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Abstract

The determination of natural rubber and resin contents of guayule (*Parthenium argentatum*) requires a long and destructive methodology to be implemented in the laboratory. In order to achieve a large number of measurements, it is necessary to determine the contents directly in the field in a fast and non-destructive way. The recent emergence of portable near infrared spectroscopy makes possible the use of this technology directly on the plants, without preparation of samples in the laboratory. The aim is to check the possibility of quantifying natural rubber and resin by using such a portable device directly on the guayule plants. Our research hypothesis is that the measure in near infrared can be performed directly on the surface of the bark. A set of 200 branches of guayule was collected randomly in a single experimental plot for two selected genotypes showing very different morphological traits. A difference between average contents of natural rubber and resin measured using a solvent-based laboratory reference method was observed with relation to the variety. Models of near infrared spectroscopy have been developed for samples in the form of powders, dry branches and fresh branches harvested in the same plot. The standard error of prediction was two times higher for branches as for powders. This was due to the fact that the powders were analyzed in controlled

conditions in the laboratory (moisture, sample homogeneity and particle size). Using near infrared spectroscopy directly on the plant allowed the determination of the average content of the plot before harvest. With a random sampling of 70 measures per plot, it was possible to estimate the average content of natural rubber and resin with a precision lower than 0.3%. Measurements made on fresh branches of very different diameters showed that the variability of the contents was lower in the plant than in the field. At the individual plant level, 40 measures were necessary to determine the average content of resin and 70 measures for natural rubber. These encouraging results obtained out of the field, with freshly cut biomass, showed that portable near infrared spectroscopy could be directly applied to the guayule bush in the field, for estimating natural rubber and resin production of a plot or for monitoring the seasonal evolution for breeding or agro-industrial production.

Keywords

Guayule, Rubber, Resin, Near infrared spectroscopy

1. Introduction

Natural rubber (NR) is a biopolymer composed of 1,4 cis-polyisoprene. It has mechanical and dynamic properties that synthetic elastomers, produced from petroleum, lack (Mooibroek and Cornish, 2000). The primary commercial source of NR is the hevea tree (*Hevea brasiliensis*), mainly cultivated on plantations in tropical climates in Malaysia, Thailand, Indonesia, and Vietnam. These countries produce 90% of the world's NR (Salvucci et al., 2009). The European Union (EU) has listed NR among the most critical materials and intends to have NR alternatives to supply its rubber industry. Guayule (*Parthenium argentatum*) is an alternative source of NR. It is an Asteraceae, a bush native to northern Mexico (Chihuaha desert) and southern Texas. Guayule grows at temperatures from -12°C to +40°C, with an average rainfall from 350-800 mm (Foster et al., 2005). Global warming emphasizes the

interest in guayule as a new crop because this species is well acclimated to dry and semi-arid climates. Moreover, it can be cultivated in poor soils, thus avoiding competition for soils suitable for food crops. Guayule is also an alternative for limiting soil erosion of fallow lands. It can be cultivated with other crops (olive, almond, argan trees) with an agro-forestry approach, and could provide additional income for farmers. Guayule rubber has physical properties identical to hevea rubber, but it is non-allergenic, unlike hevea rubber (Cornish et al., 2001; Siler et al., 1994), which is of particular interest in the medical field.

Furthermore, guayule also produces resin fractions that contain terpenes, lipids and other biomolecules (Schloman et al., 1983). Indeed, secondary metabolites such as sesquiterpenic esters (guayulins A and B) are active against insects and fungi (Romo et al., 1970), triterpenes (argentatines A and B) were successfully tested against cancer (Romo de Vivar et al., 1990); resin also contains a wide range of fatty acids from C10 to C20 (Banigan et al., 1982; Punvichai et al., 2016). Degradation products were also found (Schloman et al., 1983; Teetor et al., 2009).

The distribution of NR and resins in the various parts of the plant (leaves, branches, roots) has been studied (Curtis, 1947; Gilliland et al., 1984). In addition, the branches were cut from the trunk and divided into sub-fractions according to their length corresponding to each annual growth of the plant. NR was found mainly in the branches, and particularly in the bark (phloem). Resin concentration was also higher in the bark. Teetor et al. (2009) analysed NR and guayulins A and B in the various parts of the plant. In the branches, they found a correlation between the concentration of guayulin A and that of NR, but this correlation varies among the different lines, thus preventing use of a single calibration equation for estimating NR content in all guayule branches.

Several analytical procedures were developed to assess the rubber and resin content in guayule, using the plant biomass after drying and grinding. First Holmes and Robbins (1947), then Black et al. (1983) have described a gravimetric method with two steps: a first one with acetone to extract the resin and a second step with cyclohexane to extract the rubber, then followed by the evaporation of the solvents.

74 The rubber or resin content was calculated on a weight basis. A Soxhlet apparatus was generally used
75 for the extraction step. More recently, a new method (Accelerated Solvent Extraction, ASE) operating
76 under high pressure was successfully investigated (Teetor and Ray, 2004; Salvucci et al., 2009; Pearson
77 et al., 2013; Cornish et al. 2013). This technique was faster, uses less solvent, and reduces handling of
78 the solvents. Suchat et al. (2013) further investigated this automated method, by comparing with
79 others options found in the literature. They found no difference with the gravimetric results obtained
80 with the Soxhlet method, but they noted a higher resin yield when compared with a combined high
81 speed homogenizer and extraction protocol with acetone (often called “Polytron”; Black et al., 1983;
82 Jasso de Rodriguez and Kuruvadi, 1991). Therefore, ASE was chosen as the reference method.
83 Punvichai et al. (2016) used super critical CO_2 (SC02) to extract and separate the various resin fractions.
84 A rather polar co-solvent was necessary, and the extract yield with ethanol was about the double of
85 the one obtained with SC-CO2 or with above ASE-acetone, the yield of the so-called “resin fraction”
86 being a function of the extraction method. Other studies helped to develop spectroscopic methods
87 such as near infrared spectroscopy (NIRS). NIRS is based on vibration properties of chemical bonds of
88 organic molecules and interactions with IR wavelengths. Thus, a NIRS absorption spectrum is
89 correlated with the chemical composition of a sample. NIRS quantification is an indirect method that
90 needs a preliminarily calibration on a series of samples for which reference data are known (Burns and
91 Ciurczak, 2007). This method is largely used in the agro-food industry for quantifying macro-nutrients
92 such as fatty acids and proteins (Büning-Pfaue et al., 1998). This method is also used in the
93 pharmaceutical industry to measure moisture content and to control the concentration of active
94 components in drugs (Morisseau et al., 1995). NIRS has been used successfully with guayule to assess
95 moisture, rubber and resin contents (i) on ground dried biomass (Black et al., 1985), including coupled
96 to ASE method for accurate calibration (Suchat et al., 2013), and (ii) even on homogenized liquid
97 samples containing dispersed rubber and on purified latex (Cornish et al., 2004). However, direct NIRS
98 measurement on the whole biomass (not grinded) was not reported to date.

The recent development of portable NIRS makes possible the use of this technique for field measurement directly on plants, thus not requiring the preparation of the biomass sample in a laboratory. As an example, a portable NIRS has allowed the determination of ammonia content in Hevea latex (Narongwongwattana et al., 2015). The aim of this work was to check the link between a NIRS measurement on the surface and the content of resin and rubber of the fresh biomass. This was checked with the branches, the sampling of which being easier than for the root, and not with the leaves which contain a very low amount of rubber in comparison to resin.

2. Materials and methods

2.1 Plant material

A set of 200 guayule branch samples was harvested in February and March 2018 on an experimental plot of 700 m² located in Lansargues, France. Samples were collected at random for two varieties of guayule named CL1 (parent USDA 11591, triploid) and CLA1 (parent USDA AZ 101, tetraploid), planted in 2014 from seeds derived from USDA lines (United States Department of Agriculture), produced and harvested on Cirad guayule experimental plots since 2008, with a density of 35 000 plants/ha, no irrigation, and no fertilization. The two varieties were chosen due to their differing morphological characteristics.

2.2 Experimental protocol

Two branches (approximately 10 cm long) were collected per plant for each of the two lines (50 plants analysed per line). The leaves and flower stems were manually removed in the field. The 200 samples were stored under vacuum in plastic bags at 4°C. NIRS measurements done on 10 samples for each line (random draw) before and after storage showed that vacuum packing had little to no effect on results (similar standard errors of prediction obtained before and after storage using the NIRS models).

For the 200 samples, NIRS measurements were performed on the branches when the temperature reached 20°C at atmospheric pressure (5 different spots measured on each branch). The samples were weighed before and after drying (temperature of 70°C for 15 hours in an oven) in order to measure the moisture content of each sample (weighed at dried state and moisture calculated on humid weight). NIRS measurements were taken at the same spots on the branches as before drying.

Then, samples were crushed in a blender (Waring Avery L7162), frozen at -80°C for 15 hours, and finally ground in a Retsch ZM 200 grinder, equipped with a 1mm mesh sieve, at 18000 rpm. Only one NIRS measurement was done on each sample prepared by grinding. The same ground samples were solvent-extracted with an ASE apparatus (Accelerated Solvent Extractor, model 350, Dionex Corporation, Sunnyvale, CA USA), as detailed in the next section. After solvent evaporation and drying of various extracts, NR and resin contents of each sample were calculated.

In addition, NIRS measurements were taken randomly on branches of two plants per line (CL1 plant 1: 55 spots, CL1 plant 2: 37 spots, CLA1 plant 1: 45 spots, CLA1 plant 2: 74 spots). Diameters of the branches at the spot of NIRS measurement were recorded.

2.3 Accelerated solvent extraction (ASE)

The procedure was developed by Suchat (2012). For each dried ground sample, 2.25 g of branches were weighed and poured into stainless cells of an ASE Dionex model 350 equipped with a carousel of cells, connected to a nitrogen supply. A micro cellulosic filter (27 mm diameter, Thermo Scientific Dionex) was installed at the bottom of each cell before adding the sample. Operating conditions of extraction were as follows: the cell was filled with solvent, drying time was 5 min, duration of static extraction was 20 min with acetone and then hexane, time of purge was 90 s, and rinsing volume was 50%. Temperature of extraction was 40°C with acetone and 120°C with hexane for extracting resin and NR respectively. Three static cycles were programmed for each solvent (total extraction time with ASE was 2h15 per sample). The extracts were collected in pre-weighed glass tubes. The solvent was left

146 evaporating in open air for one week and then the extract was dried in an oven for 15h (70°C) before
147 weighing.

148 **2.4 Near infrared spectroscopy**

149 Five NIRS measurements were performed on each sample of fresh, then dried branches, and one
150 measurement on the corresponding dry powder. The portable spectrometer was a LabSpec 4
151 Standard-Res (ASD Inc.). It was equipped with a fibre cable linked to a contact probe (probe diameter
152 4 mm) to measure the branches, and with a specific device for the measurement on powders (probe
153 diameter 12 mm). The spectral range in reflectance mode was from 800 nm to 2400 nm. Absorbance
154 spectra were recorded as log (1/R), with R being the average reflectance of 32 scans (Suchat et al.,
155 2013). Each spectrum was sampled into 1601 wavelengths.

156 **2.5 Statistical analyses**

157 Statistical analyses were performed with the software Unscrambler X (v10.5, 2017, CAMO Software
158 AS, Norway) and R Studio (v0.98, 2014, RStudio Inc.). Spectra were mathematically corrected with
159 Standard Normal Variate (SNV). Such correction allowed reducing the size effect of particles and the
160 intra spectrum variability (correction of the light dispersion). The second derivative was then computed
161 using the algorithm of Savitzky Golay with a smoothing range of 11 data points and a third degree
162 polynomial. The use of this derivative allowed for separating peaks that overlap and for correcting the
163 baseline deviation of spectra. For fresh branches, the bands for water were removed in the wavelength
164 intervals of 1360-1495 nm and 1852-1950 nm (1366 wavelengths used). Equations of calibration were
165 developed with the Partial Least Squares (PLS) regression method. For cross validation, the set of NIRS
166 measurements was divided into 20 subsets with randomly selected measurements (10 measurements
167 per subset for powders and 50 measurements per subset for branches). The validation set was created
168 with a random selection of the measurements among the total population. In the case of powders, the
169 validation set included 60 measurements (total population of 200 measurements). For the branches,
170 the validation set was constituted of 300 measurements (total of 1000 measurements).

3. Results and discussion

3.1 Reference resin and natural rubber content for the entire plot

[Table 1]

The descriptive statistics for moisture, resin and NR contents after ASE extraction are shown in Table 1. The population for each variety was 100 samples. Moisture content was calculated based on wet biomass. The average moisture content for the CLA1 line was 43% (standard deviation 1.5%) and for the CL1 line was 43% (standard deviation 1.9%). Thus, moisture contents were similar for the CL1 and CLA1 lines.

The standard error of laboratory (SEL) was estimated at 0.11% for resins and 0.13% for NR (duplicate analyses; Burns and Ciurczak, 2007). The COV's found show that variability of resin contents (COV around 9%) was lower than the COV of NR content of the experimental field (COV around 15 %) (Table 1). The COV's of our experiment were much lower than those reported by Suchat et al. (2013) (COV=22.4% for resins and COV=36.8% for NR) because that author used six different lines (not the same as the two lines used in this study).

[Figure 1]

A significant difference between average resin content of CL1 and CLA1 (t = -10.83, $d.f.$ = 194.96, p-value < 10^{-3}, Figure 1-a) and average NR content (t = 23.64, $d.f.$ = 183.41, p-value < 10^{-3}, Figure 1-b) was found (t-test on unpaired samples). Samples of CL1 had a lower content of resin but a higher content of NR than CLA1 samples. This was probably due to genetic differences between the two lines since environmental background was similar (agronomic practice, soil, meteorological conditions, plot location).

Hereafter equation 1 was used to calculate the optimal sampling size to estimate the average content of a plantation with a given uncertainty (Marques De Sá, 2007).

195 $$n = t^2_{1-\frac{\alpha}{2}}(\nu)\frac{s^2}{\Delta^2} \quad (1)$$

196 where *n* = size of the population, *t* = Student variable, *α* = risk (5%), *s* = standard deviation, ν = degree
197 of freedom to estimate the standard deviation, *Δ* = uncertainty.

198 [Figure 2]

199 This equation can also be rearranged to highlight the evolution of the uncertainty depending on the
200 number of samples (Figure 2). The uncertainty decreases for a larger sampling size regardless of variety
201 and content. For a small sampling size, uncertainty tends to be ±1.5% regardless of the content and
202 line considered. For the rest of the study, the selected uncertainty was ±0.3%, which allowed
203 calculation of average contents with a relative error below 10 % (relative error is the uncertainty
204 divided by average value given in percent).

205 [Table 2]

206 Table 2 shows the calculated minimum sampling size corresponding to the chosen uncertainty. With
207 40 measurements in one plot, the uncertainty of the estimation of the average will be less than 0.3%
208 whatever the variety or content. However, the relative error will be higher for the NR than for the resin
209 content (mainly for average NR content of CLA1 of 3.89% if estimated at ±0.3%).

210

211 **3.2 Spectroscopic rubber and resin predictive models**

212 **3.2.1 Estimation of resin and natural rubber content**

213 [Table 3]

214 Table 3 shows the statistical characteristics of NIRS models for the estimation of rubber and resin
215 content according to the sample type. Each measurement was considered as an independent value
216 because five different locations were measured by NIRS on each branch. Cross validation and validation
217 results were similar. Likewise, the validation results were similar to the calibration results. Cross

validation and validation were linked to the performance of models for the estimation of individual values.

For resin content, standard errors of cross validation (SECV) were 0.37, 0.67 and 0.73 for powders, dry branches and fresh branches respectively (r^2= 0.84, 0.49 and 0.41). For NR content, the SECV found were 0.33, 0.72 and 0.83 respectively for powders, dry and fresh branches (r^2= 0.96, 0.78 and 0.71). Suchat et al. (2013) found a SECV of 0.43 for resin and NR with NIRS measurements on powder samples. Errors (SECV) in Table 3 were similar to those published earlier for powders. The coefficients of determination were a function of the variability of reference values (COV value of 11.8% for resin and 19.6% for NR with CLA1 and CL1), which might explain why the coefficients of determination for NR were higher than for resin (despite errors of models being more important for NR). The errors were approximately two times greater for branches than for powders, due to the fact that powders were dried and more homogeneous, and were analysed under optimal conditions in the laboratory. Due to the condition of branches, it was difficult to control moisture (for fresh branches), homogeneity and roughness of the bark. In the case of branches, an error was added to reference values as only one reference content was associated with the 5 measures per branch.

Both for resin and NR, the complexity of models increased when considering powders, fresh branches, and dried branches. The number of main components of the model varied respectively from 7 to 8 to 10 for resin and from 3 to 3 to 5 for NR. The number of main components was lower for NR than for resins because NR is a polymer while resins are composed of different classes of molecules.

[Figure 3] [Figure 4]

Figures 3 and 4 show the models obtained for resin and NR depending on sample type (powders, fresh or dried branches). Two distinct populations related to CL1 and CLA1 (Figure 3-b and Figure 4-b) were observed, notably for NR (CL1 in interval 5-10% and CLA1 in interval 2-5%).

The interest in developing spectroscopic models directly usable at field level is to estimate the average content of the plantation before harvesting (using equation 1). In this case, the parameter (*s*) of

equation 1 was equal to the square root of the sum of variance of the total population and of variance of the errors of the model (estimated via cross validation). For the model estimating the resin content of fresh branches, the size calculated was less than 51 measures (Table 2). Concerning the NR content estimation, the size was less than 65 measures. It was deduced that with 70 NIRS measurements in the field, it was possible to estimate the average content of resin and NR with a precision of ±0.3%.

3.2.2 Interpretation of NIRS spectra

[Figure 5]

Figure 5 shows the models coefficients (first loading) with several major absorption bands. The 970 nm band, characteristic of the resin, was associated with the O-H bond, second overtone of the R-OH link (Figure 5-a). The magnitude of the 970 nm band was highly reduced for dried samples; it was deduced that it corresponded to a volatile chemical (phenol type molecule). The bands of NR and resin at 1150 nm and 1215 nm corresponded to the C-H bond vibration, second overtone of CH_2 and CH_3 (Hans et al., 2015). The bands at 1420 nm and 1915 nm were from the vibration of the O-H bond, first overtone of H_2O. The bands of NR and resin at 1740 nm were due to the vibration of the C-H bond, first overtone of CH_2 and CH_3; and the one at 1780 nm was typical of the cellulose (Osborne et al., 1993). The band at 2260 nm was the result of the combined vibration of C-H/C=O of an aldehyde group (Black et al., 1985). The band at 2315 nm was from the vibration of the combined bonds of $CH\text{-}CH_2$ in carbohydrates of guayule bark (Black et Al., 1985). This explained why this particular band was more intense for fresh and dried branches than for powder, as well as for the 1780 nm band. The 2350 nm band was from the combined vibration of the CH-CC bond of cellulose (Osborne et al., 1993).

3.3 Prediction by NIRS of content values of fresh biomass

The branches from two plants of each variety were investigated by spectrometry. The number of measurements done on each plant varied with the size of the plant. The moisture content of each plant

267 was close to 40%. The NIRS models previously developed for fresh branches were used to estimate
268 resin and NR contents. The descriptive statistics of predicted values are given in Table 4 and the
269 associated histograms in Figure 6.

270 [Table 4]

271 [Figure 6]

272 Average resin content for the CLA1 line varied from 8.4% to 8.8% for each of the plants, with associated
273 COV of 9.3% and 10.6%. For the CL1 line, the average resin contents were 7.9% and 7.6% with COV of
274 9.1% and 9.6%. For NR, the average contents for the CLA1 line were 7.8% and 7.3% with COV of 12.0%
275 and 17.0%. For the CL1 line, the average NR contents were 6.9% and 8.2% with COV of 13.0% and
276 13.3%. The average contents found in the whole plants were in agreement with those obtained for the
277 entire plot (Table 1), except for the NR content of CLA1. The calculated COV corresponded to the
278 variability of the whole plants added to the variability induced by the errors of the spectrometry
279 models. The results were very close to the COV found for the entire plot (COV resin CLA1 = 9.5%, CL1
280 = 9.3% and COV NR CLA1 =17.0 %, CL1 = 13.9%, Table 1); the variability of the content values was thus
281 lower in the plant than at the field level.

282 [Figure 7]

283 Figure 7 shows the histogram of the diameter distribution of the guayule branches for each line. The
284 two histograms were found to overlap. The average diameter of the branches of CL1 was equal to 9.8
285 mm (SD 4.9 mm) and that of CLA1 was 10.5 mm (SD 6.6 mm). The branches of CL1 had a lesser diameter
286 than those of CLA1. This was explained by the fact that CLA1 was generally more developed and had
287 more biomass than CL1 for plants of the same age.

288 [Table 5]

289 Table 5 gives the calculated population sizes to achieve an uncertainty set at ±0.3% on the estimation
290 of the average content per plant (Equation 1 was used). The table shows that 40 measurements were

sufficient to estimate the average resin content in a plant regardless of the line. For NR, the number of measurements to obtain the average content was much higher and 70 measurements were necessary. The errors added by the models of spectroscopy increased the number of measurements needed.

4. Conclusion

The standard error of laboratory on reference measurement (Accelerated Solvent Extraction, ASE) used for calibrating the NIRS method, was fairly low for both targeted extractable components 0.11% for resin and 0.13% for natural rubber, thus providing acceptable conditions for this study. By using the ASE method, the estimation of the average content of resin and NR in a guayule field is determined with a precision of ±0.3% for a set of 40 measurements of plants chosen at random, and for both the investigated guayule lines CL1 and CLA1.

NIR Spectroscopy models, which were then developed, were of increasing complexity for samples used as powders, or as dried or "fresh" branches. Moreover, the models were more complex for resin than for NR, because the resins contain numerous classes of very different biochemical molecules, contrary to NR which is essentially composed of one type of polymer. The errors of these models were two times higher for the branches compared with the derived powders (standard error of cross validation SECV: 0.37 % for resin, 0.33 % for NR). The powders were analysed under carefully controlled conditions (moisture, homogeneity, particle size), whereas the fresh branches were used as harvested. This procedure brings unavoidable heterogeneity regarding bark surface (roughness, local chemical composition and moisture content at measured spot), in addition to variable branch diameter.

When analyzing each plant individually, a total of 40 NIRS measurements would be necessary to determine the average content of resin with ±0.3 %, and 70 NIRS measurements for the NR with the same degree of certainty. The variability of the resin or the NR content was lower within sampled

branches in a given plant than among the numerous plants sampled in the plot. This supports the targeted method, since it does not require multiple measurements per plant.

It is worth noting that this NIRS method applied here to the bush (and more specifically to branches) works well, despite varying diameters corresponding to annual growth cycles, whereas (i) it is known that the wood not sampled by NIRS also contains resin and NR, and (ii) the in-bark to wood ratio of these compounds may vary depending on branch diameter (mainly associated with branch age). A preference for this model stems from the fact that it is able to accept the heterogeneity imposed by the targeted whole-bush NIRS analysis as opposed to powdered samples used in previous trials by our team.

The two guayule lines used for this study yielded quite differing chemical data: a significant difference between the lines was observed for the average contents of resin and NR given by the branch-based model, despite measured difference of average branch diameter between the two lines (the primary reason for having selected them).

Although this spectroscopic model for field measurements may be used for estimating content in individual plants (e.g. for plant breeding), it is even better adapted for assessing the average NR and resin content in plants in an entire field, for monitoring the influence of cropping parameters and for determining the harvest date. With only 70 NIRS measurements, it would be possible to estimate the average content of resin and NR with a precision of ±0.3%. The present study was performed in a lab with harvested branches for practical reasons. The next step will be actual measurement in the field, in order to provide a simple and useful tool for breeders, agronomists and farmers.

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339

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411 **Tables**

412

413 **Table 1** Descriptive statistics of moisture, resin and natural rubber contents after ASE extraction.

414 **Table 2** Optimal sampling size to determine the average contents of the agricultural parcel depending
415 on variety with a precision of ±0.3% (reference values obtained after ASE extraction).

416 **Table 3** Statistical characteristics of the NIRS models for the estimation of resin and natural rubber
417 contents depending on sample type. RMSE: Root Mean Square Error.

418 **Table 4** Descriptive statistics of resin and natural rubber contents predicted by NIRS depending on
419 variety and plant.

420 **Table 5** Optimal sampling size to determine the average contents of one plant depending on variety
421 with a precision of ±0.3% (contents obtained using the NIRS calibrations).

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423

Variety	Parameters	Moisture content (%)	Resin content (%)	Natural rubber content (%)
CLA1	Minimum	40.14	6.43	2.65
	Maximum	47.57	10.68	5.81
	Mean	42.96	8.57	3.89
	Standard deviation	1.48	0.81	0.66
	COV%	3.45	9.45	16.97
CL1	Minimum	38.04	5.35	3.89
	Maximum	46.81	9.27	9.21
	Mean	43.24	7.43	6.53
	Standard deviation	1.89	0.69	0.91
	COV%	4.37	9.29	13.94

424 **Table 1**

425

426

	Variety	Sampling size Reference values	Sampling size NIRS models
Resin	CLA1	28	51
	CL1	21	43
Natural rubber	CLA1	19	48
	CL1	36	65

427 **Table 2**

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429

		Resin		Natural rubber	
		RMSE	r^2	RMSE	r^2
Powders	Calibration	0.21	0.95	0.28	0.97
N = 200	Cross validation	0.37	0.84	0.33	0.96
	Prediction	0.34	0.83	0.31	0.95
Dry branches	Calibration	0.62	0.56	0.64	0.83
N = 1000	Cross validation	0.67	0.49	0.72	0.78
	Prediction	0.67	0.47	0.84	0.71
Wet branches	Calibration	0.63	0.55	0.79	0.73
N = 1000	Cross validation	0.73	0.41	0.83	0.71
	Prediction	0.75	0.38	0.79	0.75

430 **Table 3**

431

	Variety	Plant	*N*	Mean	Standard deviation	COV (%)
Resin	CLA1	1	45	8.37	0.78	9.27
	CLA1	2	74	8.82	0.94	10.61
	CL1	1	55	7.90	0.72	9.12
	CL1	2	37	7.55	0.73	9.60
Natural rubber	CLA1	1	45	7.83	0.94	12.04
	CLA1	2	74	7.33	1.24	16.99
	CL1	1	55	6.90	0.90	13.00
	CL1	2	37	8.18	1.09	13.33

Table 4

436

	Variety	Plant	Sampling size
Resin	CLA1	1	27
	CLA1	2	39
	CL1	1	23
	CL1	2	24
Natural rubber	CLA1	1	40
	CLA1	2	68
	CL1	1	36
	CL1	2	54

437 **Table 5**

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440 **Figures**

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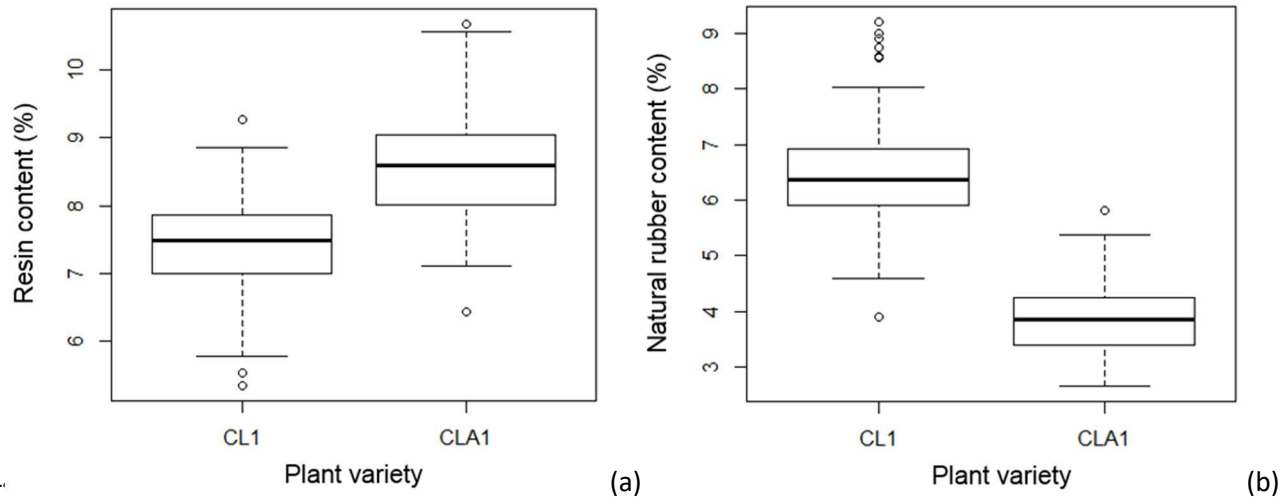

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443 **Figure 1** Box plot of resin contents (a) and natural rubber contents (b) depending on variety.

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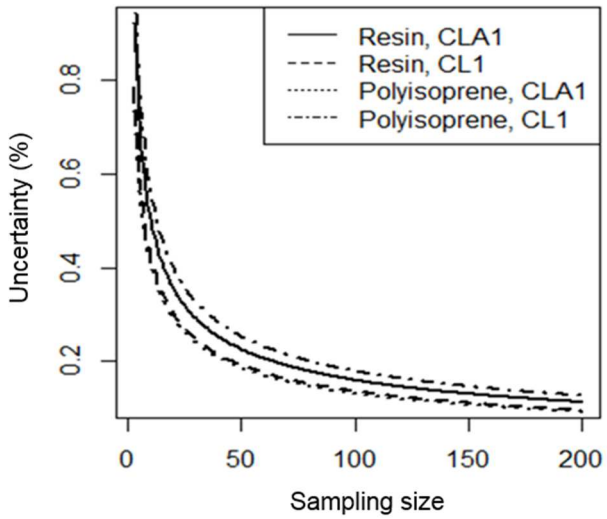

Figure 2 Uncertainty of the determination of average contents depending on sampling size and variety.

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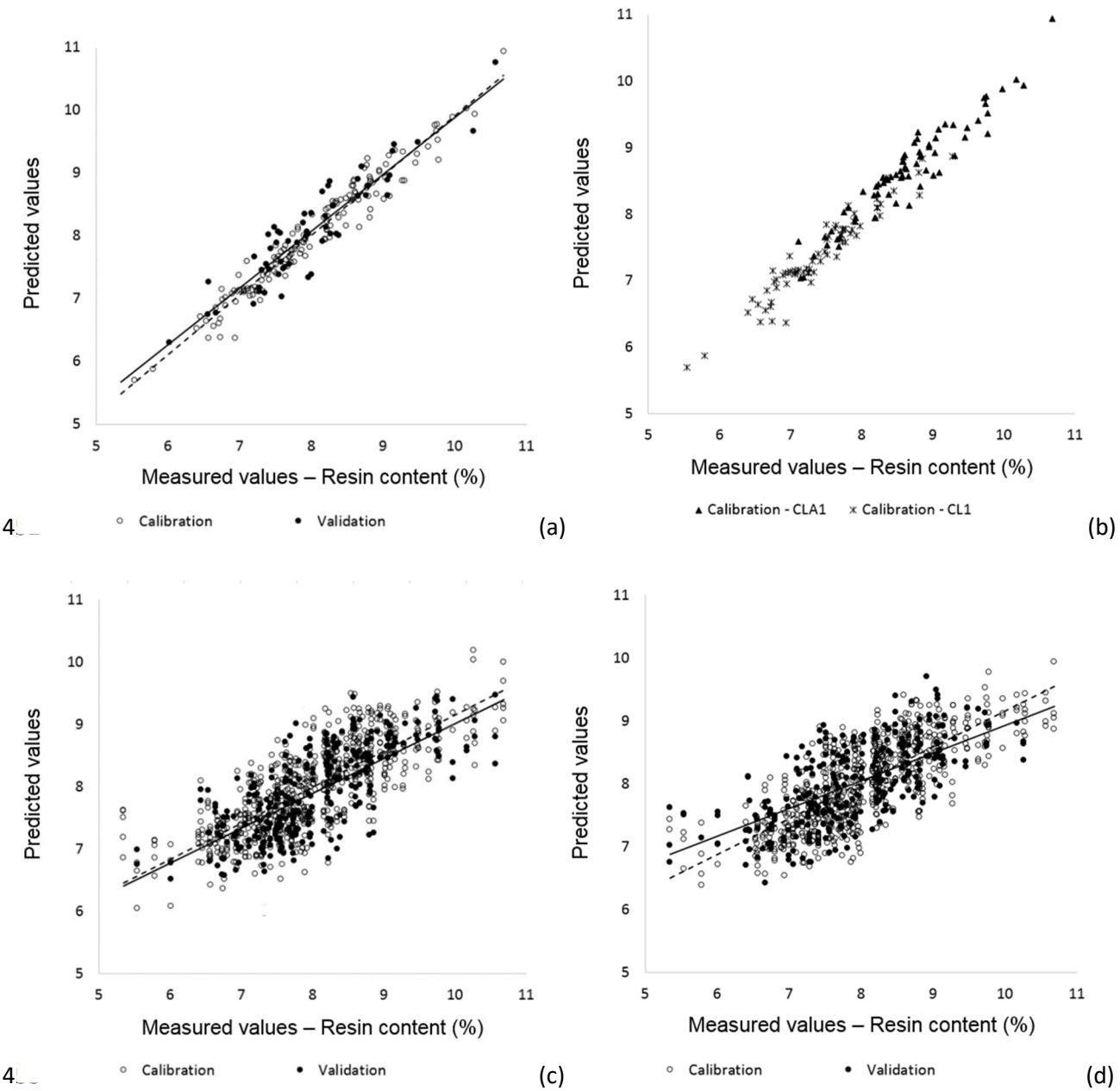

454 **Figure 3** Scatter plots between reference contents of resin and NIR predicted values depending on
455 sample type. (a) Model for powders, (b) model for powders depending on variety, (c) model for dry
456 branches, (d) model for wet branches. (- -) Calibration, (-) Validation.

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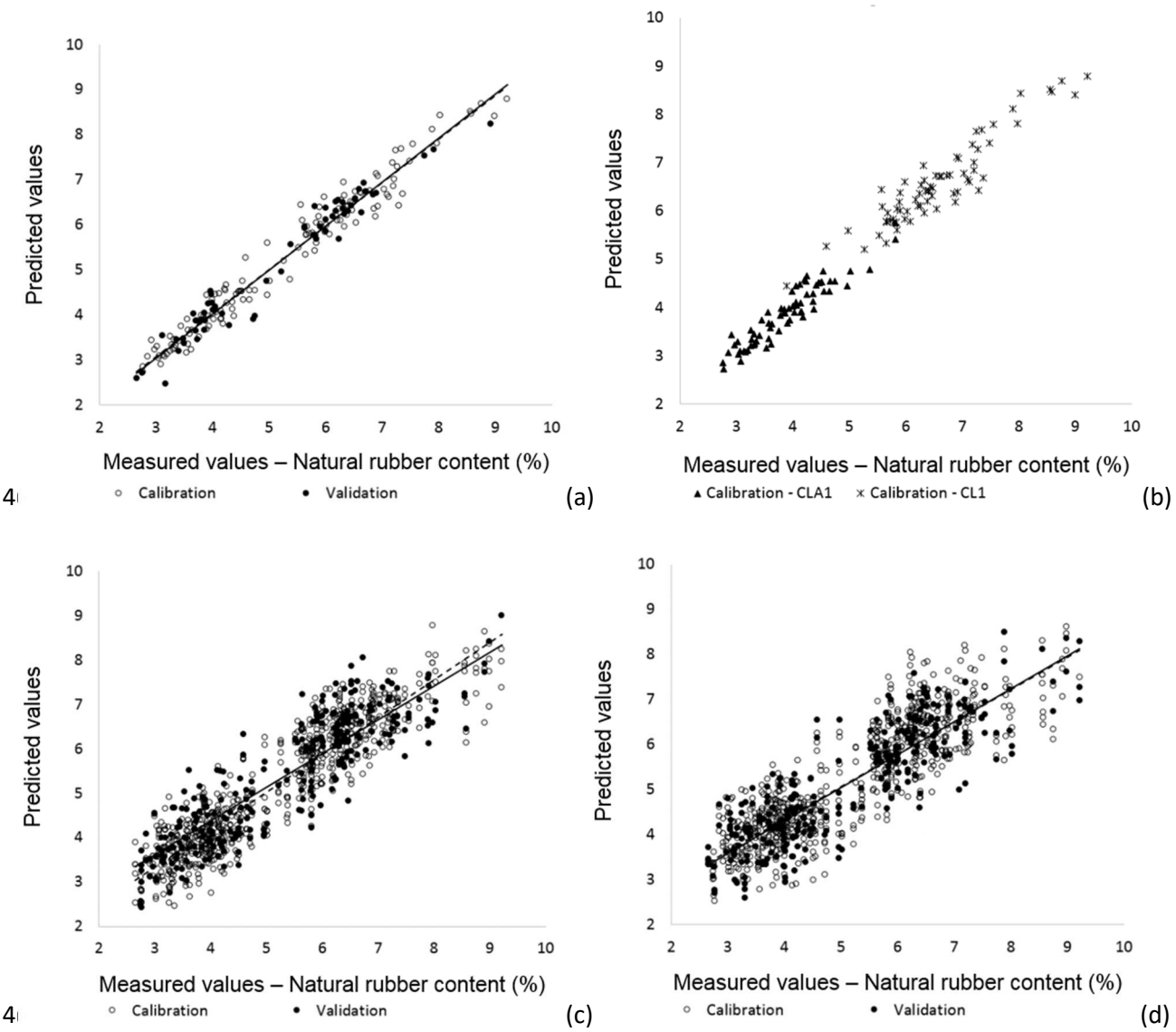

462 **Figure 4** Scatter plots between reference contents of natural rubber and NIR predicted values
463 depending on sample type. (a) Model for powders, (b) model for powders depending on variety, (c)
464 model for dry branches, (d) model for wet branches. (- -) Calibration, (-) Validation.

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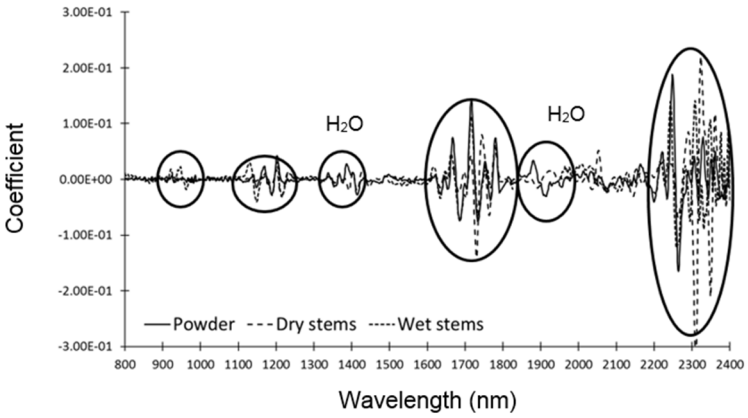
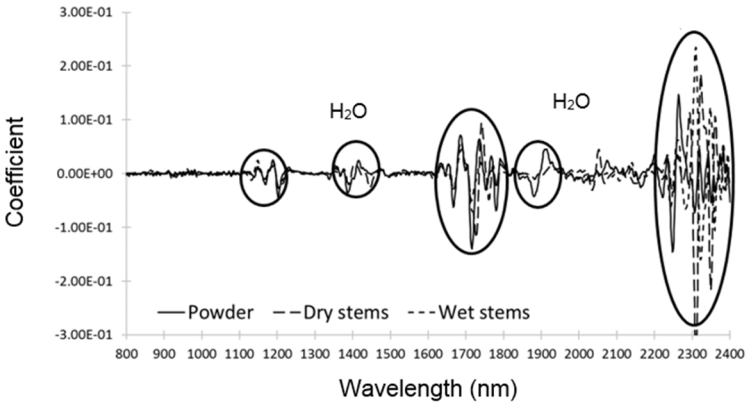


(a)



(b)

Figure 5 Resin and natural rubber NIRS models coefficients (first loading) depending on wavelength and sample type. (a) Resin model, (b) natural rubber model.

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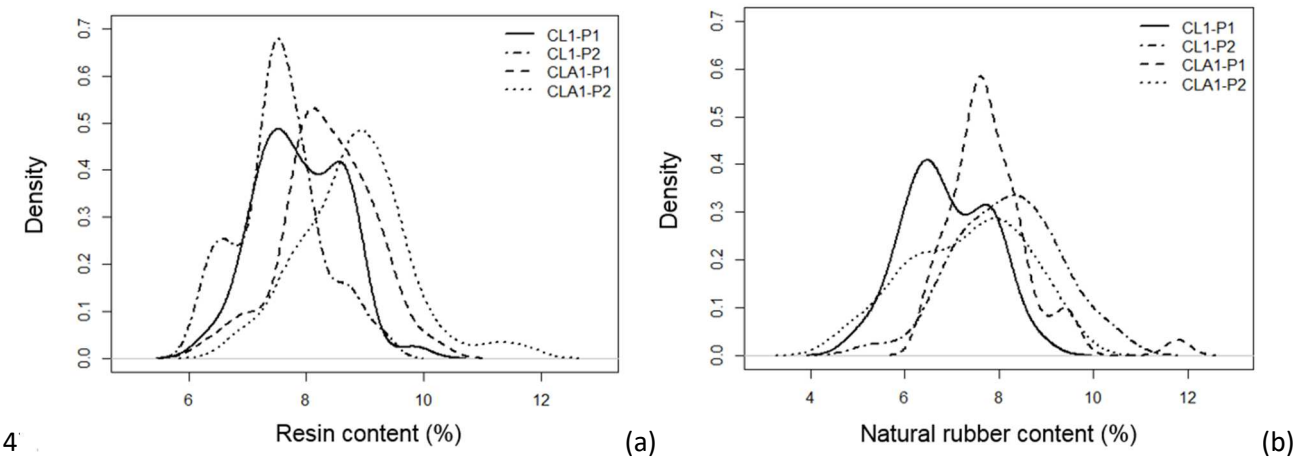

4

476 **Figure 6** Density of probability of resin content (a) and natural rubber content (b) depending on variety

477 and tested plant.

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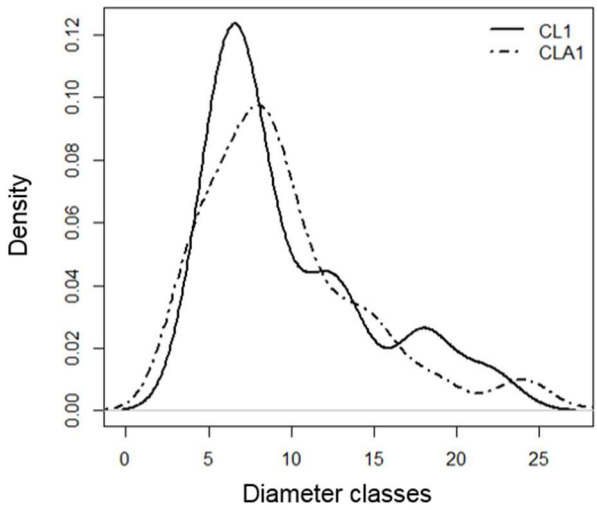

Figure 7 Density of probability of branch diameters in plants depending on variety.