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ARTICLE

Extraction and Characterization of Latex and Natural Rubber from Rubber-Bearing Plants

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Consecutive extraction of latex and natural rubber from the roots of rubber-bearing plants such as *Taraxacum kok-saghyz* (TKS), *Scorzonera tau-saghyz* (STS), and *Scorzonera Uzbekistanica* (SU) were carried out. Latex extraction was carried via two methods: Blender method and Flow method. The results of latex extraction were compared. Cultivated rubber-bearing plants contained slightly higher latex contents compared to those from wild fields. Several creaming agents for latex extraction were compared. About 50% of total natural rubber was extracted as latex. The results of the comparative studies indicated that optimum latex extraction can be achieved with Flow method. The purity of latex extracted by Blender method (~75%) was significantly lower than that extracted by Flow method (99.5%). When the latex particles were stabilized with casein, the latex was concentrated significantly. Through concentrating latex by flotation, the latex concentration of 35% was obtained. Bagasse contained mostly solid natural rubber. The remaining natural rubber in the bagasse (left after the latex extraction) was extracted using sequential solvent extraction first with acetone and then with several nonpolar solvents. Solid natural rubber was analyzed for gel content and characterized by size exclusion chromatography (SEC) for molecular weight determinations. SEC of solid natural rubber has shown that the molecular weight is about 1.8E6 and they contain less gel compared to TSR20 (Grade 20 Technically Specified Rubber), a commercial natural rubber from *Hevea brasiliensis*.

KEYWORDS: *Taraxacum kok-saghyz* (TKS); *Scorzonera tau-saghyz* (STS); *Scorzonera Uzbekistanica* (SU); natural rubber; latex; extraction; size exclusion chromatography; gel content

INTRODUCTION

Taraxacum kok-saghyz (TKS), commonly known as Russian dandelion, *Scorzonera tau-saghyz* (STS), and *Scorzonera Uzbekistanica* (SU) are perennial rubber-bearing plants discovered in the 1930s and native to the mountains of Kazakhstan and Uzbekistan. TKS and STS were industrially cultivated in 1930−1940s by the Soviets to gain rubber independence from foreign sources. TKS was also cultivated in the 1940s by Americans when the cheap rubber supplies from Southeast Asia were cut due to unstable political situations (*1*). All of these rubber-bearing plants contain significant amounts of rubber (24, 40, and 36% on a dry weight basis, respectively) and produce high-quality natural rubber comparable to that of *Hevea brasiliensis* (*2*).

These plants can be the global source of both hypoallergenic latex and solid natural rubber for the needs of the growing world economy. The idea that latex and rubber in rubber-bearing plants can be hypoallergenic has been confirmed with guayule (*3*−*5*). Therefore, the same should prove true with these rubber-bearing plants because they have similar protein makeup with guayule as a plant. Rubber-producing plants are important not only in terms of latex allergy but also in terms of solid natural rubber, which has attained higher prices because of higher oil prices and shortage of land in countries producing *Hevea brasiliensis* (**Chart 1**). The chart includes data published by the International Rubber Study Group (*6*). Current rubber consumption of the world is about 10 million metric tons, and prices have gone up 7-fold since 1997. Asian markets for natural rubber are developing very rapidly due to "Chinese" tire demand. Other rubber-producing plants such as guayule (*Parthenium argentatum*), jackfruit (*Artocarpus heterophyllus*), painted spurge (*Euphorbia heterophylla*), and fig tree (*Ficus elastica*) were also studied to meet the current needs of the world economy. Unfortunately, they produce lower molecular weight rubber (~1×10^6) with narrow unimodal molecular weight distribution (MWD) for jackfruit, whereas that obtained from *E. heterophylla* showed very broad MWD. Significant research was done to elongate the molecular weights, affecting the concentration of farnesyldiphosphate (FPP) initiator and isopentenyl diphosphate (IPP) elongation substrate (monomer) (*7*, *8*).

The previous commercialization of TKS in the 1940s by Americans confirms the possible reintroduction of TKS and STS into the northern United States and southern Canada. Another important aspect about TKS is that it contains a significant amount of inulin (up to 40%), which can be easily converted into ethanol via conventional methods (*1*). Inulin is also used as probiotic food ingredient, and there is an increased demand for it due to its health benefits. Other important bioproducts are proteins and fatty acids (*2*). Rubber-producing

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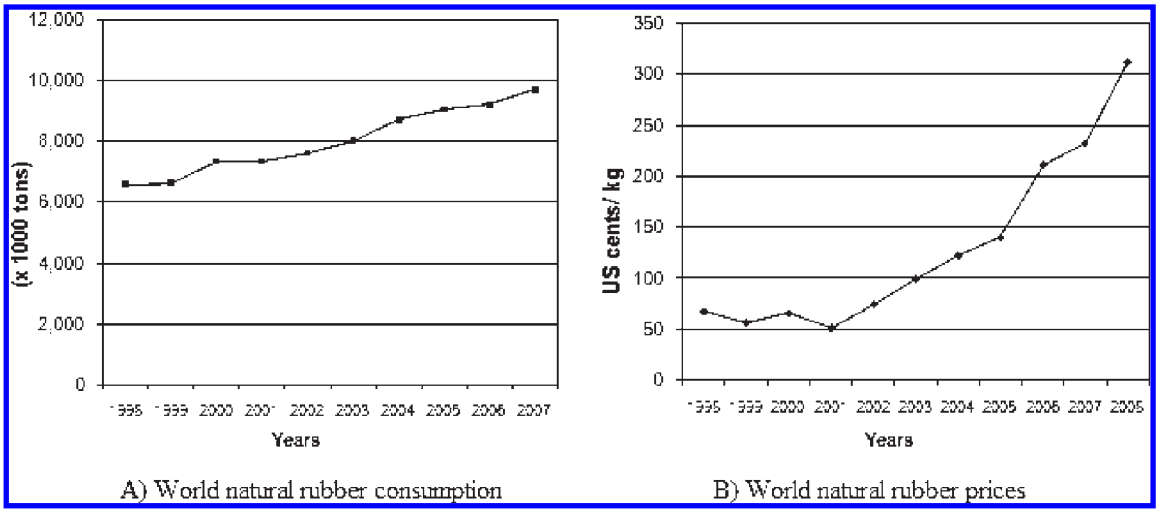
Chart 1. Worldwide Solid Rubber Consumption and Prices (www.rubberstudy.com)



plants contain significant amount of proteins (15%), which were never characterized in detail (unpublished data). TKS and STS contain also wax, resin, and fatty acids, the contents of which are lower compared to guayule (*2*). The study of biopolymers (lignin, hemicelluloses, and cellulose) and bioproducts (phenolic compounds) from the remaining bagasse (biomass) represents a big practical interest due to increased commercialization activities.

Rubber is contained in the roots of all three rubber-bearing plants in the form of both latex and solid rubber threads. The ratio of latex to solid rubber in the plants is dependent on season, cultivation, age of plants, and climate. Latex is contained in vessels (tubes), which are located in phloem tissue and anastomose to form a lacy cylinder around the stele of the plants. The latex vessel system of STS is comparable to that of TKS. Latex vessels are similar to those in *Hevea*, but unlike in *Hevea*, there is no connection between the latex vessels of adjacent ring anastomose. In TKS, solid rubber threads are sloughed off as threads in the plant tissue and as rubber sheath around the roots during April and May. The annual sloughing off of the root bark for STS was not reported, and this is accounted for by the higher accumulation of rubber found in STS. TKS can accumulate 10% of rubber in 1 year of cultivation and, therefore, it can be grown as an annual crop commercially. STS takes from 3 to 5 years to grow to commercial viability. Little information is known about SU (*9*, *10*).

Latex extraction from TKS and STS via tapping is not practical because of the small size of the roots and the viscous nature of latex. Ignat'ev (*11*) showed that latex can be extracted by flowing the latex in extraction medium. Ignat'ev developed a latex extraction method (flow method) for TKS and STS that included (1) cutting the roots, (2) putting them in the extraction medium to flow the latex, and (3) centrifugation to recover the latex (*11*−*14*). Latex in guayule is contained within individual bark parenchyma cells. Latex extraction from guayule was carried out using a Waring blender to rupture the parenchyma cells followed by filtration, purification, and centrifugation (blender method). Latex quantification methods based on the use of acids and organic solvents to coagulate the rubber were developed (*15*−*17*).

Solid rubber extraction with organic solvents can be accomplished via simultaneous and sequential solvent extraction. A polar solvent (acetone) is used first for the removal of extractives, and a nonpolar solvent (cyclohexane or hexane) is used for the extraction of rubber (*18*, *19*). Solvent processes were demonstrated at a pilot scale with guayule (*20*−*23*). However, solid natural rubber can also be extracted commercially via benign water-milling (*24*, *25*) and green dry-milling processes (*26*).

Current commercialization activities are focused on TKS. There are three independent groups in the United States and one in Canada growing TKS. The state of Ohio's Third Frontier Program has awarded $3 million to the Ohio Bioproducts Center and its partners for the selection and building of pilot-scale processing facilities. The European 7th Framework Program has allocated 8.2 million euro to several research institutions for the collaborative commercialization of TKS with Yulex Corp. (Maricopa, AZ). TKS has gained increased commercial interest in terms of both latex and rubber extraction by Delta Plant Technologies, Inc., based in Seattle, WA, after the recent re-emergence of a USDA report on commercialization activities in the 1940s (*1*). Commercialization of rubber recovery via a green dry-milling process is under development by Kok Technologies, Inc., based in British Columbia, Canada (www.koktech.com).

Guayule received a lot of attention in terms of latex extraction (*27*). However, little attention was paid to the consecutive extraction of latex and rubber. Most commercially promising rubber-bearing plants such as TKS and STS were forgotten for 60 years. The activities were not made public by either the Soviets or Americans, most probably due to the strategic nature of the activity and natural rubber. Comparison of two latex extraction methods was never carried out. Molecular properties of solid rubber from TKS and STS were not studied. SU was not known until 1950, and only biological studies were carried out. To our surprise, comparisons of these rubber-bearing plants from test plots with different climates and wild fields were never carried out. Test plots were never formed via transplantation of young plants. In this work, we will try to fill these gaps. Therefore, our research has been focused on the latex and solid natural rubber extraction from these plants grown via transplantation on test plots of two different climatic regions and harvested from wild fields. In this paper we describe latex extraction from TKS, STS, and SU from test plots and wild fields with two methods: the blender method and the flow method. The comparative results of the plants from the test plots and wild fields in terms of latex yield, latex purity, and concentration will also be discussed.

MATERIALS AND METHODS

Plant Materials (Figure 1). TKS and STS were harvested by digging from wild fields in Kegen district and on the Kara-tau Mountains in Kazakhstan and Uzbekistan. SU was harvested by digging from wild fields

Figure 1. TKS and STS samples from wild and test plots in Jizzax, Uzbekistan (May 2004): (**A**) TKS test plot in Jizzax, Uzbekistan; (**B**) TKS from wild fields (rubber sheath); (**C**) TKS plants from wild fields; (**D**) STS from wild fields (break test).

on the Chatkal Mountains in Uzbekistan. TKS roots were also harvested from the test plots in two climatic field locations in Jizzax (TKS-Jizzax) and Tashkent (TKS-Tashkent), Uzbekistan, in the fall (October) of 2004. These test plots were created via transplantation of young TKS seedlings from the wild fields that experienced at least one winter season. The roots were sorted, and aerial parts were removed from the roots, leaving 0.5 cm. The roots were dipped into 1% aqueous solution of ascorbic acid, sealed in plastic, and stored on ice in the coolers. The coolers were delivered to Tashkent, Uzbekistan, overnight and stored in a refrigerator at 4 °C until processed within 2−3 days. Grade 20 Technically Specified Rubber (TSR20), a commercial rubber sample, was used as standard for comparison purposes.

Latex Extraction with Blender Method. Latex extraction with a Waring blender was carried out according to the method of Cornish et al. (*17*) with minor modifications. Within 2 days of harvesting, 20 g of chilled roots of TKS, STS, or SU was cut into ∼0.5 mm pieces. Within 3 min of the first cut, the pieces were placed in a Waring blender (model 33BL79) containing 90 mL of ice-cold, aqueous extraction buffer (0.1% Na_2SO_3 and 0.2% NH_3) and ground for 30 s. The slurry was transferred onto a 1 mm mesh porcelain Buchner funnel without filter paper, and the homogenate was filtered through by slow vacuum suction with a water aspirator. The surface of the slurry was made even and pressed slightly with a tablespoon for efficient filtration. The remaining ground roots (bagasse) were returned to the Waring blender and reground for 30 s in another 90 mL of fresh extraction buffer. The slurry was again transferred onto the Buchner funnel and the homogenate filtered. The homogenates from both filtrations were pooled and stored in closed vessels at 4 °C until centrifugation. The homogenate (∼180 mL) was centrifuged using 30 mL centrifuge tubes for 15 min at 18368*g*. The creamy latex layers on the surface of centrifuge tubes were collected by suction-drawing with a 10 mL glass pipet and rubber filler. The tubes were then recentrifuged three more times using the same conditions, and latex layers were collected again as above. The collected latex was quantified.

Three more additional grindings (up to five in total) were carried out to determine the total extractable latex in the plants.

Latex Extraction via Flow Method. Within 2 days of harvesting, the chilled roots (250 g) of TKS, STS, and SU from a refrigerator were cut into 0.5 cm circular pieces with a knife. Within 3 min of the first cut, the pieces were immediately placed into a 1 L glass flask containing 500 mL of ice-cold extraction buffer (0.1% Na_2SO_3, 0.2% NH_3, and 0.1% casein) and

shaken for 30 min at room temperature (*11*). The homogenate was decanted into an empty 2 L glass flask, and another 500 mL of fresh extraction buffer was poured into the 1 L flask containing the root pieces. The mixture was shaken for another 30 min and decanted into a 2 L flask containing the previous homogenate. This procedure was repeated three more times. Because the roots were not ground with a blender, there was no need for filtration and contamination of latex with plant tissues was not an issue.

The homogenate was centrifuged in 30 mL centrifuge tubes for 5 min at the rate of 18369*g*. The white creamy layer of latex was collected by suction-drawing using a glass pipet and rubber filler. The remaining solution was centrifuged again twice, and the creamy layer of latex was collected again. Then, the remaining solution was centrifuged for the fourth and fifth times, and again the creamy layer of latex was collected with very good yield. Latex was quantified by coagulation with glacial acetic acid as described under Latex Quantification. The remaining bagasse was reground two more times with a blender according to Latex Extraction with Blender Method to evaluate the remaining latex in the bagasse.

Latex Quantification. The rubber in latex was quantified as follows: To 4 mL of latex was added 0.2 mL of glacial acetic acid, and the mixture was centrifuged for 15 min at 6140*g* (*15*, *17*). The coagulated rubber on the surface was collected, washed with deionized water, placed on the preweighed paper, and left in an oven overnight at 45 °C for drying to a constant weight.

Latex Purity. The coagulated and dried latex from the quantification method was dissolved in hexane (Sigma Aldrich, Germany) in capped scintillation vials, by constant shaking overnight at 25 °C (*17*). The solution was filtered through glass fiber filters (Whatman, Fairfield, NJ) to retain the hexane-insoluble contaminants (mostly tissue particles). The vials and glass filters were washed with 3 mL of fresh hexane five times. The filters were dried at 40 °C and weighed to determine the insoluble residues.

Concentrating the Latex with the Method of Centrifugation. Concentrating the latex was carried out according to the centrifugation method described by Kolesov (*28*) and Ignat'ev (*13*). To the latex collected from the first centrifugation as described in the above section were added ~1% aqueous ammonia (10:1, v/v) and creaming agents (10:1, v/v). The following creaming agents for latex separation were prepared in advance: 3% solutions of polyethylene glycol, polyvinyl alcohol, agar, pectin, and carboxymethylcellulose in distilled water. Three grams of cream separation was poured into a flask containing 97 g of distilled water, and the mixture was heated for 5−10 min in a water bath (90−95 °C). When the transparent solution was formed, it was cooled and ready for use.

The mixture was recentrifuged in 250 mL centrifuge tubes at 17200*g* for 5 min. The top creamy part was collected using a 10 mL glass pipet with a rubber filler (3 Valve Rubber, Sigma Aldrich, Germany). Collected creamy latex was recentrifuged four more times, each time collecting 50% of the top creamy layer.

The concentration of ammonia in latex was checked periodically to prevent coagulation with the following neutralization method: The latex sample (10 mL) was poured into a vial and capped. It was weighed and added to a flask containing 300 mL of distilled water. The mixture was stirred well, and 6 drops of 0.1% alcoholic solution of methyl red were added. The mixture was neutralized with a 0.1 N solution of H_2SO_4 upon titration until a weakly reddish color appeared.

Concentrating the Latex with the Method of Settlement/Flotation. The latex from the centrifugation step was further concentrated via settlement in separatory funnels. Creaming agent, 0.3% casein (1:5, v/v), was added to the latex in a separatory funnel. After settling for a day, the bottom tap was opened and 50% of the bottom phase removed. The bottom phases were pooled together, quantified, and settled for 2 more days. The top phases were also pooled together, quantified, and settled for 2 more days. The bottom phases were separated from the top phases, and latex was quantified in both phases.

Solvent-Based Sequential Rubber Extraction. A sample of air-dried bagasse left after latex extraction was ground with a Wiley mill to pass a 2 mm mesh screen (bagasse from the blender latex extraction method was used as-is). Five grams of the ground roots was placed in a round-bottom flask with reflux condenser to prevent acetone escape (HPLC grade, Sigma Aldrich, Germany) and extracted with 100 mL of acetone for 3 days with constant magnetic stirring at room temperature. Then the acetone extract was filtered using a Buchner funnel (1 mm mesh), and the roots on the funnel were washed with copious amounts of acetone. The air-dried, deresinated roots were then extracted with 300 mL of chloroform (ACS grade, Sigma Aldrich, Germany) or other nonpolar solvents (ACS grade, Sigma Aldrich, Germany) (*18*, *19*) for 24 h at room temperature with periodic shaking. Solubilized rubber was decanted slowly into a preweighed flask without disturbing precipitated plant tissue. The remaining plant tissue was washed with chloroform three times and filtered through a glass filter. The filtrates and rubber solution were pooled together. The bulk of chloroform was evaporated using a rotary evaporator. The viscous rubber in the flask was dried in the vacuum oven at 40 °C for 2 days and weighed. The rubber and resin contents of the plant were calculated on the basis of the weight of rubber and the dry weight of the plant.

Size Exclusion Chromatography (SEC) of Solid Natural Rubber. Solid natural rubber samples were cut into small pieces and dissolved in tetrahydrofuran (THF) at a concentration of about 0.2% (w/v). Natural rubber samples were allowed to swell overnight. The next day, the solutions were placed on a wrist-section shaker for about 8 h. The rubber solutions were filtered through a 0.2 μm PTFE disposable syringe filter (25 mm). The determination of molecular weights was carried out by HPLC equipped with Permagel 500, 10^3, 10^4, 10^5, and 100 Å (Column Resolution, Inc., San Jose, CA) linked in series to each other (*29*, *30*). The refractive index and light scattering detectors (Agilent Technologies, model 1100, Palo Alto, CA) were used for the monitoring the peaks. The eluent system was HPLC-grade THF (J. T. Baker Inc., Phillipsburg, NJ) a flow rate of 1.0 mL/min and column temperature of 35 °C. A series of *cis*-polyisoprene standards with molecular weights of 6.0×10^4 and 3.3×10^6 (Polymer Laboratories Ltd., Church Stretton, U.K.) was used to calibrate SEC and determine the molecular weights of natural rubber samples. All standards have nominal polydispersities of < 1.06. The signal from the RI detector was processed using PSS WinGPC Unity software (Polymer Standard Service-USA, Warwick, RI). The signal from the RI detector was processed using PSS WinGPC Unity software (Polymer Standard Service-USA). The rubber samples were compared with the commercially available technically specified rubber (TSR20) from *Hevea*, the gel fraction of which was removed prior to analysis.

Determination of Gel Content in Natural Rubber Samples. To determine the content of the natural rubber, 0.2−0.3 g of the rubber pieces was soaked in an excess of toluene for 24 h, and then the solution was passed through a wire mesh with 125 μm openings. The weight of the dried material remaining on the mesh was regarded as gel, and the gel percentage was calculated on the basis of the total original rubber weight (*29*). The rubber samples were compared with the commercially available technically specified rubber (TSR20) from *Hevea*.

RESULTS AND DISCUSSION

Identification of TKS and STS in the Wild Fields. All of these rubber-bearing plants are indigenous to Uzbekistan and Kazakhstan and are widely spread on the eastern mountains (Kara-tau and Chatkal) in large fields. Identification of rubber-bearing plants in the wild fields requires some skill to recognize the right plants. Especially, differentiating TKS from other *Taraxacum* species in the wild fields can be a real challenge for a new researcher. For the differentiation and collection of roots from rubber-bearing plants in the wild fields, the following four techniques were used: (1) leaf morphology; (2) visual observation of roots for rubber sheath; (3) checking for latex viscosity; and (4) break test for rubber threads.

TKS can be detected with leaf morphology and the availability of rubber sheath around its roots. The leaves are blue (kok-saghyz in Turkic means "blue gum"), fleshy, and glossy and have even edges with no indentations. Another difference is the latex viscosity. The volume of the latex drops from TKS does not decrease in shape when they are dried in the air. Latex from other *Taraxacum* species shrinks and reduces to 10% of original volume.

Table 1. Moisture and Total Rubber Contents of Rubber-Bearing Plants

rubber-bearing plant	moisture content (%)	rubber content (%) on dry weight
TKS from Jizzax plots	34 ± 2.1	20.6 ± 0.5
TKS from Tashkent plots	32 ± 2.5	18.4 ± 0.6
TKS from wild fields	20 ± 1.8	24.3 ± 0.5
STS from wild fields	19 ± 2.1	40.2 ± 0.5
SU from wild fields	20 ± 2.2	36.4 ± 0.6

STS and SU are mostly found in the mountains (tau-saghyz in Turkic means "mountain gum") and can be easily detected with the availability of rubber threads when the roots are broken into two. This visual observation can be used to estimate approximate rubber content in the roots as well. Break test can also be applied to TKS. However, the rubber threads in TKS are less noticeable than those in STS and SU.

The moisture of the roots was determined by drying the roots in an oven at 100 °C (**Table 1**). The total rubber contents of the dry roots were determined by sequential solvent extraction with acetone and cyclohexane (*18*).

STS contains the highest rubber among plants (40%). Moisture content was highest with the TKS plants grown in the test plots. Even though TKS contains less rubber (24%), it was previously chosen by both the Soviets and Americans as the commercial plant due to its rapid growth and significant rubber accumulation of ~10% in the first year of growth (*1*). STS took 3 years to grow to commercial viability when cultivated by the Soviets in the 1930s (*31*). Current modern plant growth stimulators may be useful to accelerate the growth of STS, which represents a big commercial opportunity. Little is known about the growth habits of SU.

Latex Extraction with Blender and Flow Methods. Latex in STS, SU, and TKS roots is contained in special canals (vessels or tubes) as in *Hevea*, the Brazilian rubber tree (*9*, *10*). Unlike *Hevea*, tapping of individual plants for latex is not viable commercially due to the small size of roots and the thicker nature of the latex. Previous Soviet research on latex extraction from these plants was based on the cutting of the plants into thin circular sections and flowing the latex in buffers with periodic shaking (*11*−*13*). The latex in guayule is contained within the parenchyma cells, and it is necessary to rupture the cells with a blender to obtain the latex without damaging the rubber particles (*17*). It is interesting to compare these methods of latex extraction and choose the optimal extraction method for current rubber-bearing plants. Therefore, latex extraction from TKS, STS, and SU was carried out with the blender method developed by Cornish (*17*) and the flow method developed by Ignat'ev (*11*−*13*).

Optimal grinding and extraction times of both methods were determined using TKS roots from wild fields (**Figure 2**). The results with the blender method indicate virtually all extractable latex was released by 30 s of grinding. Prolonged grinding did not result in the significant increase of dry latex yield. Visual inspection of the ground roots after filtration showed that all root pieces were fibrillated into pulp in 30 s. The results with the flow method indicate that it takes only 30 min of extraction to achieve latex yield similar to that with the blender method. Flowing latex in extraction buffer with periodic shaking is efficient enough and the extraction time is not too long. The approximate yields of dry latex with both methods were about ~10 and ~9%, respectively, based on the weight of dry roots.

Grinding guayule shrubs required more time (60 s) for each grind. This is due to the hardness of the shrub, which was necessary to rupture to release latex from parenchyma cells (*17*). The roots of TKS, STS, and SU are softer compared to guayule, and it is easy to fibrillate in a shorter time (30 s) using a blender. The resulting fibrils retain rubber particles, and therefore several fresh extraction buffers were required to remove the latex.

The results of grinding the roots with fresh extraction buffers every 30 s are represented in **Figure 3A**. Rubber quantification experiments have shown that the total dry latex content in STS (6.25%) and SU (5.8%) is much lower than in TKS (8%). TKS roots (TKS-Jizzax and TKS-Tashkent) harvested from test plots contained more latex (~9 and 9.5%, respectively). This might be the effect of cultivation, climate, and season. TKS grown in the cooler climate of the Jizzax region yielded more latex (9.5%). Fresh extraction buffer for each grinding had the washing effect of rubber particles. Extraction time for the flow method was 30 min, and most of the available latex was removed in 60 min (**Figure 3B**). Extraction was carried out with fresh extraction buffer every 30 min to check for any effect. No significant increase in the latex yield was observed after five extractions.

Grinding the roots of TKS, STS, and SU with a Waring blender to extract latex was very efficient and took only 30 s to rupture the roots into fibrils. However, filtration on a 1 mm Buchner funnel did not retain all of the fibrils, and therefore the latex was contaminated with small particles of root tissue. The same problem was observed with latex extraction from guayule shrubs by Cornish (*17*). Vacuum filtration using a tablecloth helped to clean the latex, but retention of the rubber particles on the tablecloth and subsequent clogging complicated the process. Purification of homogenates by settlement and centrifugation partially solved the problem.

The latex extract from the flow method was clean, and no plant tissue debris was observed. Because the roots were cut into sections (0.5 cm in thickness), no filtration was required to separate the root sections from homogenate. Decantation of homogenate worked perfectly well. The time lost for extraction was recovered because there was no need to perform a filtration step. The thickness of root sections were also investigated (results not shown). Dry latex yield decreased slightly with increasing thickness of root sections from 0.5 to 2.0 cm.

The results of total extractable latex extraction with the blender and flow methods are summarized in **Table 2**. Around 96% of the extractable latex from TKS was released after the second grinding in 60 s via the blender method. This was a little bit lower, 88%, with STS and SU. About 90% of extractable latex from TKS was removed after two extractions in 60 min via the flow method. Only 75 and 69% latex from STS and SU was removed, respectively. To achieve 99% latex extraction with the flow method, two grindings with a blender were required. Additional grinds and extractions resulted in a little bit more latex yield, but it does not seem worthwhile to consider this route in the development of a commercial process. The same conclusion was made with guayule (*17*).

It took 2 min of grinding to release 90% of extractable latex from guayule shrubs. Large errors were introduced by insufficient tissue disruption during grinding of the guayule shrubs (*17*).

The commercial processing facility for the latex extraction from TKS, STS, and SU seems to be based on the flow method. The beauty of the flow method is that it uses root sections (0.5 cm thickness), which does not require a filtration step, and there is no contamination of homogenate with fibrils, which eliminates the need for a latex purification step. Of course, the flow method takes more time for the extraction (60 min), and > 10% of total extractable latex remains in the roots. However, these disadvantages may not be a barrier for commercialization of the flow method.

Total latex and solid rubber contents of the rubber-bearing plants are illustrated in **Figure 4**. To calculate total latex content, five grindings were used. The data indicate that most of the rubber

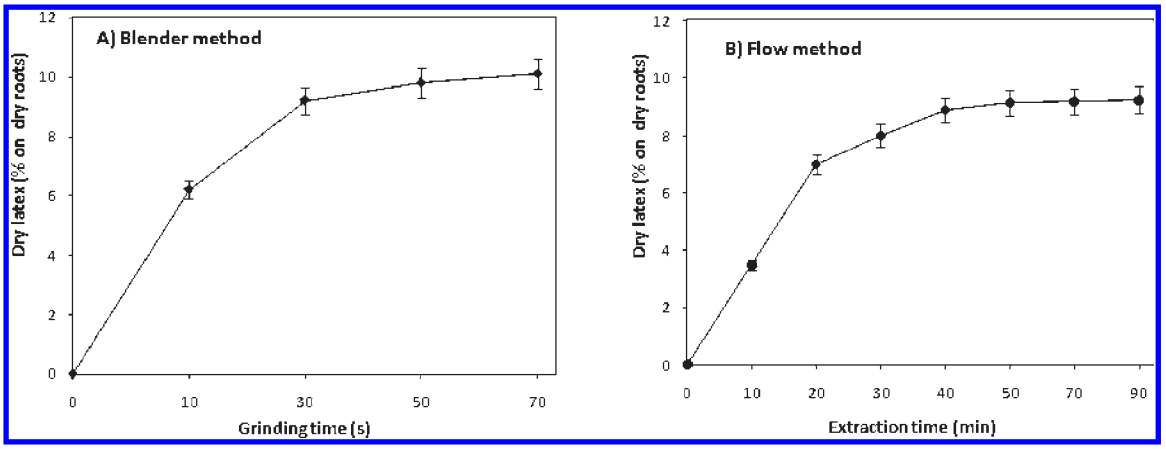


Figure 2. Comparison of latex extraction from TKS at various grinding and extraction times.

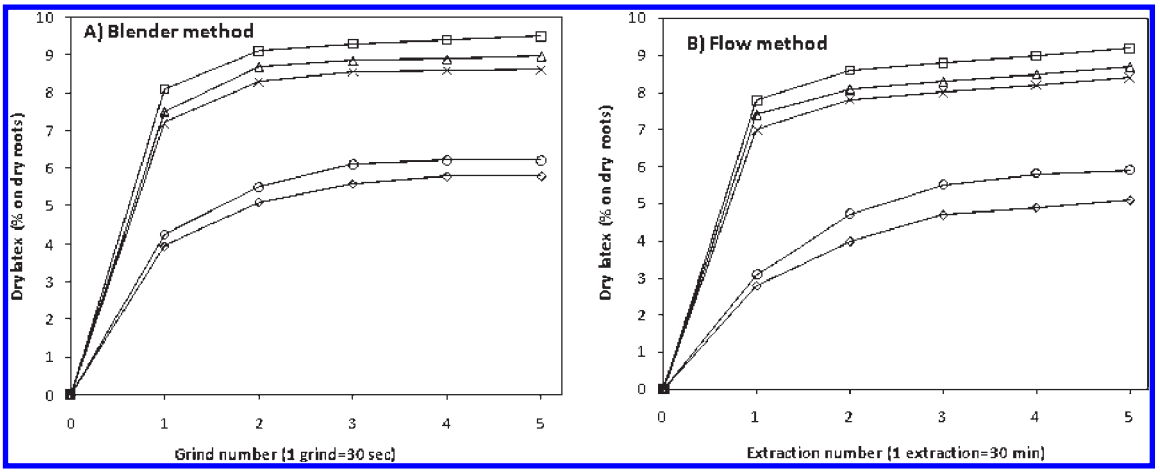


Figure 3. Comparison of the extraction of latex via blender and flow methods: roots from test plots, (□) TKS-Jizzax and (△) TKS-Tashkent; roots from wild fields, (×) TKS, (○) STS, and (◇) SU.

in the TKS from test plots (young plants) is in the form of extractable latex (~46%). The plants from wild fields (TKS, STS, and SU) contained mostly solid rubber, which is probably due to the older age of the plants.

The latex yields from STS and SU harvested from wild fields in the summer were 2 times higher (~14%) than those in the fall (~7%). This sparks an interest to study the accumulation of latex depending on the season, climate, and location. Solid rubber accumulation was observed to be highest in April and May in the case of TKS (*1*). Our results are in good agreement with the results of Siniavskii (*32*), who was able to remove 50% of rubber as latex from the roots of kok-saghyz.

Because the rubber in TKS, STS, and SU is present as latex and solid rubber threads, the potential commercial process will likely be based on the two steps of (1) latex extraction and (2) solid rubber extraction. As mentioned above, the latex extraction seems to be based on the flow method. Another option might be to recover total rubber as solid rubber if the latex was not required. Solid rubber extraction will probably be based on the dry extraction process (*26*)

Latex Purity. Latex purity is an important parameter of quality for manufacturing of final latex products. It is dependent on the extraction methods used and the softness of plant species. Latex extracted from guayule using a Waring blender was found to contain a solid component (plants tissue) of up to 10%. The solids content increased with the number of grindings and the age of the tissue ground (*17*). The purities of latex extracted via the blender method and the flow method were compared, and the results are below.

Table 2. Extraction of Latex from Rubber-Bearing Plants[a]

	plants from test plots		plants from wild fields		
grind	TKS-Jizzax	TKS-Tashkent	TKS	STS	SU
		Blender Method			
1	85.26 ± 2.2	83.52 ± 2.5	83.53 ± 3.5	68.28 ± 3.3	68.05 ± 3.8
2	95.79 ± 2.1	96.88 ± 2.0	96.29 ± 2.2	88.57 ± 2.2	88.08 ± 2.3
3	97.89 ± 1.5	98.66 ± 1.1	99.3 ± 0.5	98.23 ± 1.2	96.37 ± 2.3
4	98.95 ± 0.5	99.11 ± 0.4	99.77 ± 0.2	99.84 ± 0.1	99.83 ± 0.2
5	100	100	100	100	100
		Flow Method			
1	82.11 ± 3.3	82.41 ± 3.4	81.21 ± 3.8	49.92 ± 4.3	48.36 ± 4.5
2	90.53 ± 1.5	90.2 ± 1.2	90.49 ± 1.5	75.68 ± 2.5	69.08 ± 2.8
3	92.63 ± 1.2	92.43 ± 1.2	92.81 ± 1.3	88.57 ± 2.2	81.17 ± 1.7
4	94.74 ± 1.5	94.65 ± 1.1	95.13 ± 0.9	93.4 ± 1.1	84.63 ± 1.2
5	96.84 ± 0.5	96.88 ± 0.5	97.45 ± 0.5	95.01 ± 0.5	88.08 ± 0.6
6[b]	97.5 ± 1.3	97.8 ± 0.9	98.2 ± 0.8	97.34 ± 0.9	94.56 ± 1.1
7[b]	99.1 ± 0.2	99.12 ± 0.3	99.4 ± 0.2	99.3 ± 0.2	98.3 ± 0.4

[a] Latex yield from five grinds with blender method was accepted as 100%. [b] Grinding the root pieces after the extraction with the blender to recover the residual latex.

The latex extracted from TKS via the blender and flow methods was quantified using 4 mL of latex. Then the purity of the coagulated latex was determined, and the results are

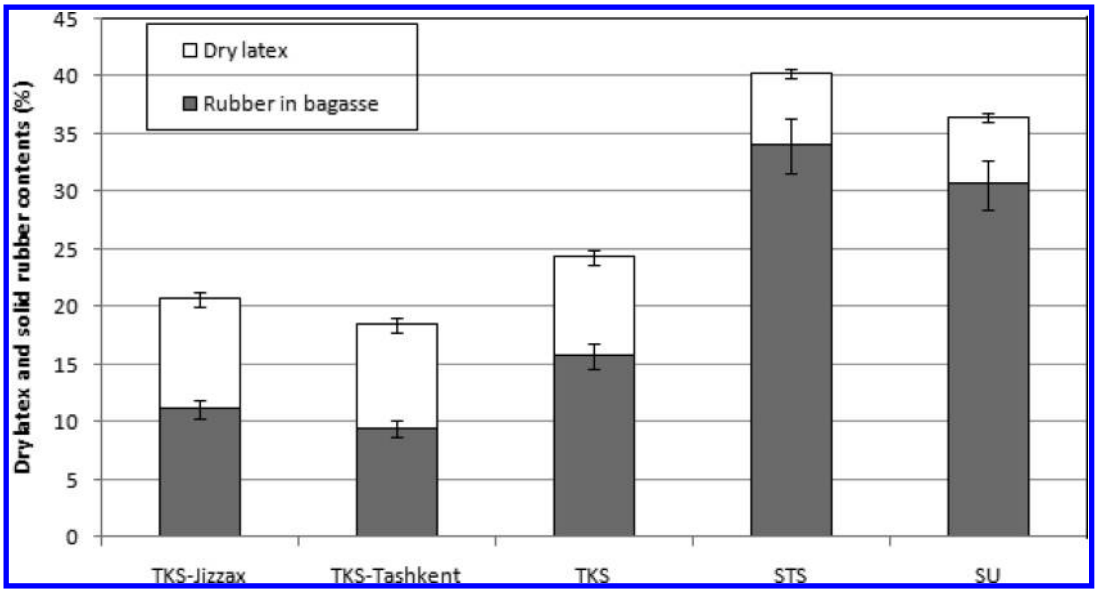


Figure 4. Dry latex and solid rubber contents in rubber-bearing plants.

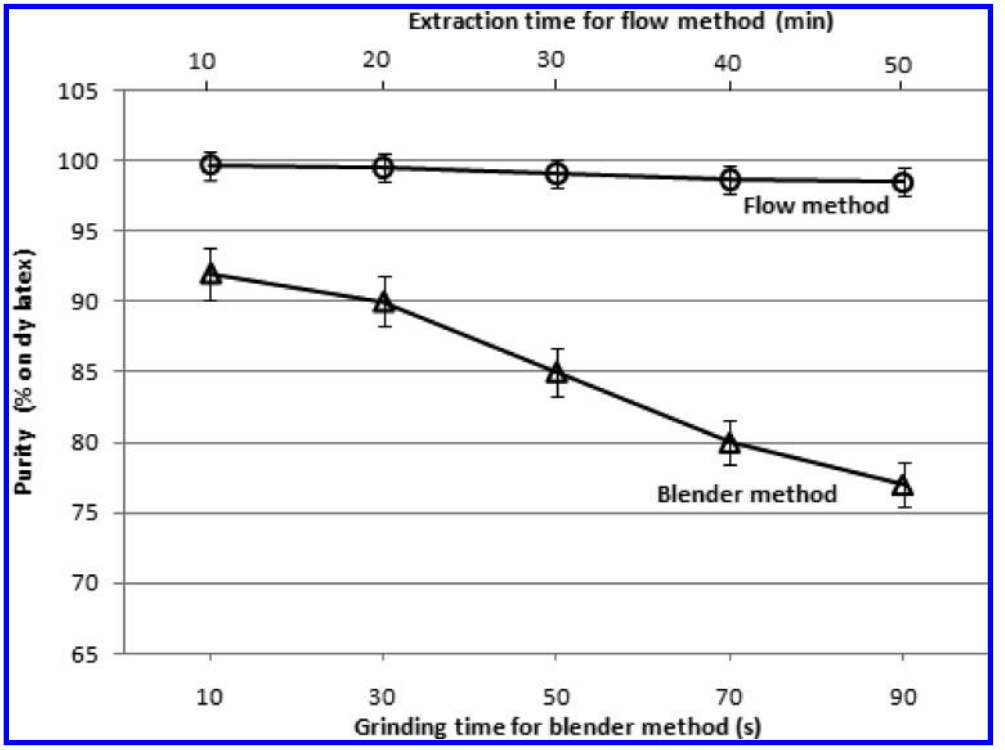


Figure 5. Purity of latex extracted from TKS via blender and flow methods.

illustrated in **Figure 5**. The content of impurities in the latex extracted via the blender method increased with grinding time due to the fibrils from the plant tissue. The purity of latex was 75% when grinding time was equal to 90 s. The latex extracted via the flow method was virtually pure (~99.5%) and contained very small amounts of insoluble residues that are most probably from root skins coming off during shaking. Increasing extraction time did not influence the purity of the latex significantly. The data clearly show that high-purity latex is obtained via the flow method.

The dependence of the coagulated latex purity on the grinding and extraction numbers using fresh extraction buffer each time is shown in **Table 3**. The latex extracted via the blender method contained significant impurities in each grinding (~8−12%). This was not the case with the flow method, by which only traces of impurities were observed (~0.3%).

Impurities in latex appeared to increase with grind number and the type of the plant. For example, impurities in TKS were less than those in STS and SU. This is might be due to the softness of the roots to form fibrils that are entrapped in latex. Similar results were observed with guayule shrubs, and the content of solids reached 20% during the second grindings for 120 s (*17*). Commercial latex extraction from guayule shrub includes filtration and latex purification steps. However, these steps were not necessary during the development of the flow method in the 1940s (*11*−*14*). Most probably, latex extraction facilities for TKS, STS, and SU will be simple and cost-effective compared to that of guayule.

Table 3. Purity of Dry Latex

	content of solids in dry latex extracted via blender method (% in extraction numbers)			contents of solids in dry latex extracted via flow method (% in grind numbers)		
rubber-bearing plant	1	2	3	1	2	3
TKS-Jizzax	8.1 ± 0.2	9.2 ± 0.1	11.2 ± 0.2	0.3 ± 0.01	0.1 ± 0.01	0.2 ± 0.02
TKS-Tashkent	8.3 ± 0.3	9.1 ± 0.2	11.4 ± 0.1	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
TKS	8.2 ± 0.2	9.0 ± 0.3	11.2 ± 0.2	0.3 ± 0.02	0.2 ± 0.02	0.2 ± 0.02
STS	10.2 ± 0.3	10.9 ± 0.3	12.8 ± 0.3	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
SU	10.5 ± 0.3	11.4 ± 0.4	12.6 ± 0.4	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.01

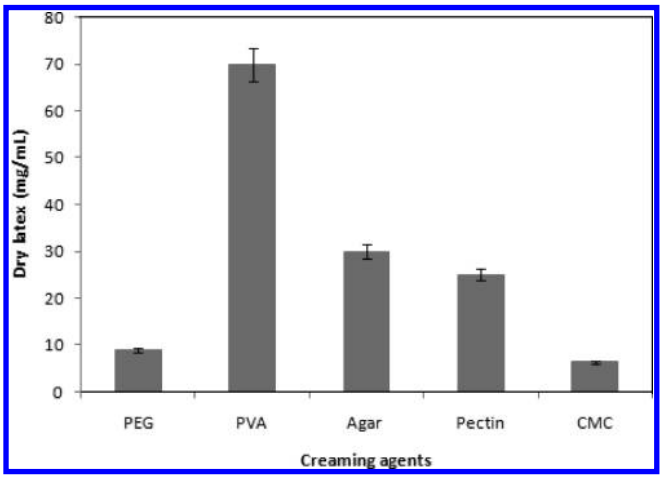


Figure 6. Dependence of dry latex yield on the agents of latex separation. PEG, polyethylene glycol; PVA, polyvinyl alcohol; CMC, carboxymethyl cellulose.

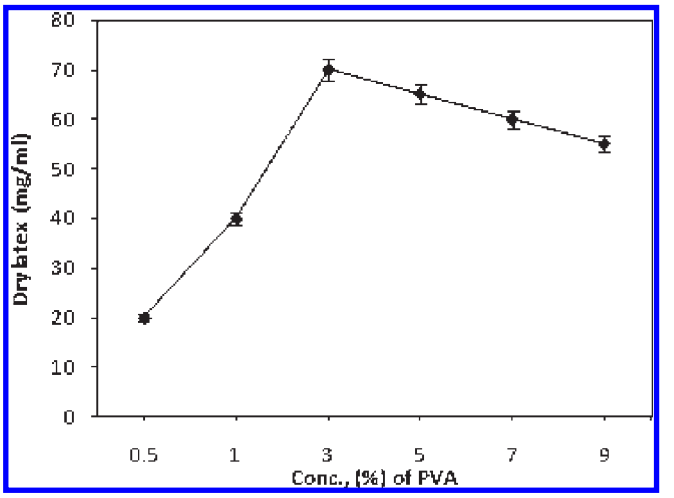


Figure 7. Dependence of PVA concentration on dry latex yield.

Purification and Concentration of TKS Latex. For the transportation of latex and production of latex products, the concentration of dry latex should be between 30 and 65%. Concentrating *Hevea* latex has been carried out through flotation and centrifugation methods for many years (*15*, *28*). Purification of guayule latex from homogenates is carried out via series of centrifugation steps and/or flotation using creaming agents (*33*). We have applied these methods for concentrating the latex and purification.

The latex was separated and purified from TKS homogenates via centrifugation using the agents of latex separation such as polyethylene glycol (PEG), polyvinyl alcohol (PVA), agar, pectin, and carboxymethyl cellulose (CMC). The results are summarized in **Figure 6**. To 80 mL of latex was added 20 mL of 3% latex separation agent, and the mixture was centrifuged at 16356*g* for 10 min. The floating latex layer on the top was removed with a pipet and filler. Latex was collected in a separatory funnel containing 5 mL of extraction buffer. This procedure was repeated six times, increasing spin speeds by 4000*g* and time by 5 min each time. The insoluble impurities and tissue fibrils of the homogenates from the blender method precipitated. No significant precipitate was observed after latex centrifugation via the flow method. The collected latex was quantified. The concentration of latex reached the highest level (75 mg/mL) with PVA. The lowest latex concentration was observed with CMC (5 mg/mL).

PVA was found to be the best agent for latex separation. The optimal concentration of PVA was found to be 3% (**Figure 7**).

To further concentrate the latex by settlement/flotation steps, creaming agent (0.3% casein) was added to the latex collected in a separatory funnel at a ratio of 1:5 (v/v) for the stabilization of rubber particles in latex. After the phase separation, the bottom layer was drained and fresh creaming agent was added. This procedure repeated five times, and the yellow color of latex disappeared. The purified and concentrated latex was quantified and stored at 4 °C under nitrogen. The concentration of latex was 12%. A film was cast with 2 mL of latex on a Petri dish. Transparent film was observed (**Figure 8**).

Figure 8. Casting a film from 2 mL of 12% latex (scratched to make noticeable).

To concentrate the latex further, the bottom layer in the separatory funnel was removed until no additional separation of two phases was observed. The final concentration was determined via casting a film and drying at 37 °C. The dry latex content was 35%. Above this concentration, the fluidity of latex slowed.

Sequential Rubber Extraction from Bagasse. Bagasse left after the latex extraction was dried at 40 °C in a vacuum oven for 2 days. The bagasse from the flow method was ground with a coffee grinder to pass the mesh screen (1 mm), and the bagasse from the blender method was used as-is. Solid rubber was extracted first with acetone and then nonpolar solvents. The results are summarized in **Table 4**.

Table 4. Solvent Effect on Rubber Yield (Percent)

plant	chloroform	extraction petroleum[a]	benzene	cyclohexane
		Blender Method		
TKS-Jizzax	6.78 ± 0.3	5.8 ± 0.2	6.1 ± 0.3	6.0 ± 0.2
TKS-Tashkent	5.48 ± 0.2	4.5 ± 0.2	5.0 ± 0.2	4.9 ± 0.3
TKS	13.68 ± 0.4	13.1 ± 0.5	13.1 ± 0.4	13.0 ± 0.3
STS	33.99 ± 0.8	32.5 ± 0.9	32.9 ± 0.7	32.8 ± 0.6
SU	30.61 ± 0.8	29.5 ± 0.9	30.1 ± 0.7	29.9 ± 0.6
		Flow Method		
TKS-Jizzax	8.1 ± 0.3	7.2 ± 0.3	7.6 ± 0.3	7.5 ± 0.2
TKS-Tashkent	6.9 ± 0.3	5.8 ± 0.2	6.3 ± 0.3	6.2 ± 0.2
TKS	14.4 ± 0.4	13.4 ± 0.4	13.7 ± 0.4	13.5 ± 0.3
STS	34.3 ± 0.8	33.3 ± 0.8	33.8 ± 0.7	33.5 ± 0.6
SU	31.3 ± 0.8	30.4 ± 0.8	30.8 ± 0.7	30.6 ± 0.6

[a] Requires several extractions (sometimes called mineral spirits).

Table 5. Molecular Properties of Solid Natural Rubber Extracted with Organic Solvents[a]

rubber sample	M_w	M_n	M_w/M_n	gel content (%)	resin content acetone extract (%)
TSR 20	2.5×10^6	1.9×10^6	1.3	55 ± 2.1	3.5 ± 0.3
TKS	1.8×10^6	1.2×10^6	1.6	34 ± 3.4	4.0 ± 0.2
STS	1.7×10^6	1.2×10^6	1.5	27 ± 2.8	4.2 ± 0.3
SU	1.8×10^6	1.4×10^6	1.3	92 ± 3.1	3.0 ± 0.2

[a] Sequential extraction was carried out first with acetone and then with chloroform.

The rubber extraction with extraction petroleum required double extraction to achieve the highest rubber yields. Chloroform was found to be the most efficient and cheapest solvent for the rubber extraction from these rubber plants. Rubber extraction was also performed with cyclohexane when it was requested. Rubber yields from the bagasse of the flow methods were slightly higher in all plant species. A 0.5 kg of rubber was extracted from each plant via sequential extraction first with acetone and then with chloroform (or cyclohexane). The MWD studies with SEC were carried out on rubber samples.

Rubber extraction with organic solvents was extensively studied using guayule shrubs (*21*, *34*, *35*). The most successful pilot plant, the Bridgestone/Firestone facility in Sacaton, AZ, produced 5.4 tons of rubber using acetone−pentane azeotrope. This rubber met all industrial specifications (*36*, *37*). However, rubber extraction with organic solvents represents only small-scale research and development interest due to environmental concerns and safety issue. Organic solvents are volatile and explosive.

SEC and Gel Content of the Solid Rubber. Molecular weight comparisons of rubber samples extracted from the bagasse left after latex extraction via the flow method have been carried out on a SEC column with HPLC using RI and light scattering detection (**Table 5**). Results indicate that weight-average molecular weights of TKS (1.8×10^6), STS (1.7×10^6), and SU (1.8×10^6) are comparable with that of TSR20 (2.5×10^6) (**Table 5**). Tires were manufactured from TKS rubber in the 1940s by Americans, and the physical tests showed that the tires from TKS rubber met all of the requirements set for those made from *Hevea* rubber (*1*). The molecular weight of the rubber from guayule significantly lower, at about 1.0×10^6 (*30*). The molecular weight of rubber is significantly decreased when processed in a blender due to the shear forces, and therefore it does not represent practical interest. Other rubber-producing plants such as jackfruit (*Artocarpus heterophyllus*), painted spurge (*Euphorbia heterophylla*), and fig tree (*Ficus elastica*) were reported to have even lower molecular weights. Significant research was done to elongate the molecular weights, affecting the concentration of farnesyldiphosphate (FPP) initiator and isopentenyl diphosphate (IPP) elongation substrate (monomer). Under these conditions, fig tree synthesized rubber polymers approximately twice the molecular weight of those made by *Hevea* and guayule (*7*, *8*).

Gel content in TKS and STS is significantly lower than in TSR20. Gel content in SU is considerably higher than in other samples, which can be removed with cold milling during industrial rubber production. The resin contents in all rubber samples are within the acceptable range (~4%).

Guayule rubber can contain as much as 20−40% resin, which leads to the additional fractionation process (*37*). Weight-average molecular weights of guayule rubber were found to be sensitive to experimental conditions such as season, age of plants, and postharvest storage conditions (*18*, *30*, *38*). The effect of these parameters on the molecular weights of rubber from TKS, STS, and SU may represent greater interest once commercial production is under way.

Our results indicate that TKS is the most promising among plants due to its rapid annual growth and 10% rubber accumulation in a year. TKS is important in terms of both latex and natural rubber production. About 50% of rubber in TKS was extracted as latex. STS can be more commercially valuable as a source of solid natural rubber if its growth habits can be accelerated.

Comparison of two latex extraction methods such as the blender and flow methods indicated the latter as a method of choice. The advantages associated with the flow method include high-purity latex and simplicity. Filtration and latex purification steps were not required. Latex concentration of 35% was achieved through flotation.

Bagasse contained mostly solid natural rubber. The remaining natural rubber in the bagasse (left after the latex extraction) was extracted using sequential solvent extraction first with acetone and then with other nonpolar solvents. STS and SU contained mostly solid rubber. Gel content was found to be highest with SU. SEC of solid natural rubber from TKS has shown that the molecular weight is about 1.8×10^6 and that it contains less gel compared with TSR20

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