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

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Abstract: *Taraxacum kok-saghyz* (TKS), 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 for the detection of TKS dry weight 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 $\leq$ 3.93%), and a swift analysis time of merely 10 min per sample, thereby enabling accurate and efficient quantification of NR dry weight. Additionally, the PY-MS system we designed can be modified for vehicular use, enabling on-site, in situ analysis, and it provides substantial support for TKS breeding and propagation efforts. 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 analysis; pyrolysis–mass spectrometry

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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 indicates 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 10^5 g/mol [8–10]. The NR derived from TKS exhibits exceptional quality, demonstrating strong resistance against bacteria and pests and low allergenicity, and it emerges as a highly promising alternative to *Hevea brasiliensis* for rubber production [6]. 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 of NR resources, there emerges a pronounced dependence on imports. The cultivation of TKS holds significant potential for addressing the significant shortage of NR resources in China [11].

The detection of NR content in TKS is fundamental for the development of TKS resources by playing a crucial role in selection, breeding, and industrialization. Table 1 compares representative methods for detecting NR. Traditional methods for assessing NR content, such as the differential weight method based on solvent extraction [12–14], nuclear magnetic resonance (NMR) spectroscopy [15], infrared (IR) spectroscopy [16], near-infrared (NIR) spectroscopy [17], and pyrolysis gas chromatography–mass spectrometry (PY-GC-MS) [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. Therefore, it is necessary to develop new NR detection methods.

Analytical pyrolysis is a technique involving the rapid heating of a sample in a well-controlled environment, thus 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) [18,19]. Portable mass spectrometers with small sizes and easily selectable quadrupoles are increasingly employed for on-site rapid analysis, establishing them as one of the most competitive in-field sampling techniques.

In the field of environmental monitoring, the demand for on-site detection of high-polymer compounds, such as microplastics, has established pyrolysis technology as a key analytical tool [20,21]. The advancements in this domain, particularly the evolution from combined pyrolysis and spectroscopy analysis to the application of PY-GC/MS techniques, represent significant progress [22–26]. Recent studies have successfully employed PY-MS for the qualitative analysis and detection of microplastics and other high-polymer compounds [27]. This methodology leverages the strengths of both pyrolysis and mass spectrometry, thus allowing for the rapid and accurate analysis of the pyrolysis products of microplastics and thereby demonstrating substantial research and application potential. Notably, the similarities in sample morphology and application scenarios between microplastics and NR in TKS provide valuable insights and precedents for the application of PY-MS technology in the detection of NR. The exploration of these similarities not only enhances our understanding and utilization of PY-MS capabilities but also promotes further development and application of this technology in environmental monitoring and NR analysis fields.

In recent years, analytical pyrolysis technology has been employed for the qualitative and quantitative analysis of NR. Researchers have explored the pyrolysis products and mechanisms of NR, demonstrating the reliability of analytical pyrolysis for the structural analysis and determination of rubber [28]. Utilizing PY-GC/MS, Takeno et al. [18,29] 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. In previous research, 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 [30]. However, PY-GC-MS instruments incur high maintenance costs and require an additional separation step, resulting in increased detection times. In contrast, the direct coupling of pyrolysis and mass spectrometry technologies for NR in TKS detection has not yet emerged. A PY-MS detection instrument designed by our team does not require chromatographic separation. The instrument can directly analyze samples through a membrane inlet mass spectrometry (MS) approach. The identification of characteristic quantitative ions aids in the rapid and quantitative determination of NR content in TKS, thus significantly reducing detection time. This development will fill a gap in the field by providing a method for on-site, rapid, high-throughput, and accurate detection of NR content in TKS.

In this study, we independently developed a mass spectrometer and designed a pre-filtration device, thus 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. Furthermore, adapting the instrument for vehicular integration allows for in situ analysis. 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.

Table 1. Summary of all representative natural rubber detection methods.

Method	**Equipment**	**Detection Capability**	**Sample Type Suitability**	**References**
Chemical Extraction Method	Grinding, stirring	Flow method (99.5%) and Blender method (approximately 75%) can be obtained using the purity of latex	TKS, *Scorzonera tau-saghyz*, *Scorzonera Uzbekistanica*	[12]
Soxhlet Extractor Method	Soxhlet extractor	Estimate of the variance for each variety with a mean of 0.069% rubber	Guayule	[13]
Accelerated Solvent Extraction	Ball mill grinding, evaporative light scattering	Linear range from 0 to 2 mg/mL R^2 = 0.995	Guayule	[14]
Alkali Boiling Method	Alkali boiling and water milling	Rough estimate with <10% variance	TKS	[31]
Bromination Method	Spectrophotometric analysis with brominating solution	Variance within 3%	Natural rubber latex	[32]
Viscometry Method	Viscometry with temperature control	Linear correlation with weight (R^2 = 0.94) and IR (R^2 = 0.99) methods	Guayule plant material	[33]
Refractive Index Method	Refractive index measurement	Average deviation of 0.29%	Natural rubber latex	[34]
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	[35]
IR Method	Fourier transform infrared spectroscopy	LOD = 12.5 µg; linear range 25–200 µg	*Eucommia* rubber	[16]
NIR Method	Portable fiber optic NIR spectrometer	R^2 = 0.95; ratio of performance to deviation (RPD) = 5.54	TKS fresh roots	[17]
NMR Method	Carbon-13 nuclear magnetic resonance	±1.0% absolute accuracy, LOD 0.5% by weight	Guayule	[15]
PY-GC-MS Method	Pyrolysis–gas chromatography–mass spectrometry detection	LOD = 2.603 mg/g Linear range from 1.20% ± 0.20% to 8.61% ± 0.28%	TKS	[30]
PY-MS Method	Pyrolysis and mass spectrometry	LOD = 0.639 µg Linear range of 12–494 µg RSD $\leq$ 3.93%	TKS and theoretically other rubber-producing plants	This article

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 h, 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 reference rubber samples or a dry weight sample analyzed through PY-MS detection. The samples collected from the TKS plantation base were cleaned to remove surface mud. A small knife was used to excise approximately 5 mg of the fresh root section, which served as the sample for PY-MS analysis to determine the fresh weight of TKS.

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: The TKS powder was weighed and transferred into a custom-made filter paper sleeve while ensuring that the filter paper holder was 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 the TKS powder. The impurities imparted a yellow color to the solution, and the reaction was allowed to proceed for approximately 8 h 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 h 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 the TKS rubber. The supernatant was decanted, the precipitated TKS rubber was dissolved in toluene and 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 pyrolysis 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 pass directly and enter the mass spectrometer system with a small online quadrupole mass spectrometer (Shanghai Limu Scientific Instrument Co., Ltd., Beijing, China). The membrane inlet material for the mass spectrometry system is Polydimethylsiloxane (PDMS), a semipermeable membrane that isolates the volatilized gases produced from the pyrolysis of TKS samples from the vacuum system of the mass spectrometer. Characterized by its high permeability to small, volatile, non-polar molecules, PDMS is an ideal choice for this application. The selectivity of the membrane primarily depends on its hydrophobicity and porous structure. Hydrophobic membranes tend to repel water molecules while adsorbing substances with hydrophobic properties. Additionally, the porous structure of the membrane allows particles of a specific size to pass through while blocking larger particles [36,37]. This setup allows small molecules, such as isoprene and limonene, to pass through the membrane into the mass spectrometer for analysis, thereby reducing the steps required for sample pre-treatment. Chromatographic separation is not needed for mass spectrometric analysis of complex samples, thus enabling direct analysis of dissolved gases or volatile compounds in gas samples. To prevent contamination of the mass spectrometer by particles that have not been fully vaporized or have condensed, a detachable and cleanable metal pre-filter is designed at the inlet to the mass spectrometer. This effectively protects the mass spectrometer from contamination and facilitates easy disassembly and cleaning.

Figure 1 displays the schematic diagram of the PY-MS. The carrier gas transports the volatilized gases from the pyrolysis of the sample through the pre-filter device and the heated transfer line. Subsequently, through dissolution–diffusion, these gases enter the mass spectrometer. Mixtures that have not dissolved and remain on the membrane surface are expelled through a split system.

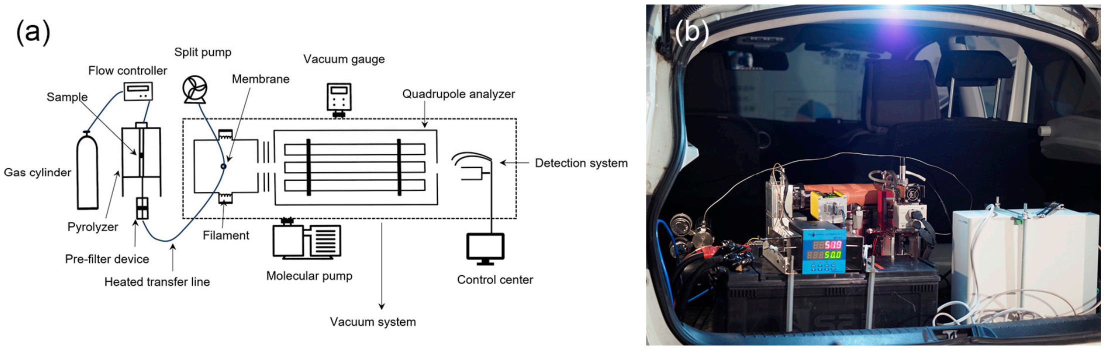

Figure 1. Schematic diagram of the structure of the PY-MS (**a**); the modified vehicle-mounted PY-MS (**b**).

The best working condition parameters for the instrument were the following. For pyrolysis conditions, the pyrolysis temperature was set at 550 °C. The pressure stabilization time was 20 s, followed by a cleaning duration of 10 s, and the pyrolysis process was conducted over 0.1 s. For mass spectrometry conditions, the insulation was kept at 80 °C, the membrane at the inlet was kept at 70 °C, and the ion source was kept at 120 °C. Electron energy from the filament was set to -65 eV, with a filament current feedback of 1.2 A. The repeller's voltage stood at 5 V, with ion lens settings at -10 V for lens 1, -120 V for lens 2, and -18 V for lens 3. The multiplier voltage reached -900 V; high-end and low-end compensation voltages were adjusted to 1.185 V and 0.175 V, respectively, and the four-pole floating voltage was -10 V. The carrier gas type was helium, the carrier gas flow rate was 50 mL/min, the vacuum environment was stable at 2×10^{-6} Pa, and the entire sampling process took 10 min.

The temperature controller for the pyrolysis reactor had dimensions of 12 × 31 × 31 cm, and the volume of the pyrolysis cup was 50 µL. The mass spectrometer measured 45 × 30 × 30 cm.

2.4. Vehicle Modification for PY-MS

To effectively address the significant technical challenge of rapidly detecting NR content in TKS on-site, we designed and customized a steel plate based on the sizes of the PY-MS instrument and the cargo space of the car. The plate securely mounts the PY-MS in the car's trunk using tools like sponge foam and cable ties. Additionally, a portable power supply and an 8 L helium gas tank were also fixed onto the steel plate by us. During field investigations, the vehicular instrument underwent rigorous testing under harsh conditions, including continuous long-distance transport, rough field terrain, and significant day-to-night temperature variations. Despite these challenges, the vehicular PY-MS instrument operated continuously with stable optimal parameters within the rubber grass cultivation base.

3. Results and Discussion

3.1. Principles of Detection Using the PY-MS Method

Upon the pyrolysis of natural rubber, the primary volatile gases produced, isoprene and limonene, accumulate on the membrane surface. Due to the permeability of the PDMS membrane to small organic molecules, all of isoprene and limonene can swiftly traverse the membrane. Other components, having concentrations much lower than isoprene and limonene, face difficulty passing through the membrane due to molecular size exclusion and face challenges in adsorption and desorption on the membrane surface within a short period, thus facilitating membrane separation. Consequently, in the mass spectrometer, the total ion current rapidly reaches its peak, which is predominantly attributed to isoprene and limonene. When the total ion current peaks, a strong fragment ion peak is observed at m/z 68. As a result, m/z 68 is selected as the quantitative 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.

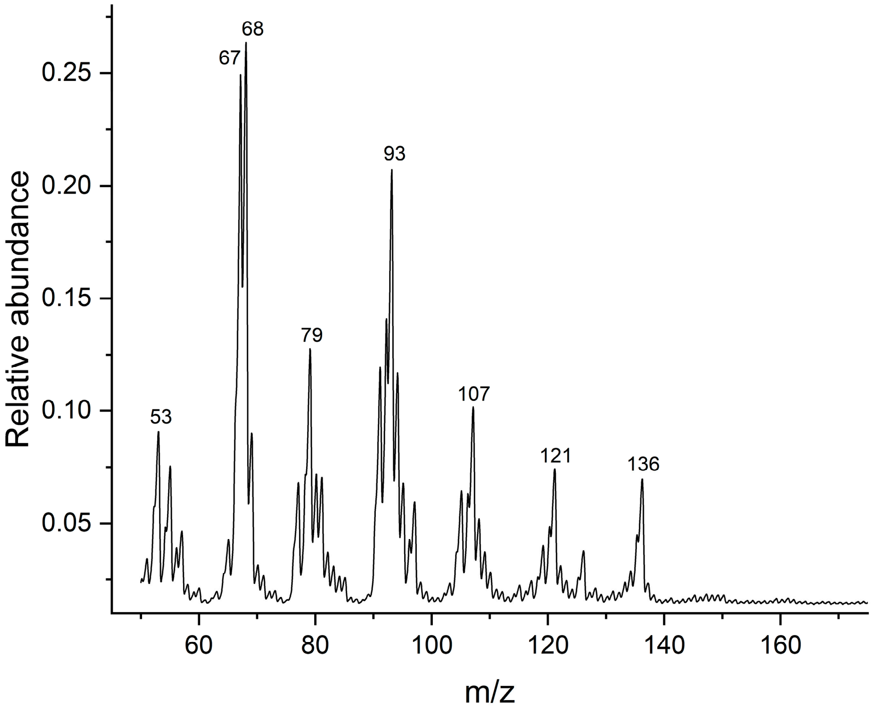

Figure 2. PY-MS spectrum of TKS reference sample.

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.

3.2. Establishing Standard Quantitative Curves for the PY-MS Method

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.

We weighed 12.35 mg of the above TKS rubber reference sample into a 10 mL volumetric flask and diluted it to volume with toluene, and the calibrator concentration was 1.235 mg/mL. We used a pipette to measure 10, 20, 40, 170, 200, 250, and 400 µL of the calibrator solution into the pyrolysis sample cup and placed it in a drying oven at 40 °C to dry until the toluene solvent completely evaporated. The known contents of reference samples in the pyrolysis sample cups are 12.35, 24.70, 49.40, 209.95, 247.00, and 494.00 μg, respectively. We repeated the measurement three times.

We put the above-mentioned dried pyrolysis sample cups into the pyrolysis injection port in sequence, performed pyrolysis–mass spectrometry membrane injection method detection, and obtained the corresponding mass spectrum. Using the manufacturer-provided software, we extracted the ion peaks with mass-to-charge ratios of 67–68 and calculated the peak areas. We drew a scatter plot of TKS rubber injection volume and peak area and performed linear fitting to obtain a linear relationship between the peak area corresponding to the ion at m/z 68 of the pyrolysis characteristic product of TKS rubber and the reference rubber injection volume of the pyrolysis cup. $y = 0.01134x + 0.6995$, and the coefficient of determination $R^2 = 0.996$ (Figure 3).

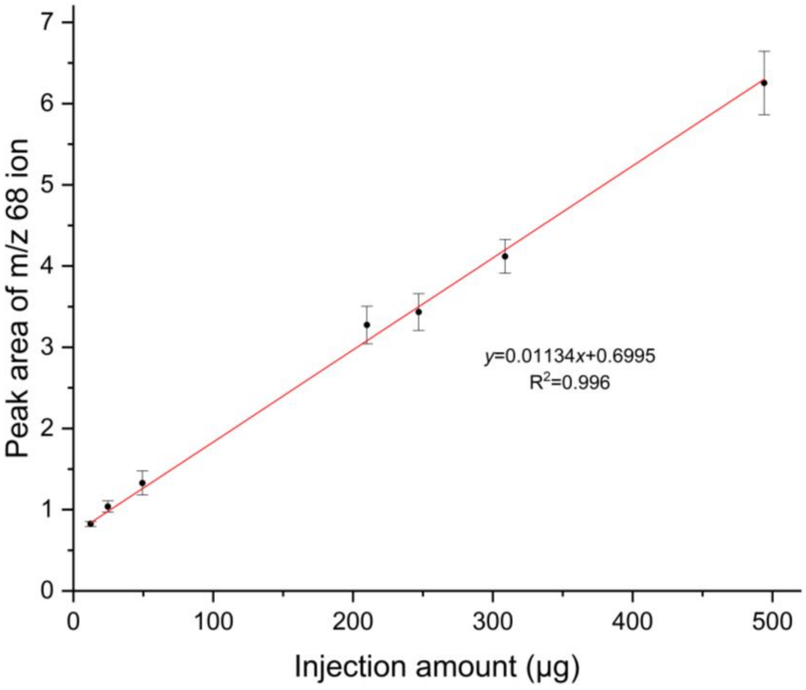

Figure 3. Calibration curve based on m/z 68 ion peak area.

3.3. Assessment of Detection Limits and Recovery in TKS Dry Weight Samples

The detection limit (limit of detection, LOD) is calculated based on the signal that is three times the standard deviation (SD) of the peak noise blank. It refers to an important indicator of the sensitivity or detection capability of an analytical method. The blank sample refers to a clean pyrolysis cup to which no sample has been added. 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.

In order to further evaluate its analytical accuracy, recovery experiments with different dry weights were performed. Recovery refers to the amount calculated based on the calibration curve and compared with TKS reference samples added. These were achieved by spiking a blank sample with TKS reference material at three amount levels (specifically, adding precise amounts of 50 μg, 150 μg, and 250 μg of TKS reference samples). PY-MS analysis was then performed. These experiments were repeated three times. As shown in Table 2, the recovery (93.21–107.83%) and RSD ($\leq$3.93%) are satisfactory. The results show that the established portable PY-MS can be used for the simultaneous identification and quantification of natural rubber.

Table 2. 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. Qualitative Analysis Results of TKS Seeds via PY-MS

TKS seeds were also subjected to qualitative analysis using PY-MS. TKS seeds from different regions were placed in the pyrolysis cup and introduced into the PY-MS. The resulting mass spectra, as shown in Figure 4, reveal that the mass spectrum of TKS seeds closely resembles that of natural rubber, but it differs in certain aspects when compared to the mass spectrum of the TKS reference material, as shown in Figure 2. Notably, the mass spectrum of TKS seeds lacks the characteristic peak of the isoprene ion at m/z 68, which is present in the spectrum of the TKS reference material shown in Figure 2. And, more importantly, throughout the process before and after sampling, the ion peak intensity at m/z 136 remained nearly unchanged. Consequently, we witnessed the absence of a characteristic ion peak at m/z 136, which would indicate dimeric limonene. Additionally, the ratio of the peaks for ions with m/z 67 and 68 in Figure 4 is approximately 0.95, which is different from the ratio in Figure 2, where it is approximately 1.74. Comprehensive analysis of the TKS seed mass spectrum reveals that under the condition of solely utilizing the PY-MS detection method, characteristic rubber ion peaks may not be present in TKS seeds. This suggests that TKS seeds might not inherently contain cells responsible for rubber synthesis. Instead, these cells are likely develop during the germination and subsequent growth of the plant. This conclusion reinforces our understanding of growth, development, and biosynthesis of NR in TKS [38]. However, it may necessitate additional verification using alternative detection methods.

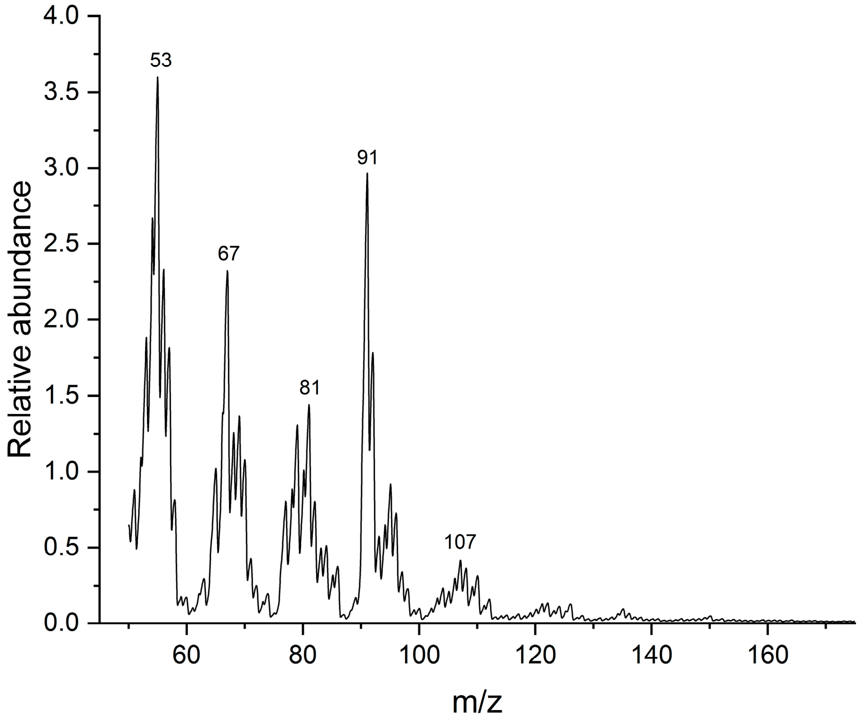

Figure 4. PY-MS spectrum of TKS seed sample.

3.5. Quantitative Analysis of NR Content in TKS Fresh Root Samples Using Vehicle-Mounted PY-MS

Field-based, vehicle-mounted PY-MS analysis of fresh roots faces more challenging control conditions compared to the stable and reproducible environment provided for dry weight samples in the laboratory. While the inherently non-reproducible nature of fresh samples might compromise some precision, it allows for in situ detection and real-time assessment of NR content, which is crucial for searching for high-yielding TKS plants.

Taraxacum officinale (TO) and TKS are both members of the dandelion genus, yet they represent distinct species. TO is the most commonly encountered species of dandelion, widely recognized as a ubiquitous wild plant in many regions. Samples from 24 different dandelions (TO and TKS) from different growth times (half-year-old and one-year-old) and from different cultivation sites (fields and greenhouses) were analyzed using the vehicle-mounted PY-MS. Relevant information is listed in Table 3. Fresh TKS samples were harvested directly from the soil, ensuring a root area of approximately 1–2 cm was thoroughly cleaned. A narrow cross-sectional segment, with a mass not exceeding 5 mg, was then promptly sectioned from the TKS root utilizing a precision scalpel. Subsequently, the fresh samples were placed in the pyrolysis cup and weighed. The mass of the TKS fresh roots was recorded, followed by direct vehicle-mounted PY-MS analysis. After pyrolysis, the peak area of the pyrolysis product at m/z 68 was inserted into the standard curve equation to calculate the NR content in the fresh samples. This allowed for further calculation of the mass percentage of NR content in fresh samples sampled on site. The results of the NR content as mass fraction percentage are shown in Figure 5. The variation in NR among TKS individuals grown in greenhouses is significant, which may be related to genetic factors. Identifying high-yielding rubber TKS is also the ultimate goal of our experiment.

Table 3. TKS and its rubber content information detected using the vehicle-mounted PY-MS.

Number	Dandelions	Growth Time	Cultivation Sites	Rubber Content, %	Average Rubber Content, %	Standard Deviation
1	TO	half-year-old	field	2.88	2.82	0.35
2	TO	half-year-old	field	2.36		
3	TO	half-year-old	field	2.87		
4	TO	half-year-old	field	3.19		
5	TO	one-year-old	field	3.25	4.00	1.07
6	TO	one-year-old	field	4.76		
7	TO	half-year-old	greenhouse	5.35	4.67	0.96
8	TO	half-year-old	greenhouse	3.98		
9	TO	one-year-old	greenhouse	5.88	6.08	0.29
10	TO	one-year-old	greenhouse	6.29		
11	TKS	half-year-old	field	4.48	5.48	1.40
12	TKS	half-year-old	field	6.47		
13	TKS	one-year-old	field	10.18	9.83	0.67
14	TKS	one-year-old	field	9.06		
15	TKS	one-year-old	field	10.26		
16	TKS	half-year-old	greenhouse	7.51	7.80	0.91
17	TKS	half-year-old	greenhouse	6.78		
18	TKS	half-year-old	greenhouse	8.96		
19	TKS	half-year-old	greenhouse	7.95		
20	TKS	one-year-old	greenhouse	17.00	10.65	4.60
21	TKS	one-year-old	greenhouse	11.26		
22	TKS	one-year-old	greenhouse	12.31		
23	TKS	one-year-old	greenhouse	7.73		
24	TKS	one-year-old	greenhouse	4.93		

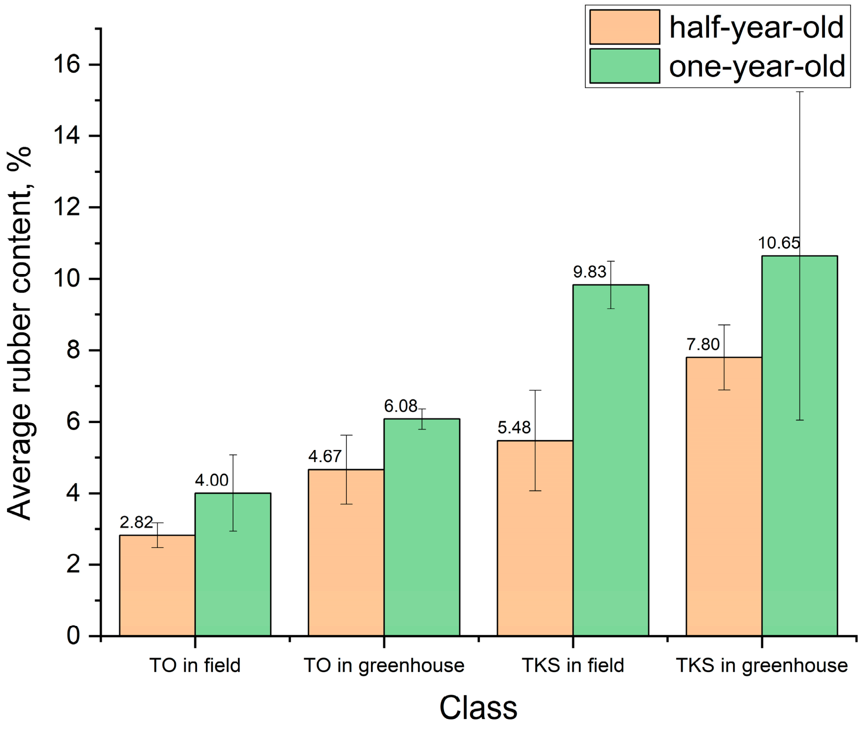

Figure 5. Bar chart of natural rubber average content variations in dandelion genus plants over growth periods and cultivation locations.

The results indicate that one-year-old TO or TKS has a higher average NR content than half-year-old ones. This is consistent with Bonner's [39] findings that NR in plants accumulates over time. From a cultivation perspective, greenhouse-grown TO and TKS showed a higher average rubber content than field-grown ones, which might be related to the well-controlled conditions in greenhouses, as environmental factors, such as sunlight, temperature, humidity, and extreme weather, can be controlled more precisely in greenhouses. Such settings offer reduced exposure to external environmental stressors, providing more ideal conditions for plant growth. In terms of variety, although TO contains NR, the average NR content in TKS is higher, with greenhouse-grown TKS having an average NR content exceeding 10% by fresh weight according to Table 3. This is similar to the average content statistics for TKS cultivated in other countries [40,41].

Given that the majority of TKS NR is concentrated in the thick roots within 2 cm of the root base, with less in fine roots far away from the root base, collecting only a portion of the fresh roots from a single TKS plant to represent the entire plant's content seems to overestimate the result. The presence of moisture in fresh TKS samples may potentially lead to lower-than-actual results. However, this does not diminish the significance of direct fresh root sampling as a breakthrough method for field investigations and rapid, relatively accurate real-time assessment of TKS fresh root NR content.

3.6. Comparison with Other Methods

With the miniaturized PY-MS prototype independently developed by our research team, we have for the first time utilized PY-MS technology for the rapid qualitative and quantitative analysis of NR content in both TKS dry weight and fresh root samples. So far, all representative methods used for natural rubber detection have been listed in Table 1. 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. Additionally, this method is also applicable to the quantification of rubber content in other rubber-producing plants [19]. Although plants may contain other small amounts of iridoids, isoprene monomers can also be produced after pyrolysis, 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.

4. Conclusions

In this study, a portable PY-MS was successfully developed, marking the first introduction of a 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 0.639 µg, with recovery rates for TKS dry weights ranging from 93.27% and 107.83%, and RSD less than 3.93%, significantly reducing test time and operational complexity. The sensitivity and accuracy of this method are superior to other existing detection techniques. Moreover, our modified vehicle-mounted PY-MS is the first to achieve on-site qualitative and quantitative analysis, meeting the demand for precise and real-time quantitative assessments in the field of rubber analysis. This work greatly facilitates the selection and breeding of TKS resources, aiding in the scaled-up industrial production of TKS.

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