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Accurate quantification of guayule resin and rubber requires sample drying below a critical temperature threshold

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ABSTRACT

To accurately quantify secondary products in plant materials, using solvent extraction, harvested plant materials must be dried and ground without altering the amount and, preferably, the composition of the compounds of interest. We examined the effect of pre-extraction drying temperatures and times on the acetone and hexane-extractable components of guayule, resin and rubber, respectively. Drying guayule samples at 50 °C prior to extraction did not alter subsequent extractions of resin and rubber. However, drying temperatures of 75 °C, and above, degraded guayule rubber into acetone-soluble fragments. The total amount of acetone and hexane extractable material remained constant up to 150 °C. However, the acetone and hexane soluble materials were progressively volatilized at 200 °C. We predict that the sensitivity of secondary products to drying temperatures in not confined to guayule and suggest that plant drying temperatures should be carefully evaluated for their impacts on the quantity and composition of analytes.

1. Introduction

Harvested plant materials are frequently dried and ground before extraction of compounds of scientific interest. However, this must be done without altering the endogenous concentrations or composition of such compounds. Some compounds, such as guayule rubber, are known to be subject to thermal and oxidative degradation (Schloman et al., 1996a; Cornish et al., 2009). In simultaneous extraction methods, the resin fraction in the rubber-producing shrub, guayule (*Parthenium argentatum* Gray), will dissolve in the organic solvents needed to extract high molecular weight rubber, and the rubber and resin fractions then need to be separated before quantification (Cornish and Schloman, 2004; Schloman et al., 1983). A simpler sequential extraction procedure is often used, where the resin is first extracted in acetone, in which high molecular weight rubber is insoluble, followed by rubber extraction using a suitable strong organic solvent, such as hexane, cylcohexane, or tetrahydrofuran (Cornish et al., 2009; Coffelt et al., 2009; Black et al., 1983).

Previous research has shown that pre-extraction sample preparation and management can have a significant effect on rubber determination (Schloman et al., 1996a). For example, sample particle size, sample settling during subsampling, and proper loading of the sample into the extraction cell can have significant effects on rubber extraction and quantification using accelerated solvent extraction under gaseous nitrogen (Pearson et al., 2010; Salvucci et al., 2009). In addition, we have found that hot acetone extraction temperatures used in Soxhlet extractions performed in air, can cause high molecular weight rubber to be degraded into acetone-soluble fragments (Cornish et al., 2009; Schloman et al., 2006, 1996b; Stumpf et al., 2001) and we hypothesize temperatures that are too high during pre-extraction will adversely affect subsequent resin and natural rubber extractions. When pre-extraction temperatures are too high the resin content may be overestimated and the rubber content underestimated.

Well characterized, stable and uniform sample preparation conditions are essential for many endeavors, including plant breeding and processing plant optimization. The objective of this study was to investigate the effect of pre-extraction heating on the apparent resin and rubber contents of guayule samples.

2. Materials and methods

2.1. Plant material

Heating Experiment #1: Dry guayule bagasse samples were obtained from the Yulex Corporation processing facility in

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Maricopa, AZ. Freshly harvested 3–4 year-old plants were partially defoliated and then ground into homogenate in aqueous hydroxide. After latex extraction, the residual bagasse was pressed to remove excess liquid then dried outdoors under ambient temperatures. Guayule plant tissue samples were stored at room temperature until they were ground. Samples were ground for 90 s in a Wiley mill (Standard Model no. 3, Arthur H. Thomas Co., Philadelphia, PA) using a 2-mm mesh screen.

Heating Experiment #2: Two year-old guayule plants, line AZ2, were hand-harvested from a commercial field at Eloy, Arizona, by clipping approximately 3 in. above soil level. Plants were immediately bagged to prevent dehydration, and chipped using a hammer mill with a 1/4 in. screen. The chipped material was bagged and shipped overnight to Fruita, Colorado. The chipped material was dried at 50 °C in air to a moisture content of 8% as determined by gravimetric calculations. The dried ground plant material was then ground for approximately 90 s in a Wiley mill using a 2-mm mesh screen. These plants were not defoliated before chipping.

Heating Experiments #3 and #4: Guayule (line AZ2) samples were collected from the Yulex processing plant in Maricopa, Arizona, in the summer and the autumn of 2008 at a stage where the plants had been chopped and partially defoliated but before latex extraction. These materials were chipped, shipped, ground, and dried as described for the hand-harvested plants above. Plant Sample #1 was sampled (~5 kg) from 3574 kg (7880 lb) of three-year old shrub, mechanically harvested from an AZ2 field grown in Urrea, Arizona by LKH Farms. The shrub was transplanted into the field on June 7, 2005 and harvested July 31, 2008. Plant Sample #2 was sampled from 17,754 kg (39,140 lb) of three-year old shrub mechanically harvested from field-grown AZ2 on the Colorado River Indians Tribes, by CRIT II Farm in western Arizona. The shrub was transplanted on May 31, 2005 and harvested on September 15, 2008.

2.2. Temperature × time studies

Ground guayule samples were thoroughly mixed and then subsampled. Samples were exposed to 200 °C for 0, 4 and 12 h, with four samples per treatment. Approximately 16 g of ground guayule per subsample was put into individual bun tin wells. Heat was applied using a 70.8 L volume capacity gravity convection oven (Precision Econotherm Oven, model 6530, Thermo Electron Corp, Marietta, OH).

Following heating in the oven, samples were allowed to cool sufficiently to allow for hand-loading into 11 ml extraction cells. Four subsamples of each experimental sample were analyzed. Loaded extraction cells were maintained at room temperature for 3–16 h until accelerated solvent extraction with an Accelerated Solvent Extractor (ASE, Model 200, DIONEX Corporation, Sunnyvale, CA) was initiated. Samples accumulated additional varying time at room temperature while they waited in queue for analysis on the ASE 200 (see Section 2.3).

Experiment #2: Two-year-old foliated guayule shrub.

Ground guayule shrub samples were subjected in quadruplicate to 50 °C, 100 °C, 115 °C, 135 °C, 150 °C and 200 °C for 0, 0.25, 0.50, 1, 2 and 4 h following a similar protocol described in Heating Experiment #1.

Experiment #3: Three-year-old summer-harvested processing plant chopped and partially defoliated guayule.

Ground partially defoliated shrub samples from Plant Sample #1 harvested on July 31, 2008, underwent the same experimental protocol as described in Experiment #2.

Experiment #4: Three-year-old summer and autumn-harvested processing plant chopped and partially defoliated guayule.

Ground subsamples from Plant Sample #1 harvested on July 31, 2008 and from Plant Sample #2 harvested on September 15, 2008 were heated in quadruplicate for up to 72 h at either 50 °C or 75 °C.

2.3. ASE extraction

At the end of the heat treatments, resin and rubber were sequentially extracted from plant tissue samples using the ASE 200 equipped with a Solvent Controller (DIONEX Corporation, Sunnyvale, CA). The solvent controller accommodates automated extractions with up to four different solvents. The ASE 200 was programmed and operated using a personal computer loaded with AutoASE 2.20 software (DIONEX Corporation, Sunnyvale, CA). All extractions with the ASE 200 were at 1500 psi. New Whatman 19.8 mm cellulose filters (VWR International, West Chester, PA) were inserted into 11 ml stainless steel extraction cells each time prior to loading samples into extraction cells.

New 40 ml TraceClean™ collection vials (VWR International, West Chester, PA) were used for collecting analyte. Collection vials equilibrated in a desiccation chamber for at least 3 h. The total time in the desiccation chamber was recorded. Vials were removed from the chamber one at a time and weighed to the fourth decimal place. The desiccant was anhydrous calcium sulfate (Drierite, W.A. Hammond Drierite Company Ltd., Xenia, OH).

Approximately 1.45 g of sample was weighed to the fourth decimal place and approximately 1.5 g of Ottawa inert sand (20–30 mesh Ottawa inert sand standard, Fisher Scientific) was mixed with the sample. Once samples were loaded into the 11-ml stainless steel extraction cells any space in the extraction cell was filled with inert sand. One blank cell was included with each ASE run (an extraction cell filled with inert sand only). Each ASE run consisted of 13 extraction cells containing twelve tissue samples and a blank. Acetone (VWR International, West Chester, PA) extractions were at 100 °C and hexane and cyclohexane (VWR International, West Chester, PA) extractions were at 140 °C (Pearson et al., 2010; Salvucci et al., 2009).

Three 5 min acetone extractions at 100 °C, followed by a 100% flush, were completed prior to extractions with hexane or cyclohexane (Pearson et al., 2010). It is not possible to complete sequential acetone and hexane extractions on a per sample basis. All acetone extractions were completed before cyclohexane extractions were performed. The ASE 200 oven does not cool fast enough at the end of a cyclohexane extraction to the lower acetone extraction temperature needed for the next sample before the ASE 200 times out. Sequential acetone extractions and the flush were collected in the same collection vial. The samples were then extracted twice for 20 min with hexane (Experiments #1 and #2) or cyclohexane (Experiments #3 and #4) at 140 °C followed by a 150% flush again collected into one vial per extracted sample. Solvents containing analytes were evaporated from collection vials in a TurboVap evaporator (Zymark Corporation, Hopkinton, MA) at a temperature below the boiling point of the solvent being evaporated. Collection vials were subsequently oven-dried at 56 °C and weighed.

Once dried, collection vials equilibrated in a desiccation chamber for several hours. Collection vials were weighed to the nearest 0.1 mg and weights recorded.

2.4. Statistical analyses

Analyses of variance were performed using Analytical Software Statistix 9 program (Analytical Software, 2008) to determine treatment effects. All statistical comparisons were at the 95% level of probability and least significant differences (0.05) were calculated for mean separation when treatment differences were significant. Because there were significant heating time × temperature