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Research article

Extraction process and characterization of *Taraxacum kok-saghyz* (TKS) latex

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ABSTRACT

Taraxacum kok-saghyz (TKS) latex is a natural latex produced from its root, and its extraction optimization process is mainly studied in the present paper. The composition of fresh roots of TKS was quantitatively analyzed, and the results showed that the moisture content of the fresh root was approximately 70 %, and the rubber content averaged to 6 % (dry weight ratio). An optimal process route for extracting the TKS latex was finally determined, making the extraction efficiency reach about 80 %, and a new latex extraction process was established and optimized and named “the process of Buffer Extraction TKS Latex (BETL)”. *Hevea* latex, extracted TKS latex and TKS latex collected directly from the broken roots were compared for study. The results showed that, like *Hevea* latex, the appearance of TKS latex was milky white; and after centrifugation, both showed four layers from top to bottom: rubber particles, Frey-Wyssling particles, C-serum and lutoids. The results of the composition analysis showed that the concentration of TKS latex ranged from 54.54 % to 68.25 %, which is close to that of concentrated *Hevea* latex; the moisture content of TKS latex was between 31.75 % and 45.46 %. The protein content of TKS latex was 13.51 mg/mL, which was lower than that of *Hevea* latex at the same rubber hydrocarbon concentration. The molecular structures and properties of *Hevea* latex, the extracted TKS latex, and the collected TKS latex were characterized by FTIR, ^{13}C NMR, GPC, TG, SEM and LPSA, and the results showed that the main components and structure of the three latexes were similar, which are all *cis*-1,4-polyisoprene, and include the proteins and lipids. The distributions molecular weights of the three latexes all showed a bimodal distribution, but the molecular weight of the latex collected from TKS was lower, which indicates the larger molecules were difficult to flow outside the root automatically. The *Hevea* latex and TKS latex rubber particles were both core-shell structure and the size distribution were bimodal, which was consistent with the GPC analysis results.

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1. Introduction

Natural rubber (NR) is widely used in the fields of the automotive industry, aerospace, rail transportation, shipbuilding, medicine and health due to its excellent properties and irreplaceability [1,2]. At present, the vast majority of NR comes from *Hevea brasiliensis* in Southeast Asia with a single tropical rainforest environment. However, the product of NR is facing some challenges, such as labor shortages, politics, climate, diseases and insect pests. These factors may cause long-term production of NR to become unsustainable. Especially the South American Leaf Blight (SALB) is a great threat to narrow genetic rubber trees in Southeast Asia, and the growth of NR production is far behind the growth of NR demand [3,4]. Therefore, there is an urgent demand to develop rubber-producing plants that can be grown outside of the tropical rainforest as a complement to *Hevea brasiliensis* to meet the growing global demand for natural rubber [5]. *Taraxacum kok-saghyz* (TKS), commonly known as Russian dandelion, is native to the Tianshan Mountains on the border between Kazakhstan and China. It was found in the 1930s and is a rubber-producing perennial herb [6]. TKS rubber is present in the roots and the rubber content increases with the growth years. TKS roots grown in the wild field contain 5 %–24 % of NR [4,5,7,8]. The structure of rubber in TKS is the same as that of NR, both of which are *cis*-1,4-polyisoprene. It is reported that the molecular weight and physical properties of TKS rubber are close to NR, especially TKS rubber also has strain-induced crystallization (SIC) effect and shows excellent performance in various applications [9,10]. Compared with *Hevea brasiliensis*, TKS has a short growth cycle, easy planting, high rubber content and excellent rubber quality, making it an ideal plant rubber resource. Therefore, TKS rubber is considered as a supplement and replacement for NR.

For fresh TKS, rubber exists in the lactiferous cell of its root in the form of latex [11]. When the root is left for a period or dried, the latex will coagulate into rubber filaments and can be stored for a long time. Because the dried roots are easier to store and transport. At present, several mature processes have been developed for the extraction of TKS rubber: (1) Hamerstrand and Montgomery developed solvent method [12] whose principle is that rubber is extracted by using organic solvents, and different solvents need to be used in order to remove different impurities before extracting rubber. (2) Eskew et al. developed wet-milling process [13,14] whose main principle is to use the density difference between rubber and non-rubber plant tissue to float rubber. The density of rubber is less than 1, so it will float on the water in a water environment. Plant tissue become loose after mechanical treatment, and their density after absorbing water is quite close to or slightly larger than water. This makes most of the plant tissue sink to the bottom. The basic process flow of the process is as follows: First, the roots are boiled in the water environment, and while removing the water-soluble impurities, the plant tissue becomes soft and the rubber is initially separated. The pebbles were then used to grind the roots. After grinding, the plant tissue was separated from the rubber using a vibrating screen. By repeating this process twice in this way, the rubber can be obtained. (3) Buranov developed dry-milling method [15] whose main principle is to use the difference between the rubber and plant tissue density and hydrophilicity, but also the difference between the rubber's viscoelasticity and the plant tissue's brittleness. The main process is divided into two stages: dry method and wet method. First, the roots were dry ground to loosen the plant tissues and rubber, and then separating rubber threads from plant root skins by air-blowing. The remaining root fragments are subjected to centrifugal leaching in a water environment after dry-milling, in which the water-soluble sugar dissolves quickly in water; under the action of centrifugation, the plant tissue is separated from the rubber, and finally the rubber is obtained by flotation. (4) Sikandar et al. developed enzymatic hydrolysis method [16]: A fungus called Thermomyces lanuginosus STm was isolated from the garden soil. The fungus can produce different hydrolases, such as cellulase, xylanase, and so on. TKS roots are depolymerized during the enzymatic hydrolysis process to obtain rubber. (5) Ramirez et al. developed an aqueous, enzyme-based method [4] for the separation and purification of TKS rubber to avoid yielding rubber with high levels of contamination by Eskew's wet-milling process and significantly improved the yield. Enzymatic digestion alone and alkaline pretreatment followed by enzymatic digestion to rid lignocellulose impurities yielded rubber with higher quality than 78 % and up to 99.4 % purity, which is significantly higher than that achieved by wet-milling process.

Researches on latex extraction are also focused by some scholars because of its simplicity, efficiency, and high purity [7,17,18]. When extracting the TKS latex, it is necessary to prevent the latex from agglomeration during the extraction process while ensuring the freshness of the roots, which further increases the difficulty of latex extraction. Therefore, few literatures on the extraction method of TKS latex have been reported. There are two types of latex extraction methods reported in the related literature. (1) Blender method [7,19]: It is a method of squeezing latex out of plants using a principle similar to juice extraction. After the harvested fresh roots are processed, they are ground in a Waring mixer containing ice-cold extraction buffer to prevent agglomeration, and the upper layer of latex is collected by solid-liquid two-phase separation. (2) Flow method [7,20]: It is a relatively gentle extraction method, which does not require breaking the roots of TKS to extract latex. It differs from the stirring method in that the extraction device and the extraction buffer composition are different. The fresh roots were processed and frozen and cut into small pieces, transferred to a conical flask containing extraction buffer, shaken, filtered with suction, and centrifuged to collect the latex [20–23]. The basic processes of the two methods are similar, but the latex purity is different due to different extraction principles. The latex obtained by the blender method is lower than the flow method. Both methods have relatively low levels of latex extraction. About 50 % of the rubber is extracted in the form of latex [7]. In this study, a new buffer latex extraction process was established and optimized to make it an industrially feasible extraction process. Compared with the extraction process in the above literature, the extraction efficiency is improved to more than 80 %, and the properties of extracted TKS latex are similar as the *Hevea* latex.

2. Materials and methods

2.1. Chemical reagents and materials

TKS from the three origins belong to one phenotype and one genotype (K445), which were cultivated from seeds, grew naturally in the field and are all one and a half years old (Provided by Hainan Rubber Institute, Xinjiang Academy of Agricultural Sciences, Heilongjiang Academy of Sciences, China). They were planted in May 2018 and harvested in September 2019. Xinjiang (long. 73°40′E ~ 96°23′E, lat. 34°22′N ~ 49°10′N) is close to the native origin of TKS. Heilongjiang (long. 121°11′E ~ 135°05′E, lat. 43°26′N ~ 53°33′N) is the northernmost province of China, and Hainan (long. 108°21′E ~ 111°03′E, lat. 19°20′N ~ 20°10′N) is the southernmost province. The general climate information of three places of origin (2018–2019) are listed in Table 1. The processing of TKS roots is in Section 3.3.1 of Results and Discussion. *Hevea* latex without the addition of ammonia is taken from the latex directly flowing from the *Hevea brasiliensis* of RRIM 600 in Xishuangbanna, China, in September 2019. The latex for the first 10 min needed to be discarded during the collection process and the subsequent fresh latex was collected in an ice-cold plastic centrifuge tube. Then they were transported to the laboratory in Beijing in a foam box with ice. Ethanol (AR, 99.8 %, Beijing Tong guang Fine Chemicals Company, China) acetone (AR, 99 %, Sinopharm Chemical Reagent Co., Ltd, China) petroleum ether (AR, Beijing Tong guang Fine Chemicals Company, China), ammonia (≥4.0 M, methanol solution, Aladin, USA), sodium sulfite (AR, 99 %, Macklin, China), casein (EP, 92 %, J&K Chemical, China), glacial acetic acid (EP, 99.8 %, 3A Chemicals, USA). The raw material roots of the process optimization and characterization part come from the annual TKS in Xinjiang.

2.2. TKS fresh root composition analysis

2.2.1. Determination of moisture content

Nine roots of TKS in Xinjiang, Heilongjiang and Hainan were selected, cleaned and marked J-1 ~ J-3, H-1 ~ H-3, Q-1 ~ Q-3 respectively. The clean fresh roots were accurately weighed and recorded as m_0 after being washed and wiped. The roots were dried to constant weight in a vacuum oven at 60 °C. Weigh accurately and record it as m_1. Each TKS root was processed in the same way. P_1 ~ P_8 are used to represent the content of different chemical components in TKS roots.

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

2.2.2. Soluble component analysis by Soxhlet extractor method

10g of washed and dried roots were shredded by a Waring blender. 100 mg were subjected to a Soxhlet extraction experiment for 48 h at the boiling point of each solvent in sequence. In this part of the experiment, the Soxhlet extraction method was used, and the dried roots were extracted with deionized (DI) water, ethanol, acetone, and petroleum ether in sequence in terms of the polarity of the soluble component in the root.

$$Water\ soluble\ content = \frac{m_2 - m_3}{m_1} \times 100\%$$

$$Pigment\ content = \frac{m_3 - m_4}{m_1} \times 100\%$$

$$Resin\ content = \frac{m_4 - m_5}{m_1} \times 100\%$$

$$Rubber\ content = \frac{m_5 - m_6}{m_1} \times 100\%$$

where m_1 is the mass of the dry root sample of constant weight; m_2 is the mass of the sample degreased filter paper bag after constant weight; m_3 is the mass of the constant weight filter paper bag after DI water extraction; m_4 is the mass of the constant weight filter paper bag after ethanol extraction; m_5 is the mass of the constant weight filter paper bag after acetone extraction; m_6 is the mass of the constant weight filter paper bag after petroleum ether extraction.

2.2.3. Determination of lignin and holocellulose content

The content of acid-insoluble lignin was determined by the sulphuric acid method according to GB/T 2677.8-1994 [24], and holocellulose by the sodium chlorite method according to GB/T 2677.10-1995 [25]. For non-wood raw materials, the formulas are as

Table 1
General climate information of three places of origin (2018–2019).

Place of origin	Annual precipitation/mm	Average temperature/°C	Maximum temperature/°C	Minimum temperature/°C
Xinjiang	403.04	9.78	46.20 (2019-07-03)	−42.34 (2018-01-07)
Heilongjiang	1028.33	3.28	39.76 (2018-06-02)	−45.50 (2018-01-25)
Hainan	1761.24	25.60	38.49 (2019-05-20)	5.03 (2018-02-05)

follows:

Lignin mass: $m_L = m_7 - m_D$

$$Lignin\ content = \frac{m_L}{m_1} \times 100\%$$

Holocellulose mass: $m_C = m_8 - m_D$

$$Holocellulose\ content = \frac{m_C}{m_1} \times 100\%$$

where m_L is the mass of lignin; m_7 is the acid-insoluble lignin mass after being dried.; m_D is the ash mass in acid-insoluble lignin; m_C is the mass of the holocellulose; m_8 is the holocellulose mass after being dried.

2.2.4. *Ash content detection*

Cleaned TKS roots were dried to constant weight. Place the sample in a high temperature box type resistance furnace (KSL-1200X-M, Hefei Kejing Material Technology Co., Ltd., China) and set the temperature to 250 °C, hold it for 30 min and then increase it to 550 °C at a heating rate of 10 °C/min for more than 3h. Weigh the remaining powder and record it as m_9;

$$Ash\ content = \frac{m_9}{m_1} \times 100\%$$

2.3. *The Buffer Extraction TKS latex process*

2.3.1. *TKS latex extraction method*

Configure the buffer solution (0.1 % Na_2SO_3, 0.2 % NH_4OH, 0.1 % casein; v/v) and store it in the refrigerator. Place a high-speed motor connected to a stirring paddle above a 250 mL beaker containing extraction buffer in advance of cutting roots. One root is used for each extraction. Then the fresh root harvested from the soil were washed and wiped up, and cut into circular small pieces of about 0.5 cm thickness in size with a semi-automatic frozen microtome (RM 2235, Leica Brand, Germany). Within 1 min, the fragments were transferred to the beaker. And the stirring paddle speed can be adjusted and inserted below the liquid level. Turn on the stirring switch, and after extracting for a certain period, the slurry was slowly filtered on a Buchner funnel with a 1 mm copper mesh. After the suction filtration was completed, collect the liquid in the suction filter bottle. Transfer the remaining solids in the Buchner funnel to the beaker, re-add the same amount of buffer solution, repeat this step for 3 times suction filtration, and collect the liquid collected from the suction filter bottle. The collected liquid in a centrifuge (LXL-2, Shanghai Anting Scientific Instrument Factory, China) was centrifuged at 12,000 rpm for 10 min, and then the upper layer of milky white latex was aspirated with a pipette and collected. After adding a certain amount of glacial acetic acid to the collected TKS latex, the mixture was centrifuged at 12,000 rpm for 10 min to obtain a coagulated rubber. Carefully pick out the coagulated rubber, place it in a watch glass, dry it in a vacuum oven at 45 °C to constant weight, and weigh it to obtain the weight of the extracted dry rubber (Actual value for short).

2.3.2. *Analysis of TKS latex extraction efficiency*

In this paper, extraction efficiency is redefined and a new method for calculating extraction efficiency was proposed to make it more accurate. The calculation formula is as follows:

$$C_E = \frac{m_R}{m_T} \times 100\%$$

where, C_E is defined as the extraction efficiency, m_R is the mass of dry rubber that was obtained by drying the latex (Actual value), and m_T is the theoretical mass of total rubber (Theoretical value). The calculation process of m_T is as follows:

$$m_T = m_F \times (100\% - C_1) \times C_R$$

where, C_1 is the moisture content, C_R is the rubber content, and m_F is the mass of TKS fresh root used for extraction.

2.4. *Characterization of TKS latex*

2.4.1. *Centrifugal stratification observation of TKS latex*

In order to observe the similarities and differences between the basic components of TKS latex and *Hevea* latex, the ultracentrifuge (Optima MAX-XL, American Beckman Coulter Co., Ltd., USA) was used to centrifuge the two samples for 60 min at 4 °C and 60,000g to treat the latex.

2.4.2. *Analysis of TKS latex concentration*

In this study, the ratio of the mass of the solids remaining after drying the latex in a vacuum oven (DZF-6030, Shanghai Hecheng Instrument Manufacturing Co., Ltd., China) at 75 °C to the mass of the latex was used to express the concentration of the latex, and compared with the concentration of *Hevea* latex.

2.4.3. Protein analysis

BCA kit (B500, Beijing LABLEAD Trading Co., Ltd., China) was used to analyze the protein content of TKS latex. The process is as follows: (1) Configure a standard protein content test solution according to the kit instructions. (2) Draw a standard curve after measuring the absorbance at different protein content. (3) According to the absorbance of the test sample, find the corresponding protein content on the standard curve. The protein content of both latexes was determined three times.

2.4.4. Thermogravimetric analyzer analysis

The thermogravimetric analyzer (TG, state system, METTLER-TOLEDO Co., Ltd., Switzerland) was used to monitor the weight loss of the dry latex under heat, and it can reflect the ingredients and the content of the dry latex. In the nitrogen atmosphere, the temperature was raised from 25 °C to 1000 °C at a rate of 10 °C/min, and then stopped.

2.4.5. Laser particle size analyzer analysis

The particle size and distribution of rubber in the TKS latex and *Hevea* latex were analyzed and compared at room temperature using a laser particle size analyzer (LPSA, Zen3600, Malvern Analysis Ltd., England). The latex was dispersed in water, and the concentration of the dispersion is diluted to one thousandth of the original. The dispersed latex was transferred and fixed at one third of the height of the cuvette. The test parameters were set as follows: the medium is water; the test object is an emulsion and the number of scans is 500/second. Scanning will start after the adjustment was completed. This experiment was repeated three times.

2.4.6. Scanning electron microscope (SEM) observation

The SEM (S4800, Hitachi Co., Ltd., Japan) was used to observe the morphology and surface morphology of the rubber particles in the TKS latex, and compared with the *Hevea* latex rubber particles. The two latexes were collected and centrifuged (section 2.4.1). The latex particles of the rubber particle layer were taken out and dispersed in an equal volume of 100 mM Tris-HCl buffer solution. The dispersion was centrifuged for 20 min and repeated twice at 10,000 rpm and 4 °C. The upper layer of latex was ultra-sonicated in pure water to make it uniformly dispersed. A diluted latex sample was dropped on the aluminum foil and the excess liquid was aspirated. The samples were quickly freeze-dried with liquid nitrogen. The dried sample was fixed on the sample stage with conductive adhesive and sputter coated with gold. The thickness of the gold layer was 5 ~ 10 nm, and the acceleration voltage is 5 kV. The magnification was adjusted according to actual needs during the test.

2.4.7. Fourier transform infrared (FTIR) spectrum analysis

After the TKS latex was coated on KBr, the moisture in the latex is dried to obtain a dry latex film. A dry latex film sample was tested using the ATR test program in an infrared spectrometer (Tensor 27, Bruker (Beijing) Technology Co., Ltd., China). A series of infrared spectra were collected in the range 500 ~ 4000 cm^{-1} with a resolution of 4 cm^{-1}. The infrared spectrum can analyze the structure and functional groups of the extract, and find the characteristic peaks of *Hevea* rubber's backbone stretching vibration to identify whether the extract is *Hevea* latex.

2.4.8. Nuclear magnetic resonance (NMR) spectrometer analysis

The ^{1}H and ^{13}C NMR spectrum (AV 400, Bruker (Beijing) Technology Co., Ltd., China) was used to analyze the molecular structure of the dry latex. The test conditions are as follows: the sample concentration is 10 mg/mL, and the scan is performed 400 times with a scan pulse of 4.8 s.

Fig. 1. Tapping of *Hevea* latex (a) and seeding and harvesting of TKS in Duolun County of Inner Mongolia Autonomous Region (China) raw material base [(b) sowing (c) artificial weeding (d) collecting seeds (e) collecting roots] Photographs courtesy of Mr. Tang Huaidong (b ~ e).

2.4.9. Gel permeation chromatography (GPC) analysis

After the extracted TKS latex was dried, 1 mg of dry latex was weighed and dissolved in 2 mL of chromatographic pure toluene to make a 0.5 mg/mL dry latex toluene solution. GPC (1260 Infinity, Agilent (Beijing) Technology Co., Ltd., China) was used to analyze the molecular weight and molecular weight distribution of the latex. The test conditions were the toluene phase as the mobile phase, the flow rate was 1 mL/min, the injection volume was 10 μL, and the temperature of the column oven and detector was 30 °C.

3. Results and discussion

3.1. Harvest and storage of TKS fresh roots

3.1.1. Harvest of TKS fresh roots and its output

Hevea latex is generally collected by tapping the *Hevea brasiliensis*, which is very time consuming and labor intensive due to it cannot be achieved by mechanized operations up to now [26], as shown in Fig. 1(a), which exhibits the tapping process of *Hevea* latex in Hainan Island of China. However, as a perennial herb, TKS can be sown and harvested by farm machinery and implements every one or two years. The complete process of TKS from sowing, weeding to harvesting the seeds and roots in Duolun County of Inner Mongolia of China can be seen in Fig. 1(b ~ e). Because Duolun County is rich in potatoes, the sowing and root harvesting processes of TKS will easily be mechanized operation by the help of the potato machinery, which will lay the foundation for large-scale planting in the future. However, at present, the weeding and seed harvesting processes of TKS still need labor to finish which will increase the costs greatly. It is believed that weeding and seeds harvesting processes will also be mechanized operations by the efforts of scientists in the future. Tapping the *Hevea* latex is very easy due to the thick trunk while tapping the TKS latex is very difficult because the roots are tiny, slender, and grown underground. But TKS can easily be mechanized operation from sowing to harvesting, so the TKS latex can be extracted through specific technology by professional manufacturers.

The structure of TKS could be divided into three parts: the green rosette leaves above ground, the very short stem near the ground and the light brown roots underground, as shown in Fig. 2, which shows the fresh 5 years old TKS from Heilongjiang province of China. The total length is approximately 60 cm and the fresh weight is approximately 0.2 kg. As a typical main root crop, the number of TKS lateral roots is not large, about 20 ~ 30 per plant. The rosette leaves are approximately 15 ~ 20 cm in length, 10 ~ 30 mm in width and the fresh weight (FW) is about 90g. The stem and roots are approximately 1 ~ 10 mm, 40 ~ 50 cm in length width and the fresh weight was about 110g, dry weight (DW) was about 35g. For freshly harvested TKS, its moisture content can reach 72 %.

In terms of rubber yield, due to differences in varieties, growth cycles and planting densities, there are large differences among countries. In the United States, Bates [27] measured the yield of TKS under different planting densities (1.24 ~ 9.88 million plants/ha) and growth cycle (6 months and 1 year). The results showed that the rubber yield was 400 ~ 1000 kg/ha, and Cornish [8] cultivated a high yield plant phenotype with a yield of 2160 kg/ha under the condition that the plant density is 1 million plants per acre (1ac = 0.4047ha) and the growth cycle is 1.5 year. In Germany, there are reports that its output reaches 150 ~ 500 kg/ha [28]. Due to the difference in phenotype and lower planting density, in some recent studies, the production of TKS has not reached this level [29,30]. In China, planting in experimental fields has already begun. After the seedlings mature (one year and a half old), the planting density is about 100 ~ 150 plants/m^2. The yield of TKS rubber is about reached 200 ~ 300 kg/ha.

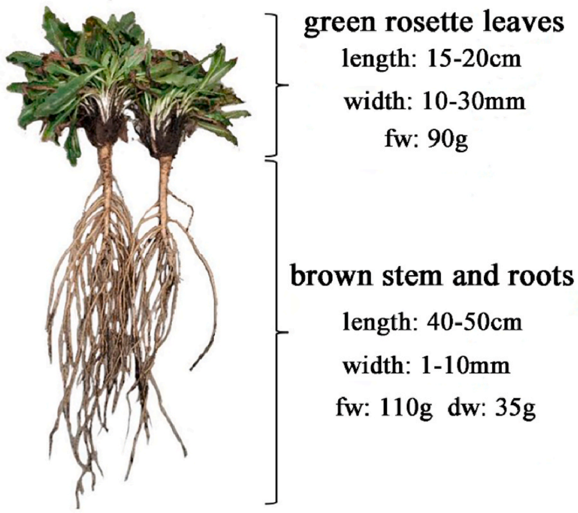

Fig. 2. The size and weight of a fresh TKS.

3.1.2. Storage of TKS fresh roots

During the process of collecting and transporting fresh NR latex, the latex is affected by bacteria and enzymes. The stability of the fresh NR latex decreases, which causes discoloration and coagulation. Latex deterioration will adversely affect a series of industrialized processes such as latex collection, processing, and quality control [31]. The *Hevea* latex produced by *Hevea brasiliensis* is collected in situ, with high latex production and a long collection time. Therefore, the collection and processing of NR latex usually do not occur at the same time and place, and research on the optimization of storage time has been focused on preservatives. As herbaceous plant, the characteristics of low biomass and low latex production of TKS determine that TKS latex collection and processing tend to be performed simultaneously. The storage time of TKS raw latex has important guiding significance for the industrialization of latex.

In view of the TKS latex will coagulate into rubber filaments within hours of the roots being dug out of the soil. So, the transportation radius of TKS fresh roots is restricted greatly, which also hinders the development of the TKS latex industry. Consequently, it is very necessary to study the effect of storage time on latex extraction so that optimal root storage time can be determined. Related literature explored the storage temperature of guayule branches. It was found that higher temperature will accelerate the decline rate of latex content and molecular weight, while storing latex at 4 °C will slow down this process [32,33], lower temperature will cause the water to freeze and cause the loss of the water phase, which may cause the rubber particles in the latex to irreversibly coagulate [33,34]. Considering that both latexes are present in plant tissues (TKS roots, guayule branches), they have a relatively high similarity. Therefore, TKS fresh root will be stored at 4 °C in this article. The washed roots were stored in a refrigerator at 4 °C for different times and then taken out, and the cross-section after cutting was shown in Fig. 3. The content of latex flowing out on the cross section decreased with the increase of storage time. When the storage time exceeded 48 h, only a small amount of latex flowed out. When the storage time exceeds 72 h, latex disappears and rubber silk appears in the TKS roots, which indicates that most of the latex has coagulated into the solid rubber. After 96 h of storage, the phenomena of rubber filamentation and TKS root decay were observed. Like *Hevea* latex [35], as the storage time increases, sugars, esters, and proteins in TKS latex are broken down by bacteria and enzymes to produce organic acids and putrefying substances. These substances neutralize the charge on the rubber particles and accelerate the coagulation of the latex [36]. Fresh roots also spoil under the action of oxygen and bacteria, shortening the time of coagulation.

In order to study the effect of storage time on the extraction efficiency of latex, the roots of different storage time will be used to extract the latex using the method of section 2.3.1. The initial conditions are: the material-liquid ratio of 1: 4 (g/mL), the extraction cycle of 3, extraction time of 20 min and extraction rotation speed (RS) of 100 rpm. As shown in Table 2, the extraction efficiency of latex gradually decreases with the storage time of fresh roots. When the storage time exceeds 48h, the extraction efficiency is less than 50 %; when the storage time reaches 96h, the extraction efficiency of latex is only 15.04 %. Within 96 h, about 50 % of the rubber was coagulated from the latex in the form of solid rubber. The experimental data are very consistent with the observation results of the fresh root section. This indicates that the longer the storage time of fresh roots, the more latex will coagulate into solid rubber, which will hinder the extraction of latex. Fresh roots must be extracted within 24 h of storage, and it also provides a basis for a suitable distance between the production line and the planting base. At present, it is not possible to perform latex extraction immediately after harvesting TKS fresh roots. Therefore, in most cases, fresh roots must go through a storage process before being processed. In industrial production, the storage time of fresh roots at 4 °C is not recommended to exceed 24 h.

3.2. Chemical composition analysis of TKS fresh root

3.2.1. Moisture analysis

Water is the most abundant component in the fresh roots of TKS. Therefore, determining the moisture content is very important for the latex extraction of TKS. A total of nine samples from different places of origin of J-1 ~ Q-3 were analyzed for moisture content. The

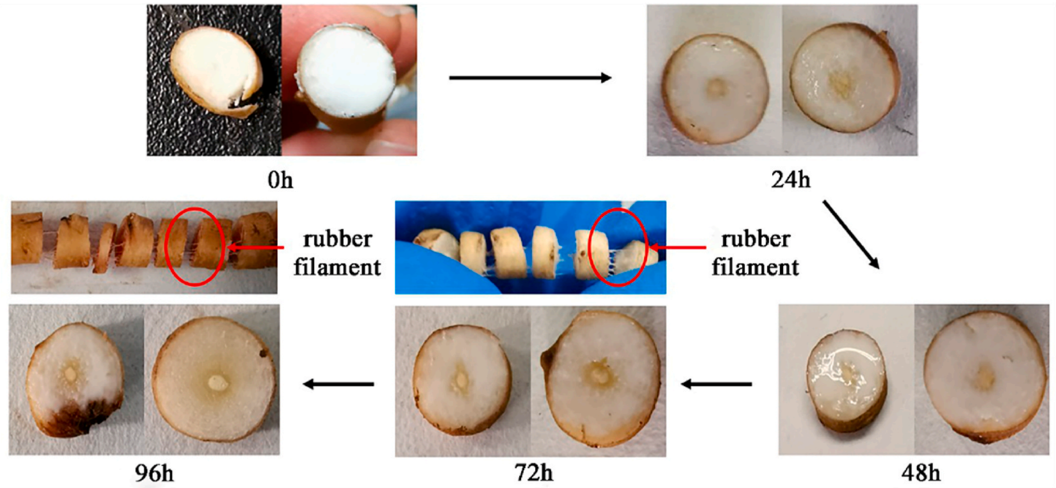

Fig. 3. TKS root section view for different storage time.

Table 2
Latex extraction data of TKS at different storage time.

Storage times/h	Fresh roots weight/g	Theoretical value/mg	Actual value/mg	Extraction efficiency/%
0	4.68	92.30	58.50	63.38
24	4.90	96.15	54.20	56.37
48	4.92	96.54	46.50	48.17
72	4.38	85.95	21.40	24.90
96	4.10	80.45	12.10	15.04

Note: The moisture content of the root used in this experiment is 71.27 %, the rubber content is 6.83 %. Calculate the extraction efficiency according to the formula in Section 2.3.2.

results are listed in Table 3. The moisture content of fresh roots of TKS in three different places of origin ranged from 70 to 75 %, of which the moisture content of TKS in Hainan is 72.9 %, 72.34 % in Xinjiang and 70.22 % in Heilongjiang averagely. It can be found that the moisture content was a little affected by the geographical conditions, it is higher in southern China and lower in northern China.

3.2.2. Soluble component analysis by Soxhlet extractor method

Soxhlet extractor method was often used to extract and determine the content of different soluble components. Since the TKS root is a complex of polar and non-polar substances, which can be extracted by polar and non-polar solvents in sequence. Water can be used as a solvent to extract water soluble molecules, such as monosaccharides and oligosaccharides. Ethanol is used to extract the pigment. Acetone is used to extract the resin. Petroleum ether is used to extract the rubber. Nine dried samples of J-1 ~ Q-3 were sequentially extracted by four different solvents to determine the content of each component. The detailed content of each component is shown in Table 4. The content of water-soluble molecules was the highest in TKS from three different places of origin, followed by the content of pigments and resins, and the content of rubber was the least. The content of water-soluble molecules (24.73 %) of TKS in Hainan is obviously higher than in the two northern provinces (around 20 %). The contents of pigments, resins, and rubbers of TKS in Heilongjiang and Xinjiang are significantly higher than those in Hainan. This may indicate that geographical conditions have a certain effect on various contents. TKS in the south needs more water-soluble molecules, while TKS in the north is more likely to produce more pigment, resin, and rubber.

3.2.3. Analysis of lignin and holocellulose

Due to the lignin and holocellulose being insoluble in water and other solvent, their contents were determining according to section 2.2.3. After determined the content of the soluble component, the remains of the nine samples of J-1 ~ Q-3 were measured for lignin and holocellulose content. The detailed content of each component is shown in Table 5. Among three different sources of TKS, the content of holocellulose was the highest (above 23 %), which was significantly higher than that of lignin (14.72 % ~ 19.72 %). In the comparison of different provinces, Hainan's TKS has the highest lignin content, while Heilongjiang's TKS has the lowest lignin content. The holocellulose content of the three provinces is similar, and the content of the two provinces in the north is slightly higher than those in Hainan. Adding the lignin content to the holocellulose content gives the total amount of lignocellulose. The total amount of lignocellulose in Hainan is significantly higher than that in the two northern provinces, while the total amount in Heilongjiang is the lowest. It is speculated that the lignin is more easily produced in TKS roots grown in the south.

3.2.4. Ash content analysis

The ash content of the TKS root was determined according to section 2.2.4. As shown in Table 6, the root ash content of the three producing areas is relatively close, the ash content of the roots from Hainan exceeds 5 %, and the rest are around 4 %, and the average content difference is about 1 %. The root ash mainly comes from the unwashed mud attached to the surface and the inorganic salts inside the root.

Table 3
Fresh root moisture content of sample from different places of origin.

Place of origin	Number	Moisture/%
Xinjiang	J-1	73.46
	J-2	71.64
	J-3	71.92
	Average value	**72.34**
Heilongjiang	H-1	68.69
	H-2	71.24
	H-3	70.73
	Average value	**70.22**
Hainan	Q-1	73.32
	Q-2	74.10
	Q-3	71.28
	Average value	**72.90**

Table 4
Content of each component from different places of origin.

Places of origin	Number	Water-soluble molecules/%	Pigment/%	Resin/%	Rubber/%
Xinjiang	J-1	17.41	14.77	13.68	6.13
	J-2	21.32	12.69	12.89	5.79
	J-3	19.67	12.95	13.02	6.27
	Average value	**19.47**	**13.47**	**13.20**	**6.06**
Heilongjiang	H-1	21.34	10.75	14.25	8.53
	H-2	20.68	12.47	13.86	8.14
	H-3	19.79	12.86	14.37	7.96
	Average value	**20.60**	**12.03**	**14.16**	**8.21**
Hainan	Q-1	24.23	9.48	11.56	4.79
	Q-2	25.37	9.25	10.83	3.65
	Q-3	24.58	10.23	11.29	4.16
	Average value	**24.73**	**9.65**	**11.23**	**4.20**

Table 5
Content of lignin and holocellulose from different places of origin.

Place of origin	Number	Lignin/%	Holocellulose/%	Total/%
Xinjiang	J-1	16.05	26.79	42.84
	J-2	17.14	24.61	41.75
	J-3	15.65	25.31	40.96
	Average value	**16.28**	**25.57**	**41.85**
Heilongjiang	H-1	14.67	22.49	37.16
	H-2	14.41	24.87	39.28
	H-3	15.07	24.13	39.20
	Average value	**14.72**	**23.83**	**38.55**
Hainan	Q-1	19.46	23.47	42.93
	Q-2	20.03	22.31	42.34
	Q-3	19.66	24.05	43.71
	Average value	**19.72**	**23.28**	**43.00**

Table 6
Content of ash from different places of origin.

Place of origin	Number	Ash/%
Xinjiang	J-1	4.85
	J-2	4.37
	J-3	5.02
	Average value	**4.75**
Heilongjiang	H-1	4.52
	H-2	3.86
	H-3	4.29
	Average value	**4.22**
Hainan	Q-1	5.66
	Q-2	6.03
	Q-3	4.93
	Average value	**5.54**

3.2.5. Analysis on climate factors and component content of TKS root

After the above analysis, it can know that the influence of climatic factors on the content of root components was significant. The composition of TKS root can be made into the histogram and pie chart (Fig. 4) to analyze the influence of geographical and climatic conditions on the overall content of all components. Heilongjiang and Xinjiang are the northern frontiers of China, and they have a typical temperate continental climate. Heilongjiang is relatively humid, while Xinjiang is dry. Both provinces have large diurnal temperature differences. Hainan is in the southernmost part of China, where it is hot and humid all year round. Lignin and holocellulose are the main components that make up the plant cell wall, and play an important role in maintaining cell morphology, cell wall rigidity, and lodging resistance [37,38]. In the limited experimental data, regular features were found through analysis. As shown in Fig. 4(a), the total amount of lignocellulose in TKS in the three places is approximately 40 %. The roots in Heilongjiang and Xinjiang have higher cellulose content. The lignin content in Hainan is significantly higher than that in the northern two places. This may be because lignin is hydrophobic and prevent the rapid evaporation of water and keep the necessary water balance in metabolism [39]. However, in the north, the evaporation of water is not so fast, so it is not necessary to maintain a high level of lignin content in TKS roots. Water-soluble molecules such as sugar and proteins play an important role in plant growth and development. The total amount of water-soluble molecules in TKS in the three places is approximately 20 %. The content of water-soluble molecules in the roots of

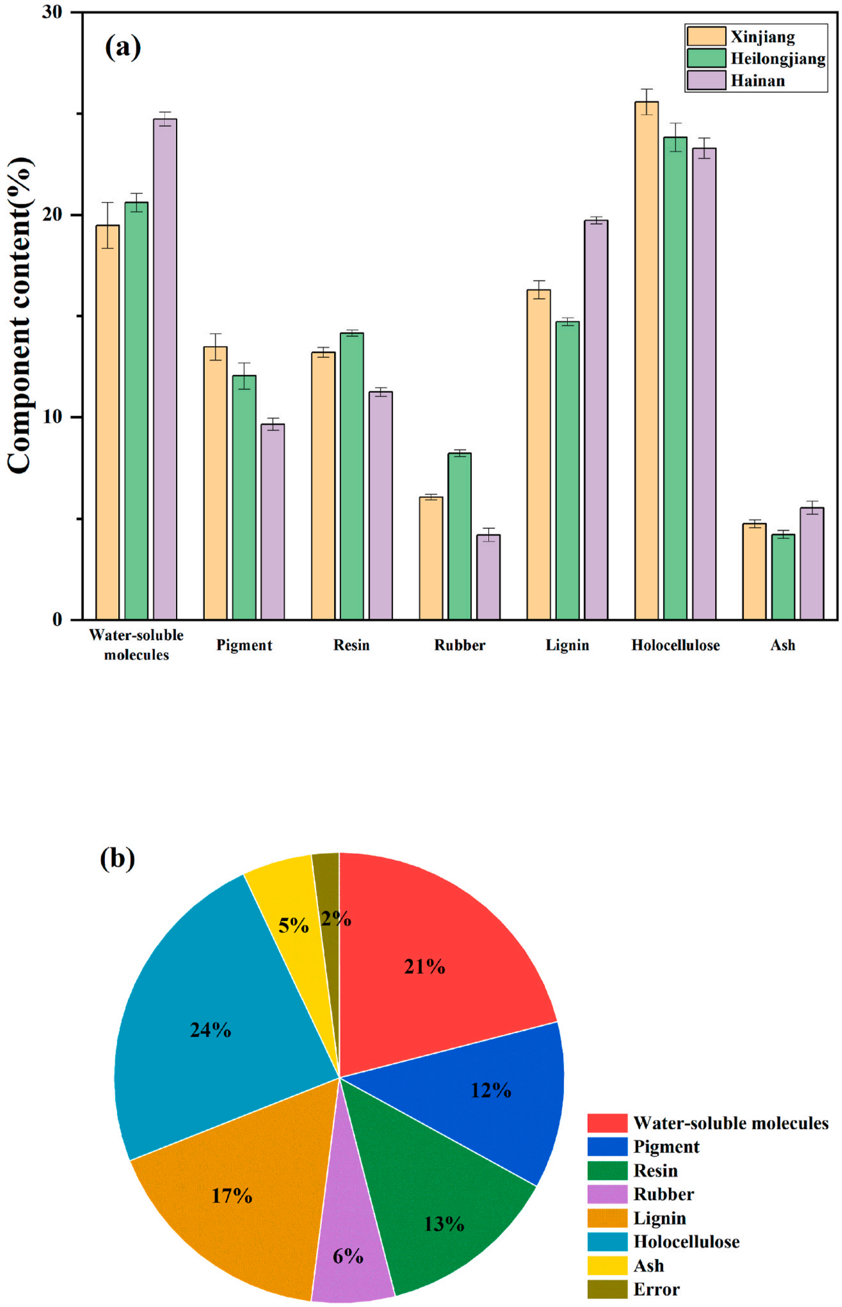

Fig. 4. (a) Contents of root components from different places of origin; (b) Proportion of each component in TKS root.

Hainan is significantly higher than that of the dry and cold north. This is because Hainan has a hot and humid climate and a faster metabolism. It may require more water-soluble molecules to participate in the regulation of plant life activities and respond to various environmental stress [40]. However, under frigid conditions, the TKS does not need so many water-soluble molecules to maintain the necessary metabolism, so part of the carbohydrate will be converted into resin and rubber to preserve energy or increase stress tolerance, such as resisting the cold climate. It seemed that the content of soluble substances of TKS was insensitive to the environmental humidity. The contents of soluble substances of TKS in Xinjiang and Heilongjiang provinces were very similar was good evidence. In short, the content of water-soluble molecules is less affected by longitude and more affected by latitude. Some resins and pigments are composed of terpenoids and their derivatives. The average total amount of resin and pigment in TKS in the three places is approximately 25 %, of which the content in Hainan is low and the content in the north is high. As an important part of the plant body, resins and pigments have special functions such as regulating physiological functions and defensing pests and diseases [41]. The synthesis of natural rubber in plants is a complex process. In some plants, rubber is thought to be mainly converted from sugar produced by plant photosynthesis [42,43] and beneficial to plant defense mechanisms [44,45]. The rubber content in the three places averages around 6 %. In a humid and hot environment, the content of water-soluble molecules is high, the metabolism of TKS is faster, and more sugar is consumed because it participates in breathing. It is found in guayule that rubber is easily produced under conditions of low temperature or large temperature difference [46]. This may also be the reason for the large rubber content of TKS in northern China.

In summary, the content of each component in the southern root is quite different from that in the northern part. Hainan is hot and humid, with the highest content of water-soluble molecules and the lowest content of rubber, resin, and pigment, which is the opposite of the northern two places. The average ash content of the three places is about 5 %, with a small gap. It can be speculated that the composition of the roots of the three places is similar when the treatment method and cleanliness process are close, all composed of trace metal ion oxides inside the root and impurities brought by the soil. The average value of the contents of all ingredients is shown in a pie chart, as shown in Fig. 4(b). The sum of the content of each component is about 98 %, and the error is only 2 %, which shows that the test results in this article are reasonable and the data are credible.

3.3. *Study on extraction process of TKS latex*

3.3.1. *TKS latex extraction process*

A new latex extraction process was established and optimized in this section. The main process is that the TKS fresh root slices are quickly transferred to the configured buffer solution, and the latex is extracted and filtered and the liquid is collected (Fig. 5.①). The collected liquid is centrifuged, and the upper milky liquid is TKS latex (Fig. 5.②). To the collected TKS latex was added glacial acetic acid and centrifuged to coagulate the rubber (Fig. 5.③). The coagulated rubber is dried to obtain a dry rubber (Fig. 5.④). The mixture was delaminated after centrifugation, and the upper milky liquid was latex. After extraction of the upper latex, the slurry became clear (Fig. 5.③). The latex is demulsified with acid and centrifuged. It was found that the latex coagulated into small pieces and floated on the upper layer of the serum.

Next, the factors that affect the efficiency of latex extraction will be optimized at room temperature. The latex extraction efficiency is used as an evaluation standard for the quality of the latex extraction process. The results will lay the foundation for the industrial extraction of TKS latex. These annual TKS root raw materials are all from Xinjiang, and stored at 4 °C for 24 h. It is necessary to explain that due to the individual differences of the plants, the rubber content of the roots varies greatly.

3.3.2. *Optimization of material-liquid ratio (M/L)*

The latex extraction process must be completed under the protection of a buffer solution. Without its protection, the latex is easily demulsification during the extraction process, which leads to the failure of the latex extraction. This group of experiments will explore and optimize the effect of the material-liquid ratio (root weight: buffer volume) on the latex extraction effect. The initial conditions are: Cs = 3, T = 20 min and RS = 100 rpm, and material-liquid ratio (M/L) was set as a variable. The experimental results are shown in Table 7. The extraction efficiency decreases with increasing buffer solution. The extraction efficiency of latex was the highest when the material-liquid ratio was 1: 2, reaching 61.72 %; the extraction efficiency was the lowest when the material-liquid ratio was 1:12 and the extraction efficiency was less than 40 %.

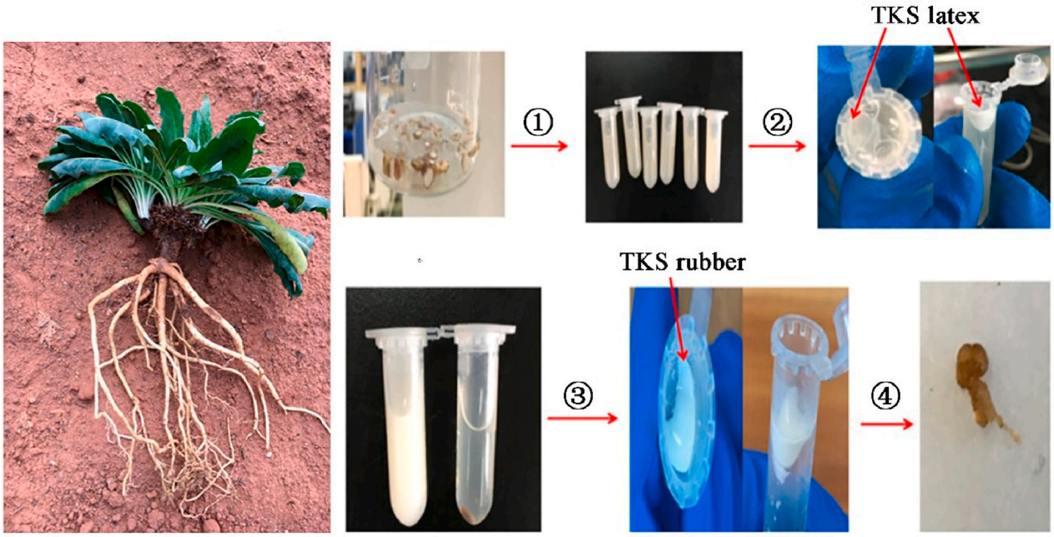

Fig. 5. Phenomenon of latex extraction experiment.

Table 7
TKS Latex extraction efficiency under different material-liquid ratio.

Material-liquid ratios/(g/mL)	Fresh roots weight/g	Moisture content/%	Rubber content/%	Theoretical value/mg	Actual value/mg	Extraction efficiency/%
1:2	4.11	70.83	2.0	23.98	14.8	61.72
1:3	3.51	72.37	1.6	15.52	9.1	58.63
1:4	4.19	71.58	2.1	25.00	14.4	57.57
1:8	3.31	73.18	2.5	22.20	10.3	46.28
1:12	2.02	73.69	3.1	16.47	6.1	37.03

3.3.3. Optimization of extraction cycles (Cs)

Rubber exists in two forms in the TKS root, namely in the form of rubber particles in latex and in the form of rubber filaments in root tissues. This group of experiments will optimize the extraction cycles based on the yield of rubber in the latex. The experiment results of the previous group show that the optimal material-liquid ratio is 1: 2. The initial conditions are: T = 20 min and RS = 100 rpm. In the case of other experimental conditions unchanged, the extraction cycles (Cs) under the three material-liquid ratios (1: 4; 1: 8; 1: 12) was set as a variable. The extracted TKS latex is dried to obtain a dry rubber and weighed. The weight of the dried rubber obtained each time is recorded. As the extraction cycles increase, the yield of rubber in the latex also increases. The experimental result is shown in Table 8. The rubber obtained in the first extraction process is the largest, accounting for about 65 % of the total mass that can be obtained four cycles. After two extractions, the yield of extracted rubber is about 90 %. When extracted three cycles, the yield of rubber reached about 96 %. After 4 extractions, increasing the number of extractions will not significantly increase the yield of rubber. Similar results have been obtained in the study of the other two material-liquid ratios (1: 8; 1: 12). In the experiments of the three material-liquid ratios, the mass of rubber obtained in the fourth extraction did not exceed 0.5 mg. This shows that the change of the material-liquid ratio will significantly affect the yield of rubber. So, continuing to increase the number of extractions has no substantial meaning for latex extraction, but it will increase the extraction cost. In summary, when this optimization is applied to industrialization, the number of latex extractions is recommended to be set to Cs = 3.

3.3.4. Optimization of actual extraction time (T)

The extraction time has a significant impact on the extraction efficiency and productivity of latex. This group of experiments uses the best parameters of the above process conditions. The initial conditions are: M/L = 1: 2 (g/mL), Cs = 3, and RS = 100 rpm, the extraction time (T) was set as a variable. The latex extraction effects of different extraction times are compared to obtain the optimal extraction time, and the final experimental results are arranged in Table 9. It can be seen from Table 9 that with the increase in extraction time, the extraction efficiency of latex will first increase and then tend to flat at 30min. When the extraction time is 30min, the extraction efficiency reaches the flat point and its value is 62.73 %. After 30min, the latex extraction efficiency stabilized at about 65 % and no longer increased. In industrial applications, the extraction time is recommended to be set to 30 min.

3.3.5. Optimization of rotation speed (RS)

The extraction rotation speed of the magnetic stirrer has a direct effect on the contact of the TKS root with the buffer solution. This group of experiments uses the best parameters of the above process conditions. The initial conditions are: M/L = 1: 2 (g/mL), Cs = 3, T = 30 min, and rotation speed (RS) was set as a variable. The experiments analyzed the effect of different extraction speeds on the effect of latex extraction. The experimental data are summarized in Table 10. It can be seen from Table 10 that the extraction efficiency of the latex first increases to reach the maximum value and then decreases with the rotation speed increase. When the rotation speed reaches 150 rpm, the extraction efficiency reaches a maximum of 67.71 %. In industrial applications, the optimal rotation speed is recommended to be set to 150 rpm.

3.3.6. Optimization of inhibiting factors (IF)

Polyphenol oxidase (PPO) is widely present in plants. It was proved by gel electrophoresis experiments that PPO does exist in TKS as well and the latex coagulation rate of wild plants is faster than plants with knockdown of PPO gene [47]. PPO is the main factor for latex coagulation, and it severely limits the efficiency of latex extraction. After researching and optimizing the above process conditions, it was found that the extraction efficiency of the latex obtained under the optimal process conditions was only about 65 %, which was at a relatively low level. This group of experiments explored and optimized the two factors that affect PPO activity, including temperature and inhibitor.

Table 8
Dry latex extraction under different extraction cycles.

Material-liquid ratios/(g/mL)	Dry rubber weight with different extraction cycles/mg			
	Cs = 1	Cs = 2	Cs = 3	Cs = 4
1:4	9.5	3.7	0.8	0.4
1:8	7.1	2.0	0.7	0.5
1:12	4.1	1.4	0.5	0.3

Table 9
Latex extraction data of TKS at different extraction time.

Extraction time/ min	Fresh roots weight/ g	Moisture content/ %	Rubber content/ %	Theoretical value/ mg	Actual value/ mg	Extraction efficiency/ %
10	5.47	74.16	5.12	72.37	26.70	36.89
20	4.88	74.16	5.12	64.56	35.80	55.45
30	6.72	72.37	5.46	101.38	63.60	62.73
40	6.36	72.37	5.46	95.95	62.70	65.35
50	5.98	72.11	4.59	76.55	49.50	64.66
60	6.13	72.11	4.59	78.47	51.40	65.50

Table 10
Latex extraction data of TKS at different extraction rotation speed.

Extraction rotation speed/ (rpm)	Fresh roots weight/g	Moisture content/ %	Rubber content/ %	Theoretical value/ mg	Actual value/ mg	Extraction efficiency/%
50	7.83	72.53	6.13	131.85	45.6	34.58
100	6.52	72.53	6.13	109.79	53.7	48.91
150	5.43	70.67	6.64	105.75	71.6	67.71
200	7.05	70.67	6.64	137.30	74.6	53.61
250	7.41	72.03	5.31	110.05	49.8	45.25

(a) Optimization of extraction temperature (ET)

This group of experiments explores the effect of different temperatures on the extraction of latex according to the previously determined optimal experimental conditions, namely: M/L = 1: 2 (g/mL), Cs = 3, T = 30 min and RS = 150 rpm, and the extraction temperature (ET) was set as a variable. The optimal extraction temperature can be obtained through this group of experiments. The experimental data are summarized in Table 11. It can be seen from Table 11 that the extraction efficiency of latex initially increases to reach the maximum value and then sharply decreases with the rise in temperature. When the temperature reaches 50 °C, the extraction efficiency of the latex reaches the maximum, which is 80.51 %. Continuing to increase the temperature, the extraction efficiency of latex dropped sharply; when the temperature was 80 °C, the extraction efficiency was only 20.56 %. In industrial applications, the optimal extraction temperature is recommended to be set to 50 °C.

When the temperature rose to 70 °C and 80 °C, the latex would demulsify and coagulate into a solid rubber, as shown in Fig. 6(a and b). The appearance of this phenomenon will affect the extraction efficiency of latex. When the temperature rose to 80 °C, the color of the rubber gradually deepened, from milky white to brown. This may be due to the discoloration caused by the oxidation of phenolic substances in the rubber [48].

(b) Optimization of inhibitor concentration (C)

Inhibitors can effectively delay the process of PPO to promote latex coagulation, which is very beneficial to latex extraction. The inhibitory effects of two inhibitors, sodium sulfite (Na_2SO_3) and dithiothreitol (DTT), will be explored and compared.

Based on the best experimental conditions previously determined, namely: M/L = 1: 2 (g/mL), Cs = 3, T = 30 min, RS = 150 rpm and ET = 50 °C. Na_2SO_3 buffers of different concentrations were configured to investigate the effect of Na_2SO_3 content on the extraction effect of latex. The experimental data are shown in Table 12. When Na_2SO_3 is not added, that is, the concentration of Na_2SO_3 is 0, the latex extraction efficiency is only 33.94 %. When the concentration of Na_2SO_3 is higher than 0.1 %, the extraction efficiency of latex is above 74 %, which is obviously improved. This indicates that the role of Na_2SO_3 in the extraction system is not only to make the system weakly alkaline and stabilize the latex, but also that its more important role is to inhibit the activity of PPO to indirectly promote the extraction of latex. In industrial applications, the optimal Na_2SO_3 concentration is recommended to be set to 0.1 %.

The results of the last group of experiments showed that changing the concentration of Na_2SO_3 could not significantly change the extraction effect of latex. In this group of experiments, Na_2SO_3 was replaced with DTT to continue the latex extraction experiment. The

Table 11
Latex extraction data of TKS at different extraction temperature.

Extraction temperature/ °C	Fresh roots weight/g	Moisture content/ %	Rubber content/ %	Theoretical value/ mg	Actual value/ mg	Extraction efficiency/ %
30	7.25	72.34	3.58	71.79	42.5	59.20
40	3.74	71.65	2.99	31.70	21.3	67.19
50	2.30	71.65	2.99	19.50	15.7	80.51
60	4.13	70.98	5.14	61.60	21.2	34.42
70	5.63	72.64	5.34	82.26	19.1	23.22
80	8.09	71.69	3.76	86.11	17.7	20.56

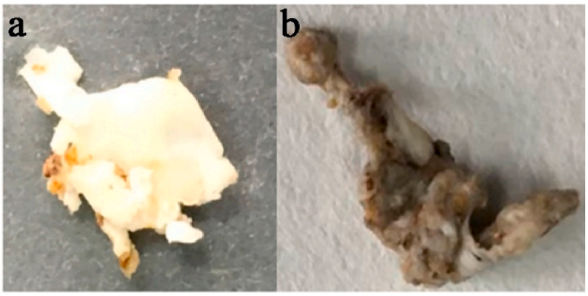

Fig. 6. Demulsification and coagulation phenomenon during latex extraction process at 70 °C(a) and 80 °C(b).

Table 12
Latex extraction data at different concentrations of Na_2SO_3.

Concentrations (v/v)/%	Fresh roots weight/g	Theoretical value/mg	Actual value/mg	Extraction efficiency/%
0	5.69	79.85	27.1	33.94
0.1	6.51	91.36	68.0	74.43
0.2	6.85	96.13	71.6	74.48
0.3	5.07	71.15	53.3	74.91
0.4	5.99	84.06	63.2	75.18
0.5	5.76	80.83	60.3	74.60
1.0	7.40	103.85	79.5	76.55

It is necessary to explain that in Section 3.3.6 (a) and (b), different results (80.51 % and 74.43 %) were obtained under two identical experimental conditions [ET = 50 °C and C(Na_2SO_3) = 0.1 %]. This is because the experimental process is cumbersome, and it is normal to have an error (only 3.04 %), which also proves that the calculation result is more accurate.

process conditions of the experiment are the same as before. The results are shown in Table 13. Similarly, the latex extraction efficiency is only 33.94 %, when the concentration of DTT is 0. When the concentration of DTT is 0.1 %, the extraction efficiency of latex reaches 60.97 %, which proves that DTT can promote the extraction of latex. With the increase in DTT concentration, the latex extraction efficiency increased slowly. Until the DTT concentration was 1.0 %, the latex extraction efficiency reached 72.97 %. This shows that DTT has a limited ability to improve the extraction efficiency of latex. The extraction efficiency of Na_2SO_3 at all measured concentrations is higher than DTT. For cost considerations, Na_2SO_3 is more suitable for industrial applications than DTT.

3.3.7. Discussion on influencing factors optimization

The influence trend and optimization conditions of each process parameter are shown in Fig. 7(a ~ f). It can be seen from Fig. 7(a), when the amount of buffer solution increased, the extraction efficiency showed a downward trend. The reason may be that the increase in the amount of buffer solution causes the latex concentration to become very low, which makes it difficult to recover the latex after centrifugation, and as the moisture content increases, more impurities such as sugar are dissolved out. These all have some influence on the extraction of latex. When this optimization is applied to industrialization, the condition of the material-liquid ratio of 1: 2 simultaneously leads to an increase in latex extraction efficiency and a decrease in cost. As shown in Fig. 7(b), when the number of extractions increases, the upward trend of the rubber yields slows down. This is because as the number of extractions increases, the latex concentration decreases, making the extraction more difficult. Each extraction will use the newly configured buffer solution, so a reasonable number of extractions will reduce the cost of the extraction process. Under the condition of the same number of extractions, the increase in the amount of buffer decreases the rubber yields. This is also consistent with the above analysis of Fig. 7(a). As shown in Fig. 7(c), when the extraction time increases, the upward trend of extraction efficiency slows down. The reason may be that when the extraction time is short, the insufficient interaction time between the extraction buffer solution and the root cuts results in inefficient extraction of the latex. The reason that the latex extraction efficiency does not continue to increase may be that the effect between the buffer solution and the root is enough, so there will be no further improvement. As shown in Fig. 7(d), when the extraction rotation

Table 13
Latex extraction data at different concentrations of DTT.

Concentrations (v/v)/%	Fresh roots weight/g	Theoretical value/mg	Actual value/mg	Extraction efficiency/%
0	5.69	79.85	27.1	33.94
0.1	6.54	92.49	56.4	60.97
0.2	5.94	84.00	54.9	65.36
0.3	6.14	89.55	59.8	66.78
0.4	6.71	97.72	67.2	68.76
0.5	5.84	93.61	65.3	70.29
1.0	6.03	96.65	70.5	72.94

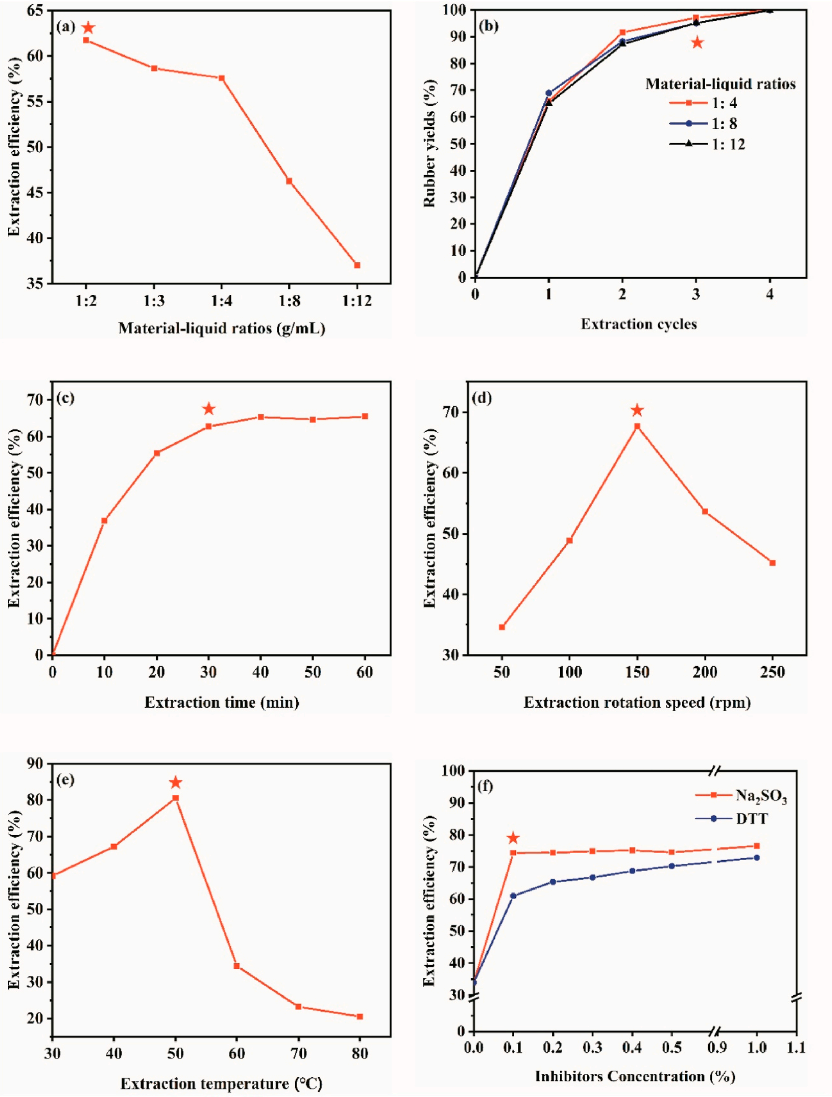

Fig. 7. Optimized parameters of "BETL" process (a) EF VS ML curve, (b) EF VS Cs curve, (c) EF VS T curve, (d) EF VS RS curve, (e) EF VS ET curve, (f) EF VS inhibitors concentration curve.

speed increases, the extraction efficiency decreases after the increase. The reason for the increase in latex extraction efficiency may be that the increase in the rotation speed makes the root slicing move faster and interact more fully with the buffer solution. When the rotation speed exceeds 150 rpm, more water-soluble molecules in the root dissolves and causes the root to become sticky. Studies on *Hevea brasiliensis* have found that high latex viscosity of the bark reduces latex flow [48]. Similarly, for TKS, an increase in the viscosity of the buffer solution during extraction is equivalent to an increase in the viscosity of the surface of the TKS root. In addition, due to the large rotational speed, the root will be thrown onto and attached to the wall of the beaker, which will prevent the root from participating in the entire extraction process. Both lead to a decrease in the extraction efficiency after the rotation speed increases. As shown in Fig. 7(e), when the extraction temperature increased, the extraction efficiency dropped sharply after rising. The rise is because increasing the temperature will inhibit the activity of PPO, thereby making the latex coagulate more slowly and facilitating the extraction of latex. And the decline is because this may be due to the dramatic thermal movement of the latex molecules as the temperature rises, which increases the possibility of colloidal particles colliding, resulting in demulsification of the latex. As shown in Fig. 7(f), the latex extraction efficiency of Na_2SO_3 as an inhibitor is higher than that of DTT, which shows that the promotion effect of Na_2SO_3 on latex extraction is better than DTT. For Na_2SO_3, when Na_2SO_3 concentration increased, the extraction efficiency showed a very slow rising trend. The reason may be that the inhibitory mechanism of Na_2SO_3 on PPO activity is short-term competitive and reversible inhibition, which is mainly manifested in the obvious inhibition of PPO activity in a short period of time; and when the reaction time is far longer than its inhibition time, it is caused by the reversible inhibition of the reaction. Its inhibitory effect on the activity of PPO decreases. This characteristic makes the effect of Na_2SO_3 with different concentrations on the reaction time much longer than its inhibition time on the enzymatic reaction is not obvious. This makes it impossible to increase the extraction efficiency of latex effectively by increasing the amount of Na_2SO_3. For DTT, when DTT concentration increased, the extraction efficiency also showed a slower rising trend compared with Na_2SO_3. This is because the inhibitory effect of DTT on PPO is mainly achieved by reducing the disulfide bonds in PPO, resulting in a change in the conformation of PPO and thereby reducing its activity. However, DTT is often unable to reduce the disulfide bonds embedded in the protein structure, which leads to certain restrictions on the denaturation effect of DTT on proteins. In the extraction process, adding Na_2SO_3 or DTT will increase the cost of recycling it. In the process of industrialized comprehensive development of TKS, the toxicity of Na_2SO_3 or DTT will adversely affect the quality of inulin in TKS.

3.3.8. Optimized route of TKS latex extraction process

A complete latex extraction process is established and drawn in Fig. 8, and named: “The Buffer Extraction TKS Latex Process, BETL process in brief”. In terms of the latex extraction process, *Hevea* latex flows directly from the cut bark. Therefore, *Hevea* latex extraction process mainly focuses on the relevant technology of tapping, which is quite different from the extraction process of other rubber-producing plants such as guayule and TKS. The extraction method of guayule and TKS latex in the literature adopts the blender

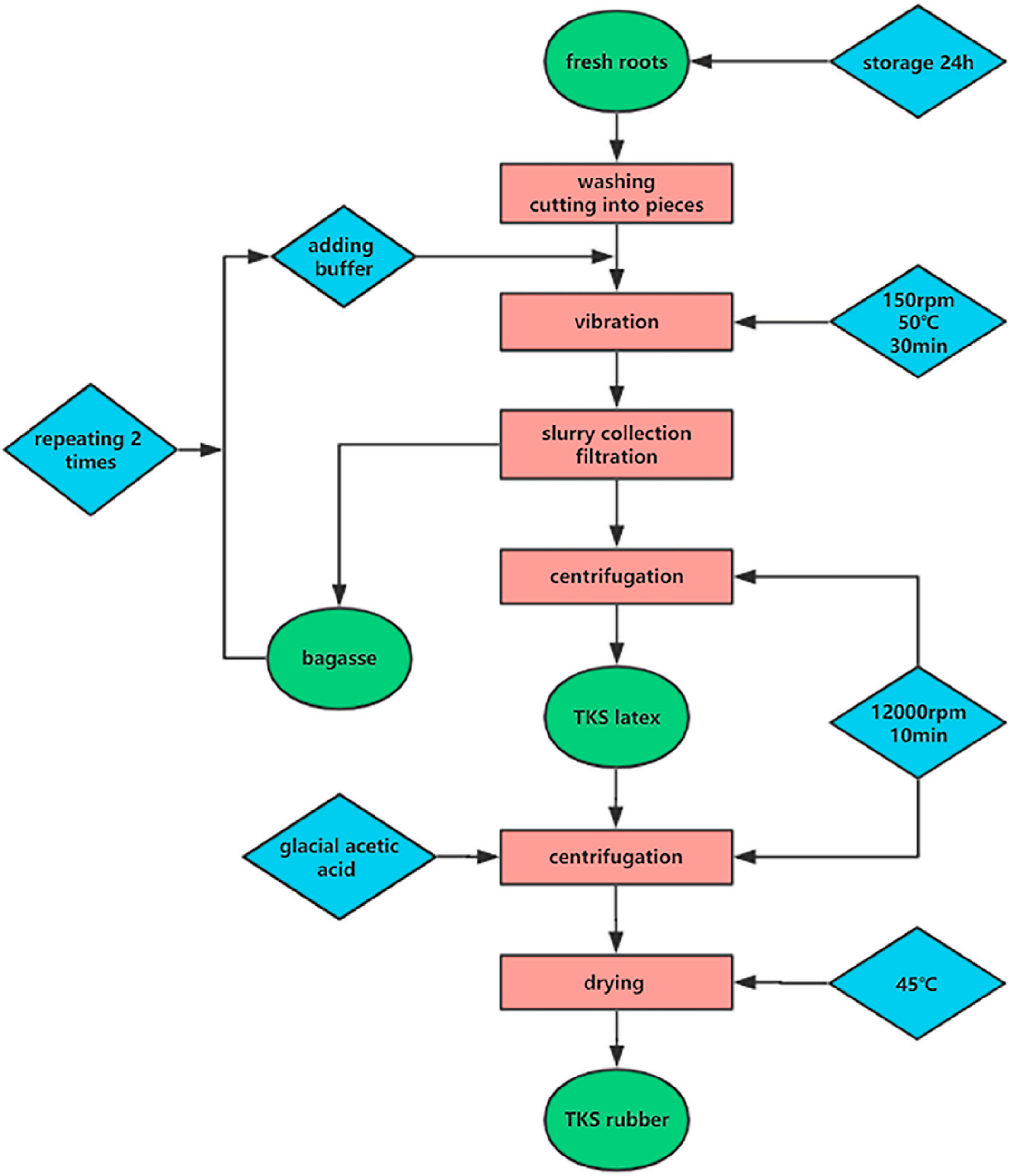

Fig. 8. Optimized “BETL” process route.

method and the flow method introduced above, and these methods only study a few influencing factors. The “BETL” process selected 6 factors that affect the extraction efficiency of latex and optimized it comprehensively, and improved the evaluation method of latex extraction efficiency, which is easier to reflect the effectiveness of the extraction method. On the storage conditions of TKS fresh root at 4 °C and 24 h, optimized process conditions of “BETL” were determined: M/L = 1: 2 (g/mL), Cs = 3, T = 30 min, RS = 150 rpm, ET = 50 °C and C = 0.1 %. Satisfying the above conditions can make the latex extraction efficiency reach about 80 %, which may promote the industrial development of TKS latex extraction.

3.4. *Characterization and comparison of NR latexes*

3.4.1. *Latex appearance and composition comparison*

The *Hevea* latex was compared with the TKS latex before and after centrifugation. It can be seen in Fig. 9(a ~ d) that there is basically no difference in appearance between TKS latex and *Hevea* latex, and both are milky white. After centrifugal separation, both the *Hevea* latex and TKS latex showed four layers with the same component. From top to bottom, the composition of the first layer is a rubber particle layer, which is the site of rubber bio-synthesis, and there are many enzymes and proteins related to the synthesis of rubber in plants on its surface [49]. The second layer is F–W particle layer, which contains lipid globules, isoprene compounds and carotenoids responsible for their orange color [35,50]. In addition, it mainly contains polyphenol oxidase and carotenoids [51]. It also affects the coagulation of latex. The third layer is the clear C-serum layer, which mainly contains carbohydrates, proteins, and active parts produced by enzymatic hydrolysis or metabolism; The fourth layer is the solid yellow body layers, vacuole-like organelles, and mainly contain carotenoids and other substances [52].

3.4.2. *Latex concentration comparison*

This section analyzes the concentration of the TKS, and uses the total solid content to represent the latex concentration. Using the optimized “BETL” process to perform latex extraction on fresh TKS roots for latex concentration analysis and comparing with that of *Hevea* latex. The five TKS plants here are from Xinjiang Province and numbered 1 ~ 5. The experimental results are shown in Table 14.

The data obtained from this group of experiments show that the distribution range of TKS latex concentration is 54.54 % ~ 68.25 % and the average value is 60.73 %. TKS latex concentration is greatly affected by factors such as variety, place of origin and plant age. The literature reports that the concentration of fresh *Hevea* latex is 30 % ~ 45 % [53]. Therefore, the concentration of TKS latex extracted by the “BETL” process is higher than that of fresh *Hevea* latex. Generally, the concentration of *Hevea* latex concentrate is approximately 60 % [54]. At the same time, there is a difference in moisture content between *Hevea* latex and TKS latex. In this experiment, the moisture content of TKS latex is about 31.75 % ~ 45.46 %, which is similar to the water content of *Hevea* latex concentrate. After analysis, the concentration of TKS latex may be higher than that of *Hevea* latex, which makes TKS latex expected to be a powerful supplement for *Hevea* latex in the latex market.

3.4.3. *Latex protein content comparison*

BCA (Poly n-butyl cyanoacrylate) kit analysis is a commonly used method for quantitative protein detection. The protein content of a sample is usually calculated by its absorbance at 562 nm. Configure the standard protein solution according to the instructions of the BCA kit used, calculate the absorbance, and draw the standard protein content curve. The protein content in *Hevea* latex and TKS latex will be calculated according to this standard curve.

The results are calculated in Table 15. The average protein content of the fresh *Hevea* latex sample measured was 11.95 mg/mL, while the average protein content of the TKS latex sample was 13.51 mg/mL. From Table 13, the average concentration of TKS latex is about 60 %. The concentration of *Hevea* latex is 30 % ~ 45 %. After normalization correction, at the same rubber hydrocarbon concentration (60 %), the protein content of *Hevea* latex is 15.89 ~ 23.90 mg/mL. The protein content of TKS latex is significantly lower than that of Hevea latex. Lower protein content is conducive to the stability of latex. Compared with *Hevea* latex, TKS latex may have lower requirements for storage conditions.

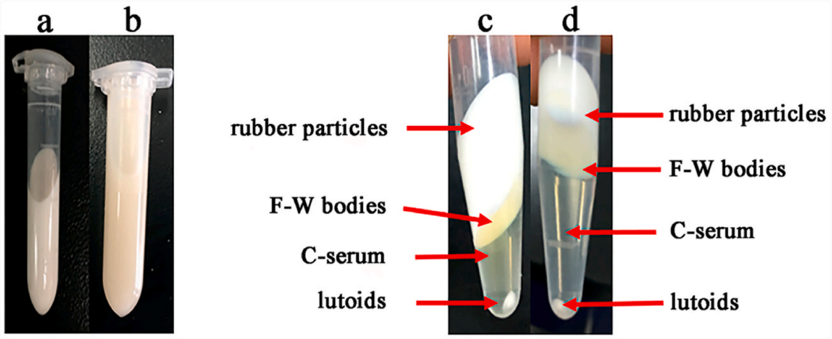

Fig. 9. Image of *Hevea* latex (a, c), TKS latex (b, d) before and after centrifugation.

Table 14
TKS latex concentration.

Number	Latex weight/mg (BETL process)	Total solid weight/mg	TKS Latex concentration/%
1	12.8	7.6	59.38
2	12.6	8.6	68.25
3	15.4	9.4	61.03
4	16.7	10.1	60.45
5	14.3	7.8	54.54
	Average		60.73

Table 15
Protein content of *Hevea* latex and TKS latex.

Sample	Average value (mg/mL)	Standard deviation (n = 3)
Hevea latex	11.95	0.58
TKS latex	13.51	0.94

3.4.4. *Thermogravimetric analysis*

In order to determine the ash content of latex, the extracted TKS latex, the collected TKS latex (latex collected directly from the roots of fresh TKS) and *Hevea* latex were dried at 75 °C and analyzed with a thermogravimetric analyzer. It can be seen from Fig. 10(a ~ c) that different dried latex samples basically start to decompose at about 270 °C. When the temperature is about 380 °C, the sample decomposition rate reaches the fastest. When the temperature reaches about 500 °C, the thermal decomposition of the sample ends. The decomposition process of TKS rubber is completely consistent with *Hevea* rubber. This also shows that the rubber contained in TKS is very similar to *Hevea* rubber. It can be seen in the figure that the three samples are composed of two components, most of which are organic phases such as rubber components, and the remaining are non-volatile ash. The rubber content in the extracted TKS latex sample is 93.20 % and the ash content is as high as 6.8 %. The rubber component content in the collected TKS latex sample exceeds 96 %, and the ash content is less than 4 %. The rubber content in the *Hevea* latex sample exceeds 98 % and the ash content is extremely low. For the extracted and collected TKS latex, the ash content was significantly greater than the *Hevea* latex. This may be caused by different latex extraction processes. *Hevea* latex and collected TKS latex are obtained by directly cutting the bark, and the fewer steps it undergoes make it less likely to be contaminated by impurities. The extracted TKS latex is obtained through the “BETL” process, after multiple steps, the introduction of impurities is unavoidable, such as the unwashed mud attached to the surface, the inorganic salts inside the root and the Na_2SO_3 in the buffer solution. Therefore, there are more impurities in the extracted TKS latex, and it can be purified to some extent during the extraction process.

3.4.5. *Characterization and comparison of rubber particle size*

The particle size of *Hevea* latex particles and TKS latex particles were analyzed using a laser particle size analyzer, LPSA. It can be seen from Fig. 11 that the rubber particles size of both are bimodal distributions, indicating that the rubber particles in the two latexes are not uniform in size. Fig. 11(a) shows that the proportion of small-sized particles in *Hevea* latex particles (45.5 %) is similarly equivalent to that of large-sized particles (54.5 %). Fig. 11(b) shows that the proportion of small-sized particles (32.5 %) in TKS latex is significantly lower than that of large-sized particles (67.5 %).

As can be seen from Table 16, the average size of large latex particles in *Hevea* latex is 983.4 nm, the average size of small latex particles is 200.1 nm; the average size of larger particles in TKS latex is 566.9 nm, and the average size of small latex particles is 139.8 nm. The average size of latex particles in TKS latex is smaller than in *Hevea* latex. This result is limited by the variety of the sample and other factors, and further determination is needed.

It can be seen from Fig. 12 that the surface morphology of the *Hevea* latex particles and the TKS latex particles are similar, and the sizes are not uniform. The core-shell structure of the latex particles can be clearly seen. The latex particles are wrapped by a “shell” with a thickness of 20 nm. The “shell” is made of proteins and phospholipids inserted from the periphery [55]. The size of *Hevea* latex particles is 1 ~ 2 μm, while the size range of TKS latex particles is between 0.5 and 2 μm, this observation corresponds to the analysis of latex particle size.

3.4.6. *Comparative analysis of molecular structure*

The FTIR spectra of *Hevea* latex, extracted TKS latex in the “BETL” process and collected TKS latex are shown in Fig. 13. It is found that the FTIR spectra of the three latexes all show a similar characteristic band. Among them, 835cm-1 is the characteristic vibration peak of *Hevea* rubber skeleton stretching. 1380 cm^{-1} is the deformation vibration peak of $–CH_3$ group. 1450 cm^{-1} is the bending vibration peak of $–CH_2$. 1663 cm^{-1} is the stretching vibration peak of C=C double bond, all of which show 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 three latexes are basically the same and the extracted product is indeed a TKS latex.

Hevea rubber contains non-isoprene components such as proteins and lipids. The main absorption peaks of the infrared spectrum of protein in *Hevea* rubber are at 3280 cm^{-1}, 1630 cm^{-1}, and 1540 cm^{-1}, which are the vibrational absorption of N–H bonds, amides I and II, respectively [56,57]. A shift of the amine absorption band was observed in the three latexes around 3334 cm^{-1}, which coincided

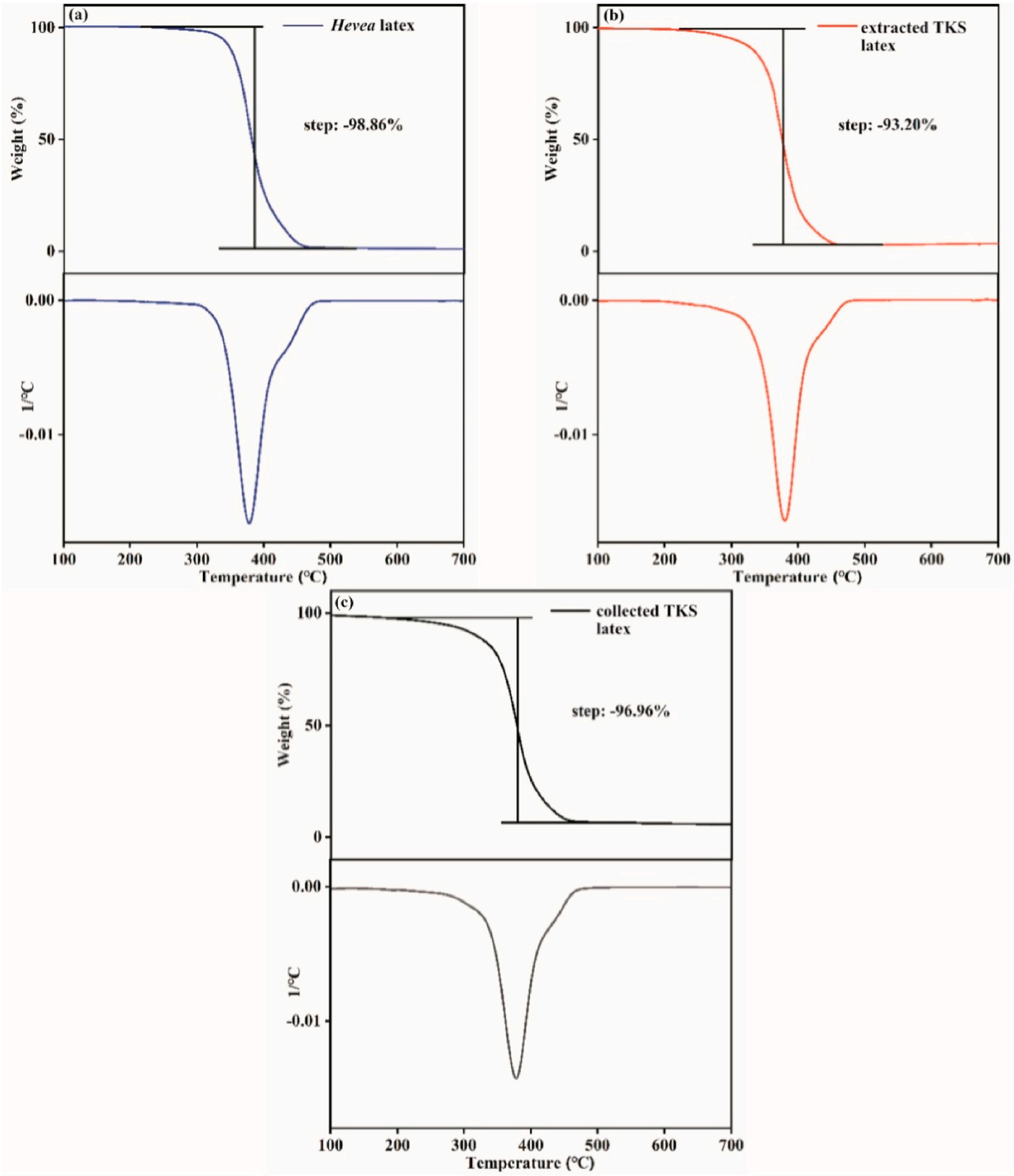

Fig. 10. TGA and DTG curve of *Hevea* latex (a), extracted TKS latex (b) and collected TKS latex (c).

with the stretching vibration peak of –OH in water molecules. The amide I peak in the three latexes shifted to around 1632 cm^{-1} (data not shown), which is close to the C=C stretching vibration absorption peak in latex. The amide II band at 1541 cm^{-1} is more obvious in the extracted TKS latex than in the collected TKS latex and *Hevea* latex, due to the presence of casein in the extraction buffer. In the lipid absorption region of *Hevea* rubber, the 1711 cm^{-1} band is associated with carboxyl groups; the second carbonyl stretching band between 1748 cm^{-1} and 1738 cm^{-1} is related to ester groups [58]. In *Hevea* latex and collected TKS latex, these two bands are more obvious. However, in the extracted TKS latex, the second carbonyl stretch band was not detected. This may be because the ester group was removed in the "BETL" process. In summary, the extraction process changes the composition of non-rubber components in latex to a certain extent.

NMR spectra of extracted TKS latex, *Hevea* latex and collected TKS latex are shown in Fig. 14. As with *Hevea* latex, the cis $-CH_3$ peak and $-CH_2$ peak in the two TKS latexes successively appear at the chemical shifts of 1.75 ppm and 2.05 ppm. Whereas the peak of extracted TKS latex is higher than that of *Hevea* latex and collected TKS latex, this shows that the content of rubber hydrocarbons in the extracted TKS latex is larger than the rest. This is consistent with the concentration of the extracted TKS latex of about 60 % and the concentration of *Hevea* latex of 30 ~ 45 %. Since the high molecular weight rubber hydrocarbons are difficult to flow out by themselves,

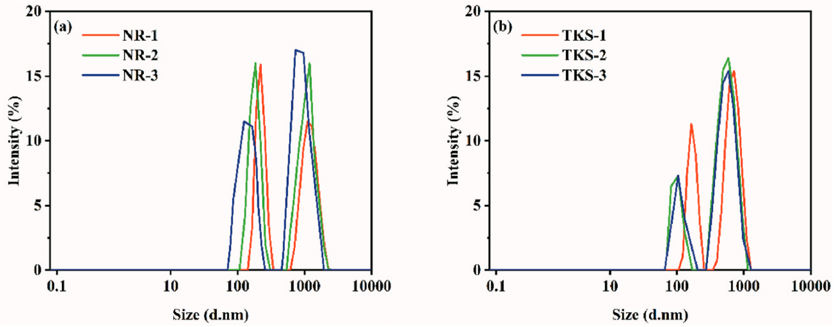

Fig. 11. Particle size analysis of *Hevea* latex particles (a) and TKS latex particles (b).

Table 16

Average size of *Hevea* latex particles and TKS latex particles.

Hevea latex			TKS latex		
Number	Average size of Peak1 (nm)	Average size of Peak2 (nm)	Number	Average size of Peak1 (nm)	Average size of Peak2 (nm)
1	1220.0	221.5	4	705.3	170.1
2	949.7	200.2	5	496.6	119.6
3	780.5	178.5	6	498.8	129.8
Average value	983.4	200.1	Average value	566.9	139.8

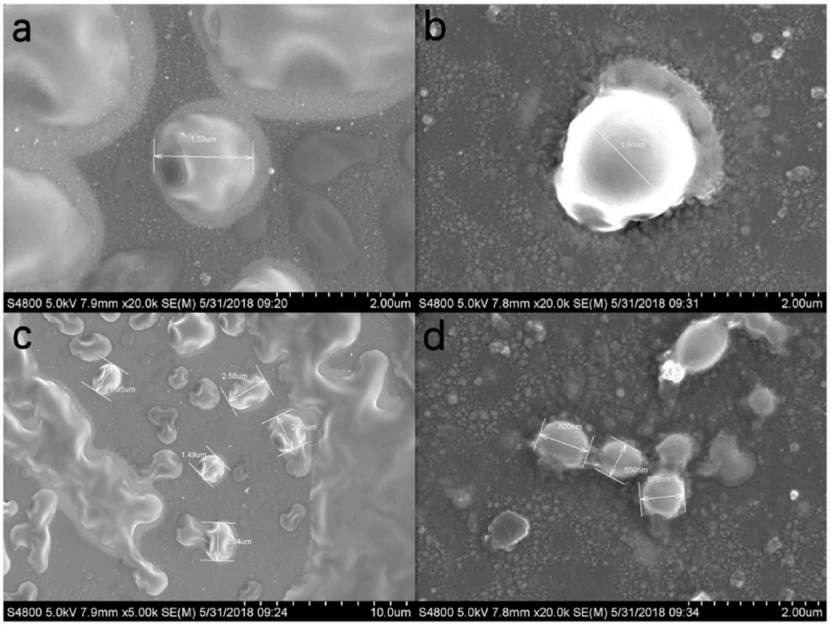

Fig. 12. SEM images of *Hevea* latex particles (a, c) and TKS latex particles (b, d).

the content of rubber hydrocarbons in the collected TKS latex is also relatively low. The –CH peaks appear at 5.15 ppm; and 1.63 ppm very weak peak of methyl proton hydrogen of *trans*-isoprene appeared; which indicates that the basic structure of the TKS latex is consistent with the *Hevea* latex, and its molecular chain structure contains not only CPI structural units, but also a small number of *trans*-1,4-polyisoprene (TPI) structural units. Since the molecular structure of *Hevea* rubber contains only one –CH group, its relative content can characterize the amount of rubber hydrocarbons in the sample. Integrating the peaks in the figure, the area of the –CH peak in the extracted TKS latex is larger than the other two samples. It can be considered that the rubber hydrocarbon content in the extracted TKS latex is higher than that of the *Hevea* latex.

Compared with extracted TKS latex, the peak shape of the collected TKS latex is relatively disordered, because there are many types of non-rubber components, which is consistent with the peak shape of the Infrared spectrum of the latex collection. This is because the

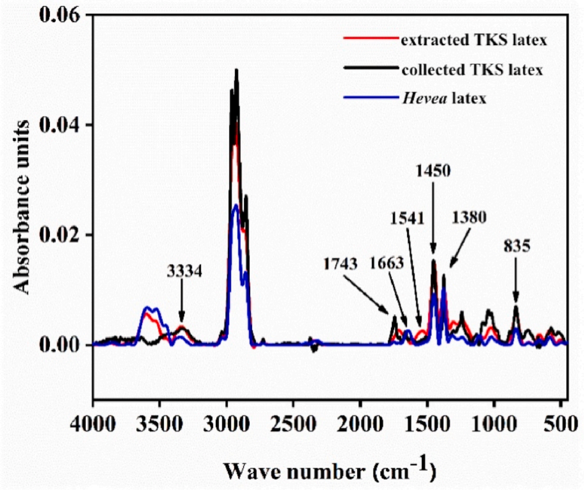

Fig. 13. Infrared spectrum of collected TKS latex, extracted TKS latex, *Hevea* latex.

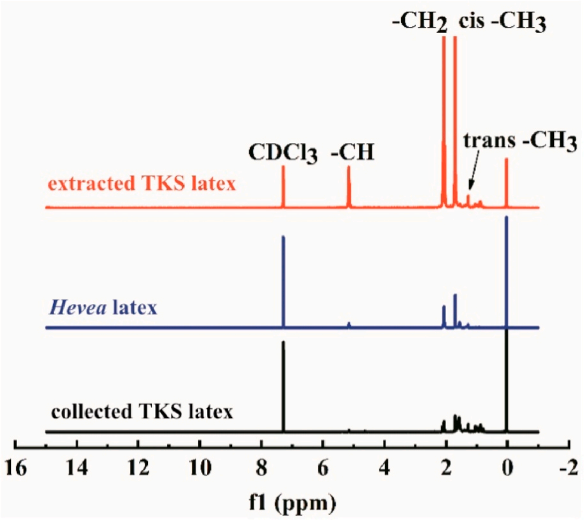

Fig. 14. ^{1}H spectra of extracted TKS latex, *Hevea* latex, collected TKS latex tested by NMR.

collected TKS latex flows directly from the washed TKS fresh roots, which will include lesser rubber hydrocarbon and more non-rubber components, such as protein and resin. However, extracted TKS latex has been purified by the "BETL" process, which will contain more rubber hydrocarbon and lesser non-rubber components. Therefore, there are many noise peaks on the spectrum of the collected TKS latex. In addition, it can be seen from the FTIR and NMR analysis that the extraction process will basically not damage the molecular structure of the latex, which also shows that various optimization processes and influencing factors in the extraction process will not negatively affect the performance of the TKS latex.

Table 17
Molecular weight of extracted TKS latex, collected TKS latex, *Hevea* latex.

	Peak	Number-average molecular weight (Mn)	Weight-average Molecular Weight (Mw)	Molecular Weight Distribution Index (Mw/Mn)
Extracted TKS latex	Peak1	1,885,285	2,270,186	1.204161
	Peak2	201,505	216,977	1.076782
Collected TKS latex	Peak1	1,379,283	1,919,729	1.391831
	Peak2	198,296	227,613	1.147845
Hevea latex	Peak1	1,943,121	2,271,958	1.169231
	Peak2	184,345	196,572	1.066327

3.4.7. Comparative analysis of molecular weight and molecular weight distribution

It can be seen from Table 17 that the GPC test results of the three samples are all bimodal molecular weights. The molecular weight of the *Hevea* latex is very close to the molecular weight of the extracted TKS latex, while the molecular weight of the collected TKS latex is less than both. This is because the latex with a large molecular weight is not easy to flow, and only a portion of the latex with a low molecular weight can be collected. GPC analysis shows that the latex extraction process does not cause the molecular weight of the TKS latex to decrease, which can ensure that the extraction process does not damage the latex structure.

It can be clearly seen from Fig. 15 that the three latexes have a bimodal distribution. The two molecular weight distributions of *Hevea* latex and extracted TKS latex are narrow and the width of the peaks are similar, which shows that the molecular characteristics of extracted TKS latex are relatively close to *Hevea* latex. The curve shape of collected TKS latex is more continuous, which shows that the molecular weight distribution of collected TKS latex is relatively wide and the latex with high molecular weight is difficult to flow out.

4. Conclusions

A series of problems in the harvesting process of fresh TKS were explored, which may play a guiding role in the cultivation and production of TKS. Within the limited scope of testing in the laboratory, some conclusions were drawn. Freshly harvested TKS roots in three provinces of China were determined for composition and the climatic factors that caused the differences in the contents of these components were also analyzed. This provides experimental data for future planting conditions and germplasm resource development. A new green latex extraction process (“BETL” process) is established and optimized, which makes the latex extraction efficiency about 80 % and enriches the extraction method of TKS rubber. This laid the foundation for the idea of integrated industrial production of TKS latex planting-harvesting-processing. The performance of TKS latex was characterized and the results show that TKS latex is very similar to *Hevea* latex. Moreover, the protein content of TKS latex is less, and it may be more advantageous than *Hevea* latex in making medical gloves. This article mainly studies the optimization process of latex, but does not involve the extraction of by-products such as inulin. In the future, the industrial development of TKS should take the path of comprehensive development to obtain maximum economic benefits.

Author contributions

Jichuan Zhang: Methodology, Conceptualization. Xiang Jie: Methodology, Conceptualization. Dongli Ma: Validation, Data curation. Xuefa Tian: Visualization, Resources, Project administration. Dongren Han: Validation, Supervision, Project administration, Investigation. Yunhan Chen: Supervision, Methodology, Investigation, Data curation. Shiqi Liu: Writing – review & editing, Writing – original draft, Investigation, Formal analysis

Question	Response
Data Availability	
Sharing research data helps other researchers evaluate your findings, build on your work and to increase trust in your article. We encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data	No

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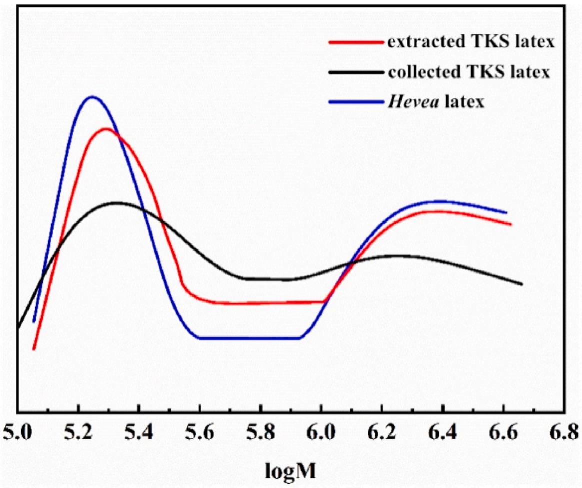

Fig. 15. Molecular weight distribution curve of extracted TKS latex, collected TKS latex and *Hevea* latex.

(*continued*)

Question	Response
Data Availability	
available will be available alongside your article upon publication. Has data associated with your study been deposited into a publicly available repository?	
Please select why. Please note that this statement will be available alongside your article upon publication. as follow-up to "**Data Availability** Sharing research data helps other researchers evaluate your findings, build on your work and to increase trust in your article. We encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data available will be available alongside your article upon publication. Has data associated with your study been deposited into a publicly available repository?"	Data will be made available on request

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] J.L. Bell, I.C. Burke, M.M. Neff, Genetic and biochemical evaluation of natural rubber from eastern Washington prickly lettuce (lactuca serriola L.), J. Agric. Food Chem. 63 (2015) 593–602, https://doi.org/10.1021/jf503934v.

[2] J.E. Puskas, K. Chiang, B. Barkakaty, 2 - natural rubber (NR) biosynthesis: perspectives from polymer chemistry, in: S. Kohjiya, Y. Ikeda (Eds.), Chem. Manuf. Appl. Nat. Rubber, Woodhead Publishing, 2014, pp. 30–67, https://doi.org/10.1533/9780857096913.1.30.

[3] A. Ahrends, P.M. Hollingsworth, A.D. Ziegler, J.M. Fox, H. Chen, Y. Su, J. Xu, Current trends of rubber plantation expansion may threaten biodiversity and livelihoods, Global Environ. Change 34 (2015) 48–58, https://doi.org/10.1016/j.gloenvcha.2015.06.002.

[4] D.A. Ramirez-Cadavid, K. Cornish, U. Hathwaik, R. Kozak, C. McMahan, F.C. Michel Jr., Development of novel processes for the aqueous extraction of natural rubber from Taraxacum kok-saghyz (TK), J. Chem. Technol. Biotechnol. 94 (2019) 2452–2464, https://doi.org/10.1002/jctb.6027.

[5] D.A. Ramirez-Cadavid, K. Cornish, F.C. Michel, Taraxacum kok-saghyz (TK): compositional analysis of a feedstock for natural rubber and other bioproducts, Ind. Crops Prod. 107 (2017) 624–640, https://doi.org/10.1016/j.indcrop.2017.05.043.

[6] G. Krotkov, A review of literature on Taraxacum koksaghyz Rod, Bot. Rev. 11 (1945) 417–461, https://doi.org/10.1007/BF02861139.

[7] A.U. Buranov, B.J. Elmuradov, Extraction and characterization of latex and natural rubber from rubber-bearing plants, J. Agric. Food Chem. 58 (2010) 734–743, https://doi.org/10.1021/jf903096z.

[8] K. Cornish, S.L. Kopicky, S.K. Mcnulty, N. Amstutz, A.M. Chanon, S. Walker, M.D. Kleinhenz, A.R. Miller, J.G. Streeter, Temporal diversity of Taraxacum kok-saghyz plants reveals high rubber yield phenotypes, Biodiversitas J. Biol. Divers. 17 (2016), https://doi.org/10.13057/biodiv/d170262.

[9] S. Musto, V. Barbera, M. Maggio, M. Mauro, G. Guerra, M. Galimberti, Crystallinity and crystalline phase orientation of poly(1,4-cis-isoprene) from Hevea brasiliensis and Taraxacum kok-saghyz, Polym. Adv. Technol. 27 (2016) 1082–1090, https://doi.org/10.1002/pat.3774.

[10] Y. Ikeda, P. Junkong, T. Ohashi, T. Phakkeeree, Y. Sakaki, A. Tohsan, S. Kohjiya, K. Cornish, Strain-induced crystallization behaviour of natural rubbers from guayule and rubber dandelion revealed by simultaneous time-resolved WAXD/tensile measurements: indispensable function for sustainable resources, RSC Adv. 6 (2016) 95601–95610, https://doi.org/10.1039/C6RA22455E.

[11] R.G. Kekwick, Latex and laticifers, in: eLS, first ed., John Wiley & Sons, Ltd, Wiley, 2002 https://doi.org/10.1038/npg.els.0000913.

[12] G.E. Hamerstrand, R.R. Montgomery, Pilot-scale guayule processing using countercurrent solvent extraction equipment, Rubber Chem. Technol. 57 (1984) 344–350, https://doi.org/10.5254/1.3536013.

[13] P. Stamberger, K. Eskew Roderick, H. Roy, S, Treatment of Rubber, 1946. US2399156A, https://patents.google.com/patent/US2399156A/en?oq=US2399156.

[14] R.K. Eskew, P.W. Edwards, Process for Recovering Rubber from Fleshy Plants, 1946 US2393035A. https://patents.google.com/patent/US2393035A/en?oq=US2393035.

[15] A.U. Buranov, Process for Recovering Rubber from Rubber-Bearing Plants with a Gristmill, US7540438B2, 2009. https://patents.google.com/patent/US7540438B2/en?oq=US7540438.

[16] S. Sikandar, V.C. Ujor, T.C. Ezeji, J.L. Rossington, F.C. Michel, C.M. McMahan, N. Ali, K. Cornish, 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. 103 (2017) 161–168, https://doi.org/10.1016/j.indcrop.2017.03.044.

[17] J. Ramsauer, P. Polzer, W. Kunz, Isolation and investigation of natural rubber latex from Taraxacum kok-saghyz with a high solid content, ACS Agric. Sci. Technol. 2 (2022) 296–301, https://doi.org/10.1021/acsagscitech.1c00206.

[18] J. Ramsauer, Isolation and Characterization of Latex from Russian Dandelion, Phd, 2022. https://epub.uni-regensburg.de/51840/.

[19] K. Cornish, M.H. Chapman, F.S. Nakayama, S.H. Vinyard, L.C. Whitehand, Latex quantification in guayule shrub and homogenate, Ind. Crops Prod. 10 (1999) 121–136, https://doi.org/10.1016/S0926-6690(99)00016-3.

[20] A.M. Ignat'ev, Vozmozhnosti polucheniia natural'nogo lateksa iz kauchukonosov [On the feasibility of obtaining natural latex from the rubber-bearing plants], Sov Kauchuk3. (1935) 22–26.

[21] N.I. Vasil'ev, Mekhanizatsiia rezaniia kornei kok-sagyza i ikh fisikomekhanicheskiia svoistva [Mechanization of the process of cutting kok-saghyz roots, and also their physical and mechanical characteristics], Kauchuk Rezina 7 (1940) 44–48.

[22] A.M. Ignat'ev, Poluchenie lateksa iz sagyzov [Extraction of latex from various saghyzes], Zhurnal Rezin. Promyshlennosti. 10 (1936) 1070–1078.

[23] A.M. Ignat'ev, Khranenie kornei kok-sagyza i poluchenie lateksa [Storage of kok-saghyz and extraction of latex], Kauchuk Rezina 1 (1940) 30–33.

[24] Fibrous Raw Material—Determination of Acid-Insoluble Lignin, GB/T 2677.8-1994, 1994. https://openstd.samr.gov.cn/bzgk/gb/newGbInfo?hcno=B78B4C8134B235B183BA7FB345DE2F80.

[25] Fibrous Raw Material—Determination of Holocellulose, GB/T 2677.10-1995, 1995. https://openstd.samr.gov.cn/bzgk/gb/newGbInfo?hcno=9B149AEEF1AC462943FA453E2B931C61.

[26] H. Huang, B. Wei, D. Zhang, Y. Gao, Development present situation and trend of rubber tapping equipment, Agric. Eng. 8 (2018) 16–20.

[27] G.M. Bates, S.K. McNulty, N.D. Amstutz, V.K. Pool, K. Cornish, Planting density and growth cycle affect actual and potential latex and rubber yields in Taraxacum kok-saghyz, Hortscience 54 (2019) 1338–1344, https://doi.org/10.21273/HORTSCI13986-19.

[28] J.B. van Beilen, Y. Poirier, Establishment of new crops for the production of natural rubber, Trends Biotechnol. 25 (2007) 522–529, https://doi.org/10.1016/j.tibtech.2007.08.009.

[29] M. Eggert, J. Schiemann, K. Thiele, Yield performance of Russian dandelion transplants (Taraxacum koksaghyz L. Rodin) in flat bed and ridge cultivation with different planting densities, Eur. J. Agron. 93 (2018) 126–134, https://doi.org/10.1016/j.eja.2017.12.003.

[30] M. Arias, J. Herrero, M. Ricobaraza, M. Hernández, E. Ritter, Evaluation of root biomass, rubber and inulincontents in nine Taraxacum koksaghyz Rodin populations, Ind. Crops Prod. 83 (2016) 316–321, https://doi.org/10.1016/j.indcrop.2016.01.023.

[31] T. Wang, H.-X. Gui, W.-F. Zhang, K.-X. Zhang, W.-Q. Yu, Y.-M. Li, R.-Z. Zeng, M.-F. Huang, Novel nonammonia preservative for concentrated natural rubber latex, J. Appl. Polym. Sci. 132 (2015), https://doi.org/10.1002/app.41749.

[32] K. Cornish, L.C. Whitehand, J.E.V. Fleet, J.L. Brichta, M.H. Chapman, B.E. Knuckles, Latex yield and quality during storage of guayule (Parthenium argentatum Gray) homogenates, Ind. Crops Prod. 22 (2005) 75–85, https://doi.org/10.1016/j.indcrop.2004.07.004.

[33] K. Cornish, M.H. Chapman, J.L. Brichta, S.H. Vinyard, F.S. Nakayama, Post-harvest stability of latex in different sizes of guayule branches, Ind. Crops Prod. 12 (2000) 25–32, https://doi.org/10.1016/S0926-6690(99)00042-4.

[34] W.G. Barb, W. Mikucki, On the coagulation of polymer latices by freezing and thawing, J. Polym. Sci. 37 (1959) 499–514, https://doi.org/10.1002/pol.1959.1203713219.

[35] J.-L. Jacob, J. d'Auzac, J.-C. Prevôt, The composition of natural latex from Hevea brasiliensis, Clin. Rev. Allergy 11 (1993) 325–337, https://doi.org/10.1007/BF02914415.

[36] G. de Oliveira Reis, P. Menut, F. Bonfils, L. Vaysse, Y. Hemar, C. Sanchez, Acid-induced aggregation and gelation of natural rubber latex particles, Colloids Surf. A Physicochem. Eng. Asp. 482 (2015) 9–17, https://doi.org/10.1016/j.colsurfa.2015.04.015.

[37] S.C. Tripathi, K.D. Sayre, J.N. Kaul, R.S. Narang, Growth and morphology of spring wheat (Triticum aestivum L.) culms and their association with lodging: effects of genotypes, N levels and ethephon, Field Crops Res. 84 (2003) 271–290, https://doi.org/10.1016/S0378-4290(03)00095-9.

[38] M. Schuetz, A. Benske, R.A. Smith, Y. Watanabe, Y. Tobimatsu, J. Ralph, T. Demura, B. Ellis, A.L. Samuels, Laccases direct lignification in the discrete secondary cell wall domains of protoxylem, Plant Physiol. 166 (2014) 798–807, https://doi.org/10.1104/pp.114.245597.

[39] B. Monties, K. Fukushima, Occurrence, function and biosynthesis of lignins, in: Biopolym. Online, John Wiley & Sons, Ltd, 2005, https://doi.org/10.1002/3527600035.bpol1001.

[40] M. Rosa, C. Prado, G. Podazza, R. Interdonato, J.A. González, M. Hilal, F.E. Prado, Soluble sugars—metabolism, sensing and abiotic stress: a complex network in the life of plants, Plant Signal. Behav. 4 (2009) 388–393, https://doi.org/10.4161/psb.4.5.8294.

[41] J. Bohlmann, C.I. Keeling, Terpenoid biomaterials, Plant J. 54 (2008) 656–669, https://doi.org/10.1111/j.1365-313X.2008.03449.x.

[42] K.M. Kelly, J.V. Staden, A comparison of transport and metabolism of [14C] sucrose and [14C] fructose in summer and winter in guayule (parthenium argentatum gray), J. Plant Physiol. 141 (1993) 436–443, https://doi.org/10.1016/S0176-1617(11)80191-4.

[43] M.G. Gilliland, J. van Staden, J.J. Mitchell, CO2 fixation and rubber deposition in the bark and leaves of guayule during winter, J. Plant Physiol. 121 (1985) 369–379, https://doi.org/10.1016/S0176-1617(85)80030-4.

[44] K. Konno, Plant latex and other exudates as plant defense systems: roles of various defense chemicals and proteins contained therein, Phytochemistry 72 (2011) 1510–1530, https://doi.org/10.1016/j.phytochem.2011.02.016.

[45] A.A. Agrawal, K. Konno, Latex: a model for understanding mechanisms, ecology, and evolution of plant defense against herbivory, Annu. Rev. Ecol. Evol. Syst. 40 (2009) 311–331, https://doi.org/10.1146/annurev.ecolsys.110308.120307.

[46] M.E. Veatch-Blohm, D.T. Ray, A. Gehrels, Night temperature, rubber production, and carbon exchange in guayule, Ind. Crops Prod. 25 (2007) 34–43, https://doi.org/10.1016/j.indcrop.2006.06.019.

[47] D. Wahler, C.S. Gronover, C. Richter, F. Foucu, R.M. Twyman, B.M. Moerschbacher, R. Fischer, J. Muth, D. Prüfer, Polyphenoloxidase silencing affects latex coagulation in Taraxacum species, Plant Physiol. 151 (2009) 334–346, https://doi.org/10.1104/pp.109.138743.

[48] P. Montoro, S. Wu, B. Favreau, E. Herlinawati, C. Labrune, M.-L. Martin-Magniette, S. Pointet, M. Rio, J. Leclercq, S. Ismawanto, Kuswanhadi, Transcriptome analysis in Hevea brasiliensis latex revealed changes in hormone signalling pathways during ethephon stimulation and consequent Tapping Panel Dryness, Sci. Rep. 8 (2018) 8483, https://doi.org/10.1038/s41598-018-26854-y.

[49] C. Bottier, in: R. Nawrot (Ed.), Chapter Seven - Biochemical Composition of Hevea Brasiliensis Latex: A Focus on the Protein, Lipid, Carbohydrate and Mineral Contents, Adv. Bot. Res, Academic Press, 2020, pp. 201–237, https://doi.org/10.1016/bs.abr.2019.11.003.

[50] A. Frey-Wyssling, Microscopic investigations on the occurrence of resins in Hevea latex, Arch Rubbercult 13 (1929) 392–412.

[51] D. Wititsuwannakul, N. Chareonthiphakorn, M. Pace, R. Wititsuwannakul, Polyphenol oxidases from latex of Hevea brasiliensis: purification and characterization, Phytochemistry 61 (2002) 115–121, https://doi.org/10.1016/S0031-9422(02)00234-0.

[52] J. Dupont, F. Moreau, C. Lance, J.-L. Jacob, Phospholipid composition of the membrane of lutoids from Hevea brasiliensis latex, Phytochemistry 15 (1976) 1215–1217, https://doi.org/10.1016/0031-9422(76)85080-7.

[53] S. Wang, J. Liu, Y. Wu, Y. You, J. He, J. Zhang, L. Zhang, Y. Dong, Micromorphological characterization and label-free quantitation of small rubber particle protein in natural rubber latex, Anal. Biochem. 499 (2016) 34–42, https://doi.org/10.1016/j.ab.2016.01.015.

[54] G. Lu, H. Yu, Changes in specification standards of natural rubber latex concentrate and its quality control, Trop. Agric. Eng. 36 (2012) 24–27.

[55] K. Nawamawat, J.T. Sakdapipanich, C.C. Ho, Y. Ma, J. Song, J.G. Vancso, Surface nanostructure of Hevea brasiliensis natural rubber latex particles, Colloids Surf. A Physicochem. Eng. Asp. 390 (2011) 157–166, https://doi.org/10.1016/j.colsurfa.2011.09.021.

[56] F.J. Lu, S.L. Hsu, A vibrational spectroscopic analysis of the structure of natural rubber, Rubber Chem. Technol. 60 (1987) 647–658, https://doi.org/10.5254/1.3536148.

[57] A. Barth, Infrared spectroscopy of proteins, Biochim. Biophys. Acta BBA - Bioenerg. 1767 (2007) 1073–1101, https://doi.org/10.1016/j.bbabio.2007.06.004.

[58] S. Rolere, S. Liengprayoon, L. Vaysse, J. Sainte-Beuve, F. Bonfils, 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. 43 (2015) 83–93, https://doi.org/10.1016/j.polymertesting.2015.02.011.