulosic biomass, including cellulase, xylanase, inulinase, and pectinase;²⁴ these hydrolases can significantly increase the purity of natural rubber up to 94%.²³ However, the strain is not universal and the cost is high. In addition to rubber, rubber grass also contains a large amount of nongum components, such as inulin. This technology has not developed a comprehensive extraction technology, and the cost of rubber extraction is too high, and cannot compete with *Hevea* rubber. In this study, we developed a new yeast fermentation method based on the understanding of the microbial fermentation method. This method uses the principle of microbial fermentation to prepare the cellulose components contained in its roots into biofuel ethanol. The principle of rubber density difference can separate rubber and nongum plant tissue, achieve the purpose of improving rubber purity, and obtain by-product ethanol at the same time, which has certain additional economic benefits so that the production cost of rubber extraction can be reduced, and the extracted *TKS* rubber has certain market competitiveness. At the same time, our extraction process has no organic solvent, which fully meets the standard of green production and is suitable for large-scale industrial development. This will be of great significance to the development of the comprehensive utilization of *TKS*.

2. MATERIALS AND METHODS

2.1. Chemical Reagents and Materials. Sulfuric acid, citric acid, sodium hydroxide, and toluene were purchased from Beijing Chemical Reagent Co., Ltd. (China). Cellulase and IMXS81 yeast were provided by the Microbiology Laboratory of Beijing University of Chemical Technology. NR (Standard Chinese Rubber Whole Field) was bought from Yunnan rubber Co., Ltd. (China).

2.2. *TKS* Roots. The *TKS* roots used in the experiment came from the *TKS* planting base of the Heilongjiang Academy of Sciences in China, and its rubber content was distributed

between 3.72 and 5.04%. The fresh roots of *TKS* that were grown in the field for one year were harvested and dried to form dried roots at room temperature. Dry *TKS* roots were frozen in liquid nitrogen. Then, it was left to dry at room temperature, and finally, the dried *TKS* roots were ground into powder with a high-speed universal pulverizer (ZG-HTY-800 g), and the particle size distribution is 0.075 mm ~ 0.4 mm.

2.3. *TKS* Rubber Extraction Process. **2.3.1. Pretreatment Process.** The basic routes of the pretreatment process are shown in Figure 1. First of all, the raw dandelion roots were cleaned and ground with a waring mixer to obtain powdered raw materials. Then, the dilute acid was added for treatment, water was added and stirred, and allowed to stand after stirring for a while. After standing, the crude rubber was collected, the supernatant was poured out in the container after picking up the crude rubber, and then, water was added to continue stirring and sedimentation. The above process was repeated until the top layer has solid glue again. Then, the remaining solid–liquid mixture is filtered by suction with a vacuum water circulation pump to separate the solid precipitate from the liquid at the bottom of the glass container. The collected solid precipitate was dried in an oven at 60 °C, and finally, the desired product was obtained.

2.3.2. Enzymatic Degradation Process. A citric acid–sodium hydroxide buffer solution with a pH value of 4.8 was configured. This buffer solution (100 mL), 10 g of solid precipitate, and 1 g of crude rubber collected after pretreatment were added to a 500 mL Erlenmeyer flask. On the ultra-clean bench, 2 mL of cellulose was added to the Erlenmeyer flask, after the Erlenmeyer flask was sterilized at 121 °C for 20 min. The Erlenmeyer flask was moved to a shaking incubator with a rotation speed of 180 rpm at 50 °C for enzymatic degradation for 72 h. During the enzymatic degradation, 2 mL of enzymatic degradation solution was taken every 12 h for the subsequent determination of sugar concentration. After the enzymatic degradation was completed, the rubber floating above the solution was collected. After the bottom residue was filtered out, the hydrolyzed solution was collected and placed in a –20 °C refrigerator for the next experiment.

2.3.2.1. Determination of the Concentration of Sugar. The concentration of sugars was determined by high-performance liquid chromatography (Dionex ultimate 3000, Dian Co., Ltd., USA). Test instrument: Aminex HPX-87P column. Test conditions: The mobile phase was 5 mmol/L sulfuric acid, the flow rate was 0.6 mL/min, the column temperature was 65 °C, the detector temperature difference was 50 °C, and the sample volume was 20 µL at each injection.

2.3.3. Fermentation Process. **2.3.3.1. Seed Liquid Culture.** IMX581 yeast stored in glycerin at –70 °C needed to be activated before fermentation. First, the YPD medium [Yeast Extract Peptone Dextrose Medium (1 L)] (composition: 4 mL of deionized water, 40 mg of yeast, 80 mg of glucose, 80 mg of peptone) was sterilized at 121 °C for 20 min. After melting the yeast and glycerin mixture stored at –70 °C, 50 µL was added to the sterilized YPD medium on the ultra-clean workbench, and finally, the medium was placed in a shaking incubator at 30 °C for 12 h for shaking.

2.3.3.2. Fermentation. After melting the cryopreserved enzymolysis solution, 75 mL was poured into a 300 mL Erlenmeyer flask. Yeast (0.75 g), 1.5 g of glucose, 1.5 g of peptone, and enzymatically purified rubber were added to the Erlenmeyer flask. The Erlenmeyer flask was sterilized at 121 °C for 20 min, and after sterilization, the seed solution that

accounts for 1.5% of the volume of the enzymolysis solution was added to the Erlenmeyer flask on the ultra-clean workbench. Finally, the Erlenmeyer flask was transferred to a shaking incubator at 30 °C, and the rotation speed was adjusted to 180 rpm for the anaerobic fermentation experiment. During the fermentation process, the fermentation liquid was taken at regular intervals to determine the growth of yeast and the concentration of ethanol. After the fermentation was completed, the purified rubber floating at the interface of the fermentation liquid was collected.

2.3.3.3. Concentration Determination of Ethanol and Yeast. A gas chromatograph (TRACE1300, Thermo Fisher Scientific Co., Ltd., USA) equipped with a Porapack Q column was used to determine the ethanol concentration. The column temperature was 180 °C, the detection chamber temperature was 180 °C, the gasification chamber temperature was 180 °C, and the carrier gas hydrogen flow rate was 30 mL/min. The ultra-micro spectrophotometer (model N60, Implen Co., Ltd., Germany) was used to evaluate the growth of yeast by measuring the absorbance of the fermentation solution at 600 nm.

2.4. Determination of the Purity and Extraction Efficiency of *TKS* Rubber. In this study, toluene was used as a solvent to determine the purity of crude rubber. The method was as follows: crude rubber and toluene were mixed at 70 °C and stirred at 200 rpm for 24 h. *TKS* rubber was filtered after completely dissolved. The undissolved material remaining at 80 °C was dried and weighed in a vacuum oven (DZF-6030, Shanghai Hecheng Instrument Manufacturing Co., Ltd., China). The formula for calculating the purity of *TKS* rubber was as follows:

$$P = \frac{m_0 - m_1}{m_0} \times 100\%$$

P is the purity of *TKS* rubber, m_0 is the mass of crude *TKS* rubber, and m_1 is the mass of the undissolved material.

The extraction efficiency of *TKS* rubber was defined as the percentage of the mass of pure rubber and the mass of rubber contained in the dry *TKS* root.

2.5. Characterization of *TKS* Rubber. **2.5.1. Pretreatment Process Statistical Analysis.** An orthogonal experiment was used to analyze the results of the *TKS* roots pretreatment process. Latin orthogonal analysis software was used to perform range calculations and to judge and analyze the primary and secondary factors that affect the experiment.

2.5.2. Scanning Electron Microscopy. Scanning electron microscopy (S4800, Hitachi Co., Ltd., Japan) was used to observe the dry *TKS* roots, and the test voltage was adjusted to 5 kV. The dry *TKS* roots were put in liquid nitrogen for freezing. Then, the freeze-dried *TKS* roots were sliced horizontally and vertically respectively, and the thickness of the slices was about 2 mm. SEM was also used to observe the samples of dry *TKS* root before and after pretreatment.

2.5.3. Fourier Transform Infrared Spectroscopy. A dry rubber sample was tested using the ATR test program in an infrared spectrometer (Tensor 27, Bruker (Beijing) Technology Co., Ltd., China). The test conditions were as follows: the background scan 32 times and the sample scan 32 times. A series of infrared spectra were collected in the range 400~4000 cm^{-1} with a resolution of 4 cm^{-1} .

2.5.4. Nuclear Magnetic Resonance Spectrometry. The ^1H NMR spectrum (AV 400, Bruker (Beijing) Technology Co., Ltd., China) was used to analyze the molecular structure of the

dry rubber. The test conditions were as follows: the sample concentration is 10 mg/mL, and the scan was performed 400 times with a scan pulse of 4.8 s.

2.5.5. Gel Permeation Chromatography. Rubber (1 mg) was weighed and dissolved in 2 mL of chromatographic pure toluene to make a 0.5 mg/mL rubber–toluene solution. GPC (1260 Infinity, Agilent (Beijing) Technology Co., Ltd., China) was used to analyze the molecular weight and molecular weight distribution of the rubber. The test conditions were the toluene phase as the mobile phase, the flow rate was 1 mL/min, and the injection volume was 10 μ L. The temperature of the column oven and detector was set to 30 $^{\circ}$ C.

2.5.6. Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) (STARe system, METTLER TOLEDO, Switzerland) was used to detect and analyze the glass transition temperature of rubber. DSC tests were performed using 8 mg rubber samples under a nitrogen atmosphere. The rubber samples were first scanned from 25 to 100 $^{\circ}$ C at a heating rate of 10 $^{\circ}$ C/min and held at 100 $^{\circ}$ C for 5 min to eliminate the thermal history of the samples and then cooled the sample from 100 to -100 $^{\circ}$ C at a cooling rate of 10 $^{\circ}$ C/min and scanned a second time. After a 5 min hold, the sample was reheated to 100 $^{\circ}$ C at a heating rate of 10 $^{\circ}$ C/min and scanned a third time. The whole process was scanned three times to get more accurate results.

2.5.7. Thermogravimetry Analysis. A thermogravimetric analyzer (TG, STARe system, METTLER-TOLEDO Co., Ltd., Switzerland) was used to monitor the weight loss of the rubber under heat, and it could reflect the ingredients and the content of the rubber. In the nitrogen atmosphere, a 10 mg rubber sample was raised from 25 to 800 $^{\circ}$ C at a rate of 10 $^{\circ}$ C/min and then stopped.

3. RESULTS AND DISCUSSION

3.1. Dry TKS Root Morphology Observation. The dry roots of TKS were brown and varied in shape, weight, length, and width. As shown in Figure 2, their lengths and widths were



Figure 2. Dry TKS roots morphology and rubber drawing phenomenon.

distributed between 80–200 mm and 30–100 mm. Their weights are distributed between 10–50 g.²⁴ As an herbaceous plant, the TKS root was composed of main roots and lateral roots of different thicknesses. Both main roots and lateral roots were found to contain rubber, and their content was not related to the type of root. After the root was broken, there would be an obvious rubber thread drawing phenomenon.

Dry TKS root was usually composed of three parts from the inside to the outside: root core, root flesh, and root bark (Figure 3a). The adhesive force of rubber filaments and plant

tissues is clearly shown in Figure 3b. Increasing the magnification made it easier to find that as the root bark and root flesh were separated, the coagulated rubber in the laticifer connected the two parts. The rubber filaments and the laticifer cells were separated and were not bonded together (Figure 3c). This was because as a nonpolar molecule (*cis*-1,4-polyisoprene), natural rubber was found in plants in the form of latex in laticifers. Whereas laticifers are composed primarily of cellulose,¹⁸ this polysaccharide with a large number of hydroxyl groups was a typical polar molecule.²⁵ In the photograph of the longitudinal section of the root flesh (Figure 3d), it was observed that the rubber filaments are cylindrical, and the diameter of the rubber filaments was in the range of 1 to 2 μ m. In the transverse and longitudinal sections of the root core (Figure 3e,f), many honeycomb fiber bundle tubes were visible, but the distribution of rubber filaments was not found.

In summary, through the distribution of rubber in the dry TKS roots, it could be found that the rubber filaments and the laticifer would not stick together due to polar differences in plant tissues (Figure 3c). This was because the rubber filaments were nonpolar, and the contracted laticifer was polar. Based on this, it should be very easy to extract rubber from the roots of TKS. However, the physical entanglement of rubber filaments and plant tissue increased the difficulty of separating rubber from the TKS root tissue. When extracting rubber, the binding force between plant tissue and rubber needed to be broken, but the adhesion of plant tissue cannot be avoided. Many small plant tissue fragments attached to the surface of the rubber filaments (Figure 3d) also confirmed this phenomenon.

3.2. Chemical Composition Analysis of Dry TKS Root.

The composition of TKS dry roots has been determined in previous research by our group.²⁶ Soxhlet extraction was used to determine the content of water-soluble molecules, pigments, resins, and rubber. The NREL²⁷ (National Renewable Energy Laboratory) method was used to determine the content of cellulose, hemicellulose, lignin, and ash. The HPLC (high-performance liquid chromatography) method²⁸ was used to determine the content of inulin and to reduce sugar in water-soluble molecules. In this article, the composition of water-soluble molecules and hemicellulose has been refined. The specific determination method was not repeated^{27,28} here; only the results were explained. The proportion of each component is shown in Table 1. The rubber content accounts for about 4% of the total weight of dry root.

The average content of water-soluble molecules was 26%, the content of inulin was approximately 22%, and the content of reducing sugar was approximately 4%. A large amount of inulin in the roots of TKS indicated that TKS had huge market potential in the food and pharmaceutical fields, and the production cost of TKS rubber would be reduced. The average content of pigments, resins, and rubbers was 10, 13, and 4%, respectively. The total amount of lignin and total cellulose accounted for about 40% of the dry root content, of which the average lignin content was 20%. The total amount of total cellulose was about 20%, of which the average content of cellulose was 13% and the average content of hemicellulose was 7%. The roots of TKS contained a lot of woody structures, so weakening cellulose-based plant tissue became one of the goals of the rubber extraction process below. At the same time, enriching the cellulose content made it easier to prepare bioethanol. The average ash content was 4%, which showed

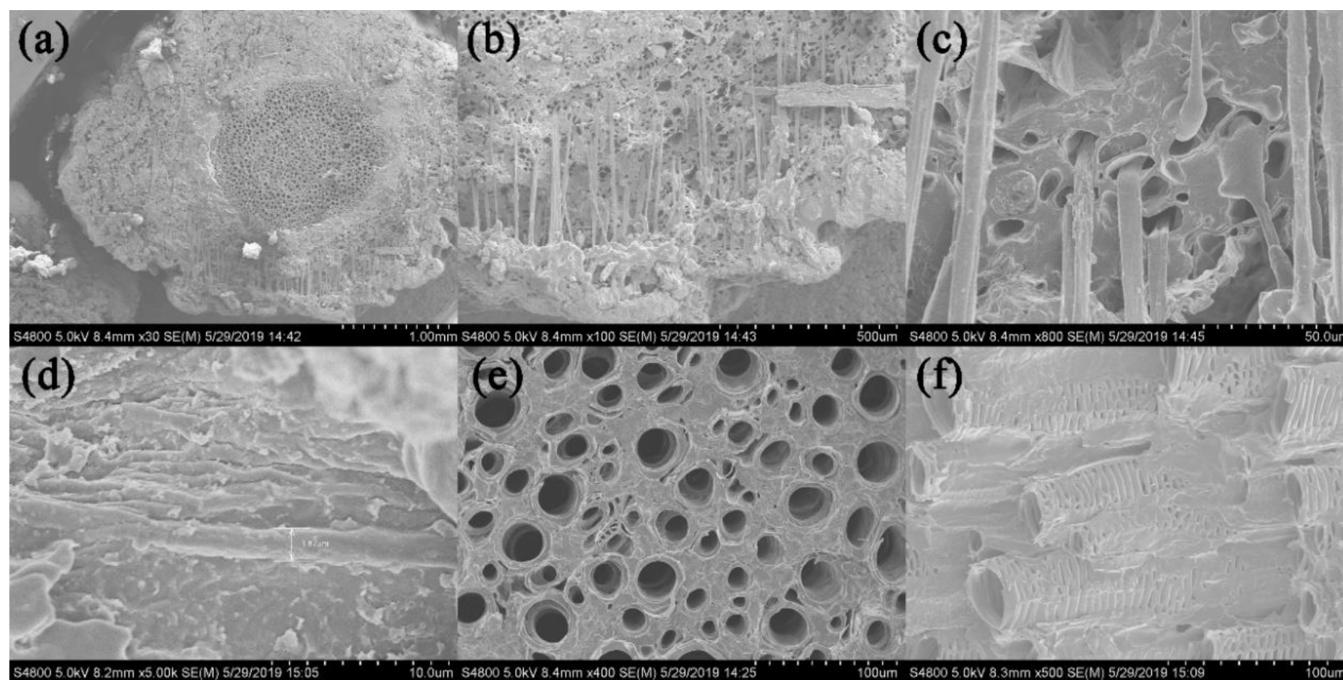


Figure 3. SEM images of *TKS* root slices (a, b, and c are transverse slices of a lateral root; b and c are amplification of the root flesh; d is the longitudinal slice of root flesh; e and f are the transverse and longitudinal slices of the root core, respectively).

Table 1. Chemical Composition of Dry *TKS* Root

	inulin	reducing sugar	pigment	resin	rubber	cellulose	hemicellulose	lignin	ash
content	22%	4%	10%	13%	4%	13%	7%	20%	4%

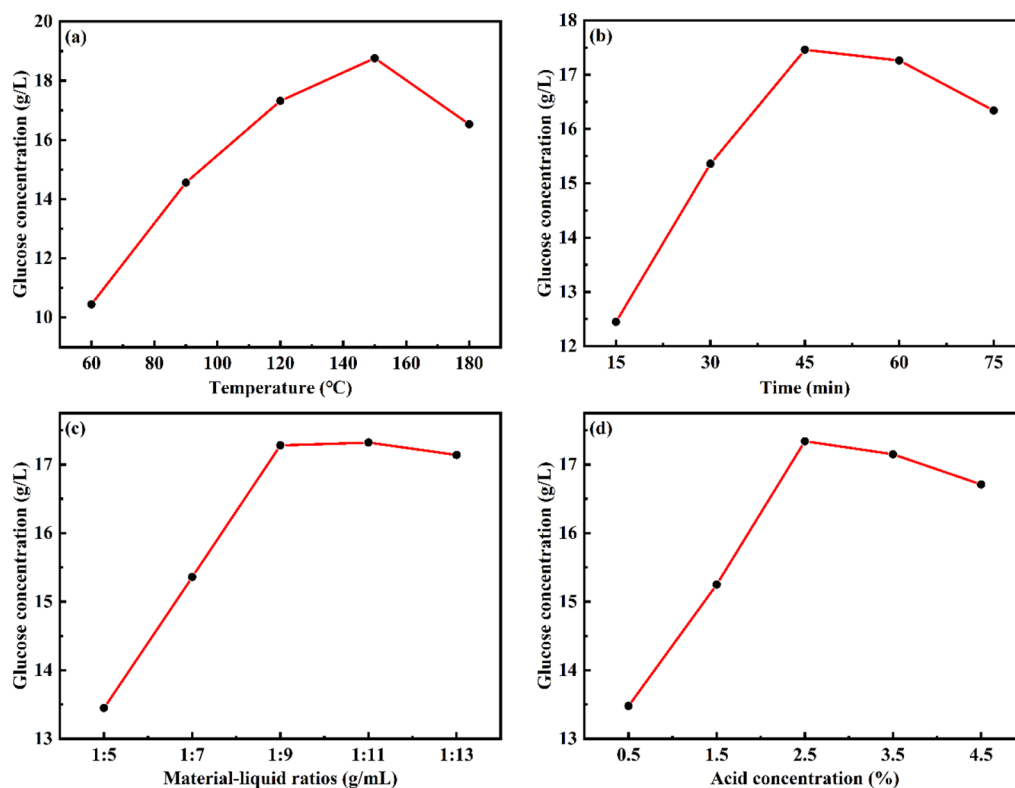


Figure 4. Pretreatment experiment operation steps.

that the results in this article were reasonable and the data were credible.

3.3. Study of the Extraction Process of *TKS* Latex. Cellulose was one of the sugar sources for preparing biofuel

ethanol. The composition analysis above showed that *TKS* root contains 13% cellulose and 7% hemicellulose, so ethanol, a by-product, would be prepared while extracting *TKS* rubber. Inulin could also be used as a raw material for ethanol fermentation, but considering that the value of inulin was higher than that of ethanol, crude inulin would be directly extracted as a raw material for purification. This research was aimed at the extraction of rubber and the fermentation of cellulose–ethanol, so the purification of inulin would not be expanded on in this article, and the SEM analysis showed that the rubber and plant tissues were entangled with each other. Therefore, the process idea is to first destroy the dense structure of lignocellulose in *TKS* through pretreatment so that the cellulose can be removed from the protection of lignin so that the exposed cellulose can have a good enzymatic hydrolysis effect. The purity of the rubber harvested after the *TKS* pretreatment is improved and ethanol is prepared in the process; finally, we can obtain high-purity rubber products and also harvest the by-product biofuel ethanol. The plant residues can also be used to make fertilizers to reduce process costs in rubber production. The established gum extraction process route is mainly divided into three links, namely, the pretreatment process of *TKS*, the enzymatic hydrolysis process, and the fermentation process.

3.3.1. Pretreatment Process. Studies have shown that dilute sulfuric acid could break the glycosidic bonds between hemicellulose. Therefore, dilute sulfuric acid was used to destroy the dense lignocellulose structure of the root of *TKS* so that the fiber bundle skeleton was exposed, which facilitated the full contact between cellulose and cellulase and increased the yield of by-product ethanol. Besides, the pretreatment process can make the root rubber get rid of the binding of nonrubber plant tissue to the greatest extent.

The four influencing factors of temperature (T), pretreatment time (PT), material–liquid ratio (M/L), and acid concentration (AC) would be analyzed and optimized in the pretreatment process. The sample after the pretreatment was subjected to an enzyme degradation experiment, and the sugar concentration of the enzymatic hydrolysate was sampled three times for determination. The average value of the sugar concentration was used as a criterion for evaluating the effect of the pretreatment process. The initial conditions of the pretreatment process were as follows: $T = 120\text{ }^{\circ}\text{C}$, PT = 45 min, M/L = 1:11 (g/mL), and AC = 2.5%. Each condition was optimized and analyzed below. Finally, orthogonal experiments were used to determine the optimal pretreatment process parameters.

3.3.1.1. Influence and Optimization Temperature. The initial conditions of this group of experiments were as follows: PT = 45 min, M/L = 1:11 (g/mL), AC = 2.5%, and the temperature (T) was set as a variable. The experimental results are shown in Figure 4a. When the temperature was within 60–150 $^{\circ}\text{C}$, the sugar concentration of the enzymatic hydrolysate gradually increased with the increase in temperature. When the temperature exceeded 150 $^{\circ}\text{C}$, the sugar concentration in the enzymatic hydrolysate showed a downward trend. This was because *TKS* roots produced some substances that inhibited cellulase at high temperatures. When the temperature was too low, the lignocellulose network structure in the root of *TKS* cannot be completely broken, so the cellulose component cannot be fully degraded and the sugar concentration was low. Therefore, according to the results of the single-factor experiment, $T = 120\sim 160\text{ }^{\circ}\text{C}$ was

selected as the temperature range of the subsequent orthogonal experiment.

3.3.1.2. Influence and Optimization Pretreatment Time. The initial conditions of this group of experiments were as follows: $T = 120\text{ }^{\circ}\text{C}$, M/L = 1:11 (g/mL), AC = 2.5%, and the pretreatment time (PT) was set as a variable. The experimental results are shown in Figure 4b. When the time was within 15–45 min, as the reaction time increased, the sugar concentration in the enzyme degradation solution gradually increased. When the reaction time exceeded 45 min, the sugar concentration showed a slow downward trend. This was because, as the reaction time prolonged, part of the cellulose component of the *TKS* root was decomposed by dilute acid, which caused the sugar concentration in the enzyme degradation solution to decrease. Also, if the reaction time was too short, the root hemicellulose cannot be fully decomposed and the sugar concentration would be low; if the reaction time was too long, some cellulose components would be degraded, which would also reduce the sugar concentration. Therefore, according to the results of the single-factor experiment, PT = 30–60 min was selected as the time range of the subsequent orthogonal experiment.

3.3.1.3. Influence and Optimization of the Material-to-Liquid Ratio. The initial conditions of this group of experiments were as follows: $T = 120\text{ }^{\circ}\text{C}$, PT = 45 min, AC = 2.5%, and the material-liquid ratio (M/L) was set as a variable. The experimental results are shown in Figure 4c. When the ratio of material to liquid was lower than 1:9, as the solution gradually increased, the sugar concentration also gradually increased. The sugar concentration started to decrease when the solid–liquid ratio exceeded 1:11. When the feed liquid was relatively low, the insufficient reaction of *TKS* roots with dilute acid would lower the sugar concentration. Too high a material-to-liquid ratio would also reduce the sugar concentration because the *TKS* roots and the solution might be in full contact, which might lead to the degradation of some cellulose components. When the material-to-liquid ratio was greater than 1:9, the difference in sugar concentration in the enzymatic hydrolysis solution was small, and too much solution would increase the consumption of water resources, increasing the amount of wastewater. Therefore, according to the results of the single-factor experiment, M/L = 1:5–1:9 was selected as the range of the subsequent orthogonal experiment.

3.3.1.4. Influence and Optimization of Acid Concentration. The initial conditions of this group of experiments were as follows: $T = 120\text{ }^{\circ}\text{C}$, PT = 45 min, M/L = 1:11, and the acid concentration (AC) was set as a variable.²⁷ The experimental results are shown in Figure 4c. When the acid concentration was lower than 2.5%, as the acid concentration increased, the sugar concentration in the enzymatic hydrolysis solution increased. When the acid concentration exceeded 2.5%, the sugar concentration showed a slow downward trend. This was because the cellulose component of the *TKS* root was removed by excessive acid, which caused the sugar concentration to decrease. When the acid concentration was too low, the root reaction was not sufficient, which made the cellulose components in the lignocellulose network structure not completely exposed, resulting in a low sugar concentration. When the acid concentration was too high, it would cause the degradation of some cellulose components, which also led to a decrease in the sugar concentration. Therefore, according to the results of the single-factor experiment, AC = 1–3% was

Table 2. Orthogonal Experiment Results and Range Analysis^a

experimental indicators		<i>T</i> (°C)	PT (min)	M/L (g/mL)	AC (%)	rubber purity (%)	sugar concentration (g/L)
①		120	30	1:5	1	88.28	10.52
②		120	45	1:7	2	89.33	12.46
③		120	60	1:9	3	84.99	17.15
④		140	30	1:7	3	87.68	15.56
⑤		140	45	1:9	1	84.88	13.67
⑥		140	60	1:5	2	83.69	14.38
⑦		160	30	1:9	2	92.75	14.25
⑧		160	45	1:5	3	82.88	13.12
⑨		160	60	1:7	1	77.02	11.45
mean and range of rubber purity	K1	87.55	89.57	84.95	83.39	order of impact levels: PT > AC > T > M/L	
	K2	85.42	85.70	84.68	88.59		
	K3	84.22	81.90	87.54	85.18		
	R	3.33	7.67	2.86	5.20		
mean and range of sugar concentration	K1	13.38	13.44	12.67	11.88	order of impact levels: AC > M/L > T > PT	
	K2	14.53	13.08	13.16	13.70		
	K3	12.94	14.32	15.02	15.28		
	R	1.59	1.24	2.35	3.40		

^aNote that K1, K2, and K3 are the average values; R is the extreme difference range.

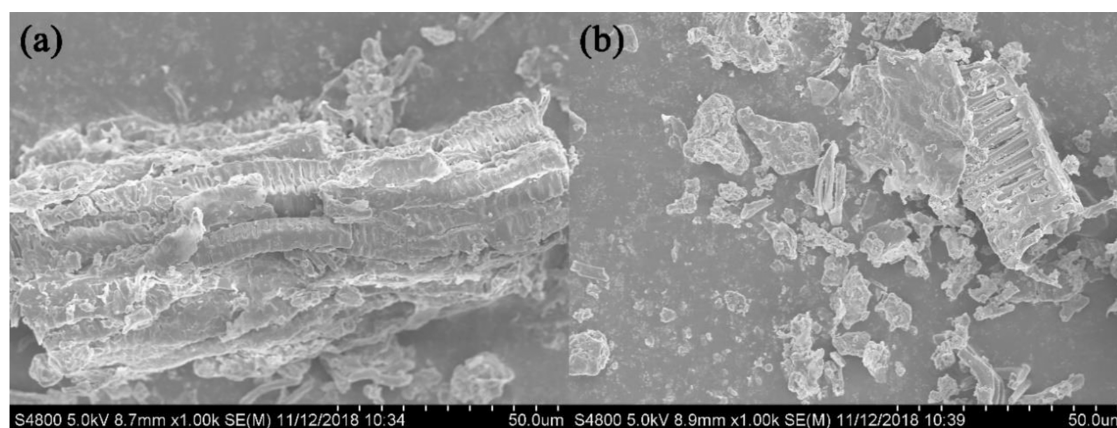


Figure 5. SEM images of dry TKS roots before and after pretreatment (a is before pretreatment, b is after pretreatment).

selected as the acid concentration range of the subsequent orthogonal experiment.

3.3.1.5. Analysis and Optimization of the Results of Orthogonal Experiments. According to the processing results of the single-factor experiment above, sugar concentration and crude rubber purity were used as experimental indicators to carry out a four-factor and three-level orthogonal experiment. The results are shown in Table 2.

The results of the orthogonal experiment and range analysis of the TKS root pretreatment process are shown in Table 2. The primary and secondary order of the influencing factors of rubber purity was PT > AC > T > M/L, which showed that the best process parameters for rubber purity were *T* = 120 °C, PT = 30 min, M/L = 1:9, and AC = 2%. The primary and secondary order of the influencing factors of sugar concentration was AC > M/L > T > PT, which showed that the best process parameters for sugar concentration were *T* = 140 °C, PT = 60 min, M/L = 1:9, and AC = 3%. The optimal parameter combinations corresponding to the two experimental indicators were inconsistent, so the comprehensive balance method was used to determine the optimal value of each factor. When the temperature was *T* = 120 °C, the purity of rubber was increased by 2.49% compared with that when *T* = 140 °C, and the sugar concentration was decreased by 7.91%

compared with that when *T* = 140 °C. The decreased sugar concentration was much greater than the increased rubber purity, so *T* = 140 °C was determined as the optimal temperature. Comparing the two average values, the pretreatment time was the primary factor affecting the purity of the rubber and the second factor affecting the sugar concentration, so PT = 30 min was selected as the best pretreatment time. The effect of the material-liquid ratio was the same for the two experimental indicators, so M/L = 1:9 was selected as the best material-liquid ratio. AC = 2 and 3% were the best acid concentration parameters under the two experimental indicators. The acid concentration was the primary factor that affected the sugar concentration and the second factor that affected the purity of the rubber, so AC = 3% was selected as the optimal acid concentration. The process of alkaline pretreatment was mentioned in Cornish's article: roots were washed, cut into small pieces (20–40 mm), and dried in a 45 °C oven. TKS roots (50 g) were mixed with 500 mL of 10% NaOH (pH 7) and boiled in a water bath at 100 °C for 35 min to loosen the lignocellulosic components and extracted the inulin. In the orthogonal experiment, the ratio of solid to liquid and the concentration of sugar were explored, and a more suitable pretreatment method was obtained, which improved

the extraction yield and purity, which was also one of the innovations of this study.

In summary, the optimal pretreatment process parameter combination of *TKS* roots was determined as $T = 140\text{ }^{\circ}\text{C}$, $\text{PT} = 30\text{ min}$, $\text{M/L} = 1:9$, and $\text{AC} = 3\%$. The final rubber purity of the best pretreatment process was 90.1%, and the rubber extraction efficiency was between 65.2 and 75.7%. Compared with the traditional solvent method, the rubber purity of the pretreatment process needs to be further improved.

3.3.1.6. SEM Observation of Pretreatment Samples. The SEM photographs before and after pretreatment are shown in Figure 5. The dense lignocellulose structure of the *TKS* root was destroyed after pretreatment, leaving the fiber bundle skeleton contained inside exposed. Therefore, the enzymatic degradation effect would be significantly improved after the pretreatment process, and it was also beneficial to the purification of crude rubber.

3.3.2. Analysis and Optimization of the Enzyme Degradation Process. Crude rubber and solid residues were obtained after the pretreatment process was completed. The main components of crude rubber include rubber and plant tissues attached to the rubber, which were mainly composed of lignocellulose. Considering that cellulase had a degrading effect on plant tissues, the solid residue and crude rubber were further degraded to purify the crude rubber and ferment to prepare sugar liquid. Cellulase mainly acted on cellulose and hemicellulose in crude rubber and cellulose in solid residues. In the enzyme degradation experiment, the sugar concentration in the enzyme degradation solution was measured at intervals.

Figure 6 represents the change curve of the sugar concentration in the enzyme degradation solution over time.

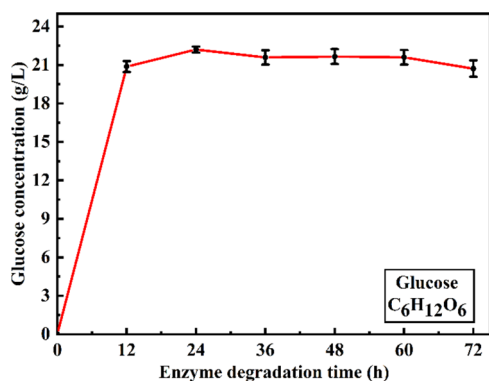


Figure 6. Relationship between glucose concentration and enzyme degradation time.

Within 0–24 h, the enzyme degradation time increased significantly with the increase of sugar concentration; when the enzyme degradation time reached 24 h, the sugar concentration reached the maximum. When the enzyme degradation time exceeded 12 h, the sugar concentration almost tended to a stable state, and the sugar concentration had a slight downward trend with the extension of the enzyme degradation time. This might be due to the degradation of some monosaccharides produced during the enzymatic hydrolysis process, resulting in a slight decrease in sugar concentration. Therefore, due to time cost considerations, 12 h was considered the best time for the enzyme degradation process, which would also greatly shorten the time for *TKS* rubber extraction. After measurement, the purity of *TKS* rubber was up to 93.8%, which was 4.1% higher

than the pretreatment process. This was because cellulase had a degrading effect on impurities and the impurities in crude rubber were shaken off in an aqueous environment, resulting in increased rubber purity. The *TKS* rubber floating on the surface of the enzyme degradation solution was collected and dried and weighed. Compared with the pretreatment process, the yield of the *TKS* rubber prepared by the enzyme degradation process increased by 2.1%. This was because the small amount of rubber embedded in the solid residue was released during the enzymatic degradation process.

3.3.3. Fermentation Process. **3.3.3.1. Determination of Yeast Growth.** To explore the influence of rubber on ethanol yield during fermentation, a separate enzymatic degradation solution (TY1) and an enzymatic degradation solution added with rubber (TY2) were selected as fermentation substrates. The enzyme degradation liquid and rubber are both collected in the enzyme degradation process and the fermentation conditions of the two substrates were the same. The growth of yeast in the two fermentation solutions was measured at intervals. The growth of yeast is shown in Figure 7a. With the increase in fermentation time, the concentration of yeasts in the two fermentation solutions increased significantly and decreased slightly in the later stages of fermentation. The growth rate of yeast in TY1 was higher than that in TY2. This was because rubber had an inhibitory effect on the growth of yeasts, resulting in more severe conditions for yeast activities.

3.3.3.2. Extraction of Rubber and Preparation of Ethanol. The change curve of ethanol content with fermentation time is shown in Figure 7b. Within 24 h of fermentation, the ethanol concentration stabilized, which also indicated that 24 h can be used as the best fermentation time. The ethanol content in the late fermentation period decreased significantly, which might be due to the volatile nature of ethanol that caused the ethanol in the fermentation bottle to volatilize during sampling and determination. Or because in the late fermentation stage, ethanol was consumed as a nutrient by the life activities of yeast, resulting in a decrease in ethanol content. According to calculations, the highest yield of ethanol in the rubber extraction process was 2.05% (100 g of *TKS* dry roots produced 2.05 g of ethanol).

After fermentation, the purity of the harvested rubber can reach up to 95.54%, which was improved compared to the purity of the rubber harvested by the enzyme degradation process. Considering that the lignocellulose doped in rubber cannot be decomposed by yeast, this slight increase might be caused by two reasons. There were fine particles at the bottom of the flask used in the fermentation process, which might be caused by the mechanical vibration. This led to an increase in the purity of the rubber. The second reason was that as Figure 7b shows, after 36 h of fermentation, the ethanol content in TY2 was higher than that in TY1. Figure 6 also shows that the growth rate of yeast in TY1 was much higher than that in TY2. This indicated that in the late fermentation stage, the nonrubber components in the rubber were consumed by the life activities of yeasts, increasing rubber purity. To verify this statement, the enzymatically degraded rubber was placed in deionized water for the same time as the fermentation solution with rubber added in a shaking incubator. The results showed that the highest rubber purity in deionized water was 94.03%, which was lower than the rubber purity in the fermentation solution. Therefore, this explanation can be established in theory, but its mechanism has not yet been proven.

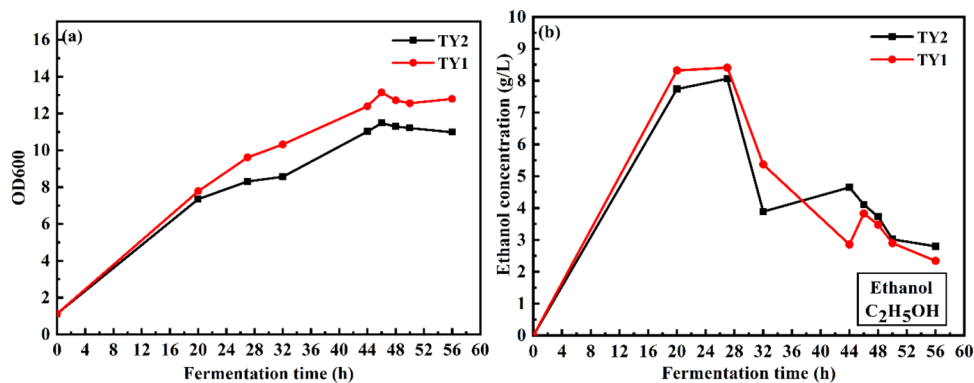


Figure 7. (a) Growth of yeast in TY1 and TY2 with fermentation time and (b) curves of ethanol concentration with fermentation time in TY1 and TY2.

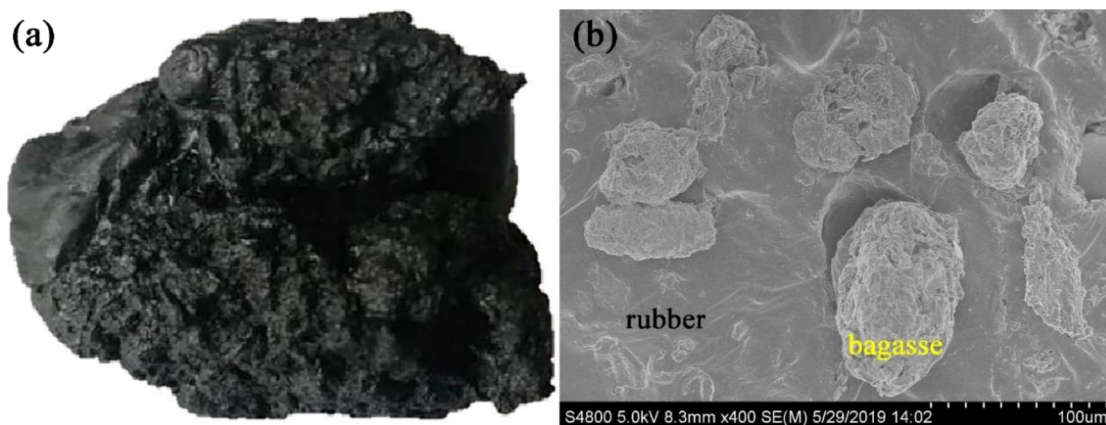


Figure 8. TKS rubber and its SEM images.

3.3.4. Observation of the Appearance of TKS Rubber. The TKS rubber prepared by the ME process is shown in Figure 8a. The surface of the rubber was black with a few impurities. To further understand the form of accumulation of these impurities, the TKS rubber was subjected to scanning electron microscopy (SEM). As shown in Figure 8b, a large number of micron-sized fine particles were predominantly widely distributed on the surface of the rubber. These impurities are mainly composed of fine lignocellulosic structures embedded in the rubber in the form of particles. Similar to black liquor (BL), the main components of impurities are lignin, cellulose, and hemicellulose,²⁹ and some phenolics can cause blackening of dandelion rubber during processing.³⁰ Although these impurities decreased the purity of the rubber, small-sized agro-industrial residues have been shown to reinforce the rubber in terms of mechanical properties.³¹ Thus, microscopic impurities were used as fillers, which might also improve the mechanical properties of TKS rubber and increased its value in use.

3.3.5. Optimized Route of the TKS Latex Extraction Process. A complete rubber extraction process was established and drawn in Figure 9 and named microbial extraction process, ME process in "brief". The enzyme digestion and extraction process of TKS rubber had been mentioned and developed in many studies. However, related research involving the analysis and optimization of process-influencing factors was not common. Divided into three subprocesses, the "ME" process comprehensively analyzed and optimized multiple factors affecting rubber purity. Besides, the extraction of by-products

had also been preliminarily explored in this article, to provide ideas for future research, while saving the cost of industrial production.

In summary, the optimized process conditions of "ME" were determined, and the optimal parameters of the pretreatment process are $T = 140\text{ }^{\circ}\text{C}$, $\text{PT} = 30\text{ min}$, $\text{M/L} = 1:9$, and $\text{AC} = 3\%$. The optimal processing time of the enzyme degradation process and fermentation processes were 12 and 24 h, respectively. Satisfying the above conditions can make the rubber purity and ethanol yield reach 95.54 and 2.05%, respectively, which may promote the industrial development of TKS rubber extraction.

3.4. Characterization of TKS Rubber. **3.4.1. Comparative Analysis of Molecular Weight and Molecular Weight Distribution.** As shown in Table 3, the molecular weights of TKS rubber and commercial natural rubber were measured. The test results showed that the TKS rubber extracted by the "ME" process had a similar or higher molecular weight and molecular weight distribution index to natural rubber. This indicated that the operations included in the "ME" process, such as root smashing, dilute acid treatment, enzyme degradation, and fermentation, had little adverse effects on the quality of rubber. Therefore, the TKS rubber obtained by this process was considered to have a certain use value.

3.4.2. Comparative Analysis of the Molecular Structure of TKS Rubber. The TKS rubber and NR extracted by the ME process were tested by FTIR spectroscopy. As shown in Figure 10, the FTIR spectra of the two rubbers showed similar characteristic bands. Among them, 835 cm^{-1} was the

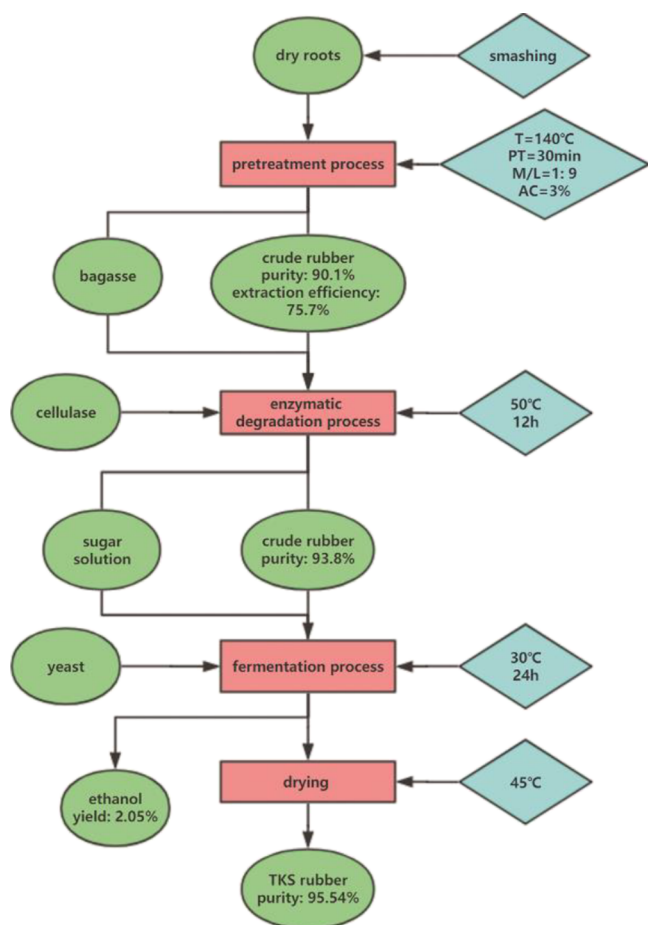


Figure 9. Optimized "ME" process route.

Table 3. Molecular Weight of TKS Rubber and Natural Rubber

samples	M_w ($\times 10^6$)	M_n ($\times 10^6$)	M_w/M_n
TKS rubber	1.88	1.42	1.34
NR	1.80	1.40	1.29

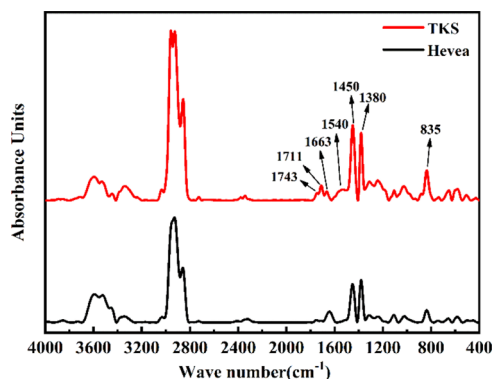


Figure 10. FTIR spectrum of TKS rubber and Hevea.

characteristic vibration peak of natural rubber skeleton stretching. The deformation vibration peak of the $-\text{CH}_3$ group was 1380 cm^{-1} . The bending vibration peak of $-\text{CH}_2$ was 1450 cm^{-1} . The stretching vibration peak of the $\text{C}=\text{C}$ double bond was 1663 cm^{-1} , all of which showed the characteristics of *cis*-1,4-polyisoprene. By analyzing the groups represented by the above characteristic peaks, it can be

considered that the structures of the two rubber were basically the same and the extracted product was indeed TKS rubber.

Natural rubber contains non-isoprene components such as proteins and lipids. The main absorption peaks of the infrared spectrum of protein in natural rubber were at 3280 , 1630 , and 1540 cm^{-1} , which are the vibrational absorption of N–H bonds, amides I and II, respectively. A shift of the amine absorption band was observed in the two rubber around 3350 cm^{-1} , which coincided with the stretching vibration peak of $-\text{OH}$ in water molecules. The amide I peak in TKS rubber shifted to around 1663 cm^{-1} , which overlapped with the $\text{C}=\text{C}$ stretching vibration absorption peak. The amide II band at 1540 cm^{-1} in TKS rubber was observed. In the lipid absorption region of natural rubber, the 1711 cm^{-1} bands were associated with carboxyl groups; the second carbonyl stretching band 1743 cm^{-1} was related to ester groups. The lack of protein and phospholipid peaks in the commercial NR might be caused by too little content. This was because NR was prepared by acid coagulation, and some proteins and lipids may be lost with the wastewater, so the remaining content was not enough to be measured by spectroscopy. In short, the difference between the two infrared spectral information bands had no significant effect on the structure and properties of TKS rubber, which made the extracted TKS rubber have a certain commercial value.

^1H NMR spectra of TKS rubber and NR are shown in Figure 11. The *cis* $-\text{CH}_3$ peak, $-\text{CH}_2$ peak, and $-\text{CH}$ peaks in the two rubbers successively appeared at the chemical shifts of 1.75, 2.05, and 5.15 ppm, respectively,³⁷ which indicated that the basic structure of TKS rubber was consistent with NR, and its molecular chain contained CPI structural units. The peak shapes of the two rubbers were similar. Compared with NR, there was little difference between the two, which was the same. Also, it can be seen from the FTIR and NMR analysis that the ME process would basically not damage the molecular structure of the rubber and negatively affected the performance of the TKS rubber.

3.4.3. Thermal Properties of TKS Rubber. TKS rubber and NR were tested by TGA and DSC to determine their thermal properties. Figure 12a shows the TG curves of the two rubbers. TKS rubber and NR started to decompose at about 230 and 250 °C, respectively. When the temperature was around 380 °C, the decomposition rate of the two reached the maximum. The thermal decomposition of the two ended almost simultaneously when the temperature was about 500 °C. During the whole process, the decomposition rate of TKS rubber was lower than that of NR. This was because the impurities in TKS rubber made the structure of TKS rubber denser than NR, which made the decomposition of TKS rubber more difficult. It can be seen from the figure that part of the weight loss of TKS rubber had occurred before the weight loss of NR. This was because nonrubber components such as lignin and resin remained inside the rubber. After the decomposition was complete, the ash content of TKS rubber was significantly higher than that of NR, which was because the impurities contained in it had more inorganic components.

The DSC heating curve of the two rubbers is shown in Figure 12b. For these two samples, the glass transition temperatures (T_g) measured by DSC were similar to about -60 °C . In short, the decomposition process and glass transition temperature of TKS rubber are the same as those of NR, which also showed that TKS rubber and natural rubber have similar thermal properties. The lower T_g might be

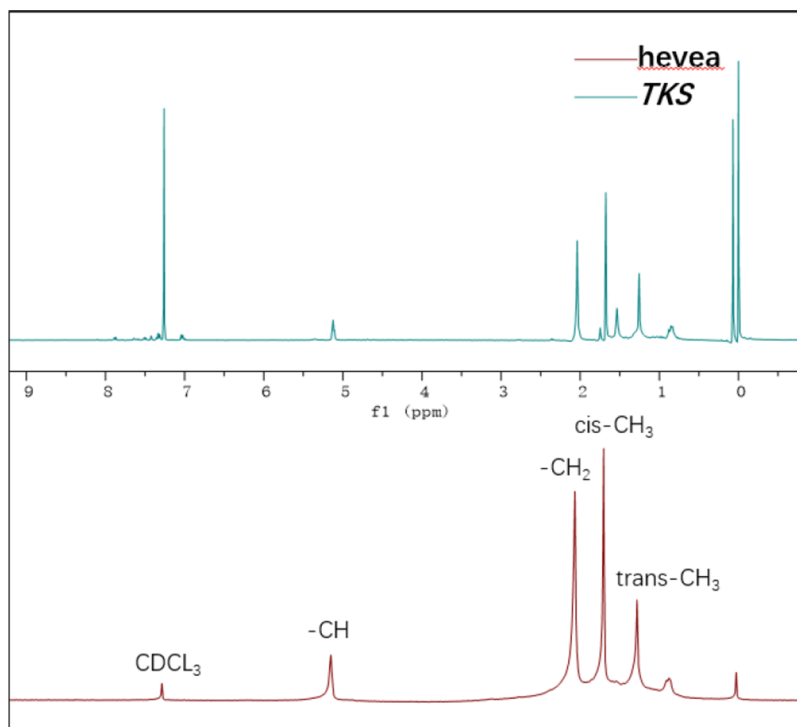


Figure 11. NMR ^1H spectrum of TKS rubber and Hevea.

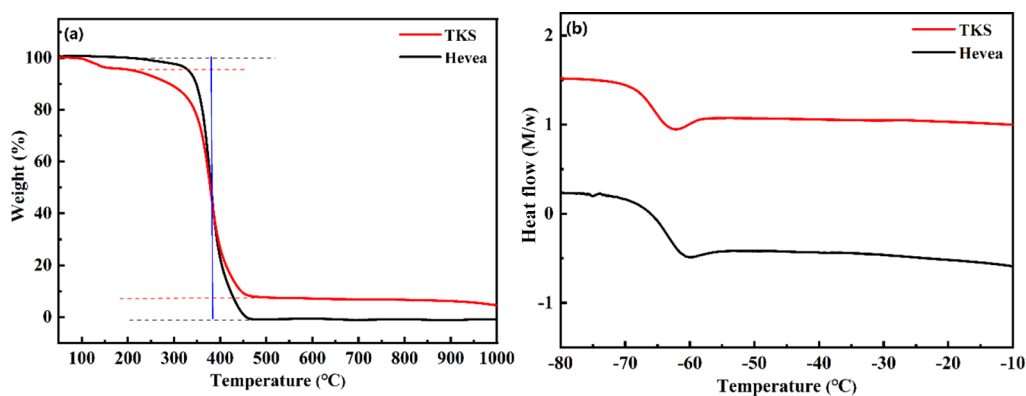


Figure 12. TGA curve (a) and DSC heating curve (b) of TKS rubber and Hevea.

beneficial to the application of TKS rubber at low temperatures.

3.4.4. Mechanical Properties of TKS Rubber. Figure 13 shows the stress–strain curves of TKS rubber and NR. In both vulcanized and unvulcanized NR, the mechanism of SIC was thought to be that the short chains between the network crosslinking points are fully stretched and become the oriented crystal nuclei. The coiled chains around them were then partially aligned parallel to the nucleus to form a highly oriented crystallite.^{38–42} Unlike NR, unvulcanized TKS rubber did not exhibit the SIC phenomenon. When both rubbers were purified with acetone under the same conditions and vulcanized, TKS rubber exhibited mechanical properties and SIC comparable to NR. The reason for the inability of raw TKS rubber to exhibit SIC might be that it did not form a natural cross-linked network and cannot form an oriented nucleus. Besides, TKS rubber had very high elongation at break, good processability, and soft texture, so it was very suitable for snow tire manufacturing.

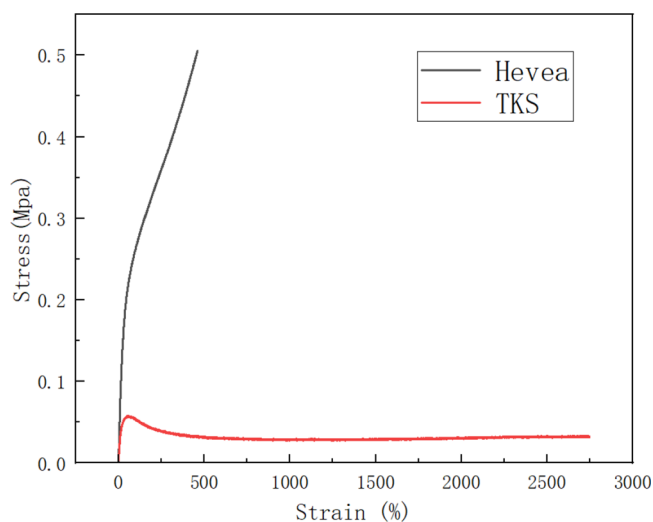


Figure 13. Stress–strain curves of TKS rubber and Hevea.

4. CONCLUSIONS

The morphological characteristics of TKS dry roots were studied. As a result, it was found that many tiny plant tissue debris adhered to the rubber at the roots, which was one of the difficulties in limiting the improvement of rubber purity. The chemical composition of TKS dried roots was determined. The results showed that inulin was the main water-soluble substance of TKS roots, accounting for 22% of the dry root mass, and lignocellulose was the most abundant impurity (40%). Cellulose was the substrate to produce biofuel ethanol by fermentation, so the microbial extraction process ("ME" process) that simultaneously produces ethanol was proposed to be optimized. The final purity of TKS rubber can reach 95.54%, and the productivity of ethanol can reach about 2.1%. The purity of the rubber obtained by this process was relatively high, and it is considered that it can effectively reduce the finished products of industrial production. The performance of the rubber harvested by the "ME" process was characterized. The results showed that the molecular weight of TKS rubber reached the commercial NR molecular weight standard and its chemical structure and thermal properties were close to NR, which could replace NR to a certain extent. There were still lignocellulosic impurities with a particle size of micrometers in the rubber, and these impurities were used as fillers to have little effect on rubber quality. In the future, more diverse TKS rubber extraction technologies needed to be developed to make more effective use of impurities such as inulin and lignocellulose.

AUTHOR INFORMATION

Corresponding Authors

Zheng Wang – College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, China; Email: wangzhengbio@163.com

Jichuan Zhang – Center of Advanced Elastomer Materials, College of Material Science & Engineering and Energy Conservation and Resource Utilization Engineering Research Center of Elastomer Materials, Ministry of Education, Beijing University of Chemical Technology, Beijing 100029, China; orcid.org/0000-0002-8584-5891; Email: dandelion@mail.buct.edu.cn

Authors

Shuai Zhao – Center of Advanced Elastomer Materials, College of Material Science & Engineering, Beijing University of Chemical Technology, Beijing 100029, China

Xiang Jie – Center of Advanced Elastomer Materials, College of Material Science & Engineering, Beijing University of Chemical Technology, Beijing 100029, China

Zhe Ma – Center of Advanced Elastomer Materials, College of Material Science & Engineering, Beijing University of Chemical Technology, Beijing 100029, China

Yushi Li – Shandong Linglong Tyre Co., Ltd, Zhaoyuan 265406, China; orcid.org/0000-0003-3802-3171

Qiuhai Nie – Shandong Linglong Tyre Co., Ltd, Zhaoyuan 265406, China

Yong Ma – Shandong Linglong Tyre Co., Ltd, Zhaoyuan 265406, China

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acsomega.2c07870>

Author Contributions

[†]S.Z. and X.J. contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are deeply grateful to the financial support granted by the National Key Research and Development Program of China and the National Natural Science Foundation of China (Grant No. 2022YFB3704800, 2022YFB3704803, and 52273051).

REFERENCES

- (1) Dedruktip, N.; Leelawanachai, W.; Tangboriboon, N. Natural Rubber-Whisker Alumina Fiber Composite Materials for Mechanical and Thermal Insulation Applications. *Key Eng. Mater.* **2018**, *789*, 221–225.
- (2) Phinyocheep, P. Chemical modification of natural rubber (NR) for improved performance. *Chemistry, Manufacture and Applications of Natural Rubber*; Woodhead Publishing Limited, (2014), 68–118.
- (3) Mullins, L. Challenges facing natural rubber at the beginning of the second hundred years. *J. Rubb. Res. Inst. Sri Lanka* **1977**, *54*, 422–432.
- (4) Huang, Z. Challenges and Development Strategies of China's Natural Rubber. *Chin J. Eng. Sci.* **2001**, *3*, 28–32.
- (5) YuanYuan, Y. The Development and Tendency of Natural Rubber Industry in Malaysia. *Southeast Asian and South Asian Studies* **2012**.
- (6) Sundar, D.; Reddy, A. R. Interactive influence of temperature and growth light intensity on rubber accumulation and rubber transferase activity in guayule (*Parthenium argentatum* Gray). *J. Plant Physiol.* **2001**, *158*, 1291–1297.
- (7) Oghenekome, U. O. Natural rubber, *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg, germplasm collection in the Amazon Basin, Brazil: A retrospective. *Econ. Bot.* **2004**, *58*, 544–555.
- (8) Goncalves, P. D. S.; Martins, A. L. M.; Gorgulho, E. P.; Bortoletto, N.; Bermond, G. Influence of six rootstocks on growth of six scion clones of rubber tree. *Pesqui. Agropecu. Bras.* **1994**.
- (9) Quesada-Méndez, I.; Quintero-Barrera, L.; Aristizábal, F.; Olga, R.-A. Assessment of the genetic diversity of natural rubber tree clones of the SINCHI Institute's clone collection, using of morphological descriptors. *Colomb.* **2011**, *14*, 69–79.
- (10) Zhongqi, Z. Development of Laticifers of Brazil Rubber Trees under Different Ecological Conditions. *Chin. J. Ecol.* **1987**, 1–3.
- (11) Vieira, S. R.; Pierre, L. H.; Grego, C. R.; Siqueira, G. M.; Dafonte, J. D. *J. Geospat. Modelling.* **2010**, *16*, 255–264.
- (12) Righi, C. A.; Bernardes, M. S. The potential for increasing rubber production by matching tapping intensity to leaf area index. *Agrofor. Syst.* **2007**, *72*, 1–13.
- (13) Soumahin, E. F.; Obouayeba, S.; Anno, P. A. Low tapping frequency with hormonal stimulation on *Hevea brasiliensis* clone PB 217 reduces tapping manpower requirement. *J. Anim. Sci.* **2009**, *2*, 109–117.
- (14) Sainoi, T.; Sdoodee, S. The impact of ethylene gas application on young tapping rubber trees. *J. Agric. Sci. Technol.* **2012**, *8*, 1497–1507.
- (15) Fließ, H.; Rugewehling, B.; Eickmeyer, F.; Wehling, P. Breeding of Russian dandelion (*Taraxacum koksaghyz*) – From the wild type to a new resource for a sustainable rubber production. *Dev. Brain Res.* **2013**, *132*, 33–45.
- (16) Artschwager, E.; Mcguire, R. C. Contribution to the Morphology and Anatomy of the Russian Dandelion (*Taraxacum kok-saghyz*). *Tech. Bull.* **1943**, 24.
- (17) Eskew, R. K. Natural Rubber from Russian Dandelion. *Rubber Chem. Technol.* **1946**, *19*, 856–864.
- (18) Kekwick, R. G. *Latex, and Laticifers*; eLS, 2002.

- (19) Cornish, K.; Xie, W.; Kostyal, D.; Shintani, D.; Hamilton, R. G. Immunochemical Analysis of Latex Proteins from Rubber Dandelion *Taraxacum kok-saghyz*. In *International Latex Conference 2015*, 05.
- (20) Buranov, A. U.; Elmuradov, B. J. Extraction and characterization of latex and natural rubber from rubber-bearing plants. *J. Agric. Food Chem.* **2010**, *58*, 734–743.
- (21) Ramirez-Cadavid, D. A.; Valles-Ramirez, S.; Cornish, K.; Michel, F. C., Jr. Simultaneous quantification of rubber, inulin, and resins in *Taraxacum kok-saghyz* (TK) roots by sequential solvent extraction. *Ind. Crops Prod.* **2018**, *122*, 647–656.
- (22) Eskew, R. K.; Edwards, P. W. Process for recovering rubber from fleshy plants. Google Patents 1946.
- (23) Sikandar, S.; Ujor, V. C.; Ezeji, T. C.; Rossington, J. L.; Michel, F. C.; McMahan, C. M.; Ali, N.; Cornish, K. *Thermomyces lanuginosus* STm: A source of thermostable hydrolytic enzymes for novel application in extraction of high-quality natural rubber from *Taraxacum kok-saghyz* (Rubber dandelion). *Ind. Crops Prod.* **2017**, *103*, 161–168.
- (24) Ramirez-Cadavid, D. A.; Cornish, K.; Michel, F. C., Jr. *Taraxacum kok-saghyz* (TK): Compositional analysis of a feedstock for natural rubber and other bioproducts. *Ind. Crops Prod.* **2017**, *107*, 624–640.
- (25) Hernández, M.; Lima, E.; Guzmán, A.; Vera, M.; Novelo, O.; Lara, V. A small change in the surface polarity of cellulose causes a significant improvement in its conversion to glucose and subsequent catalytic oxidation. *Appl. Catal. B: Environ.* **2014**, *144*, 528–537.
- (26) Zhuo, Y.; Liao, X.; Liao, L.; et al. Analysis of Extraction and Structure of *Taraxacum kok-saghyz* Rubber. *Chin. J. Trop. Sci.* **2021**, *42*, 3617–3624.
- (27) Sluiter, J. B.; Ruiz, R. O.; Scarlata, C. J.; Sluiter, A. D.; Templeton, D. W. Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. *J. Agric. Food Chem.* **2010**, *58*, 9043–9053.
- (28) Cengiz, S.; Kışmıroğlu, C.; Cebi, N.; Çatak, J.; Yaman, M. Determination of the most potent precursors of advanced glycation end products (AGEs) in chips, crackers, and breakfast cereals by high performance liquid chromatography (HPLC) using precolumn derivatization with 4-nitro-1, 2-phenylenediamines. *Microchem. J.* **2020**, *158*, No. 105170.
- (29) Wang, H.; Cai, J.; Liao, Z.; Jawad, A.; Iftikhar, J.; Chen, Z.; Chen, Z. Black liquor as biomass feedstock to prepare zero-valent iron embedded biochar with red mud for Cr (VI) removal: Mechanisms insights and engineering practicality. *Bioresour. Technol.* **2020**, *311*, No. 123553.
- (30) Wahler, D.; Gronover, C.; Foucu, F.; Twyman, R.; Moerschbacher, B.; Fischer, R.; Muth, J.; Prüfer, D. Polyphenol oxidase Silencing Affects Latex Coagulation in *Taraxacum* Species. *Plant Physiol.* **2009**, *151*, 334–346.
- (31) Barrera, C. S.; Cornish, K. Novel mineral and organic materials from agro-industrial residues as fillers for natural rubber. *J. Polym. Environ.* **2015**, *23*, 437–448.
- (32) Anttila, R.; Alanko, S.; Horneman, V. M. The C–H bending vibration ν_4 of chloroform CH_2Cl_2 . *Mol. Phys.* **2004**, *102*, 1537–1542.
- (33) Rolere, S.; Liengprayoon, S.; Vaysse, L.; Sainte-Beuve, J.; Bonfils, F. Investigating natural rubber composition with Fourier Transform Infrared (FT-IR) spectroscopy: A rapid and non-destructive method to determine both protein and lipid contents simultaneously. *Polym. Test.* **2015**, *43*, 83–93.
- (34) Preghenella, M.; Pezzotti, G.; Migliaresi, C. Comparative Raman spectroscopic analysis of orientation in fibers and regenerated films of *Bombyx mori* silk fibroin. *J. Raman Spectrosc.* **2007**, *38*, 522–536.
- (35) Song, X.; Fagiani, M. R.; Debnath, S.; Gao, M.; Maeda, S.; Taketsugu, T.; Gewinner, S.; Schollkopf, W.; Asmis, K. R.; Lyalin, A. Excess charge driven dissociative hydrogen adsorption on Ti_2O_4 . *Phys. Chem. Chem. Phys.* **2017**, *19*, 23154–23161.
- (36) Kongkaew, C.; Poonsrisawat, A.; Champreda, V.; Loykulnant, S. Maillard reaction in natural rubber latex: Improvement of concentration process. *J. Appl. Polym. Sci.* **2017**, *134*, 45224.
- (37) Musto, S.; Barbera, V.; Maggio, M.; Mauro, M.; Guerra, G.; Galimberti, M. Crystallinity and crystalline phase orientation of poly(1,4-cis-isoprene) from *Hevea brasiliensis* and *Taraxacum kok-saghyz*. *Polym. Adv. Technol.* **2016**, *27*, 1082–1090.
- (38) Ikeda, Y.; Yasuda, Y.; Hijikata, K.; Tosaka, M.; Kohjiya, S. Comparative Study on Strain-Induced Crystallization Behavior of Peroxide Cross-Linked and Sulfur Cross-Linked Natural Rubber. *Macromolecules* **2008**, *41*, 5876–5884.
- (39) Suzuki, T.; Osaka, N.; Endo, H.; Shibayama, M.; Ikeda, Y.; Asai, H.; Higashitani, N.; Kokubo, Y.; Kohjiya, S. Nonuniformity in Cross-Linked Natural Rubber as Revealed by Contrast-Variation Small-Angle Neutron Scattering. *Macromolecules* **2010**, *43*, 1556–1563.
- (40) Valentín, J. L.; Posadas, P.; Fernández-Torres, A.; Malmierca, M. A.; González, L.; Chassé, W.; Saalwächter, K. Inhomogeneities and Chain Dynamics in Diene Rubbers Vulcanized with Different Cure Systems. *Macromolecules* **2010**, *43*, 4210–4222.
- (41) Karasevska, O. P.; Mordiyuk, B. N.; Markovsky, P. E.; Shyvanyuk, V. M.; Skyba, I. O. Effect of strain-induced $\beta \rightarrow \omega$ transformation on mechanical behaviour of β -titanium and β -zirconium alloys. *Metallofiz. Noveishie Tekhnol.* **2009**, *31*, 1573–1587.
- (42) Liu, J.; Tang, Z.; Huang, J.; Guo, B.; Huang, G. Promoted strain-induced-crystallization in synthetic cis-1,4-polyisoprene via constructing sacrificial bonds. *Polymer* **2016**, *97*, 580–588.