

Reduction of tapping frequency associated with stimulation of two *Hevea brasiliensis* clones in Thai non-traditional rubber area. 2. Effect on biochemical composition and properties of latex films and rubber samples

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ABSTRACT

This study, Part 2 of a two-part series, was conducted over four years in Udon Thani, Thailand – a non-traditional rubber-growing area – to evaluate how shifting from a non-stimulated, alternate-day tapping system (T1 S/2 d2) to a stimulated (2.5% ethephon), every-third-day system (T2 S/2 d3 Stim) affects the biochemical composition, physical properties and mesostructure of natural rubber. Two clones (RRIM600 and RRIT251) were studied using dry latex films and technically specified rubber (TSR5-like and TSR10-like) samples. All indicators varied significantly with sample type. Within each sample type, clone had a stronger effect than tapping system. RRIM600 latex films had lower protein content than RRIT251, though T2 S/2 d3 Stim increased protein levels for both clones. Lipid content in films remained stable across clones and tapping systems. While the tapping system did not affect latex film properties, RRIT251 films showed higher plasticity and Mooney viscosity, while RRIM600 had higher PRI. In TSR-like samples, protein content was unaffected by clone or tapping system. However, lipid content varied: in TSR5, the highest levels were in RRIM600/T1 S/2 d2 and RRIT251/T2 S/2 d3 Stim, and the lowest in RRIM600/T2 S/2 d3 Stim and RRIT251/T1 S/2 d2; in TSR10, RRIT251/T2 S/2 d3 Stim had the highest lipid content and RRIM600/T2 S/2 d3 Stim the lowest. Tapping had limited effect on overall TSR properties, while clone significantly influenced Mooney viscosity and gel content. Principal Component Analysis confirmed that clone-driven variability was the main factor influencing sample clustering. Overall, the results emphasize the dominant role of clone selection over tapping system in determining rubber quality in non-traditional growing areas.

1. Introduction

Natural rubber (NR), derived from the latex of *Hevea brasiliensis*, is an indispensable material used in diverse applications, from tires to surgical gloves. As global climate change intensifies, agricultural systems are increasingly expanding into marginal or non-traditional areas, while

persistent labor shortages continue to challenge the sustainability of conventional production practices. At the same time, NR sector is expected to experience sustained growth in demand, with the International Rubber Study Group (IRSG) projecting an annual increase of approximately 2%. In this context, the NR sector faces mounting pressure to ensure sustainable and cost-effective production systems without

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compromising material quality.

The quality of NR products is partly driven by the biochemical composition of the latex and its processing into various dry forms, such as Technically Specified Rubber (TSR). NR end-use products must meet rigorous mechanical, thermal and structural performance standards, which are influenced by agronomic, post-harvest and processing practices (Wisunthorn et al., 2008, Chambon et al., 2020, Nambiathodi and Varghese, 2024). Understanding how these practices affect product quality is critical for ensuring the sustainability and competitiveness of the NR industry.

Latex composition is sensitive to several factors including geographical location, seasonal variations (Le Roux et al., 2000), *Hevea* genotype (Liengprayoon et al., 2013, Sant'Anna et al., 2021, Silva et al., 2021), fertilization (Chotiphan et al., 2019), and tapping system, such as tapping frequency (Aji et al., 2022, Gohet et al., 2024) and the use of ethylene stimulation (Lacote et al., 2010). While stimulation compensates for yield reductions associated with lower tapping frequency by enhancing latex flow duration and latex regeneration (Coupé and Chrestin, 1989, Jacob et al., 1989), it can also alter the levels of proteins, lipids, and other non-isoprene components, which are key determinants of NR product properties (D'Auzac et al., 1997). These changes may influence critical performance indicators, including elasticity, plasticity, and storage stability of TSR. Although reducing tapping frequency is increasingly considered a promising strategy to decrease labor requirements, limit tree stress, and reduce production costs, it remains essential to ensure that such systems do not compromise the quality of NR-derived products.

While several studies have examined the relationship between reduced tapping frequencies and latex production or tree physiology (Kudaligama et al., 2010, Aji et al., 2022, Chotiphan et al., 2023, Hernández-Arredondo et al., 2023, Purwaningrum et al., 2023, Gohet et al., 2024), far fewer have investigated their impact on latex composition and NR material properties. To our knowledge, one of the earliest studies addressing the relationship between reduced tapping frequencies (d2, d4, d7) and NR quality dates back to 2002 (Ferreira et al., 2002), assessing parameters such as ash and nitrogen contents, plasticity and viscosity across four clones. In the early 2010s, work from the Rubber Research Institute of Sri Lanka further explored both the physiological response under d4 systems (Rodrigo et al., 2011) and the impact of d2, d3, d4 and d6 tapping frequencies on NR properties including initial plasticity (P_0), plasticity retention index (PRI), Mooney viscosity, nitrogen and ash contents (Kudaligama et al., 2012). More recently, Zhao et al. compared NR quality (P_0 , PRI, Mooney viscosity, nitrogen and ash contents, molar mass distribution) obtained from d4 and d6 tapping systems in China and suggested that reduced tapping frequency could enhance both labor productivity and NR quality, while also emphasizing that such conclusions may not be universally transferable across different environmental contexts (Zhao et al., 2025).

The present study (Part 2) builds on the findings of Part 1 (Kum-ourm et al., 2025), which focused on fresh latex structure and biochemical composition, by investigating the downstream effects of reduced tapping frequency in a non-traditional rubber-growing area on processed NR materials and dry latex films. Specifically, it examines how transitioning from tapping every two days (T1 S/2 d2) to an ethephon-stimulated once-every-three-days system (T2 S/2 d3 Stim) affects the biochemical composition physical properties and mesostructure of dry latex films and TSR-like samples (TSR5 and TSR10). The study was conducted over a four-year period (three sampling campaigns per year, i.e. 12 campaigns in total) on two *Hevea brasiliensis* clones, RRIM600 (parentage TJIR1 X PB86) and RRIT251 (primary clone), in Udon Thani province, Thailand. Several variables were analyzed, including biochemical composition (protein and lipid contents and profiles), material properties (plasticity, storage hardening, viscosity) and structural parameters (gel content and mesostructure). By explicitly addressing whether a reduced-frequency, low-input tapping system can reconcile labor efficiency, agronomic adaptation to marginal

environments and material performance, this study contributes to the development of more sustainable rubber production systems while maintaining the industrial value of NR products.

2. Material and methods

This study carried out in a non-traditional area for rubber cultivation (Udon Thani, Thailand) investigates how the transitioning from tapping every two days (T1 S/2 d2) to once-every-three-days tapping associated with stimulation (T2 S/2 d3 Stim) affects the composition (proteins, lipids, phospholipids, glycolipids, neutral lipids and free fatty acids), the physical properties (P_0 , P_{30} , PRI, PH, ΔP ASHT, Mooney viscosity) and the mesostructure (gel content, M_n , M_w , M_w/M_n) of latex films as well as TSR5-like and TSR10-like rubber samples. This study was limited to two clones (RRIM600, RRIT251) and did not monitor environmental factors such as soil or humidity, which constrains generalization. Despite these limitations, the four-year dataset provides valuable insights into tapping practices in non-traditional cultivation areas.

2.1. Experimental design and field conditions

The field experiment was conducted in Udon Thani province, Thailand (17.40713°N, 102.619°E; 200 m above sea level), a non-traditional rubber-growing conditions. Two certified *Hevea brasiliensis* clones (RRIM600 and RRIT251), opened for tapping in 2018, were studied. RRIM600 is a reference and the most planted clone in Thailand with stable production and moderate metabolic activity, whereas RRIT251 is known for its higher metabolic intensity and greater responsiveness to tapping practices, making these two clones suitable for comparative physiological studies.

The trial followed a randomized complete block design including two tapping systems: (i) T1 S/2 d2 (tapping every two days without hormonal stimulation) and (ii) T2 S/2 d3 Stim (tapping every three days with stimulation using 2.5% ethephon applied five times per year). Each treatment was applied to homogeneous groups of trees under identical agronomic practices. Stimulation was applied with a brush to a 1 cm-wide band on the panel just above the tapping cut, using 0.7 g of ready-to-use Ethephon diluted in water at a concentration of 2.5%. The application was performed 24 h prior to the next tapping. The frequency of stimulation was defined on an annual basis according to clone characteristics (Gohet et al., 2003). In this study, both clones (RRIM600 and RRIT251) received the same treatment, consisting of five stimulations per year (June, July, August, September, October) with 2.5% ethephon, applying 0.7 g of stimulant on a 1 cm band of the tapping panel: ET 2.5% Pa 0.7(1) 5/y.

Fertilization followed the standard recommendations of the Rubber Authority of Thailand (NPK 21–7–14, 500 g tree⁻¹ applied twice annually in June–July and August–September). No fertilizer was applied during sampling periods to avoid transient effects on latex composition.

2.2. Latex collection and sampling

Latex sampling was carried out three times per year (June, August, and October), corresponding to relatively stable phenological stages, in order to minimize seasonal variability.

Trees were tapped early in the morning (~4 AM) by one single tapper. For each experimental unit, latex from multiple trees was pooled to obtain representative samples. The first drops were discarded, and fresh latex was collected immediately after tapping. Samples were maintained on ice and rapidly transported to the laboratory for further processing to prevent biochemical degradation.

Part of the fresh latex was directly used for dry rubber preparation, while the remaining fraction was processed for biochemical analyses.

2.3. Preparation of dry rubber samples

Three types of rubber samples were prepared from fresh latex: (i) dry latex films and rubber sample similar to Technically Specified Rubber of (ii) grade 5 (TSR5-like) and (iii) grade 10 (TSR10-like). TSR-like sample preparation protocols were adapted from industrial process. The main difference was the drying step that was done on creped rubber in a laboratory oven. The drying conditions (temperature and duration) were set for each grade after a preliminary test taking volatile matter, P_0 and PRI as criteria.

2.3.1. Latex film preparation

A volume of 250 mL of latex was poured on a $25 \times 33.5 \times 2 \text{ cm}^3$ tray. The tray was placed in a ventilated oven at 70°C for approximately 18–20 h. Regular visual verifications were made every 5 h and the film considered dry by the total disappearance of white spot.

2.3.2. TSR5-like preparation

TSR5-like rubber samples were prepared from 1 L of fresh latex. On day 1, fresh latex was collected and its Total Solid Content (TSC) was measured. The estimated Dry Rubber Content (DRC) was calculated as 90% of the TSC. Latex was precisely weighed and poured in a coagulation tank ($27 \times 42 \times 10 \text{ cm}^3$). The volume of distilled water to be added to latex to reach a DRC of 20% was calculated. After adding this volume of distilled water to latex, the mixture was gently mixed with a plastic mixing plate and the pH of diluted latex was measured. Using a 10 mL micropipette, a 4.7% solution of formic acid was gradually added into diluted latex until a pH of 4.8 was reached. Acidified latex was covered with another coagulation tank to prevent dirt and dust and was let to mature for 16–20 h. On day 2, latex coagula were creped under water shower using an industrial creper (minimum roll gap 3 mm, front roll (diameter 40.5 cm) and rear roll (diameter 45.72 mm) operated at speeds of 82.85 rpm and 106.37 rpm, respectively). The coagula were creped with 1 single pass and 3 double passes to get 6–10 mm thick fresh crepe. The obtained crepes were dried in a hot-air oven (Memmert, UFE 550, Germany) at 115°C for 3h30, cooled down at room temperature and weighed.

2.3.3. TSR10-like preparation

TSR10-like rubber samples were prepared from $2 \times 500 \text{ mL}$ of fresh latex. On day 1, for each elementary plot, the latex was collected, and $2 \times 500 \text{ mL}$ were poured in 2 plastic collection cups and weighed. A volume of 4.7% formic acid solution was added to latex to reach a final concentration of 0.25% and the mixture was gently mixed with a glass rod. The cups with coagulated latex were placed on a flat surface. On day 2, the coagula were removed from the cups, transferred in large container with holes at the bottom and stored outside in ventilated area for 30 days. On day 30, latex coagula were creped under water shower using an industrial creper (same specification as for TRS5-like). The coagula were creped with 1 single pass and 11 double passes to get 6–10 mm thick fresh crepe. The obtained crepes were dried in a hot-air oven (Memmert, UFE 550, Germany) at 120°C for 2h30, cooled down at room temperature and weighed.

2.4. Homogenization of rubber samples

Dry films and TSR-like crepes were homogenized prior to biochemical, structural, and physical property analyses. The homogenization method used in this study followed SMR Bulletin No.7, Part B.2, 1992 standard.

2.5. Biochemical composition of rubber samples

2.5.1. Lipids

2.5.1.1. Extraction of lipids from dry rubber samples. Lipids were extracted from homogenized dry rubber samples with the method previously developed for dry rubber (Liengprayoon et al., 2008). The pieces of 2.5 g of freeze-dried fractions were ground under liquid nitrogen and transferred into 50 mL of chloroform: methanol (2:1; v/v). After 6 h of agitation at 200 rpm, rubber was removed by filtration and total lipids were concentrated using a rotary evaporator. Total extract was redissolved with 4 mL of the same solvent and water-soluble components were removed with 1 mL of 0.9% NaCl solution. The lipid-containing bottom layer was taken and evaporated to obtain the lipid extract.

2.5.1.2. Characterization of lipids from dry rubber. Lipid profiles were analyzed by thin-layer chromatography (TLC) after dilution of lipid extracts in chloroform according to their lipid content in rubber, in order to visually represent individual lipid classes (concentration range: 5–10 mg/mL). A volume of 8 μL of each solution was deposited onto silica gel 60 G TLC plates ($10 \times 20 \text{ cm}$; 0.25 mm thickness, Merck, Darmstadt, Germany). Lipid migration was performed using different mobile phases adapted to neutral lipids (NL), glycolipids (GL), and phospholipids (PL), followed by detection using specific reagents and/or heating conditions, as previously described (Liengprayoon et al., 2008).

Total lipid extracts were further fractionated into three classes NL, GL, and PL by solid-phase extraction (SPE) according to Wadeesirisak et al. (2017). Briefly, SPE cartridges were activated by successive rinsing with 2.7 mL of methanol and 2.7 mL of chloroform. A total of 30 mg of lipids dissolved in 0.5 mL of chloroform was loaded onto the cartridge, and lipid classes were sequentially eluted using 8.1 mL of chloroform (NL), 8.1 mL of acetone/methanol (9:1, v/v) (GL), and 8.1 mL of methanol (PL). The flow rate was maintained at approximately 3 mL/min using a vacuum pump. Each fraction was collected, evaporated, and weighed, and the purity of lipid classes was verified by TLC as described above.

Free fatty acids (FFAs) in lipid samples were quantified according to the method described by Van Autryve et al. (1991), which is based on the specific complexation of FFAs with rhodamine 6 G. The resulting complex was measured by spectrophotometry at 513 nm.

2.5.2. Proteins

2.5.2.1. Total nitrogen content. Total protein content was estimated from nitrogen content measured on dry rubber samples using a CHN analyzer, applying a conversion factor of 6.25.

2.5.2.2. Extraction of proteins from rubber samples. Proteins were extracted from homogenized dry rubber samples as previously described (Srisomboon et al., 2021). Briefly, the dry samples were solubilized in cyclohexane/ethanol 9:1 v/v for 3 days at 35°C at a ratio of 1/400 w/v rubber/solvent. Solubilized samples were centrifuged for 30 min (10,000 g, 20°C) and the pellet was let dried under fume hood for 1 day. The dry pellet was put in Laemmli 1X buffer (62.5 mM Tris, 10% w/v glycerol, 2% w/w SDS, 0.01% bromophenol blue, pH 6.8) in a ratio of 8/100 w/v pellet/buffer. The dry pellets from latex films and TSR-like samples were let to incubate in Laemmli buffer for 3 and 7 days, respectively, before running the SDS-PAGE electrophoresis under denaturing conditions.

2.5.2.3. SDS-PAGE electrophoresis of protein extracts. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a Bio-Rad Mini-Protean Tetra Cell system. Gels consisting of 14% acrylamide/bis-acrylamide were prepared with a comb forming 10 wells per gel (volume capacity: 44 mL per well). Prior to loading, protein

extracts were supplemented with 2- β -mercaptoethanol to a final concentration of 4% (v/v) and heated at 95°C for 10 min. Glycerol was then added to reach a final concentration of 10% (v/v). Each well was loaded with 44.0 μ L of protein extract, and electrophoresis was carried out at a constant voltage of 200 V.

Gels were stained for 2 h in a solution containing 40% ethanol, 10% acetic acid, and 0.1% Coomassie Blue R-250, followed by destaining in 10% acetic acid for 2–3 h until complete background removal.

Gel images were acquired using a GS-900 calibrated densitometer (Bio-Rad) and analyzed with Phoretix 1D software (version 1.0.628, TotalLab Ltd., Newcastle Upon Tyne, UK) in automatic detection mode. Lanes of uniform width were defined for analysis. Molecular weights of protein bands were determined by calibration against a standard containing 10 proteins with known molecular weights (250, 150, 100, 75, 50, 37, 25, 20, 15, and 10 kDa; Precision Plus Protein #1610363, Bio-Rad).

The relative protein distribution was quantified as the percentage of total lane density across three molecular weight regions: high (250–33 kDa), medium (33–16 kDa), and low (16–10 kDa). In addition, the relative densities of two main bands were specifically measured. These bands, located at approximately 21–23 kDa and 13–15 kDa, correspond to the proteins SRPP1 (Small Rubber Particle Protein; theoretical MW: 22.4 kDa) and REF1 (Rubber Elongation Factor; theoretical MW: 14.7 kDa), respectively (Bottier et al., 2019).

2.6. Properties of rubber samples

Initial Wallace plasticity (P_0) and plasticity retention index (PRI) were determined according to ISO 2930:2009 and ISO 2007:2007, respectively. Wallace accelerated storage hardening test (WASHT) was measured according to SMR Bulletin N°7 Part C.1, 1992 standard. Mooney viscosity was determined according to ISO 289-1:2015.

2.7. Structure of rubber samples

2.7.1. Mesostructure

The molecular weight (M_n , M_w) and molar mass distribution (MMD) of homogenized rubber samples were determined by size exclusion chromatography (Water-e2695) using columns in series packed with polystyrene based H-type TSK-GEL (TOSOH) having the exclusion limits of 4.0×10^6 and 4.0×10^8 . The rubber solution was prepared by dissolving dried rubber into tetrahydrofuran, THF (LabScan, HPLC grade) at 0.05% (w/v) concentration and filtered through nylon membrane filters of 0.45 μ m. The THF was used as an eluent with a 1 mL/min flow rate at 40 ± 0.01 °C, monitoring with a refractive index as a detector (2414 RI Detector). A calibration curve of elution time versus molar mass was obtained using ten synthetic *cis*-1,4 polyisoprene standards (Polymer Standards Service Inc.: Standards Kit No. WAT035708).

2.7.2. Gel content

Homogenized rubber samples dissolved in toluene at a concentration of 0.1% w/v were kept in the dark without stirring for one week at room temperature. The solution weighing approximately 30 g was centrifuged at 25,000g at 20 °C for 30 min to separate the gel and sol fractions. The precipitated gel fraction was recovered by coagulation with methanol and dried in vacuum at 40 °C for 24 h. The gel content was calculated from the ratio between the weight of the dried gel fraction and the weight of the rubber sample.

2.8. Statistical analysis of the dataset

The software JMP® 12.2 Windows was used for all statistical analysis. A mean comparison was carried out between treatments (RRIT251/T1 S/2 d2, RRIT251/T2 S/2 d3 Stim, RRIM600/T1 S/2 d2, RRIM600/T2 S/2 d3 Stim) using the Tukey-Kramer HSD (honestly significant difference) test. A multiple linear model was applied to evaluate the effects of

the two studied parameters (*i.e.* clone and tapping system) and their possible interaction on measured indicators. The effects of clone, tapping system and their interactions on measured indicators were assessed by p-Value of Tukey-Kramer test and considered to be very significant when $p < 0.0001$, significant when $p < 0.05$ or not significant when $p > 0.05$.

A Principal Component Analysis (PCA) was performed using data from both Part 1 (Kum-ourm et al., 2025) and Part 2 (present study) to explore linear relations between variables. To ensure temporal consistency in the PCA dataset, LD data were from LD measurement made on the day of sampling of the latex used for dry rubber sample manufacture while the production data was the cumulated yield of the month of the sampling. For each PCA analysis, the sample size exceeded by far the number of considered variables.

3. Results and discussion

Fresh latex from two rubber tree clones, *i.e.* RRIM600 and RRIT251, cultivated under two tapping systems, *i.e.* T1 S/2 d2 and T2 S/2 d3 Stim, was prepared in the form of dry latex films as well as TSR5-like and TSR10-like rubber samples. Several indicators were measured on those three sample types including: protein, lipid, phospholipid (PL), glycolipid (GL), neutral lipid (NL), and free fatty acid (FFA) contents, as well as physical properties (P_0 , P_{30} , PRI, PH, ΔP ASHT, Mooney viscosity) and mesostructure (gel content, M_n , M_w , M_w/M_n). Among physical properties, P_0 (initial plasticity, also called Wallace plasticity) illustrates the flowing ability of the material, P_{30} (aged plasticity after a treatment at 140°C for 30 min) and PRI (plasticity retention index = $(P_{30}/P_0) \times 100$) reflect the resistance of NR to thermo-oxidation, PH (value of Wallace plasticity P_0 after 24 h under P_2O_5 at 60°C) and ΔP (= PH – P_0) indicate the ability of NR to harden during storage and finally, Mooney viscosity, defined as the torque required to rotate a disc (rotor) embedded in the NR sample, is used to predict the NR processing behavior. High Mooney viscosity indicates a stiffer material, making it more challenging to process, whereas low Mooney viscosity corresponds to a softer material, resulting in easier processing. The mesostructure of NR samples was assessed by measurement of gel content (part of the sample that is still visible and insoluble after several days in toluene) and average molecular weight of polymer chains contained in soluble fraction, weighted by the number of molecules (M_n) and the mass of the molecules (M_w), and finally the polydispersity index defined as M_w/M_n .

3.1. The sample type strongly influences all indicators

As shown in Table 1, all biochemical indicators including proteins, total lipids, PL, GL, NL and FFA are very significantly impacted by the sample type (*i.e.* latex film, TSR5-like, TSR10-like). Similarly, all properties and mesostructure indicators are very significantly influenced by the sample type, except P_0 , PRI and M_n (yellow color) that are significantly impacted. As compared to TSR-like samples, films are richer in proteins and polar lipids; they display higher plasticity, PRI and gel content, lower viscosity and are more susceptible to storage hardening (ASHT).

Representative molar mass distributions (MMD) of latex films, TSR5-like and TSR10-like are shown in Fig. 1A and Fig. 1B for RRIM600 and RRIT251, respectively. These MMD obtained from tapping system T1 S/2 d2 are also representative of T2 S/2 d3 Stim, as the tapping system showed no noticeable effect on this indicator (Fig. S1). All MMD consist of two populations: one of short chains (molar mass $< 4.10^5$ g/mol) and another of long chains (molar mass $> 4.10^5$ g/mol). As reported in recent studies (Hayemasae et al., 2024, Huang et al., 2024), MMD of both clones show distinct trends for each sample type: latex film (green), TSR5-like (blue), and TSR10-like (orange). Both clones follow a similar trend of shifting toward lower molar mass and narrower distribution as the material transitions from latex film to TSR5-like and then to TSR10-like. RRIT251 clone exhibits sharper peaks for TSR-like samples,

Table 1

Effect of sample type (latex film, TSR5-like, TSR10-like), assessed by p-Value of Tukey-Kramer test, on biochemical indicators (proteins, lipids, phospholipids, glycolipids, neutral lipids, free fatty acids), physical properties (P_0 , P_{30} , PRI, PH, ΔP ASHT, Mooney viscosity) and structure (gel content, M_n , M_w , M_w/M_n). The number of observations is indicated. The very significant effects ($p < 0.0001$) are written in bold characters. The trends of the effects are detailed.

Indicator	Number of observations	Effect of Sample Type (p-value of Tukey-Kramer test)	Ranking of sample types
Proteins (% w/w dry matter)	302	< 0.0001	Film > TSR5 > TSR10
Total lipids (% w/w dry matter)	302	< 0.0001	Film > TSR10 > TSR5
Phospholipids (% w/w dry matter)	300	< 0.0001	Film > TSR5 > TSR10
Glycolipids (% w/w dry matter)	300	< 0.0001	Film > TSR5 > TSR10
Neutral lipids (% w/w dry matter)	300	< 0.0001	TSR10 > TSR5 > Film
Free fatty acids (% w/w dry matter)	302	< 0.0001	TSR10 > TSR5 > Film
P_0 (1/100 mm)	300	0.0003	Film > TSR10 > TSR5
P_{30} (1/100 mm)	300	< 0.0001	Film > TSR10 > TSR5
PRI (%)	300	0.0002	Film > TSR10 = TSR5
PH (1/100 mm)	300	< 0.0001	Film > TSR10 > TSR5
ΔP ASHT (1/100 mm)	300	< 0.0001	Film > TSR5 > TSR10
Mooney Viscosity (a. u.)	299	< 0.0001	TSR10 > TSR5 > Film
Gel (% w/w dry matter)	302	< 0.0001	Film > TSR5 > TSR10
M_n (kg mol ⁻¹)	267	0.0058	TSR10 > TSR5 > Film
M_w (kg mol ⁻¹)	302	< 0.0001	Film > TSR5 > TSR10
M_w/M_n	302	< 0.0001	Film > TSR10 > TSR5

indicating a slightly more uniform molecular weight distribution compared to RRIM600.

For RRIM600 clone, the distribution of latex film is bimodal and exhibits a peak at a lower molar mass ($\sim 1.10^5$ g/mol) indicating a high proportion of short polymer chains. For TSR5-like, the large peak centered around $\sim 1.10^6$ g/mol shifts toward lower molar masses compared to the latex film reflecting a decreased proportion of long polymer chains. TSR10-like has a slightly narrower distribution than TSR5-like and is centered around a similar molar mass ($\sim 1.10^6$ g/mol).

For RRIT251, the MMD of latex film is bimodal but the peak of short chains visible at lower molar mass ($\sim 1.10^5$ g/mol) is much less

pronounced than for RRIM600. Compared to the latex film, the large peak of TSR5-like ($\sim 1.10^6$ g/mol) is shifted towards lower molar masses, similar to the trend observed in RRIM600. TSR10-like has the narrowest distribution among the samples centered around $\sim 1.10^6$ g/mol. This indicates greater homogeneity in molar mass compared to TSR5-like.

The effect of sample type is also visible on the SDS-PAGE gels of protein extracts from fresh latex, latex film, TSR5-like and TSR10-like shown in [Supplementary material](#) (Fig. S2). As compared to fresh latex protein extracts, the ones of rubber samples display much less protein bands (Fig. S2A). While 26 bands are detected in protein extract from fresh latex, only 5 and 4 bands are detected in protein extracts from latex film and TSR samples, respectively (Fig. S2B). [Table S3](#) in [Supplementary material](#) provides the molecular weights of the bands detected in SDS-PAGE gels of fresh latex and rubber samples. The band located at 42.1 kDa in fresh latex is still visible in latex film (although less intense) but not anymore in TSR-like samples. Four bands present in fresh latex are visible in latex films, TSR5-like and TSR10-like samples. These bands are located at 35–36, 27–28, 21–23 and 13.6–14.6 kDa, likely corresponding to β -1–3 glucanase, hevine, small rubber particle protein (SRPP1) and rubber elongation factor (REF1), respectively ([Srisomboon et al., 2021](#)). The most intense bands in films are the ones attributed to REF1, SRPP1 and hevine. REF1 and SRPP1 are the two most intense bands in TSR5-like while only REF1 band is clearly visible in TSR10-like. The more processed the sample (creping and maturation), the less visible the protein bands.

Similar to proteins, the effect of sample type on lipid profiles was clearly observed on TLC (Fig. S4). Between fresh latex and rubber samples (films, TSR5-like and TSR10-like), the detected compounds for neutral lipids (NL) (Fig. S4A) were similar. They were all composed of free sterols, free fatty acids (FFA), γ -tocotrienol, triglycerides of furan fatty acids and esterified lipids on the top of the TLC plates with different relative intensity of the spots. It was obviously observed that the intensity of free fatty acids spot was more important in rubber samples than in fresh latex. Among rubber samples, the FFA spot appeared and increased with the processing condition. The release of FFA can be mainly attributed to the hydrolysis of polar lipids (glycolipids (GL)) and phospholipids (PL)) and TSR10-like processing resulted in the highest FFA proportion. This result is consistent with the disappearance of MGDG and DGDG from the 4 detected GL families in latex (MGDG, DGDG, SG and ESG) upon processing. Mainly ESG and SG were found left in TSR5-like and TSR10-like samples respectively (Fig. S4B). For PL, phosphatidyl choline (PC), the major PL in natural rubber, decreased rapidly and resulted in LPC and FFA (Fig. S4C). The hydrolysis seemed to occur more rapidly in PL than in GL as no PL was visually observed on TLC for TSR10-like sample which was not the case for GL. It was also noticed that film sample prepared under the condition of this study

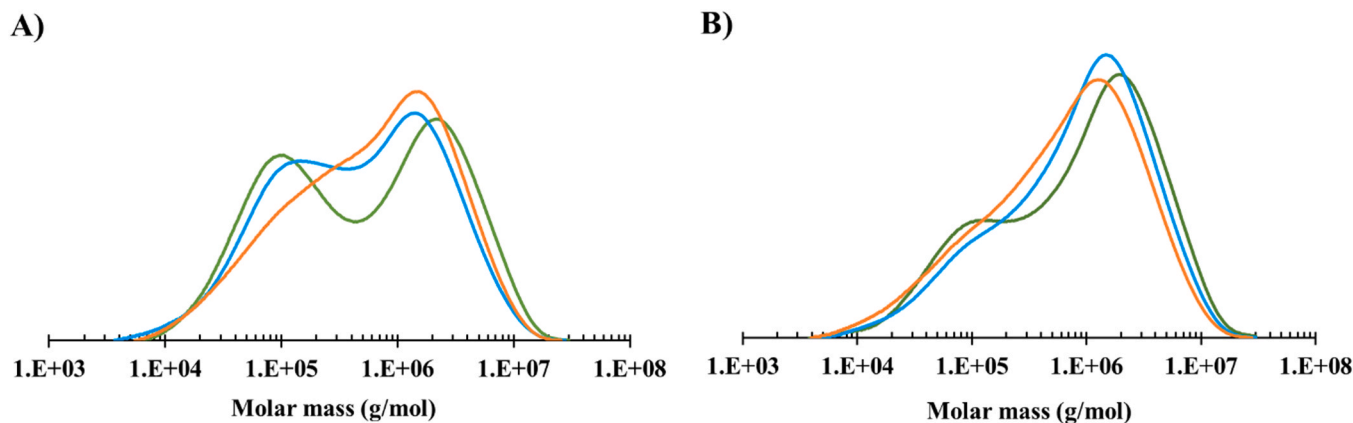


Fig. 1. Representative molar mass distributions (tapping system T1 S/2 d2) of *cis*-1,4 polyisoprene chains for RRIM600 (A) and RRIT251 (B) clones of latex film (green), TSR5-like (blue) and TSR10-like (orange).

provided a similar lipid profile as compared to fresh latex.

The sample type was shown to have a strong influence on measured indicators as well as on MMD of poly-isoprene chains, SDS-PAGE profiles of protein extracts and TLC profiles of lipid extracts. These results highlight the strong influence of process (films vs TSR-like) and maturation time (TSR5-like vs TSR10-like) on the biochemical composition and properties of raw NR. In the following paragraphs, results will be detailed by sample type.

3.2. For all sample types, clone has stronger effect than tapping system on measured indicators

Table 2 details, by sample type, the number of observations, the mean and the standard error of measured indicators as well as the statistical effects of the clone, the tapping system and the interaction clone $\times$ tapping system. An overview of Table 2 quickly highlights the strong effect of clone as compared to tapping system.

The clone had a very significant impact on various indicators across all sample types. For latex films, the protein and glycolipid contents, P_0 , P_{30} , PRI, PH, Mooney viscosity, gel content, M_n , M_w and M_w/M_n were all very significantly influenced by the clone. In the case of TSR5-like samples, P_0 , P_{30} , PH, Mooney viscosity, gel content, M_n , M_w and M_w/M_n were similarly affected. TSR10-like samples exhibited very significant changes in P_0 , P_{30} , ΔP ASHT, Mooney viscosity, gel content and M_w . Additionally, the clone had a significant impact on other indicators including PL and NL contents of latex films, GL content, FFA content and PRI of TSR5-like, as well as GL content, FFA content, PRI, M_n and M_w/M_n of TSR10-like.

In contrast to the clone, the tapping system had a limited effect on measured indicators. For latex films, protein and PL contents were significantly affected by the tapping system, while in TSR5-like samples, only the PRI showed a significant change. Interestingly, some indicators that were not significantly influenced by either the clone or the tapping system individually, were significantly impacted by their interaction (clone $\times$ tapping system). This interaction primarily affected lipid indicators. In TSR5-like samples, the interaction had a particularly strong influence on total lipid content. Significant clone $\times$ tapping system effects were also observed in latex films (total lipid and NL contents), TSR5-like samples (NL content) and TSR10-like samples (total lipid and NL contents). These findings underscore the complex interplay between genetic factors (clone), agricultural practices (tapping system) and their combined effects on the biochemical composition and properties of NR samples.

Beyond statistical significance, the magnitude and consistency of clonal effects observed across all sample types strongly suggest that rubber properties are predominantly genetically determined. The systematic differences between RRIM600 and RRIT251 in terms of Mooney viscosity, gel content, and molecular weight distribution point toward inherent variations in polymer biosynthesis and structuration mechanisms within the laticifer cells. Such stability across processing conditions and tapping systems may reflect a form of metabolic homeostasis in the rubber tree, whereby the biosynthesis of polyisoprene and associated non-rubber components is tightly regulated. This buffering capacity could explain why moderate agronomic changes, such as tapping frequency and stimulation, have limited impact compared to genetic factors. From a materials science perspective, these results indicate that clone selection is a primary lever for tailoring NR properties, potentially more influential than processing conditions at early stages.

3.3. Latex films: clone influences biochemical contents and properties, while tapping system has minimal impact

Table 3 gathers the biochemical composition, physical properties and mesostructure indicators of latex films derived from both studied clones (RRIM600 and RRIT251) harvested by two tapping systems: T1 S/2 d2 and T2 S/2 d3 Stim.

Latex films from clone RRIM600 show significantly lower protein content than RRIT251 under both tapping systems. This trend was also observed on fresh latex as shown in Part 1 of this study (Kum-ourm et al., 2025). T2 S/2 d3 Stim results in higher protein content for both clones, with RRIT251/T2 S/2 d3 Stim recording the maximum (4.78%). Total lipid values are broadly similar between clones RRIM600 and RRIT251, with no marked differences or clear trends. For RRIM600, values fluctuated slightly from 2.79% (T1 S/2 d2) to 2.74% (T2 S/2 d3 Stim), indicating a slight decrease (although not significant) with the stimulation system (T2 S/2 d3 Stim). For RRIT251, a slight increase in total lipid content is observed under T2 S/2 d3 Stim (2.77–2.91%), but the difference remains not significant. Regarding the 3 lipid classes, the tapping system has no significant impact on PL, GL and NL contents of latex films while the clone significantly influences both GL and NL contents. As compared to RRIT251, latex films from RRIM600 clone display lower and higher contents of GL and NL, respectively. Neither clone nor tapping system has a significant impact on FFA content of latex films.

The tapping system has no significant effect on the properties (P_0 , P_{30} , PRI, PH, ΔP ASHT, Mooney viscosity) nor on the mesostructure indicators of latex films (gel, M_n , M_w , M_w/M_n). In contrast, the clone significantly impacts all those parameters. RRIT251 latex films exhibit higher P_0 , P_{30} , PH and Mooney viscosity values as compared to films from RRIM600 clone. Inversely, PRI values of RRIT251 films were significantly lower as compared to those of RRIM600. The increase in plasticity after ASHT (ΔP) was of the same order of magnitude for both clones. As compared to RRIM600 films, RRIT251 films display higher values of gel, M_n and M_w and a lower value of M_w/M_n .

The higher Mooney viscosity, gel content, and molecular weights observed in RRIT251 latex films suggest a more structured polymer network, likely resulting from stronger intermolecular interactions and/or a higher degree of chain entanglement. These features may be associated with differences in the composition and organization of non-rubber components, particularly proteins and lipids known to stabilize rubber particles and influence chain growth. Conversely, RRIM600 films, characterized by lower viscosity and gel content but higher PRI, may reflect a polymer system with lower structural constraints but enhanced resistance to thermo-oxidative degradation. This distinction highlights a trade-off between processability, mechanical integrity and storage stability.

3.4. TSR5-like: lipid composition is influenced by clone and tapping system, while properties are solely impacted by clone

Table 4 gathers the biochemical composition, physical properties and mesostructure indicators of TSR5-like rubber samples derived from two rubber tree clones (RRIM600 and RRIT251) harvested by two tapping systems: T1 S/2 d2 and T2 S/2 d3 Stim.

The protein content of TSR5-like is comparable and not significantly different across clones and tapping systems, ranging from 2.77 (RRIM600/T1 S/2 d2) to 2.86% w/w dry matter (RRIT251/T1 S/2 d2). This is in agreement with a previous study showing that the tapping system (d2, d3, d4, d6) did not affect the nitrogen content of crepe rubber (Kudaligama et al., 2012). Both clone and tapping system significantly influence the total lipid content. Interestingly, the effect of the tapping system is reversed between the two clones. In RRIM600, the highest lipid content was observed under T1 S/2 d2 (2.62%) and the lowest under T2 S/2 d3 Stim (2.39%), whereas in RRIT251, the opposite trend was seen, with higher values under T2 S/2 d3 Stim (2.56%) compared to T1 S/2 d2 (2.42%). PL levels are consistent across all samples (~0.21–0.23%) with no effect of clone nor tapping system. GL content shows minor variability, with higher values observed in RRIT251 (0.50%–0.53%) compared to RRIM600 (0.48%). Tapping system has no significant effect on GL while a significant effect of clone is observed, but only for T2 S/2 d3 Stim tapping system (RRIT251 > RRIM600). TSR5-like contain 1.70–1.91% of NL. Significant effects are

Table 2

Number of observations, mean and standard error of measured indicators detailed by sample type (latex film, TSR5-like, TSR10-like) and effects (assessed by p-Value of Tukey-Kramer test) of clone (*i.e.* RRIM600, RRIT251), tapping system (*i.e.* T1 S/2 d2, T2 S/2 d3 Stim) and their interaction on these indicators. The very significant effects ($p < 0.0001$) are written in bold characters while the notation 'NS' indicate non-significant effects. Trends of these effects are indicated for clone and tapping system. The trends of the effect of the interaction clone $\times$ tapping system are detailed in footnotes below.

Indicator	Sample Type	Number of observations	Mean from all data	Standard error	Effect of Clone	RRIM600 > RRIT251	RRIT251 > RRIM600	Effect of Tapping System	T1 S/2 d2 > T2 S/2 d3 Stim	T2 S/2 d3 Stim > T1 S/2 d2	Effect of Interaction Clone $\times$ Tapping System
Proteins (% w/w dry matter)	Film	111	4.38	0.05	< 0.0001		X	0.0041		X	NS
	TSR5	80	2.82	0.04	NS			NS			NS
	TSR10	111	2.49	0.04	NS			NS			NS
Total lipids (% w/w dry matter)	Film	111	2.82	0.02	NS			NS			0.0336 ^a
	TSR5	80	2.50	0.02	NS			NS			< 0.0001 ²
	TSR10	111	2.55	0.02	NS			NS			0.0101 ^a
Phospholipids (% w/w dry matter)	Film	111	0.66	0.01	0.0007		X	0.0237		X	NS
	TSR5	79	0.22	0.01	NS			NS			NS
	TSR10	110	0.16	0.01	NS			NS			NS
Glycolipids (% w/w dry matter)	Film	111	0.75	0.01	< 0.0001		X	NS			NS
	TSR5	79	0.50	0.01	0.0098		X	NS			NS
	TSR10	110	0.41	0.01	0.0223		X	NS			NS
Neutral lipids (% w/w dry matter)	Film	111	1.41	0.02	0.0402	X		NS	X		0.0143 ^c
	TSR5	79	1.78	0.02	NS			NS			0.0002 ^b
	TSR10	110	1.98	0.02	NS			NS			0.0003 ^a
Free fatty acids (% w/w dry matter)	Film	111	0.16	0.01	NS			NS			NS
	TSR5	80	0.43	0.01	0.0002		X	NS			NS
	TSR10	111	0.77	0.01	0.0004		X	NS			NS
P ₀ (1/100 mm)	Film	109	53.3	1.2	< 0.0001		X	NS			NS
	TSR5	80	47.5	1.2	< 0.0001		X	NS			NS
	TSR10	111	52.5	0.5	< 0.0001		X	NS			NS
P ₃₀ (1/100 mm)	Film	109	45.1	0.7	< 0.0001		X	NS			NS
	TSR5	80	39.2	1.0	< 0.0001		X	NS			NS
	TSR10	111	43.1	0.4	< 0.0001		X	NS			NS
PRI (%)	Film	109	86.7	1.1	< 0.0001	X		NS			NS
	TSR5	80	82.9	0.6	0.0002	X		0.0281		X	NS
	TSR10	111	82.4	0.5	0.0043	X		NS			NS
PH (1/100 mm)	Film	109	74.2	1.2	< 0.0001		X	NS			NS
	TSR5	80	59.7	1.2	< 0.0001		X	NS			NS
	TSR10	111	61.2	0.5	NS			NS			NS
Δ P ASHT (1/100 mm)	Film	109	20.9	0.8	NS			NS			NS
	TSR5	80	12.2	0.3	NS			NS			NS
	TSR10	111	8.7	0.4	< 0.0001	X		NS			NS
Mooney Viscosity (a. u.)	Film	108	70.7	1.6	< 0.0001		X	NS			NS
	TSR5	80	74.5	2.3	< 0.0001		X	NS			NS
	TSR10	111	91.6	0.9	< 0.0001		X	NS			NS
Gel (% w/w dry matter)	Film	111	12.7	0.6	< 0.0001		X	NS			NS
	TSR5	80	11.9	0.5	< 0.0001		X	NS			NS
	TSR10	111	9.5	0.5	< 0.0001		X	NS			NS
M _n (kg mol ⁻¹)	Film	101	197	8	< 0.0001		X	NS			NS
	TSR5	70	233	9	< 0.0001		X	NS			NS
	TSR10	96	250	17	0.0219		X	NS			NS
M _w (kg mol ⁻¹)	Film	111	1767	25	< 0.0001		X	NS			NS
	TSR5	80	1627	27	< 0.0001		X	NS			NS
	TSR10	111	1597	20	< 0.0001		X	NS			NS
M _w /M _n	Film	111	10.1	0.3	< 0.0001	X		NS			NS
	TSR5	80	7.4	0.2	< 0.0001	X		NS			NS
	TSR10	111	7.7	0.2	0.0019	X		NS			NS

^a RRIT251/T2 S/2 d3 Stim > RRIM600/T1 S/2 d2 > RRIT251/T1 S/2 d2 > RRIM600/T2 S/2 d3 Stim

^b RRIM600/T1 S/2 d2 > RRIT251/T2 S/2 d3 Stim > RRIT251/T1 S/2 d2 > RRIM600/T2 S/2 d3 Stim

^c RRIM600/T1 S/2 d2 > RRIT251/T2 S/2 d3 Stim > RRIM600/T2 S/2 d3 Stim > RRIT251/T1 S/2 d2

Table 3

Biochemical composition (proteins, lipids, phospholipids (PL), glycolipids (GL), neutral lipids (NL), free fatty acids (FFA)), physical properties (P_0 , P_{30} , PRI, PH, ΔP ASHT, Mooney viscosity) and mesostructure (gel content, M_n , M_w , M_w/M_n) of latex films ($\pm$ standard error). Values not connected by same letter are significantly different according to Tukey-Kramer test ($P = 5\%$).

Sample type: Latex films				
Clone	RRIM600		RRIT251	
Tapping system	T1 S/2 d2	T2 S/2 d3 Stim	T1 S/2 d2	T2 S/2 d3 Stim
Proteins (% w/w dry matter)	3.97 $\pm$ 0.08 ^c	4.17 $\pm$ 0.10 ^{b,c}	4.37 $\pm$ 0.09 ^b	4.78 $\pm$ 0.10 ^a
Lipids (% w/w dry matter)	2.79 $\pm$ 0.06 ^{a,b}	2.74 $\pm$ 0.04 ^b	2.77 $\pm$ 0.03 ^{a,b}	2.91 $\pm$ 0.04 ^a
PL (% w/w dry matter)	0.61 $\pm$ 0.02 ^b	0.64 $\pm$ 0.02 ^{a,b}	0.67 $\pm$ 0.02 ^{a,b}	0.71 $\pm$ 0.01 ^a
GL (% w/w dry matter)	0.69 $\pm$ 0.03 ^c	0.70 $\pm$ 0.02 ^{b,c}	0.76 $\pm$ 0.01 ^{a,b}	0.79 $\pm$ 0.02 ^a
NL (% w/w dry matter)	1.49 $\pm$ 0.03 ^a	1.40 $\pm$ 0.03 ^{a,b}	1.34 $\pm$ 0.02 ^b	1.41 $\pm$ 0.03 ^{a,b}
FFA (% w/w dry matter)	0.14 $\pm$ 0.02 ^a	0.15 $\pm$ 0.02 ^a	0.16 $\pm$ 0.01 ^a	0.18 $\pm$ 0.01 ^a
P_0	39.6 $\pm$ 0.5 ^b	39.0 $\pm$ 0.6 ^b	62.7 $\pm$ 1.2 ^a	62.8 $\pm$ 1.3 ^a
P_{30}	38.4 $\pm$ 0.8 ^b	36.7 $\pm$ 0.7 ^b	50.4 $\pm$ 0.7 ^a	49.9 $\pm$ 0.8 ^a
PRI (%)	97.5 $\pm$ 2.7 ^a	94.8 $\pm$ 2.6 ^a	80.8 $\pm$ 0.9 ^b	79.8 $\pm$ 0.9 ^b
PH	63.0 $\pm$ 1.7 ^b	61.1 $\pm$ 1.9 ^b	81.9 $\pm$ 1.1 ^a	83.0 $\pm$ 1.1 ^a
ΔP ASHT	23.4 $\pm$ 1.7 ^a	22.1 $\pm$ 1.6 ^a	19.2 $\pm$ 1.3 ^a	20.3 $\pm$ 1.5 ^a
Mooney Viscosity	52.1 $\pm$ 1.2 ^b	53.4 $\pm$ 1.8 ^b	81.4 $\pm$ 1.9 ^a	81.8 $\pm$ 1.8 ^a
Gel (% w/w dry matter)	9.2 $\pm$ 0.9 ^b	10.5 $\pm$ 1.0 ^{a,b}	14.5 $\pm$ 0.9 ^a	14.5 $\pm$ 1.2 ^a
M_n (kg mol ⁻¹)	130 $\pm$ 5 ^b	138 $\pm$ 9 ^b	243 $\pm$ 11 ^a	232 $\pm$ 15 ^a
M_w (kg mol ⁻¹)	1558 $\pm$ 32 ^b	1520 $\pm$ 45 ^b	1936 $\pm$ 38 ^a	1898 $\pm$ 33 ^a
M_w/M_n	12.2 $\pm$ 0.5 ^a	11.8 $\pm$ 0.6 ^a	8.6 $\pm$ 0.3 ^b	9.1 $\pm$ 0.4 ^b

observed on NL: effect of tapping system for RRIM600 (T1 S/2 d2 > T2 S/2 d3 Stim) and clonal effect for T1 S/2 d2 (RRIM600 > RRIT251). FFA content is higher in RRIT251 (0.46%–0.48%) than in RRIM600 (0.35%–0.41%), with one statistically significant difference noted for T2 S/2 d3 Stim.

The tapping system has no significant effect on TSR5-like properties (P_0 , P_{30} , PRI, PH, ΔP ASHT, Mooney viscosity) and mesostructure indicators (gel, M_n , M_w , M_w/M_n). These results agree with a previous study showing that P_0 , PRI and Mooney viscosity of crepe rubber were not significantly different between d2, d3, d4 and d6 tapping frequencies (Kudaligama et al., 2012). In contrast, the clone has a significant influence on all properties and mesostructure indicators, except for ΔP ASHT and PRI that are not affected for T1 S/2 d2. As compared to RRIT251, RRIM600 displays lower values of P_0 , P_{30} , PH, Mooney viscosity, gel, M_n and M_w while both PRI and polydispersity index M_w/M_n are higher.

The persistence of clonal effects after processing into TSR5-like materials indicates that the initial biochemical composition of latex continues to influence the final material properties. In particular, the higher Mooney viscosity and gel content of RRIT251-derived samples suggest that structural features established during biosynthesis are only partially altered by processing. This supports the hypothesis that non-rubber components, especially proteins and polar lipids, play a structuring role by promoting interactions between polyisoprene chains, thereby contributing to gel formation and increased viscosity.

Table 4

Biochemical composition (proteins, lipids, phospholipids (PL), glycolipids (GL), neutral lipids (NL), free fatty acids (FFA)), physical properties (P_0 , P_{30} , PRI, PH, ΔP ASHT, Mooney viscosity) and mesostructure (gel content, M_n , M_w , M_w/M_n) of TSR5-like rubber sample ($\pm$ standard error). Values not connected by same letter are significantly different according to Tukey-Kramer test ($P = 5\%$).

Sample type: TSR5-like rubber				
Clone	RRIM600		RRIT251	
Tapping system	T1 S/2 d2	T2 S/2 d3 Stim	T1 S/2 d2	T2 S/2 d3 Stim
Proteins (% w/w dry matter)	2.77 $\pm$ 0.07 ^a	2.78 $\pm$ 0.07 ^a	2.86 $\pm$ 0.05 ^a	2.84 $\pm$ 0.07 ^a
Lipids (% w/w dry matter)	2.62 $\pm$ 0.07 ^a	2.39 $\pm$ 0.03 ^b	2.42 $\pm$ 0.02 ^b	2.56 $\pm$ 0.03 ^a
PL (% w/w dry matter)	0.23 $\pm$ 0.02 ^a	0.21 $\pm$ 0.02 ^a	0.21 $\pm$ 0.01 ^a	0.22 $\pm$ 0.01 ^a
GL (% w/w dry matter)	0.48 $\pm$ 0.02 ^{a,b}	0.48 $\pm$ 0.01 ^b	0.50 $\pm$ 0.01 ^{a,b}	0.53 $\pm$ 0.01 ^a
NL (% w/w dry matter)	1.91 $\pm$ 0.06 ^a	1.70 $\pm$ 0.03 ^b	1.71 $\pm$ 0.02 ^b	1.81 $\pm$ 0.03 ^{a,b}
FFA (% w/w dry matter)	0.41 $\pm$ 0.03 ^{a,b}	0.35 $\pm$ 0.02 ^b	0.46 $\pm$ 0.02 ^a	0.48 $\pm$ 0.02 ^a
P_0 (1/100 mm)	35.2 $\pm$ 0.8 ^b	34.2 $\pm$ 0.9 ^b	55.2 $\pm$ 1.0 ^a	56.0 $\pm$ 0.9 ^a
P_{30} (1/100 mm)	29.4 $\pm$ 0.6 ^b	29.6 $\pm$ 0.7 ^b	44.2 $\pm$ 1.2 ^a	46.0 $\pm$ 1.0 ^a
PRI (%)	83.8 $\pm$ 1.4 ^{a,b}	87.0 $\pm$ 1.3 ^a	80.0 $\pm$ 1.3 ^b	81.9 $\pm$ 0.7 ^b
PH (1/100 mm)	47.2 $\pm$ 1.0 ^b	47.3 $\pm$ 1.2 ^b	66.9 $\pm$ 0.8 ^a	68.1 $\pm$ 0.9 ^a
ΔP ASHT (1/100 mm)	12.1 $\pm$ 0.6 ^a	13.1 $\pm$ 0.6 ^a	11.7 $\pm$ 0.5 ^a	12.1 $\pm$ 0.5 ^a
Mooney Viscosity (a.u.)	54.1 $\pm$ 1.9 ^b	52.4 $\pm$ 1.3 ^b	86.8 $\pm$ 2.5 ^a	89.0 $\pm$ 2.7 ^a
Gel (% w/w dry matter)	8.9 $\pm$ 0.5 ^b	9.5 $\pm$ 0.5 ^b	13.4 $\pm$ 0.9 ^a	13.6 $\pm$ 0.9 ^a
M_n (kg mol ⁻¹)	168 $\pm$ 8 ^b	167 $\pm$ 5 ^b	288 $\pm$ 11 ^a	266 $\pm$ 14 ^a
M_w (kg mol ⁻¹)	1435 $\pm$ 37 ^b	1423 $\pm$ 35 ^b	1795 $\pm$ 39 ^a	1724 $\pm$ 40 ^a
M_w/M_n	8.6 $\pm$ 0.2 ^a	8.6 $\pm$ 0.2 ^a	6.5 $\pm$ 0.2 ^b	6.7 $\pm$ 1.4 ^b

3.5. *TSR10-like: clone impacts some properties under both tapping systems as well as lipid content under T2 S/2 d3 Stim, while tapping system has no effect*

Table 5 gathers the biochemical composition, physical properties and mesostructure indicators of TSR10-like rubber samples derived from two rubber tree clones (RRIM600 and RRIT251) harvested by two tapping systems: T1 S/2 d2 and T2 S/2 d3 Stim.

Although not significantly different, slight variations are observed regarding the protein content of TSR10-like samples. T2 S/2 d3 Stim tapping system displays higher protein content than T1 S/2 d2 while TSR10-like samples from RRIM600 are richer in proteins than RRIT251. The total lipid content of TSR10-like is significantly influenced by the clone, but this effect is evident only under the T2 S/2 d3 Stim tapping system, where TSR10-like from RRIM600 contain lower lipid levels compared to RRIT251. Conversely, under the T1 S/2 d2 tapping system, an opposite (though not statistically significant) trend is observed, with TSR10-like from RRIM600 exhibiting higher lipid levels than those from RRIT251. While the impact of the tapping system on total lipid content is not significant, its effect varies between clones: as compared to T2 S/2 d3 Stim, T1 S/2 d2 results in higher and lower lipid content in RRIM600 and RRIT251, respectively. Among the total lipids, GL and PL contents remain consistent across all TSR10-like samples, showing no significant variation induced by either the clone or the tapping system. However, NL content in TSR10-like of both clones responds differently to the tapping system. In RRIM600, T1 S/2 d2 results in higher (but not significantly different) NL content compared to T2 S/2 d3 Stim, whereas in RRIT251, the opposite trend is observed, with T2 S/2 d3 Stim yielding significantly higher NL content than T1 S/2 d2. FFA content of TSR10-

Table 5

Biochemical composition (proteins, lipids, phospholipids (PL), glycolipids (GL), neutral lipids (NL), free fatty acids (FFA)), physical properties (P_0 , P_{30} , PRI, PH, ΔP ASHT, Mooney viscosity) and mesostructure (gel content, M_n , M_w , M_w/M_n) of TSR10-like rubber sample ($\pm$ standard error). Values not connected by same letter are significantly different according to Tukey-Kramer test ($P = 5\%$).

Sample type: TSR10-like rubber				
Clone	RRIM600		RRIT251	
	T1 S/2 d2	T2 S/2 d3 Stim	T1 S/2 d2	T2 S/2 d3 Stim
Proteins (% w/w dry matter)	2.45 $\pm$ 0.08 ^a	2.56 $\pm$ 0.08 ^a	2.40 $\pm$ 0.07 ^a	2.55 $\pm$ 0.06 ^a
Lipids (% w/w dry matter)	2.57 $\pm$ 0.06 ^{a,b}	2.46 $\pm$ 0.04 ^b	2.52 $\pm$ 0.03 ^{a,b}	2.64 $\pm$ 0.04 ^a
PL (% w/w dry matter)	0.16 $\pm$ 0.01 ^a	0.16 $\pm$ 0.01 ^a	0.16 $\pm$ 0.01 ^a	0.16 $\pm$ 0.01 ^a
GL (% w/w dry matter)	0.40 $\pm$ 0.02 ^a	0.39 $\pm$ 0.01 ^a	0.42 $\pm$ 0.01 ^a	0.43 $\pm$ 0.01 ^a
NL (% w/w dry matter)	2.01 $\pm$ 0.04 ^{a,b}	1.91 $\pm$ 0.03 ^b	1.94 $\pm$ 0.02 ^b	2.05 $\pm$ 0.03 ^a
FFA (% w/w dry matter)	0.72 $\pm$ 0.02 ^b	0.71 $\pm$ 0.02 ^b	0.78 $\pm$ 0.03 ^{a,b}	0.83 $\pm$ 0.02 ^a
P_0 (1/100 mm)	49.6 $\pm$ 1.5 ^b	49.0 $\pm$ 1.2 ^b	55.2 $\pm$ 0.6 ^a	54.1 $\pm$ 0.8 ^a
P_{30} (1/100 mm)	41.7 $\pm$ 1.0 ^{b,c}	40.7 $\pm$ 0.6 ^c	44.9 $\pm$ 0.6 ^a	43.9 $\pm$ 0.6 ^{a,b}
PRI (%)	84.5 $\pm$ 1.1 ^a	83.5 $\pm$ 1.2 ^a	81.5 $\pm$ 0.8 ^a	81.7 $\pm$ 0.6 ^a
PH (1/100 mm)	62.1 $\pm$ 1.7 ^a	61.7 $\pm$ 1.2 ^a	61.7 $\pm$ 0.7 ^a	59.8 $\pm$ 0.8 ^a
ΔP ASHT (1/100 mm)	12.5 $\pm$ 0.7 ^a	12.7 $\pm$ 0.7 ^a	6.5 $\pm$ 0.4 ^b	5.7 $\pm$ 0.3 ^b
Mooney Viscosity (a.u.)	85.2 $\pm$ 2.5 ^b	86.2 $\pm$ 1.7 ^b	96.4 $\pm$ 1.2 ^a	94.9 $\pm$ 1.4 ^a
Gel (% w/w dry matter)	7.4 $\pm$ 1.0 ^a	9.3 $\pm$ 1.0 ^a	9.6 $\pm$ 0.8 ^a	10.6 $\pm$ 1.0 ^a
M_n (kg mol ⁻¹)	187 $\pm$ 9 ^a	218 $\pm$ 27 ^a	260 $\pm$ 20 ^a	303 $\pm$ 47 ^a
M_w (kg mol ⁻¹)	1527 $\pm$ 45 ^{b,c}	1482 $\pm$ 36 ^c	1677 $\pm$ 30 ^a	1647 $\pm$ 37 ^{a,b}
M_w/M_n	9.1 $\pm$ 0.6 ^a	8.1 $\pm$ 0.5 ^{a,b}	7.5 $\pm$ 0.4 ^{a,b}	6.9 $\pm$ 0.3 ^b

like samples ranges from 0.71% to 0.83%. For both clones, the tapping system has no significant effect on FFA content. RRIT251 is richer in FFA

than RRIM600 (significantly for T2 S/2 d3 Stim, but not for T1 S/2 d2).

The clone significantly influences some properties and mesostructure indicators including: P_0 , P_{30} , ΔP ASHT, Mooney viscosity and M_w . In contrast, the tapping system has no significant effect on properties nor on mesostructure indicators of TSR10-like. As compared to RRIT251, RRIM600 displays lower values of P_0 , P_{30} , Mooney viscosity and M_w while its ΔP ASHT value is higher.

The reduced clonal discrimination observed in TSR10-like samples compared to TSR5-like highlights the significant impact of maturation processes. Prolonged storage before processing likely induces biochemical and structural rearrangements, such as lipid hydrolysis and protein degradation, which may partially erase the initial clonal signature. This suggests that processing conditions, particularly maturation time, can modulate or attenuate genetically determined properties, although not completely override them.

3.6. PCA reveals clone-driven variability and process influence on sample clustering

Fig. 2 presents the results of a principal component analysis (PCA) performed on the dataset including data from both Parts of this study, i.e. Part 1 on fresh latex (Kum-ourm et al., 2025) and Part 2 on rubber samples (present work). The obtained score plot and circle of correlation of the dataset are presented on the left and right, respectively. Both principal components 1 (21.6% variance explained) and 2 (15.8% variance explained) together account for 37.4% of the total variance. The score plot (left) clearly highlights distinct clustering between clones. This separation indicates significant differences between the two clones, along horizontal component 1. The circle of correlation (right) displays the contributions of variables to the two principal components. Since the large number of variables can make it difficult to read this correlation circle, Table S5 in Supplementary material gathers the Pearson linear correlation coefficients between variables of the whole dataset, i.e. variables measured on all sample types. The correlation circle (right) indicates that the variables most strongly associated with this component 1 (PC1) are the initial plasticity (P_0) values of film ($P_{0,F}$) and TSR5 ($P_{0,5}$). It is consistent with the difference of mean values of the variable P_0 of film that is 39.3 for RRIM600 and 62.8 for RRIT251. Within each

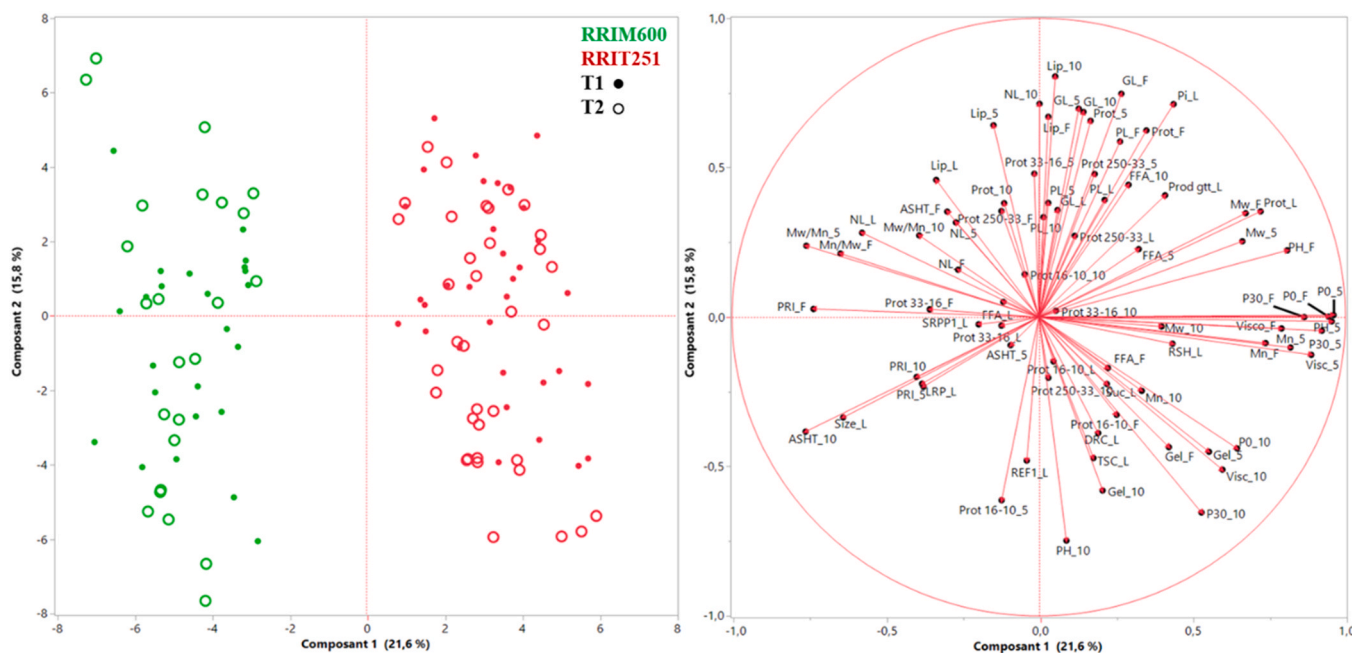


Fig. 2. Score plot (left) and circle of correlation (right) of principal components including all sample types (fresh latex, latex films, TSR5-like and TSR10-like). In the circle of correlation, the abbreviated variables are coded by _L, _F, _5 and _10 corresponding to fresh latex, latex film, TSR5-like and TSR10-like samples, respectively. The list of variables and their abbreviations is shown in Table S5.

group (RRIM600 and RRIT251), the filled circles (T1 S/2 d2) and open circles (T2 S/2 d3 Stim) show some dispersion on the score plot. However, no clear separation appears between both tapping systems (T1 S/2 d2 vs T2 S/2 d3 Stim) along any of the 2 first principal components, confirming that the primary variation arises more from clone rather than from tapping system.

Fig. 3 presents the results of four PCA performed by sample type, *i.e.* fresh latex, dry latex film, TSR5-like and TSR10-like. The obtained score plots and circles of correlation are presented in the upper and the lower row, respectively. Both principal components 1 and 2 together account for 38.1%, 49.8%, 54.2% and 42.9% of the total variance for fresh latex, dry latex film, TSR5-like and TSR10-like, respectively. All these values are higher than the 37.4% of the total variance of the PCA applied on the whole dataset including all sample types. Interestingly, the score plots of fresh latex and TSR10-like are much more scattered than the ones of latex film and TSR5-like, which appear more tightly clustered. As observed for the whole dataset, the separation of samples for latex film and TSR5-like is mainly driven by the clone.

The contrasting expression of biochemical variables, relative to fresh weight in fresh latex and dry matter in latex film, captures different aspects of the same dataset: analysis of fresh latex provides insights into its metabolic and physiological state while the one of dry latex film highlights material properties. These differences should be carefully considered in any comparative analysis, as they underscore the dual nature of the dataset: one reflecting physiology, and the other reflecting material science. This context-dependent interpretation enriches our understanding of how the same variables manifest differently across sample types. It also highlights the importance of the ratio TSC/DRC of the latex which appears as an important driver of dry sample properties. We previously showed that biochemical components are not homogeneously distributed within the centrifugation fractions of latex (rubber fraction, luteoids, C-serum) (Liengprayoon et al., 2021). Both luteoid and C-serum fractions are much richer in non-isoprene molecules (lipids, proteins, minerals) than rubber fraction. Thus, each variation of DRC, even slight, results in significant enrichment or depletion of non-isoprenes in dry samples what might partly drive the dry sample properties.

The clustering of the samples by clones observed in latex films is even more pronounced for TSR5-like samples. In contrast, this effect is much

less for TSR10-like samples where the dataset is more scattered and looks as the one of fresh latex. This result stresses out the important of the process in TSR manufacturing. While fresh was quickly acidified after collection for both TSR grades, the chronology of manufacturing after acidification differs between both grades. For TSR5-like, latex coagula were creped and dried less than 24 h after acidification, while for TSR10-like, latex coagula were let to mature for 30 days before creping and drying. The maturation time of TSR10-like seems to mitigate the impact of the clone in the data clustering.

The comparison of the circles of correlation shown in the right panel of Fig. 2 (whole dataset) and in the lower row of Fig. 3 (dataset separated by sample type) shows that the contributions of individual variables to the principal components vary according to the data considered for PCA. Some correlations that may have appeared on the whole dataset are no longer valid when the data are analyzed by sample type, and vice versa. Tables S6, S7, S8 and S9 in Supplementary material gather the Pearson linear correlation coefficients between variables measured on fresh latex, dry latex film, TSR5-like and TSR10-like, respectively. The correlations are described by sample type under each table.

From an application perspective, the distinct property profiles of the two clones suggest different end-use potentials. The higher Mooney viscosity, gel content, and molecular weights of RRIT251 rubber are indicative of stronger intermolecular interactions and enhanced green strength, making this clone particularly suitable for applications requiring high mechanical integrity in the uncured state. In contrast, RRIM600 rubber, characterized by lower viscosity and higher PRI, may be better suited for applications requiring improved processability and resistance to thermo-oxidative aging during storage. These findings reinforce the importance of clone selection as a strategic parameter in the rubber value chain, enabling the production of materials tailored to specific industrial requirements.

4. Conclusion

This study demonstrates that clonal identity is the primary factor governing the biochemical composition, physical properties and meso-structure of NR, whereas the tapping system exerts only a limited influence. Across all sample types, most indicators were predominantly driven by genetic differences between clones, while variations in

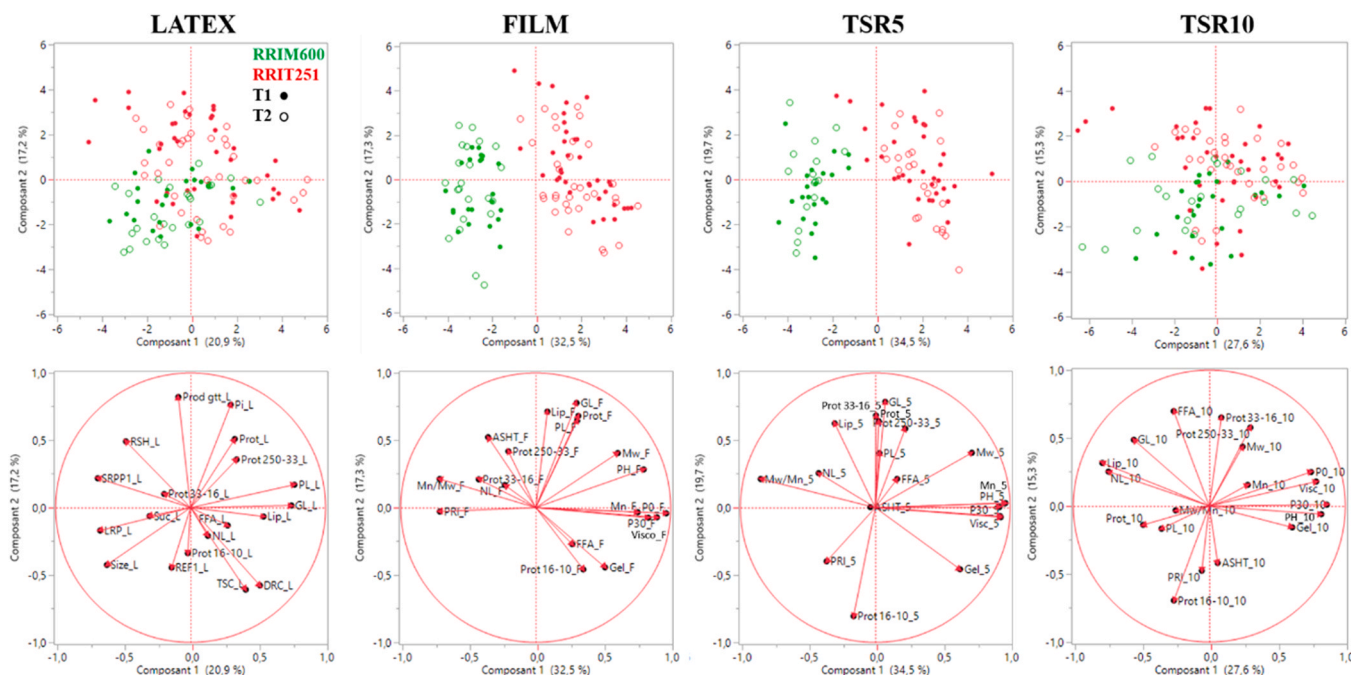


Fig. 3. Score plot (top) and circle of correlation (bottom) of principal components detailed by sample type (fresh latex, latex films, TSR5-like and TSR10-like).

tapping frequency and stimulation had minimal impact. Principal component analysis further confirmed that clone, together with processing conditions, represents the main source of variability.

These findings suggest that NR quality traits are strongly genetically determined and relatively stable under moderate changes in tapping practices, supporting the feasibility of reduced tapping frequency combined with stimulation in non-traditional rubber-growing areas. From an applied perspective, the contrasted properties observed between clones highlight the importance of clone selection as a key lever to tailor rubber performance for specific industrial applications.

It is important to stress that the results of this study are specific to the described experiment and cannot be generalized, as temporal and environmental effects were intentionally not addressed. Therefore, future research should expand the range of clones and environmental conditions, and further investigate the interactions between environment and genetic factors, agronomic practices, and processing steps to optimize both yield and material performance in marginal production zones.

Abbreviations

ASHT Accelerated Storage Hardening Test
DRC Dry Rubber Content
FFA Free Fatty Acid
GL Glycolipids
kDa kilo Dalton
M _n average molecular weight of polymer chains weighted by the number of molecules
M _w average molecular weight of polymer chains weighted by the mass of the molecules
MW Molecular Weight
NL Neutral Lipids
NR Natural Rubber
P ₀ Wallace plasticity
P ₃₀ aged plasticity after a treatment at 140°C for 3 h
PH Wallace plasticity P ₀ after 24 h under P ₂ O ₅ at 60°C
PL Phospholipids
PRI Plasticity Retention Index
PCA Principal Component Analysis
SDS-PAGE Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis
SPE Solid Phase Extraction
TLC Thin Layer Chromatography
TSC Total Solid Content

CRediT authorship contribution statement

Lucksanaporn Tarachiwin: Supervision, Project administration, Funding acquisition, Conceptualization. **Siriluck Liengprayoon:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Noriyuki Onozuka:** Supervision, Project administration, Funding acquisition, Conceptualization. **Christine Char:** Formal analysis. **Céline Bottier:** Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Jatuporn Chaityut:** Methodology, Formal analysis. **Yukino Miyagi-Inoue:** Supervision, Project administration. **Régis Lacote:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **Yumi Sakaguchi-Kitaura:** Project administration, Funding acquisition. **Laurent Vaysse:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Ai Matsuura:** Supervision, Project administration, Funding acquisition. **Hathainat Kum-ourm:** Methodology, Formal analysis, Data curation. **Vincent Ferrer:** Writing – review & editing, Methodology. **Frédéric Gay:** Writing – review & editing, Methodology, Conceptualization. **Tucksin Lerksamram:** Methodology, Formal analysis. **Saowalak**

Jantarasunthorn: Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.indcrop.2026.123695](https://doi.org/10.1016/j.indcrop.2026.123695).

Data availability

The data that has been used is confidential.

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