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Rapid Determination of *Taraxacum kok-saghyz* Rubber Content by Pyrolyzer Hyphenated with a Miniaturized Mass Spectrometer

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Abstract: *Taraxacum kok-saghyz*, a rubber-producing plant with excellent potential, emerges as a viable substitute for rubber tree (*Hevea brasiliensis*). While natural rubber is a desirable material, conventional techniques for assessing rubber content have faced challenges in meeting practical production requirements. To address this issue, we have developed a pyrolysis-mass spectrometry (PY-MS) instrument for the quantitative evaluation of natural rubber (NR) content in rubber-producing plants. The derived standard curve equation, established through external standard calibration, demonstrates a correlation coefficient (R^2) surpassing 0.99. The method exhibits commendable recovery rates (93.27%-107.83%), relative standard deviations (RSD≤ 3.39%), and a swift analysis time of merely 10 minutes per sample, thereby enabling accurate and efficient quantification of NR. This approach possesses significant potential for extensive utilization in the assessment of rubber content in rubber-producing plants other than TKS. The integration of pyrolysis-mass spectrometry for the identification of polymers with high molecular weight offers a valuable pathway for the examination of diverse polymers.

Keywords: *Taraxacum kok-saghyz*; natural rubber; quantitative; pyrolysis-mass spectrometry

1. Introduction

Natural rubber (NR) is an irreplaceable raw material for industrial production and a strategic material for international trade[1,2]. The primary source of NR is the rubber tree (*Hevea brasiliensis*), but increased worldwide demand necessitates that alternative sustainable sources are urgently required[3,4]. *Taraxacum kok-saghyz* (TKS), a member of the Taraxacum genus within the Asteraceae family, is primarily distributed in Kazakhstan and the Tianshan Valley-Tex River Basin in Xinjiang, China[5]. The roots of TKS contain rubber with a mass fraction ranging from 2.89% to 27.89%[6–8], characterized by a high molecular weight exceeding 10^5g/mol[9–11]. The NR derived from TKS exhibits exceptional quality, demonstrating strong resistance against bacteria and pests, low allergenicity, and emerges as a highly promising alternative to *Hevea brasiliensis* for rubber production[12]. China has been the world's largest NR consumer for several years. However, China's domestic NR production capacity can only meet less than half of the domestic market demand. In the wake of a scarcity in NR resources, there emerges a pronounced dependence on imports. The cultivation of TKS holds significant potential in addressing the significant shortage of NR resources in China[13].

The detection of NR content in TKS is fundamental for the development of TKS resources, playing a crucial role for selection, breeding, and industrialization. Traditional methods for assessing natural rubber content, such as the differential weight method based on solvent extraction[14–16], alkali boiling method[17], and gel chromatography[18], are time-consuming, inaccurate, costly, and environmentally unfriendly. These drawbacks have rendered them inadequate for meeting the evolving needs of modern TKS development of NR related industrial.

Analytical pyrolysis is a technique involving the rapid heating of a sample in a well-controlled environment, inducing the swift breakdown of the sample into volatile small molecular fragments. In the field of rubber engineering, the application of analytical pyrolysis involves the comprehensive and systematic analysis of polymer materials through the combination of pre-treatment extraction methods and modern analytical techniques such as gas chromatography and/or mass spectrometry (PY-GC/MS)[19,20]. Portable mass spectrometers with small sizes and easily selectable quadrupoles are increasingly employed for on-site rapid analysis, establishing themselves as one of the most competitive in-field sampling techniques.

The coupling of pyrolysis-mass spectrometry (PY-MS) is a challenging method in mass spectrometry that combines online sample pre-processing, however, because of the ease of less pretreatment and the excellence of quantitation capacity, PY-MS coupling has broad market and application prospects in various fields[21], including polymer analysis, environmental science, food science, organic geochemistry, energy science, forensic analysis, and tobacco science.

In recent years, PY-MS technology has been employed for the qualitative and quantitative analysis of high molecular weight polymers such as microplastics[22]. Researchers have explored the pyrolysis products and mechanisms of NR, demonstrating the reliability of analytical pyrolysis for structural analysis and determination of rubber[23]. Utilizing PY-GC/MS, Shinya et al[24,25]. conducted the first quantitative study of rubber in plant leaves, determining the content of polyisoprene and polybutadiene and correlating the release of isoprene and limonene to their molecular weights. Our team applied PY-GC/MS to quantify NR in TKS, establishing a stable and environmentally friendly method based on the linear relationship between the peak area of the main pyrolysis product, limonene, and the injected rubber standard quantity[26]. However, mobile pyrolysis-GC/MS instruments incur high maintenance costs and an additional separation step, resulting in increased detection times. In contrast, PY-MS with no need of chromatographic separation allows direct analysis through membrane inlet mass spectrometry, with characteristic quantifiable ions facilitating the quantitative determination of NR content in plants promptly.

In this study, we independently developed a mass spectrometer (MS) and designed a pre-filtration device, successfully integrating the pyrolysis instrument as PY-MS. Utilizing the PY-MS instrument, we established a novel method for the quick and precise quantification of TKS rubber content. The results indicate that the method's standard curve has an R^2 value exceeding 0.99, demonstrating high recovery rates and low relative standard deviations (RSD). This method proves suitable for rapid, high-throughput detection, offering a new avenue for the quantitative determination of rubber content in TKS.

2. Materials and methods

2.1. Materials

The samples utilized in this research were gathered from the TKS plantation base in Liaoyuan County, Jilin Province, China. The collected samples from the TKS plantation were wiped clean of surface mud, dried in a forced-air drying oven at 60 °C for 24 hours, and subjected to constant weight to remove moisture. Subsequently, the TKS dry root samples were pulverized into a powdered state using a ball mill, sealed in plastic bags, and stored in a dry environment for use as an extraction raw material for high-purity standard rubber samples or a thermal pyrolysis mass spectrometry detection sample.

The employed chemical reagents, namely acetone, toluene, and ethanol, were all of analytical grade quality and purchased from Tongguang Fine Chemicals Co., Ltd., Beijing, China.

2.2. Preparation of TKS rubber reference samples

The high-purity TKS natural rubber extracted in-house is regarded as a proprietary standard reference sample, because of the absence of commercially available standard reference materials for

TKS. Plant pigments, resins, and other polar compounds are readily soluble in highly polar solvents, whereas TKS rubber is easily soluble in non-polar compounds. According to this principle, a two-step Soxhlet extraction method can be employed to extract and prepare reference samples of TKS rubber.

Step 1: Weigh the TKS powder and transfer it into a custom-made filter paper sleeve, ensuring that the filter paper holder is filled to approximately one-third of its total volume. Silica gel, at a volume of 2~3 times the volume of the filter paper sleeve, was added to increase the contact area of TKS powder, thereby enhancing the extraction efficiency. The mixture in the filter paper sleeve was uniformly blended and placed in a 150 mL Soxhlet extractor. Utilizing the principle of like dissolves like, acetone, a highly polar solvent, was initially used to extract impurities such as pigments and resins from TKS powder. The impurities imparted a yellow color to the solution, and the reaction was allowed to proceed for approximately 8 hours or until the solvent exhibited no significant change in color. Subsequently, the filter paper sleeve was removed and placed in a forced-air drying oven at 60°C until a constant weight was achieved.

Step 2: The filter paper sleeve containing the mixed sample was then placed in a 150 mL Soxhlet extractor, and toluene, a low-polarity solvent, was used to extract TKS rubber for approximately 8 hours until the solution became colorless and transparent. The toluene-extracted flask was then placed in a rotary evaporator to concentrate the extraction to a volume of approximately 5 mL, which was transferred to a 50 mL beaker. Ethanol, at approximately 5 times the volume of the solution, was added to precipitate TKS rubber. The supernatant was decanted, and the precipitated TKS rubber was dissolved in toluene, re-precipitated with ethanol, and the process was repeated until the precipitate solution became clear and transparent, yielding a milky-white TKS rubber. The precipitate was dried in a drying oven at 40°C until a constant weight was obtained, resulting in the TKS rubber reference sample. This reference sample can be used for subsequent quantitative analysis using the instrument standard curve method and instrument calibration.

2.3. Experimental apparatus

The PY-MS used in the experiment consists of a PY3030S single-shot thermal cracking device (Frontier, Tokyo, Japan). The instrument has good reproducibility and can enable stable thermal decomposition of TKS powder into volatile gases and solids at high temperatures. The pyrolyzed products passes directly and enters the mass spectrometer system with a small online quadrupole mass spectrometer (Shanghai Limu Scientific Instrument Co., Ltd., Beijing, China). The membrane injection material of the MS system is polydimethylsiloxane (PDMS), and this semipermeable membrane separates isoprene and limonene from the gas phase system, enabling mass spectrometry analysis of complex samples without chromatographic separation.

In order to prevent incomplete vaporized or condensed solid particles from contaminating the mass spectrometer, we have designed a removable and cleanable metal pre-filter in front of the mass spectrometer entrance (Figure 1), which can effectively protect the mass spectrometer from contamination and facilitate disassembly and cleaning.

The best working condition parameters of the instrument: Pyrolysis conditions: pyrolysis temperature 550°C, injection port temperature 300°C. Mass spectrometry conditions: combined insulation temperature 80°C, inlet membrane temperature 70°C, ion source temperature 120°C, filament electron energy -65eV, filament current feedback 1.2A, repeller voltage 5V, ion lens 1 voltage -10V, ion lens 2 Voltage -120V, ion lens 3 voltage -18V; The multiplier voltage is −900 V, the high-end compensation voltage is 1.185 V, the low-end compensation voltage is 0.175 V, and the four-pole floating voltage is −10.00 V; The carrier gas type is helium, the carrier gas flow rate is 50 mL/min, the vacuum environment is stable at 2×10−6 Pa, and the entire sampling process takes 10 min.

The temperature controller for the pyrolysis reactor has dimensions of 12×31×31cm, while the mass spectrometer measures 45×30×30cm.

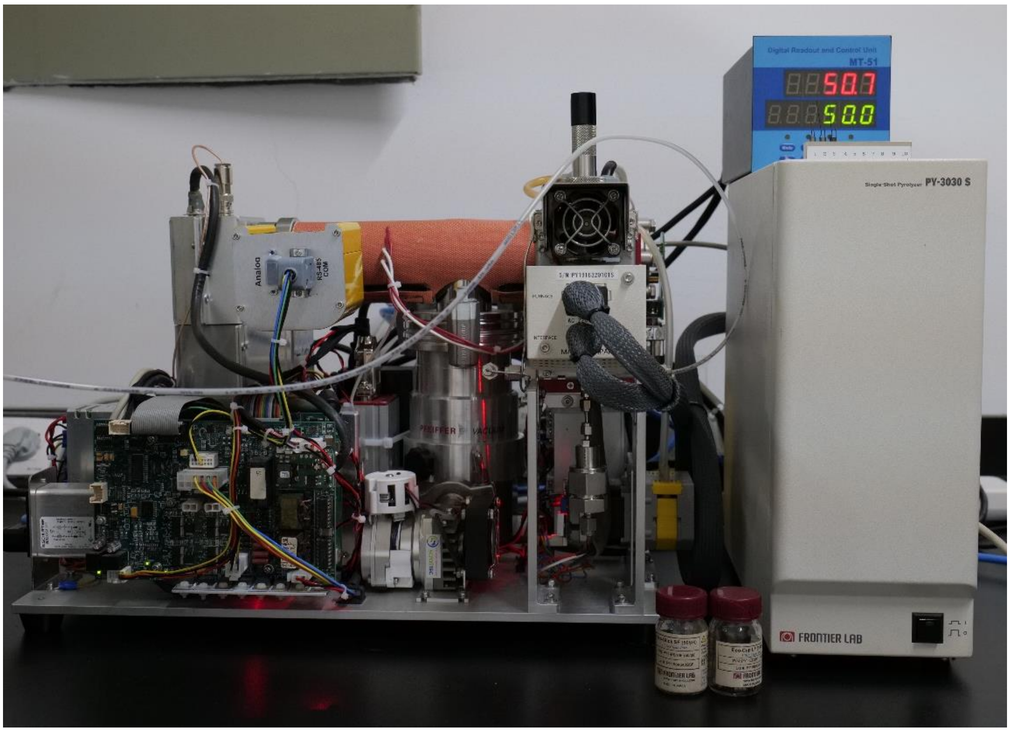

Figure 1. PY-MS instrument and pre-filter structure.

3. Results and discussion

3.1. Detection principle

A PY-MS was used to quantify the NR content in TKS. Thermal decomposition of TKS generates a substantial amount of isoprene monomer and limonene dimer, resulting in the production of a high-intensity ion with m/z 68 in the mass spectrum. Consequently, m/z 68 is selected as the quantification characteristic ion for TKS (Figure 2). The linear relationship is observed between the rubber mass of TKS and the peak area of the quantitative characteristic ion under identical PY-MS conditions.

Employing an external standard method, Initially, TKS rubber reference samples of varying masses are prepared for sampling, and the peak area of the quantitative characteristic ion produced in the mass spectrometer for each reference sample is calculated. A TKS quantitative calibration curve is plotted with sample mass as the abscissa. Subsequently, a variety of TKS real samples are tested using PY-MS. Finally, the rubber content in TKS is calculated by substituting the peak area data into the calibration equation.

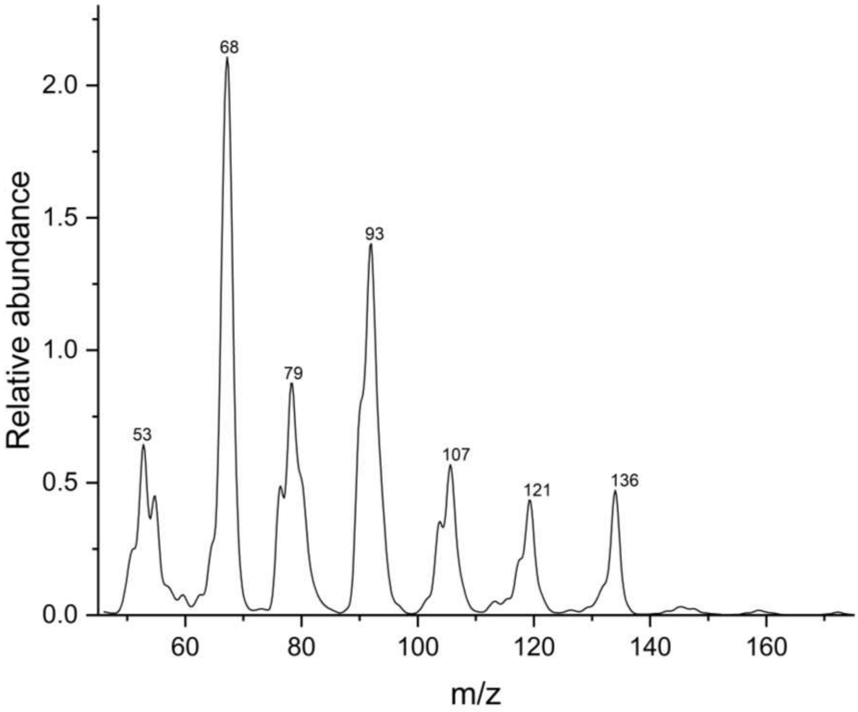

Figure 2. PY-MS spectrum of TKS reference sample.

3.2. Establish standard quantitative curve

According to the linear relationship between the mass of rubber in TKS and the peak intensity and area of quantitative characteristic products, a quantitative relationship standard curve was established.

Weigh 12.35 mg of the above TKS rubber reference sample into a 10 ml volumetric flask, dilute to volume with toluene, and the calibrator concentration is 1.235 mg/mL. Use a pipette to measure 10, 20, 40, 170, 200, 250, and 400 μL of the calibrator solution into the thermal cracking sample cup, and place it in a drying oven at 40°C to dry until the toluene solvent completely evaporates. The known contents of reference samples in the lysis sample cups are 12.35, 24.70, 49.40, 209.95, 247.00, and 494.00 μg respectively.

Put the above-mentioned dried thermal cracking sample cups into the thermal cracking injection port in sequence, perform thermal cracking-mass spectrometry membrane injection method detection, and obtain the corresponding mass spectrum. Extract the ion peaks with mass-to-charge ratios of 67-68, and calculate the peak areas respectively. Draw a scatter plot of TKS rubber injection volume and peak area, and perform linear fitting to obtain a linear relationship between the peak area corresponding to the ion at m/z68 of the thermal cracking characteristic product of TKS rubber and the reference rubber injection volume of the thermal cracking cup. $y = 0.01134x + 0.6995$, the correlation coefficient R^2=0.996 (Figure 3).

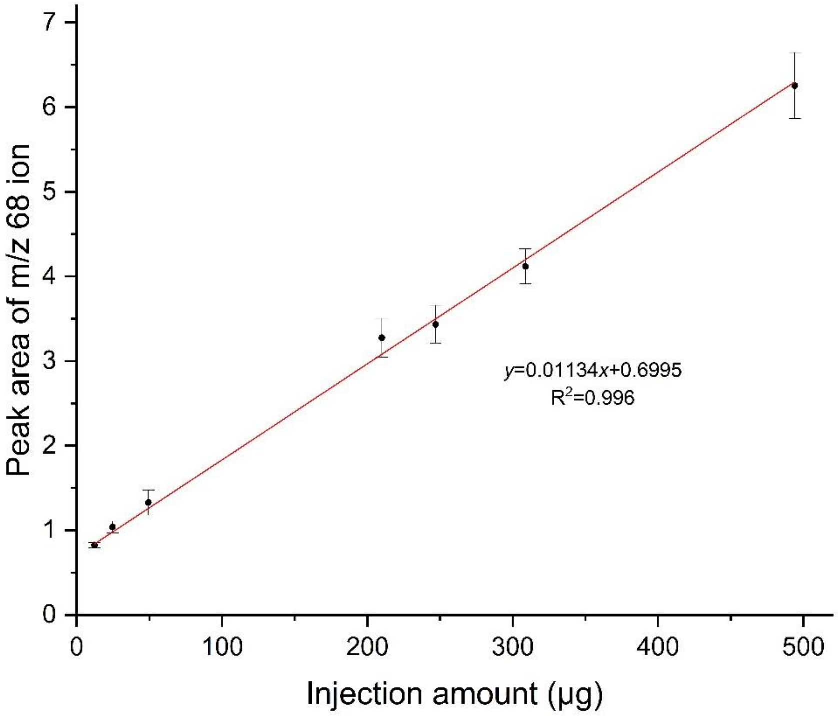

Figure 3. Calibration curve based on m/z 68 ion peak area.

3.3. Limit of detection and recovery experiment

The signal which corresponds to three times the peak noise blank SD is utilized to compute the detection limit (limit of detection, LOD). It refers to an important indicator of the sensitivity or detection capability of an analytical method. The response values of blank samples were measured ten times. The m/z 68 ion was extracted, and peak areas were calculated to obtain the standard deviation of peak area intensities. Subsequently, the method's LOD was determined by dividing three times the standard deviation by the slope of the calibration curve, resulting in an LOD of 0.639 μg/mg.

In order to further evaluate its analytical performance, recovery experiments with different weights were performed. These experiments were repeated 3 times. As shown in Table 1, the recovery (93.21%-107.83%) and RSD (≤ 3.39%) are satisfactory. The results show that the established portable PY-MS can be used for the simultaneous identification and quantification of natural rubber.

Table 1. Recovery results of TKS spiking experiment.

TKS spiked amount, μg	Confidence interval for peak area mean, 95%	Quantity recovered, μg	Recovery, %	RSD, %
50.00	1.60±0.11	53.92±7.36	107.83	2.70
150.00	2.86±0.28	139.82±19.14	93.21	3.93
250.00	4.60±0.27	259.08±18.68	103.63	2.39

3.4. Comparison with other methods

With the portable PY-MS prototype independently developed by our research team, we used PY-MS technology for the first time to achieve rapid and quantitative detection of rubber content in TKS. So far, all representative methods used for natural rubber detection have been listed in Table 2. Compared with this method, there is no other detection method that can simultaneously achieve high-throughput, rapid, accurate, lower-cost, and miniaturized qualitative and quantitative analysis and detection of natural rubber. Although plants may contain other small amounts of iridoids, isoprene monomers can also be produced after thermal cracking, but the content of these substances is relatively low compared to rubber and can be ignored. This method will provide new insights into natural rubber analysis.

Table 2. Summary of all representative natural rubber detection methods.

Method	**Equipment**	**Detection capability**	**Sample Type Suitability**	**References**
Solvent Extraction Method	Grinding, stirring, chemical extraction	Flow method (99.5%) and Blender method (approximately 75%) can be obtained Purity of latex.	TKS, *Scorzonera tau-saghyz, Scorzonera Uzbekistanica*	[14]
Soxhlet Extractor Method	Soxhlet extractor	Estimate of the variance for each variety mean 0.069 % rubber	Guayule	[15]
Accelerated Solvent Extraction	Ball mill grinding, evaporative light scattering	Linear range 0 to 2 mg/ml. $R^2 = 0.995$.	Guayule	[16]
Alkali Boiling Method	Alkali boiling and water milling	Rough estimate with <10% variance	TKS	[17]
Bromination Method	Spectrophotometric analysis with brominating solution	Variance within 3%	Natural rubber latex	[27]
Viscometry Method	Viscometry with temperature control	Linear correlation with weight ($R^2 = 0.94$) and IR ($R^2 = 0.99$) methods	Guayule plant material	[28]
Refractive Index Method	Refractive index measurement	Average deviation of 0.29%	Natural rubber latex	[29]
Gel Chromatography	Size Exclusion Chromatography	LOD is 0.58 mg/mL for leaves and 0.47 mg/mL for fruit; the linear range is 2–10 mg/mL for leaves and 0.5–10 mg/mL for fruit.	*Eucommia* leaves and fruits	[18]
IR Method	Fourier Transform Infrared Spectroscopy	LOD =12.5 μg, Linear range 25-200 μg	*Eucommia* rubber	[30]
NIR Method	Portable fiber optic NIR spectrometer	R^2= 0.95, ratio of performance to deviation (RPD)= 5.54	TKS fresh roots	[31]
NMR Method	Carbon-13 Nuclear Magnetic Resonance	±1.0% absolute accuracy, LOD 0.5% by weight	Guayule	[32]

PY-GC-MS Method	Thermal pyrolysis-gas chromatography-mass spectrometry detection	LOD=2.603 mg/g Linear ranged from 1.20% ±0.20% to 8.61% ±0.28%	TKS	[26]
PY-MS Method	Thermal pyrolysis and mass spectrometry	LOD=0.639 μg/mg. Linear range 10-500 μg/mg RSD ≤ 3.39%	TKS and theoretically other rubber-producing plants	This article

4. Conclusions

In this study, a portable PY-MS was developed successfully, marking the first introduction of PY-MS technique for determining rubber content in TKS. This technology enables high-throughput, rapid, and real-time screening of NR. The LOD for TKS is 0.639μg/mg, with recovery rates ranging from 93.27% and 107.83%, significantly reducing test time and operational complexity. The sensitivity and accuracy of this method are superior to other existing detection techniques, meeting the demands for precise and real-time quantitative assessment in the rubber analysis field. The work facilitates greatly the selection and breeding of TKS resources, aiding in the scaled-up industrial production of TKS consequently.

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