FRACTIONNATION OF *HEVEA BRASILIENSIS* LATEX BY CENTRIFUGATION: (ii) A MEAN TO LOCATE THE DRIVERS OF NATURAL RUBBER UNIQUE STRUCTURE AND PROPERTIES?

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Abstract

The biochemical composition of Fresh *Hevea* latex fractions (cream, skim, C-serum, lutoids) was comprehensively described in the first part of this publication. The fractions obtained from the same latex were remixed. The rebuilt latex was used to prepare ADS (Air Dried Sheet) rubber sheets. The possible quantitative effects of the components of each fraction on the structure and properties of the obtained rubber ADS samples were studied. This protocol was performed on lattices harvested from 2 clones (RRIM600 and PB235). The obtained rubber samples made from latex containing various proportions of serum and/or lutoid fractions, were submitted to a panel of analytical characterizations: lipid extract, mineral composition, mesostructure (SEC-MALS), Initial Plasticity P_0, Plasticity Retention Index (PRI), and Accelerated Storage Hardening Test (ASHT). Moreover, non-accelerated 2-month storage in controlled conditions was also studied. While the proportion of serum and/or lutoid did not influence much the structure and properties of freshly obtained dry ADS rubber, an important effect of their presence was evidenced on the change of structure and properties during storage. For example, ADS rubber samples made from lutoid- and serum-deprived latex were those that showed the lowest ΔP (ASHT) and the lowest Δgel and ΔP_0 after 2-month storage. In addition, clonal differences in terms of lipid extract, rubber structure and properties were underlined : when compared to RRIM600 rubber, PB235 rubber showed higher lipid content, P_0, M_n, M_w and lower mineral content, ΔP (ASHT) and ΔP_0(2-month non accelerated storage).

Keywords : Hevea brasiliensis; Latex Fractionation; storage hardening.

INTRODUCTION

Natural Rubber (NR), a biopolymer produced from the latex of *Hevea brasiliensis*, exhibits very specific properties never mimicked by synthetic rubbers. Compared to synthetic counterpart, natural rubber still has better mechanical properties such as wear resistance, high elasticity and low internal heat build-up. The main difference between natural rubber and synthetic rubber is non-isoprene components. *Hevea brasiliensis* is known to contain 30-45% of rubber hydrocarbon (poly(*cis*-1,4-isoprene)) and around 3-5 % of non-isoprene [1]. These non-isoprene components consist of various compounds such as

proteins, carbohydrates, lipids, amino acids, nucleic acids and inorganic constituents. However, NR presents important drawbacks including: i) the rather non consistency or variability of its properties, and ii) a dynamic of structuration or "storage hardening" the mechanisms of which are still not fully understood.

Storage hardening is a phenomenon specific to natural rubber, which leads to an increase in bulk viscosity, usually characterized by the Mooney viscosity (MV) and Wallace plasticity (P_0) [2]. The increase in the viscosity upon storage is not a desirable property of natural rubber as a raw material because this means a change in its processing behavior. Though the mechanisms of storage hardening remain to be conclusively explained, the hardening process has been postulated as linked with the reactions between the polyisoprene chains and some non-isoprene components such as proteins and phospholipids [3] and/or abnormal groups such as aldehyde, epoxide, carbonyl, and lactone [4; 5]. Moreover, other factors possibly involved in storage hardening of natural rubber were reported, such as the effect of sugar [5], metal ions [6], short polyisoprene chains [7] and humidity and temperature [8]. The storage hardening of NR was studied mainly by physical properties and mesostructural (macromolecular structure and aggregates or gel). Wisunthorn *et al.* [9] studied the dynamic structuring of natural rubber by SEC-MALS. They underlined that the gel formation is the key parameter for storage hardening of NR. It depends on genotype as different clones have different number of short chains and non-isoprene components and these two components are associated with the gel formation.

In this framework, an international project supported by French National Research Agency (ANR) called "RUBBex" was launched in 2014. One of the objectives of this on-going project is to identify and localize the main biochemical components of latex that drive NR quality consistency and the ability of raw NR structure to continue to evolve during storage. The idea is to target new treatments before processing that would allow a better control of the properties property variability and of the dynamics of structuration along time.

To better understand the role of non-isoprene component in this phenomenon, high speed centrifugation was used to separate the main fractions of natural rubber latex: cream fraction (large rubber particles), skim (small rubber particles), C-serum and bottom fraction (or lutoids). Each fraction was recombined to make "reconstituted" latex and mini ADS. Thereafter, we aimed to study and identify the main biochemical components of each fraction (poly(*cis*-1,4-isoprene), proteins and lipids) and their possible involvement in the structuration dynamics of NR.

MATERIAL AND METHODS

1. Fresh latex collection

Hevea brasiliensis trees of certified RRIM600 and PB235 clones were tapped early morning and the latex was collected from a plantation of Visahakit Thai Rubber Co.,Ltd in Chantaburi Thailand. Each independent repetition of latex lot was made of the mix of collected latex from a group of 10-13 trees that were labelled on the field. The genotype conformity was systematically checked for all sampled trees (microsatellites DNA Analysis, Rubber ID, CIRAD). Two sampling campaigns were organized: October 2016 and January 2017.

Ice was put in the collection cups. Rubber trees were taped and the first 10 drops of latex were discarded. A plastic collecting cup one was placed on ice and latex was collected by pouring latex through the sieve into the bottle around 1.5 hour after tapping. Latex bottle was kept in an ice box during collection and transportation.

2. Fractionation by centrifugation

Fresh latex was centrifuged at 10,000 rpm for 45 min at 4 ˚C (centrifugation C1). After C1, serum and skim were separated from the bottom fraction (*i.e.* lutoids) and the upper layer of cream by suction with a syringe. Cream layer was collected with a spatula and kept in a plastic bottle. Remaining cream on the C1 pot wall was removed with a tissue

paper. The left bottom fraction (lutoids) was weighted and NaCl solution with the same osmolality as the initial latex was added.

The obtained skim and serum mix was centrifuged at 17,000 rpm for 45 min at 4°C (centrifugation C2). After C2, serum was sucked by syringe and kept in a plastic bottle. Skim (upper fraction after C2) was collected and added to the cream obtained after C1. The obtained mix of cream and skim was called "Skream".

Lutoids in NaCl solution was centrifuged at 10,000 rpm for 30 min at 4°C (centrifugation C3). After C3, NaCl solution was removed with syringe. The upper layer (remaining cream) was added to the cream obtained after C1. Washed Lutoids fraction was added with the same NaCl solution (1:1 w:w).

3. Reconstituted latex and mini air dried sheet (mini ADS)

ADS samples were prepared from 9 combinations of latex centrifugation fractions. The details of the composition of the 9 reconstituted latices are shown in Table 1 which indicates, for each fraction, the relative quantity (fresh weight) used in reconstituted latex compared to the initial one in latex. For example, M5 is a latex that contains 50% of the initial quantity of serum, and 50% of the initial quantity of lutoids. The missing quantities were replaced by mineral water (Minéré brand, Nestlé).

The mini-ADS were made from 100-mL of DRC 20 reconstituted latex. The tank was a plastic box (8×6×3.5 cm^3). The latex was acidified by a 1.5% formic solution until pH 4.8. After latex coagulated, the coagulum was manually pressed and a mini-ADS was made with flat (3 passes) and crossed (2 passes) handmangle to get a final thickness of 2-3 mm. Mini-ADS was washed with water during all process and dried in the oven at 50°C until the absence of white spot (4-5 days).

Table 1. Detail of the composition of the 9 combinations (for each fraction (serum or lutoid), the figure indicates the relative quantity (fresh weight) used in reconstituted latex compared to the initial one in latex).

Combination	SERUM	LUTOID
M1	100%	100%
M2	50%	100%
M3	0%	100%
M4	100%	50%
M5	50%	50%
M6	0%	50%
M7	100%	0%
M8	50%	0%
M9	0%	0%

4. Homogenization, Initial Plasticity, Plasticity retention index and Accelerated Storage hardening test

International standard were followed to homogenize the ADS rubber sample, measure Initial plasticity (P_0), plasticity retention index (PRI) and to perform Accelerated storage hardening test (ASHT). The references of the standard are indicated in Table 2.

Table 2 Reference of international standard

Method	**Reference**
Rubber homogenization	SMR bulletin No.7 part B.2; 1992
Initial plasticity (P_0)	ISO 2007
Plasticity Retention index (PRI)	ISO 2930
Accelerated storage hardening test (ASHT)	SMR bulletin No.7 part C.1; 1992

5. Lipid extraction

Lipids of ADS rubber were extracted using a chloroform/methanol (2:1) mixture extraction method as previously described by Liengprayoon *et al.* [10]

6. Mineral analysis

Minimum 50 mg of fractions in 10 ml of 50% nitric were mineralized using microwave reaction systems with 3 successive cycles: 1) 30 min at 140 □C; 2) 30 min at 170°C and 3) 30 min at 190°C. After mineralization, samples were diluted to reach 10% nitric acid concentration. Sulfur (S), phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) in the samples were analyzed using Inductively coupled plasma atomic emission spectroscopy (ICP-AES).

7. ATR-FTIR analysis

FTIR spectra were recorded with a Nicolet 6700 spectrometer, equipped with a MCT detector, with a spectral resolution of 4 cm^{-1} and one-level zero filling. A hundred interferograms, with an acquisition time of 3.5 min, were added. For each ADS rubber sample, P0 pellet was sectioned and ATR-FTIR spectrum was recorded on three zones of the freshly open section. The areas of three bands were measured after baseline correction. To characterize lipid band, carboxyl + ester stretching mode were used at 1770 – 1700 cm^{-1}.. Proteins were identified using the amide II band (1600 – 1500 cm^{-1}). Band between 1500 – 1400 cm^{-1} is characteristic of rubber. The lipid and amide II band areas were normalized by the rubber band area. For example, "normalized lipid area" is made by the ratio of lipid band area by the rubber band area.

8. Characterization of the NR mesostructure by size exclusion chromatography (SEC)

Sample were analyzed by size exclusion chromatography (SEC) following the method described by Dubascoux *et al.* [11]. The SEC equipment consisted of an on-line ERC 3112 degasser (ERC), a SHIMADZU LC-20AD pump, auto sampler (717 plus, Waters), 2 columns with a guard column , a refractive index detector (RID20A, SHIMADZU) and a multi-angle light scattering detector (DAWN DSP, Wyatt Technology). The columns, maintained at 45°C, are two PL Gel mixed ALS (Agilent, 20 µm, 300 mm × 7.5 mm I.D.) with a guard column. The mobile phase was Tetrahydrofuran (THF) stabilized with 2,6-di-tert-butyl-4-methylphenol (BHT, 250 mg/L) at a flow rate of 0.65 mL/min; the injected volume was 100 µL, run time was 40 min. All diode detectors at all 18 angles in the Multi-angle light scattering (MALS) detector were normalized using a THF solution of low polydisperse polystyrene standard (M_W = 34 kg/mol, Polymer Standards Service). The same solution was used to determine the interconnection volume between the two detectors (0.175 mL).

The gel superior to 1 µm (Gel>1µ), is made of all aggregates (macrogel and microaggregates superior to 1 µm) removed from the solution by the filtration before injection in SEC or AF4. The concentration (C2) of the soluble fraction after filtration was calculated from the quantity of material eluting from the columns (SEC) by integrating the chromatogram. The percentage of Gel>1µ in the material was calculated from the difference between the exact initial concentration of the NR solution (C1) and the concentration C2 (Eq. (1)).

$$Gel_{>1\mu} = ((C1 - C2)/C1) \times 100 \quad (1)$$

9. Non-accelerated Storage hardening test

In addition to accelerated storage hardening test (ASHT), ADS rubber (pellet) samples were submitted to two conditions of "non-accelerated" storage in France for an approximate 2-month period. First condition was called "European condition". Sample were stored in laboratory at room temperature. Temperature (°C) and % humidity were recorded during all storage time (17-25°C, 24-68% relative humidity). Second condition is called "Tropical condition". Sample were stored in a climate chamber (Memmert, UNE 200 programmable oven) at 33°C and 75% humidity. Three pellets of each ADS sample were used to measure P_0 before storage. Initial plasticity (P_0) measurement and SEC-MALS analysis were performed before and after storage.

RESULT AND DISCUSSION

Lipid content and FTIR analysis

Nine combinations of fractions were made to reconstitute lattices that were used to prepare ADS samples. The lipid content of these ADS and FTIR normalized area are presented in Table 3. M1 (composition identical to initial latex) was considered as a control. The lipid content varied with *H. brasiliensis* genotypes. Clone PB235 ADS rubber samples gave significantly higher lipid extraction yields than those of RRIM600 clone. The lipid quantity in RRIM600 rubber varied from 1.82% to 2.46% of dry rubber weight while it varied from 2.95% to 3.25 % in PB235 rubber. This clonal effect was described earlier by Liengprayoon *et al.* [12]. FTIR normalized lipid area were consistent with the gravimetric measurement of lipid extract. The observation of normalized protein area indicates that RRIM600 rubber is richer in protein than that of PB235 clone which is consistent with the nitrogen content of the latex reported in the first part of this paper [13].

The lipid content varied also with the composition of the samples. For RRIM600, lipid extraction yield seemed to increase with lutoid proportions. Statistically only lutoid deprived sample were found to contain lower lipid extract (M8 & M9) or to have lower FTIR normalized lipid area (M7, M8 & M9) than M1. Same tendencies were observed for PB235 clone though differences were not found significant. This is consistent with the first part of this paper that found that lutoid fraction is rich in lipid. Concerning normalized Protein area, for RRIM600 clone, only two serum-deprived samples (M6 and M9) showed significantly lower values than that of M1 which suggests that hydrosoluble proteins contained in serum are taking part in the structure of ADS natural rubber material.

Table 3. Lipid extracts (% w/w dry rubber) and FTIR peak area (lipid and amide II) from combination samples.

Samples	Proportion (skream : serum : lutoid)	RRIM600			PB235		
		Lipid (% of dry rubber)	FTIR Normalized Lipid area	FTIR Normalized Protein area	Lipid (% of dry rubber)	FTIR Normalized Lipid area	FTIR Normalized Protein area
M1	**(1:1:1)**	**2.46**	**0.058**	**0.074**	**3.25**	**0.119**	**0.037**
M2	(1:0.5:1)	2.48	0.071	0.053	3.15	0.102	0.032
M3	(1:0:1)	2.34	0.061	0.055	3.19	0.099	0.031
M4	(1:1:0.5)	-	-	-	3.04	0.117	0.041
M5	(1:0.5:0.5)	2.05	0.049	0.050	2.97	0.105	0.039
M6	(1:0:0.5)	2.08	0.051	0.042*	3.00	0.095	0.031
M7	(1:1:0)	2.12	0.035*	0.073	3.21	0.092	0.039
M8	(1:0.5:0)	1.85*	0.028*	0.053	3.02	0.093	0.037
M9	(1:0:0)	1.82*	0.027*	0.043*	2.95	0.102	0.033

* significantly different from corresponding **M1** sample (control) (Dunnett test with control, P<0.005).

Mineral composition

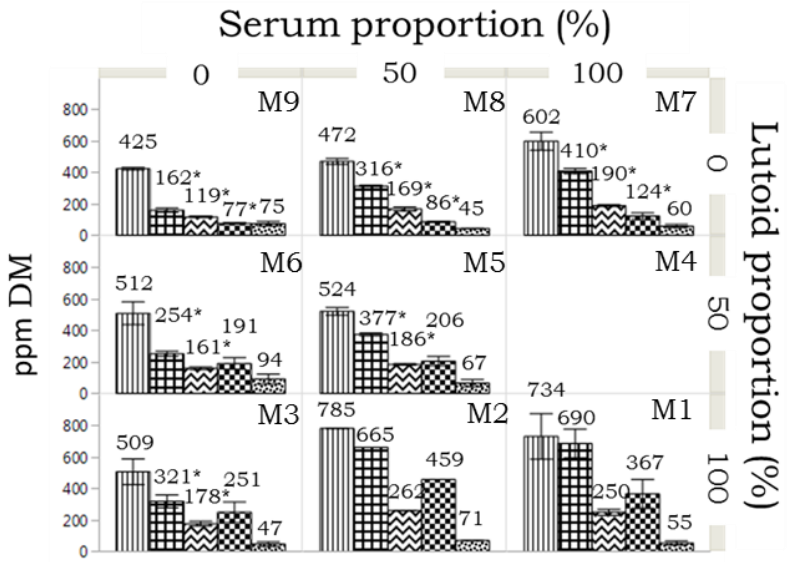
The mineral composition of ADS samples made from reconstituted latex is presented in Figure 1. Phosphorus, potassium, sulfur, magnesium and calcium were analyzed. For all elements and all combinations, samples from RRIM600 clone had higher mineral contents than the ones from PB235 clone. Phosphorus was found to be the element present in the highest quantity in rubber (425 – 734 ppm for RRIM600 clone and 329 – 512 ppm for PB235 clone). Mineral composition of the water used to dilute latex or compensate fraction absence was considered negligible (<10 ppm for P, K, S and Mg).

M9 rubber samples (only skream, top left of figures 1a and 1b) from both clone lattices were those with the lowest mineral content. P, K, S and Mg increased when serum and lutoids proportion increased which confirms that minerals are preferentially located in water soluble fractions of latex (including C- and B-sera).

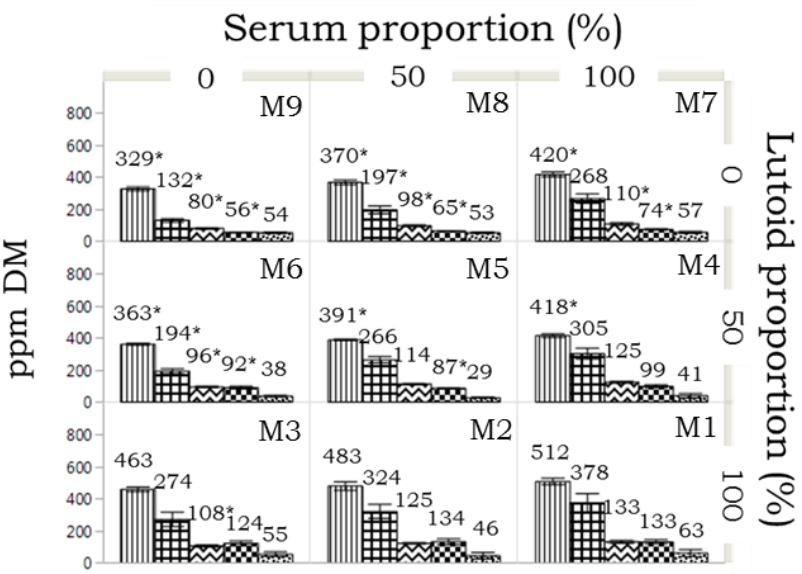
As described before in the first part of this publication [13], Mg is mainly located in lutoid fraction. This specific location had consequences on the Mg content on the rubber.

Indeed, in RRIM600 clone, when observing lutoid-less and serum-less samples (top left of figures 1a), adding 50% of lutoid proportion resulted in an increase by 2.5 times of Mg content in rubber while adding 100% of lutoid proportion increased that quantity by 3.3 times. Same observation can be made for PB235 clone (1.6- and 2.2-time increases, respectively).

K content of rubber seemed to mainly originate from serum fraction of latex. Indeed, the increase of serum proportion (read the figure horizontally from left to right) is a more important driver of K content than the increase of the lutoid proportion (read the figure from top to bottom). This is consistent with the fact that K was reported to be mainly located in serum fraction of latex [13].



(a) RRIM600



(b) PB235

Figure 1. Mineral composition of ADS samples from (a) RRIM600 and (b) PB235 (ppm Dry Matter (DM)); ||||| : P ; ⊞ : K ; ≋ : S ; ▩ : Mg ; ⁙ : Ca ;

*significantly different from corresponding M1 sample (control) (Dunnett test with control, P<0.005) on each mineral composition.

Physical properties

The P_0 varied depending on *H. brasiliensis* clones (Table 4). As described before [12], rubber from clone PB235 has a significantly higher P_0 than that from RRIM600 clone.

For both clones, none of the combination gave a rubber that has a P_0 value significantly different from that of M1 rubber sample (control). However, for RRIM600 clone only, removing serum tended to decrease the P_0 value.

Rubber samples from clone RRIM600 have a higher PRI than those from PB235 clone. Moreover, the P_{30} and PRI varied with lutoid and/or serum proportions. P_{30} and PRI in both clones increased when serum proportion increased. However, these 2 indicators decreased when lutoids proportion increased. Though those differences were not significant, we can infer that lutoids may contain factors that will favor scission and/or lower crosslinking extent during heating part of P_{30} test (30 min, 140°C). Accordingly, PRI of rubber issued from M7 lutoid-less RRIM600 latex was significantly higher than that of the control M1 and has a value exceeding 100 which is a marker of crosslinking.

The minimum value of ΔP was observed for rubber made from lutoid- and serum-deprived latex (M9) which confirmed the presence of structuring factors in both lutoid and serum fractions. However, serum role seemed to prevail as maximum values of ΔP were obtained with the rubber issued from latex with 100% proportion of serum (M1, M4, M7) for both clones.

Table 4. Physical properties (P_0, P_{30}, PRI and ΔP) of combination samples from both clones.

Sample	Proportion (skream : serum : lutoid)	**RRIM600**				**PB235**			
		P_0	P_{30}	PRI	ΔP	P_0	P_{30}	PRI	ΔP
M1	(1:1:1)	36.6	34.1	94.7	39.0	64.4	43.8	68.0	18.1
M2	(1:0.5:1)	34.5	33.0	95.7	37.5	63.4	42.9	67.7	15.6
M3	(1:0:1)	31.4	26.7*	85.5	25.2*	63.5	41.0	64.6	15.6
M4	(1:1:0.5)	-	-	-	-	63.6	46.5	73.3	21.5
M5	(1:0.5:0.5)	33.3	33.0	99.6	29.7	63.2	45.7	72.6	17.7
M6	(1:0:0.5)	33.2	29.7	89.2	28.5	64.1	44.2	69.1	14.7
M7	(1:1:0)	37.8	40.2	107.0*	43.3	67.6	50.9*	75.4	19.7
M8	(1:0.5:0)	35.2	36.5	103.5	31.2	67.4	49.0*	72.9	17.2
M9	(1:0:0)	32.1	31.9	99.7	19.5*	64.9	41.7	64.3	12.9

* significantly different from corresponding M1 sample (control) (Dunnett test with control, P<0.005).

Mesostructure: Gel, Mn, Mw

Table 5 presents the gel superior to 1 µm ($Gel_{>1\mu}$), the number-averaged molar mass (M_n) and the weight-averaged molar mass (M_w) obtained from the SEC-MALS analysis of the ADS rubber samples. A clear clonal difference is known for those indicators and was reported earlier [9]. PB235 clone produces a rubber with a reduced number of short chains (higher M_n) and a high number of long chains (higher Mw). It explains both the higher P_0 and the lower ability of PB235 rubber to harden during storage. The involvement of short chains in storage hardening was indeed described earlier [7; 9; 14].

No clear trend was observed regarding the difference of mesostructure indicators as a function of proportions of lutoid and/or serum in the latex. But, it can be noticed that samples M9 had the lowest M_n, especially the sample from RRIM 600. For RRIM600, the M_n of sample M1 and M9 are 944 and 686 kg mol^{-1} respectively. This 27% difference, though not significant, can be attributed to a lower abnormal elution for sample M9 compared to sample M1 (Figure 2). As explained by Kim *et al.* [14], the more microaggregates eluting with short polyisoprene chains, the more abnormal elution is observed on the molar masses (M_i) elution profiles. As it can be seen on Figure 2, the only difference in molar masses profile (M_i) is for elution volume between 14 and 16 mL. It can be assumed that less

microaggregates (or lower sizes) co-eluted with short chains for M9 sample compared to M1 sample.

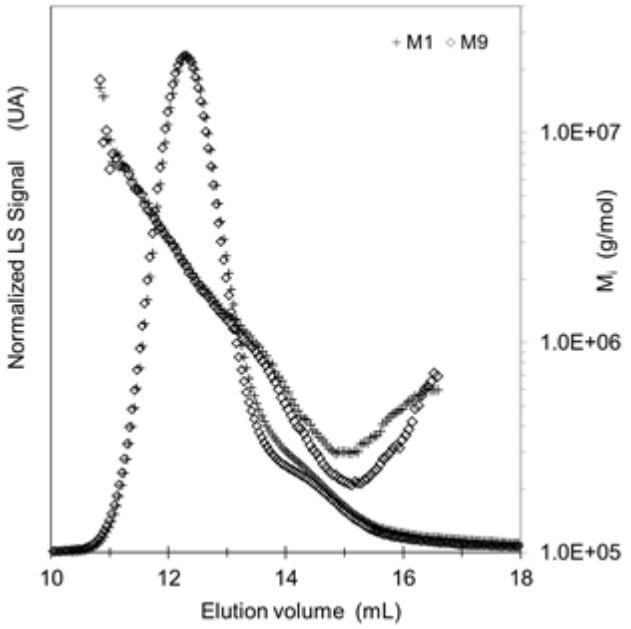


Figure 2. Chromatograms showing the light scattering (LS) signal and molar masses (M_i) as a function of elution volume for fresh NR samples M1 (control) and M9 (only skream).

Table 5. Gel>1µ, Mn and Mw of combination samples from RRIM600 and PB235 clones.

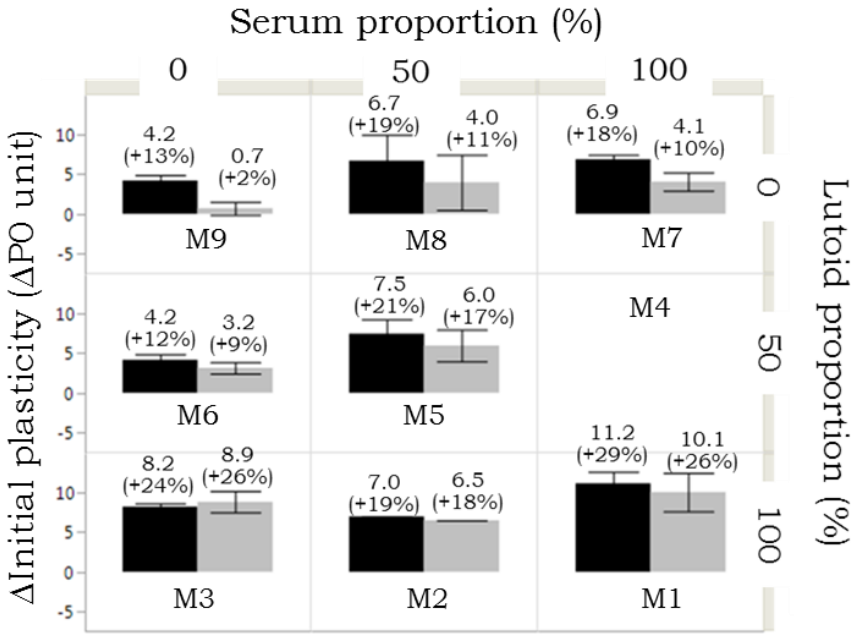
Sample	Proportion (skream : serum : lutoid)	$Gel_{>1\mu}$ (%)	Mn (kg/mol)	Mw (kg/mol)
RRIM600				
M1	**(1:1:1)**	**24.70**	**944**	**1777**
M2	(1:0.5:1)	20.80	984	1791
M3	(1:0:1)	26.72	754	1623
M4	(1:1:0.5)	-	-	-
M5	(1:0.5:0.5)	23.93	880	1779
M6	(1:0:0.5)	19.80	854	1726
M7	(1:1:0)	27.30	865	1806
M8	(1:0.5:0)	25.10	781	1736
M9	(1:0:0)	22.56	686	1633
PB235				
M1	**(1:1:1)**	**29.48**	**1491**	**1935**
M2	(1:0.5:1)	29.57	1493	1907
M3	(1:0:1)	29.28	1500	1948
M4	(1:1:0.5)	30.87	1627	1968
M5	(1:0.5:0.5)	29.43	1631	2006
M6	(1:0:0.5)	30.95	1582	1967
M7	(1:1:0)	28.15	1511	1973
M8	(1:0.5:0)	35.62	1577	1997
M9	(1:0:0)	27.63	1378	1859

Variation of P_0 and $Gel_{>1\mu}$ after 2-month storage

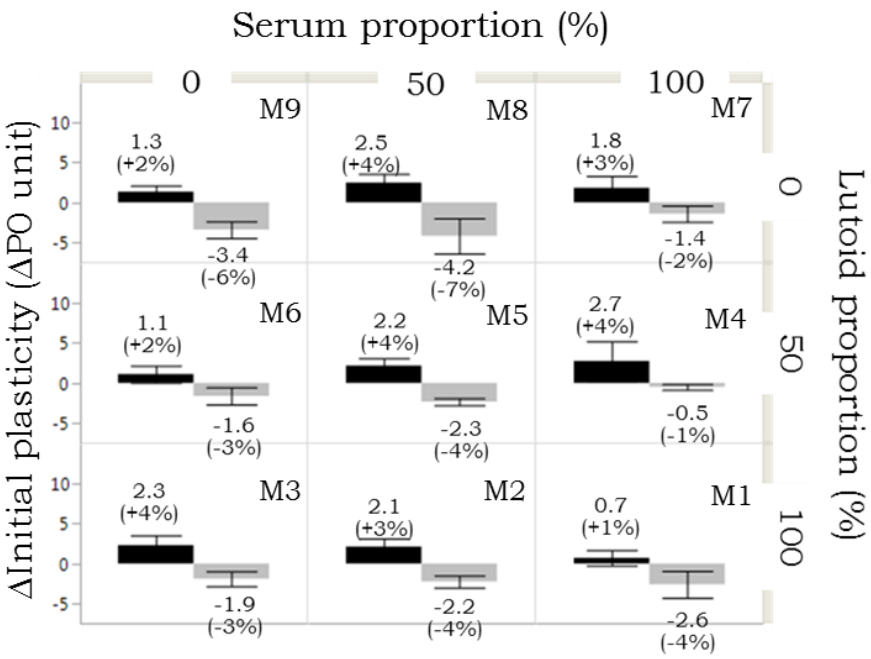
Initial plasticity (P_0) of pellet samples (ADS) before and after storage was measured. The differences (ΔP_0) between P_0 from stored samples (2 months) and P_0 from fresh samples from reconstituted latex from both clones are shown in Figure 3. For RRIM600 samples, as expected, P_0 increased more when stored in European conditions (by 13 to 29%) than stored in Tropical conditions (2 – 26%), though the differences between the two conditions were not found to be significant. Lutoids and serum seemed both to contain important factors

that favor increase of P_0 after storage (see figure 3a : increase of ΔP_0 both from left to right and from top to bottom).

For PB235, the evolution was very different. A rather small increase of P_0 was observed (<4%) in European condition while a small decrease was observed in Tropical conditions (-1% to -7%). However, those variations (positive or negative) were in the range of 1-3 points of P_0 which is very low. The clonal ranking of ΔP_0 is consistent with that of ΔP (ASHT) reported earlier (Table 4): RRIM 600 rubber was much more prone to hardening over storage (accelerated or not) than that of PB235 [9]. However, the prevalence of serum that was observed in ASHT test was not observed anymore in the 2-month storage test. Therefore, the phenomena occurring under ASHT test may not rely on the same mechanisms as the ones occurring during non-accelerated storage.



(a) RRIM600



(b) PB235

Figure 3. Delta of initial plasticity (ΔP0) in different storage conditions black bars: European condition (17-25°C; 24-68%RH) ;gray bars: Tropical condition (33°C; 75%RH) on (a) RRIM600 and (b) PB235.

As for ΔP_0, $Gel_{>1\mu}$ of RRIM600 samples increased during storage while $Gel_{>1\mu}$ of PB235 samples decreased mostly (Table 6). For RRIM600, $\Delta Gel_{>1\mu}$ of reconstituted samples stored in European conditions tended to increase more than that of samples stored in Tropical conditions (not significantly different). Concerning Gel>1µ, the only significant differences, between M1 (control) and other samples, were observed on tropical storage conditions for two lutoid-deprived samples (M7 and M9).

PB235 clone mesostructure evolution differed from that of RRIM600 clone. For most of combinations, $Gel_{>1\mu}$ mostly decreased after storage in both conditions.

Table 6. $\Delta Gel_{>1\mu}$ of combination samples from RRIM600 and PB235 in European and Tropical storage conditions.

Sample	Proportion (skream : serum : lutoid)	$\Delta Gel_{>1\mu}$ (%)	
		European storage conditions	Tropical storage conditions
RRIM600			
M1	(1:1:1)	10.3	10.6
M2	(1:0.5:1)	4.9	9.1
M3	(1:0:1)	4.7	2.6
M4	(1:1:0.5)	-	-
M5	(1:0.5:0.5)	4.3	5.9
M6	(1:0:0.5)	5.1	4.2
M7	(1:1:0)	2.9	1.6*
M8	(1:0.5:0)	8.4	1.7
M9	(1:0:0)	4.3	0*
PB235			
M1	(1:1:1)	1.1	-9.5
M2	(1:0.5:1)	-0.9	-1.5
M3	(1:0:1)	-5.4	-1.9
M4	(1:1:0.5)	0.7	-1.6
M5	(1:0.5:0.5)	0.5	-1.9
M6	(1:0:0.5)	-4.5	-5.5
M7	(1:1:0)	-2.8	0.5
M8	(1:0.5:0)	-13.4*	-11.0
M9	(1:0:0)	-2.1	-10.3

* significantly different from corresponding M1 sample (control) (Dunnett test with control, $P<0.005$).

CONCLUSION

PB235 and RRIM600 clones were chosen because they were expected to produce different rubber materials in terms of biochemical composition, mesostructure and bulk properties. The obtained results confirmed those differences: when compared to RRIM600 rubber, PB235 rubber showed higher lipid content, P_0, M_n, M_w and lower mineral content ΔP (ASHT) and ΔP_0 (2-month non accelerated storage).

Concerning the effect of the proportions of serum/lutoid in the latex, it was found that:

1. In terms of lipid and mineral composition of ADS rubber, the observed differences were consistent with the composition of each fraction in the latex (*e.g.* additional lipid and Mg brought by lutoid, additional K brought by serum).
2. P_0 measured on ADS rubber were not found to vary significantly with the lutoid and/or serum proportions.
3. Mesostructure indicators ($gel_{>1\mu}$, M_n, M_w) of ADS rubber measured rapidly after their preparation were not found to vary significantly with the lutoid and/or serum proportions. However, the samples deprived in both lutoids/serum tended to have a

lower M_n showing a different structuration (lower content or lower size microaggregates).

4. Significant effects of the proportions of lutoid and/or serum were found on structure ($Gel_{>1\mu}$) and properties (P_0) of 2-month stored samples according to the type of storage: i) accelerated storage or ASHT (60°C, 24h, P_2O_5) and ii) 2-month storage in European or Tropical conditions. Indeed, the relative influence of each fraction were found different (prevalence of serum role in ASHT, while both fractions (serum & lutoids) seems to contribute to the 2-month storage hardening).
5. Significant effects of the proportions of lutoid and/or serum were also found on P_{30} values measured during PRI test (140°C, 30 min) that could also be considered as an accelerated storage under high oxidative conditions. Indeed, absence of lutoids and presence of serum (M7) led to the highest P_{30} and PRI values for both clones. This prevalence of serum role echoes that observed in ASHT test.

The results reported in this paper confirm that the structuration of NR during storage rely on numerous and complex mechanisms. The involvement of non-isoprene components contained in serum and lutoid centrifugation fractions of latex was clearly confirmed, though most of the observed trends need to be confirmed by further experimental repetitions. Accumulating more data will also allow quantifying the involvement of each fraction and possibly identifying and focus on specific biochemical actors. Protein analyses of the rubber samples are under progress for this purpose to confirm the first information provided by ATR-FTIR analyses.

ACKNOWLEDGEMENT

The authors thank ANR agency for financial support of the RUBBex-ANR 14-CE07-0026-02 project and Alfabet scholarship. Thailand International Cooperation Agency (TICA) is acknowledged for financial support of the field trips. The authors would like to thank Visahakit Thai Rubber Co., Ltd. for kindly providing samples. Agropolis Foundation is thanked for the support to the creation of LipPolGreen-Asia platform in Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), Bangkok. This work was undertaken as part of the Hevea Research Platform in Partnership (HRPP).

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