Effect of Microorganisms During the Initial Coagulum Maturation of *Hevea* Natural Rubber

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ABSTRACT: The involvement of microorganisms in the initial stage of maturation of natural rubber coagula was assessed with five latex treatments that varied in the initial quantity of microorganisms; the treatments ranged from latex added with an antimicrobial agent (3.4×10^4 CFU/mL) to strongly inoculated latex (2.4×10^7 CFU/mL). After 0–6 days of maturation, the obtained rubber was characterized with respect to its physical and structural properties. The Wallace plasticity (P_0) and plasticity retention index (PRI) remained constant during maturation with the antibiotic-added treatment. PRI decreased with the maturation time, and the rate was proportional to the initial microorganism concentration. P_0 of all inoculated rubber increased for the first 2 days of maturation and decreased after 6 days of maturation. With respect to structural parameters, a higher initial microorganism content corresponded to a higher gel content and a lower weight-average molar mass after maturation, drying, and storage. The inoculated rubber showed a stable value for the number-average molar mass (M_n), in contrast to the noninoculated samples, for which an increase in M_n during maturation was observed. The quantity of microorganisms significantly affected the physical properties and structure of the processed dry rubber. The mechanisms occurring during the initial stage of maturation are complex, and microorganisms are involved not only in the increase in sensitivity to thermooxidation but also in the crosslinking phenomenon between isoprene chains.
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Key words: crosslinking; molecular weight distribution/molar mass distribution; rubber; structure-property relations

INTRODUCTION

TSR10 and TSR20 are the most produced commercial grades of raw technically specified natural rubber (NR). The main raw materials for producing these two grades are the coagula or cuplumps, which are obtained by natural (or auto) coagulation of latex in a collection container a few hours after tapping. The time between the tapping of the trees and the processing of the coagula for TSR10 and TSR20 raw NR can reach several weeks: this is called the maturation time. During this period, the coagula undergo intrinsic biochemical modifications in which microorganisms are supposed to play an important role.[1,2] This maturation time is crucial for the processors as it affects the quality of the processed rubber. Indeed, the quality of NR obtained from coagula available to processors from the grower sector is inconsistent, especially in terms of its sensitivity to thermooxidation.[3]

NR latex is the cytoplasm of laticiferous cells located in the bark of the *Hevea brasiliensis* tree. Its composition, rich in mineral salts, sugars, amino acids, proteins, and lipids, is a highly favorable medium for microbial growth. Taysum[4] identified about 100 species of microorganisms in *Hevea* latex and in its commercial derivatives. Bacteria in fresh latex may come from several sources (the bark and cuts of tapped trees, collection cups, etc.). According to Taysum,[5] the primary infection can reach, according to cases, 10^6 to 10^7 total bacteria per milliliter, and coagulation starts when the bacterial population reaches 10^9 to 10^{10} bacteria per milliliter. Microorganisms can account for the spoilage of latex and an ammoniated latex concentrate. Some, such as *Gordonia* sp.,[6] *Nocardia* sp.,[7] several bacteria species,[8,9] and fungi,[10] are known to degrade dry rubber and rubber products.

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Few researchers have studied the effects of microorganisms in latex on the properties of raw NR. Soewarti and Moh[11] showed that coagula obtained after the inoculation of skim latex with some microorganisms lead to rubber of lower quality in comparison with the rubber obtained when coagulation is provoked by the addition of formic acid. Increasing the maturation time usually increases the sensitivity of NR to thermooxidation.[1,2,12,13] Hasma and Othman[14] also attributed the lower resistance to thermooxidation of autocoagulated coagula to the bacterial decomposition of proteins and other nonrubber components by bacteria, which leads to the release of strong prooxidants such as free copper.

The storage conditions for coagula between the day of delivery to the factory and the day of processing was studied recently by Intapun et al.[15] It was observed that coagula arriving in the factory after a few weeks of maturation at the farms were rather sensitive to thermooxidation [plasticity retention index (PRI) = 22–28]. It was demonstrated that the industrial storage conditions had a significant impact on the properties of dry rubber. After 24 days of storage in a 3-m-high pile, the sensitivity to thermooxidation of the coagula interestingly decreased, as illustrated by an increase in PRI up to 46. Soaking the crumb rubber in a phosphoric or oxalic acid solution before drying is another industrial method for increasing PRI.[1]

The initial quantity of microorganisms in latex before coagulation and its effect on the structure and properties of rubber have not been reported. This was the main purpose of this study, which was performed under controlled maturation conditions on a laboratory scale. The maturation of coagula was studied from 0 to 6 days after inoculation with different quantities of microorganisms sampled in the rubber field. The main bulk properties assessed during maturation were PRI and the initial Wallace plasticity (P_0). Furthermore, analyses of the mesostructure (macromolecular structure and gel content) of raw rubber were performed to understand the evolution of properties during maturation.

EXPERIMENTAL

Clean latex

Five liters of latex was collected from 32 *Hevea brasiliensis* trees from an RRIM600 clone located in the rubber plantation of Prince of Songkla University (Surat Thani, Thailand). A special tapping procedure was developed for the collection of latex samples with a level of microbial contamination as low as possible. The collection material was autoclaved at 121°C for 15 min before use. The sterilized materials included a knife, a spout, a plastic tube connected to a collection plastic bag (15 cm × 23 cm), a plastic sheet (60 cm × 70 cm), a metallic blade (5 cm × 12 cm), and water (1 L). The bark was slightly scrapped with the metallic blade downward from the cut to 3 cm below it. The scrapped area was cleaned successively with water, ethanol, and water with commercial sterile cotton. The clean panel was covered with a plastic sheet fixed to the tree by a rubber band 10 cm above the tapping cut. Before the tapping, a new spout was fixed to the tree. Latex was directed to flow through the spout and the plastic tube into the collection bag. The bag was placed inside an ice-containing cup. When the latex flow stopped (ca. 3 h after tapping), bags containing latex were transferred to a laminar flow cabinet in a nearby laboratory. The latex harvested with this special tapping system is called clean latex in this article.

Inoculum

Thirty-two trees, identical to those used for latex collection, were tapped according to the traditional procedure, and the harvested latex was allowed to coagulate naturally. Three days after the tapping, the 32 coagula were squeezed, and approximately 250 mL of serum containing suspended microorganisms was collected and filtered through a metallic sieve (1-mm pore size). Microbial cells were then centrifuged and washed as follows: 80 mL of serum was centrifuged at 10,000*g* for 15 min (8 tubes × 10 mL). For each tube, the supernatant was removed, and the cell pellet was resuspended in 10 mL of 0.9% NaCl (w/v). The washing operation was repeated twice. The cell suspensions were then pooled in a capped flask, and the obtained inoculum was stored at 4°C until further use. The 2.5× concentrated inoculum was obtained with the same procedure, except that the last resuspension was performed with 4 mL instead of 10 mL of 0.9% NaCl (w/v). The microbial population in the inoculum was estimated as described in the section "Counting of Microorganisms."

Latex preparation: five treatments

The amount of microorganisms in the latex was controlled either through the volume of the inoculum added to the latex or the addition of sodium azide as an antimicrobial agent. Fresh, clean latex was split into five different lots of 1 L each, which corresponded to the five treatments. An antibiotic (16 mL of a 10% sodium azide solution) was added to the first lot, whereas the others received different amounts of inoculum, as detailed in Table I. Preliminary tests had shown that sodium azide provided antimicrobial activity without interfering with the rubber's properties.

TABLE I
Preparation of the Applied Treatments

Treatment	M0+N	M0	M1	M2	M5
Clean latex (mL)	1000	1000	1000	1000	1000
10% sodium azide solution (mL)	16	—	—	—	—
Inoculum (mL)	—	—	14	28	—
2.5× concentrated inoculum (mL)	—	—	—	—	28
Sterile 0.9% NaCl (mL)	12	28	14	—	—
Total volume (mL)	1028	1028	1028	1028	1028

Counting of microorganisms

The microbial population in the inoculum was characterized with selective agar media. An approximately 10× dilution was performed before it was spread on the agar plate. The following categories were numbered: gram-positive bacteria, gram-negative bacteria, lactic acid bacteria, total aerobic and total anaerobic bacteria, and yeasts. Microorganisms present in the inoculum are indicated in Table II.

The counting of microorganisms in the latex before coagulation was limited to the total aerobic population on plate count agar media (Table II).

Preparation of the coagula

Approximately 9 mL of a 5% formic acid solution was added to each lot of latex to reduce the pH to 5.2. The pH change was monitored with a disinfected pH probe (Sentix SP S7, VWR GmbH, Weilheim, Germany) connected to a Multi 350 data logger (VWR). Each latex mixture lot, corresponding to one treatment, was poured into 45-mL sterile glass cups (15 minicups per treatment). Minicoagula in glass cups were stored in sterile, hermetically closed boxes in an incubator (BD series 53, Binder GmbH, Tuttlingen, Germany) at 40°C for 3 h. Under a laminar flow hood, coagula were retrieved, placed on sterile stainless steel trays, and kept in sufficiently large hermetically sealed plastic boxes.

Maturation of the coagula

Coagula with different microbial populations were matured under saturated humidity in closed, sterile plastic boxes (one maturation box per treatment) and incubated at 40°C. This temperature was chosen with respect to that prevailing in the maturation piles of coagula. To evaluate the effect of the maturation time, three coagula from each box were randomly sampled and weighed from the start (day 0) to the 6th day after coagulation.

Creping and drying of the coagula

A laboratory-scale creper (minicreper) was built with the following specifications: a gap between nip rolls of 0.04 mm, a front-roll speed of 190 rpm, a friction ratio of 1 : 1.3, a roll length of 20 cm, a roll diameter of 10 cm, and an engine power of 2.2 kW. Each cup of coagulum was creped by 16 double passes in this minicreper (the crepe was folded end to end between each pass) followed by 1 single final pass.

The obtained crepes were dried in a hot-air oven (UE700, Memmert GmbH & Co., Schwabach, Germany) at 125°C for 2 h, cooled in a desiccator, and weighed. The creping and drying process parameters were chosen after a comparative study with the industrial STR20 process used at the factory of Von Bundit Co., Ltd. (Surat Thani, Thailand).

Bulk characterization of the coagula and rubber

Because during creping only water-soluble components were leached, the dry rubber content of coagula (DRC_c) was assessed as the percentage ratio of the weight of the crepe to the fresh weight of the coagula.

TABLE II
Microbial Population in the Inoculum

Microorganism	Specific medium	Culture conditions	Total count (CFU/mL)
Total aerobic bacteria	PCA (Difco, United States)	35–37°C for 24–48 h	8.7×10^8
Total anaerobic bacteria	PCA (Difco, United States)	35–37°C for 24–48 h in an anaerobic jar	1.1×10^9
Gram-positive bacteria	MSA (Difco, United States)	35–37°C for 24–48 h	4.2×10^8
Gram-negative bacteria	MacConkey agar (Merck, Germany)	35–37°C for 24–48 h	3.2×10^8
Lactic acid bacteria	MRS (Difco, United States)	35–37°C for 24–48 h	4.1×10^7
Yeasts and molds	MEA (Difco, United States)	25°C for 72 h	8.5×10^7

MEA = malt extract agar; MRS = de Man, Rogosa, and Sharpe agar; MSA = mannitol salt agar; PCA = plate count agar.

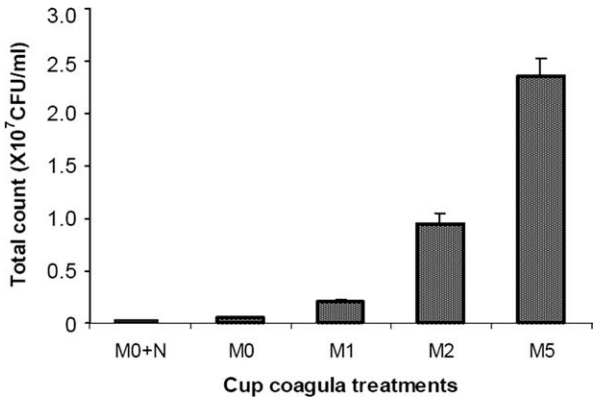

Figure 1 Initial amount of the microorganisms in the latices for each treatment. The error bars are SEMs of three replicates.

About 20 g of the crepe was homogenized according to Standard B2 of SMR Bulletin 7 (1992). P_0 and PRI were measured in accordance with Standard B8 of SMR Bulletin 7 (1992). P_0 and PRI measurements were performed twice: within 24 h and 80 days after drying.

Mesostructure and gel content testing

The method described by Kim et al.[16] was followed with slight modifications. A 25 ± 2 mg sample of homogeneous rubber was dissolved in 40 mL of tetrahydrofuran stabilized with 3,5-di-*tert*-butyl-4-hydroxytoluene for 2 weeks. The rubber solutions were stored at 30°C for 7 days without stirring and then were gently stirred for 1 h daily for 7 more days. The solution was filtered [Acrodisc (1 μm, glass fiber), Pall, NY] and injected into a size exclusion high-performance liquid chromatography system consisting of an online degasser (Elite, Alltech Associates Inc., Deerfield, IL), a pump (Waters 515, Waters Corp., Milford, MA), a Waters 2410 refractive-index detector, and a multi-angle laser light scattering detector (Dawn DSP, Wyatt Technology Corp., Santa Barbara, CA). The columns were three inline PLgel mixed beds (Varian, Inc. (ex Polymer Laboratories), Palo Alto, California CA; 20 μm, 7.8-mm i.d. × 30 cm) with a guard column. The columns were thermostated at 45°C. The mobile phase was tetrahydrofuran stabilized with 3,5-di-*tert*-butyl-4-hydroxytoluene. The flow rate was 0.65 mL/min. The injected volume was 0.15 mL. The number-average molar mass (M_n) and weight-average molar mass (M_w) were calculated with ASTRA software (Wyatt Technology). Fourteen angles, from angle 3 (32°) to angle 16 (134°), were used for the calculation using the Zimm method. The differential refractive-index increment (dn/dc) value was 0.130 mL/g.

For a given sample injected into the size exclusion chromatography/multi-angle laser light scattering chain, the refractive-index increment of the solvent and sample solution was measured with a refractive-index detector. This represents the incremental refractive-index change (dn) of the solution for an incremental change of the concentration (dc). ASTRA software was used for the calculation of the injected quantity of NR after filtration by integration of the whole NR peak on the chromatogram. Thus, as the concentration of the solution (0.625 mg/mL) and the injected volume (0.15 mL) were known before and after filtration, the fraction eliminated by filtration, that is, the percentage of total gel, could be calculated as follows:

$$\text{Total gel content}(\%) = \left(\frac{m_0 - m_1}{m_0}\right) \times 100 \qquad (1)$$

where m_0 is the mass of the sample in 0.15 mL before filtration and m_1 is the mass of the injected sample (calculated with size exclusion chromatography/multi-angle laser light scattering).

RESULTS AND DISCUSSION

Initial amount of microorganisms in the inoculated latex

The clean latex (M0) contained a concentration of total aerobic microorganisms of 4.82×10^5 CFU/mL, whereas the addition of sodium azide (M0+N) reduced this amount to 3.41×10^4 CFU/mL. The addition of inocula (M1, M2, and M5) raised this amount to 2.16×10^6, 9.52×10^6, and 2.36×10^7 CFU/mL, respectively (Fig. 1). The quantity of counted aerobic microorganisms in the inoculated latex was therefore proportional to the quantity of added inoculum.

Evolution of DRC_c during maturation

On the first day of maturation, the average dry rubber content of all inoculated coagula was 40.5%. After 6 day of maturation, DRC_c increased up to a range of 62–72%; this depended on the initial number of microorganisms (Fig. 2). Indeed, increasing the quantity of microorganisms proportionally decreased the dry rubber content during maturation as well as the level of the obtained plateau. The physical aspects of coagula after 6 days of maturation were very different (Fig. 3). The presence of growing microorganisms was clearly illustrated by the generation of bubbles inside the coagula. From a qualitative point of view, the size and number of bubbles seemed to increase with the initial quantity of microorganisms.

The difference in DRC_c between treatments was mainly explained by the difference in the fresh weight before drying (results not shown); this illustrated a higher serum retention from the coagula containing larger amounts of microorganisms.

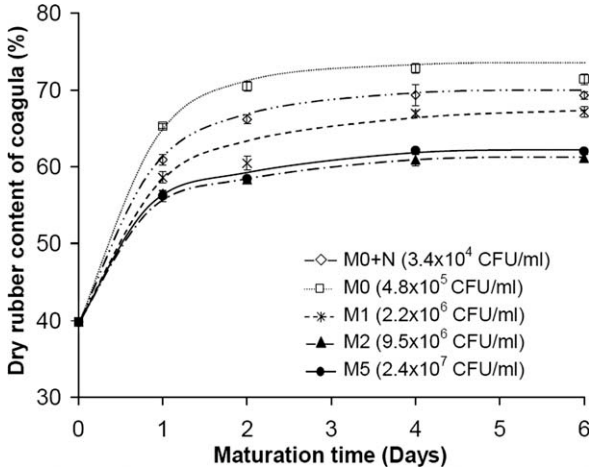

Figure 2 Evolution of DRC_c with the maturation time. The error bars are SEMs of three replicates.

Superior water retention in the inoculated coagula can be explained in two ways. First, gas produced by the microbiological metabolism formed bubbles inside the coagula (Fig. 3); the cavities formed by the bubbles, bigger and more numerous with the quantity of microorganisms, could act as a reservoir and trap water. Second, microorganism activity may have degraded non-isoprene compounds, especially proteins, which are known to help water migration in the hydrophobic rubber medium.[17] Water may thus have been retained inside the coagula because of a lack of a carrier.

Evolution of PRI and P_0 during maturation

The initial PRI values of rubber originating from all coagula processed 3 h after coagulation were similarly high, with an average value of 97.6 [standard error of the mean (SEM) = 1.4; Fig. 4(A)]. The evolution of PRI during maturation was clearly dependent on the initial quantity of microorganisms in the latex. Indeed, when microbial growth was prevented (M0+N), no change in PRI was observed. For the three inoculated treatments (M1, M2, and M5), the drop rate of PRI was proportional to the initial microorganism concentration, even though the PRI value reached after 6 days was similarly found to be very low (<10). For the noninoculation treatment (M0), which contained a low initial number of microorganisms, a less important drop was observed during maturation, the PRI value after 6 days being 70. The presence of microorganisms in the latex before coagulation clearly led to a proportional increase in the sensitivity to thermooxidation (a decrease in PRI). This may be explained by two phenomena: (1) microorganism activity released prooxidant molecules such as free metallic ions[14] or fatty acids,[18] and (2) this activity degraded or inactivated some of the native latex antioxidants such as tocotrienols.[14] Ongoing studies of this antioxidant are in progress, but it has already been observed from these samples that the disappearance of free tocotrienols is associated with a decrease in PRI (data not shown).

Figure 4(B) presents the evolution of P_0 during the maturation time (0–6 days) for all treatments described previously, the main difference between the treatments being the initial quantity of microorganisms. The control sample for all treatments (0 days of maturation) displayed a P_0 value of approximately 34.1 (SEM = 0.35), whatever the quantity of microorganisms was. When observed during maturation, the evolution of P_0 was clearly dependent on the quantity of microorganisms. Indeed, when microbial activity was prevented (treatment M0+N), P_0 did not change significantly, whereas for the noninoculated treatment (M0), P_0 increased up to 45 after 6 days of maturation. With inoculated ones (M1, M2, and M5), an increase in P_0 was observed for the first 2 days of maturation and was followed by a drop to 30.5, 27.5, and 27.0, respectively, after 6 days of maturation. The observed variations of P_0 could be due to a balance between the hardening of rubber by the crosslinking of polyisoprene chains and softening by oxidative scission of the polyisoprene chains during drying. In inoculated samples (M1, M2, and M5), despite a decrease in PRI from the first day, crosslinking seemed to counteract and even to exceed scissions during the first 2 days, and this led to an

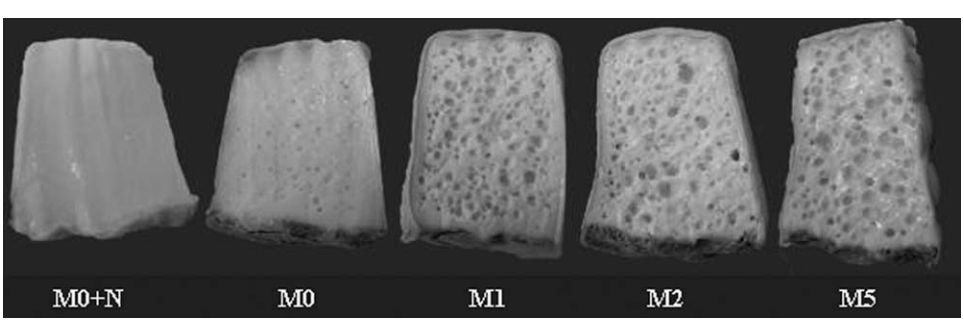

Figure 3 Central sections of cup coagula after 6 days of maturation with different initial levels of microorganism inoculation.

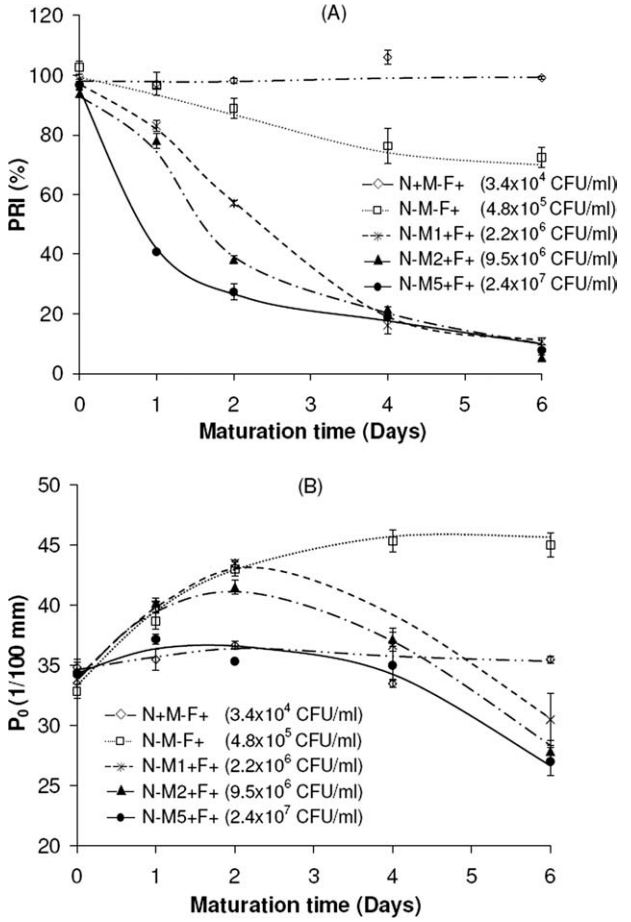

Figure 4 Evolution of (A) PRI and (B) P_0 with the maturation time. Measurements were performed within 24 h after drying. The error bars are SEMs of three replicates.

increase in P_0. Later, after the 4th day, scission became prominent and led to a decrease in P_0. For noninoculated samples (M0), the crosslinking phenomenon was greater than scission during the 6 days of maturation. Rubber added with an antimicrobial agent (M0+N) showed a constant value of P_0, which illustrated the equilibrium (or nonexistence) of these two phenomena. The involvement of microorganisms in the crosslinking phenomenon is difficult to assess. It can be supposed that microbial activity interacts with polymer chain abnormal groups as well as non-isoprene constituents, and this leads to new interactions between rubber chains. Microorganisms interestingly seem to promote both phenomena, crosslinking and scission, which have contrary effects on P_0. Mesostructural information (total gel and molar masses), which is discussed later, is needed to complete these assumptions.

Evolution of P_0, PRI, and the mesostructure of rubber after storage

For all treatments but M2 (considered similar to M1), measurements of P_0 and PRI were repeated before structural analysis by size exclusion chromatography analysis after storage of the samples for 80 days at room temperature. Because of the low variance among repetitions for P_0 and PRI measured just after drying and because three repetitions of the size exclusion chromatography analysis were realized, only one repetition of each treatment and each maturation time was performed for the remeasurement of P_0 and PRI and for the assessment of the polyisoprene molar masses and total gel.

Figure 5(A,B) presents the remeasured PRI and P_0 values (plain line) and the initial measurements just after drying (dotted line). The obtained results showed the same global trends as those obtained without storage, but P_0 was clearly increased because of storage hardening. This hardening, assessed by ΔP_0 (P_0 measured 80 days after drying minus P_0 measured within 24 h after drying) was in the range of 10–15 P_0 units for noninoculated samples (M0 and M0+N). With the inoculated samples (M1 and M5), the shift was more important (15–22) and increased with the maturation time. This is shown in Figure 6, which presents the evolution of ΔP_0 with the maturation time for treatments M0+N, M1, and M5. A good linear correlation between the maturation time and the increase in P_0 was observed for inoculated samples ($p < 0.01$). Therefore, the

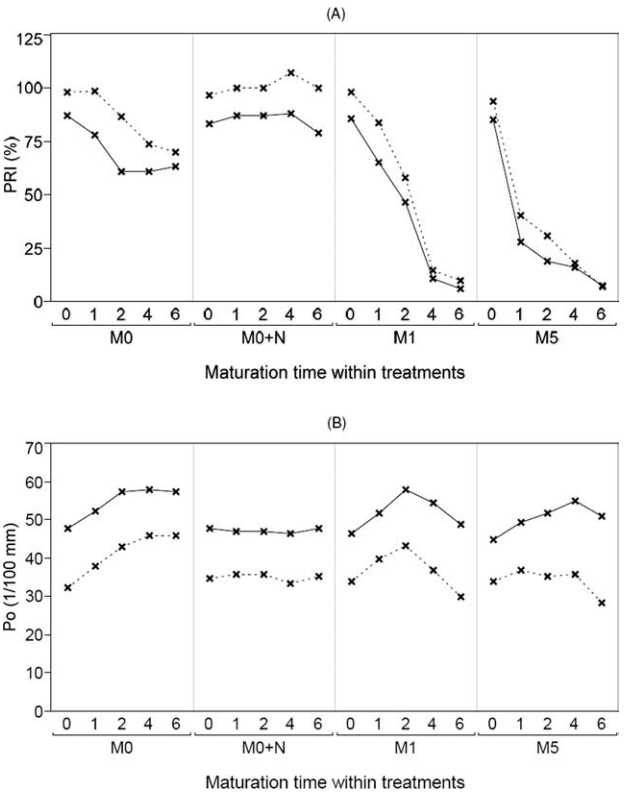

Figure 5 Evolution of (A) PRI and (B) P_0 with the maturation and storage time. Measurements performed after 80 days of storage (solid lines) were compared with initial measurements (dashed lines).

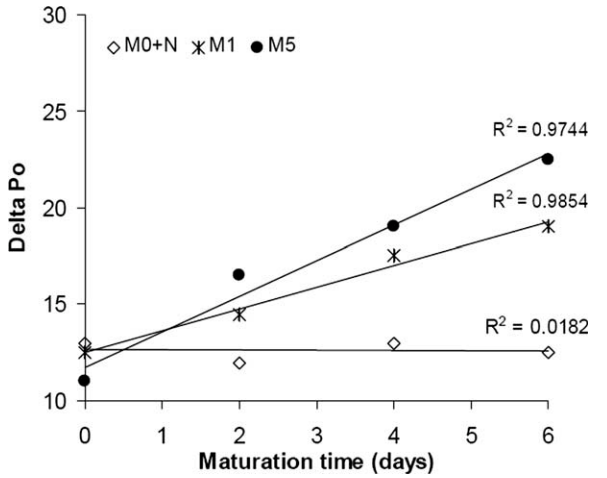

Figure 6 Effect of the maturation time on the increase in P_0 during 80 days of storage. The solid lines are linear regressions; the coefficient of determination (R^2) is indicated for each corresponding straight lines.

presence of microorganisms in latex promoted storage hardening, and the increase in P_0 was more marked for inoculated samples that matured for 4 or 6 days. This is again an illustration of the impact of microbial activity during maturation on the crosslinking of rubber chains even after drying, that is, during the storage time.

The mesostructure of the rubber after storage was also studied. With respect to M_w and M_n, it is worth noting that, in contrast to the other measured parameters, the initial values of the molar masses from samples processed 3 h after coagulation were slightly different between treatments, inoculated rubber showing slightly lower values of M_n and M_w (Fig. 7). M_n followed a different trend than that of M_w during maturation. Indeed, inoculated rubber showed a stable value of M_n staying around 700 kg/mol, whereas noninoculated samples (M0+N and M0) showed an increase in M_n over the maturation time up to 900 kg/mol. M_n data are delicate to interpret. Indeed, on the one hand, scissions would logically produce more short chains and decrease M_n, but on the other hand, short chains may be involved in the gel and therefore may not be counted in the M_n assessment; this could lead to its overestimation.[19] A slight decrease in M_w was observed with the maturation time for the antibiotic-treated rubber (M0+N). M_w values dropped from 1500 to 1400 kg/mol after 6 days of maturation. M_w from the M0 treatment (no inoculum, no antibiotic) showed a similar trend with a faster drop. For inoculated treatments (M1 and M5), this drop was more important, M_w reaching a minimum value of approximately 1150 kg/mol. Interestingly, the drop rate was proportional to the quantity of microorganisms, the minimum value being reached after 1 day for M5 and after 4 days for M1. M_w, which preferentially illustrates the high-molecular-mass isoprene population, confirmed that the presence of microorganisms in latex before coagulation promoted scissions. Because of the similarity of M_w and PRI evolution, those scissions were most likely due to a thermooxidative phenomenon that occurred during drying.

Concomitantly, it is obvious that microorganisms are also involved in crosslinking interactions between isoprene chains during and/or after maturation (drying and/or storage), as illustrated by the total gel evolution (Fig. 8). This total gel included both the microgel and the macrogel as defined by Allan and Bristow,[20] in contrast to other authors who determined only the macrogel by centrifugation when they were studying the mechanisms of gel formation during storage hardening of NR.[21–23] The control samples for all treatments (0 days of

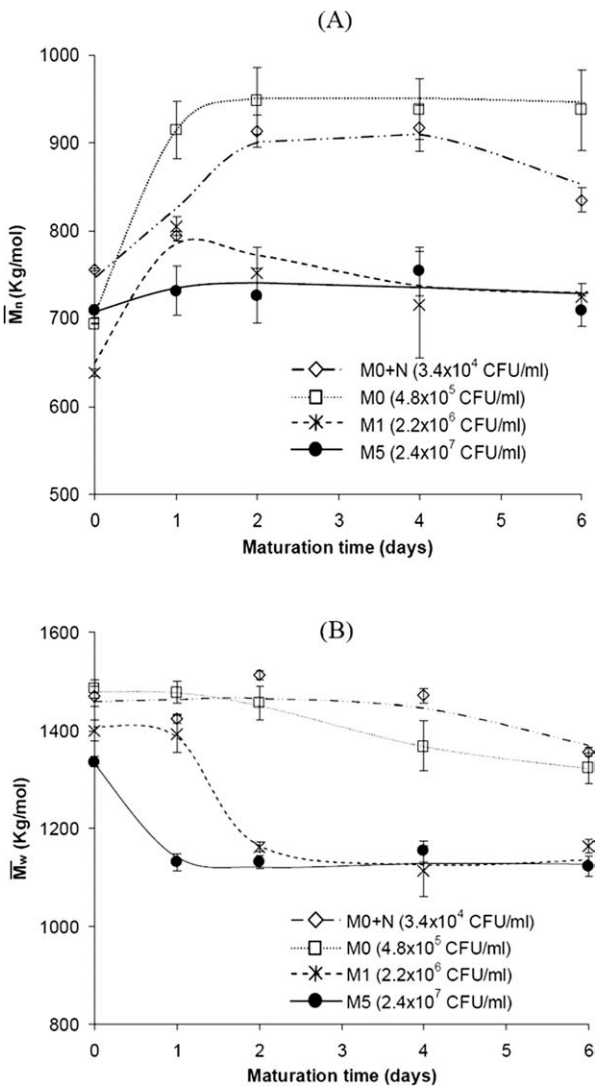

Figure 7 Evolution of (A) M_n and (B) M_w with the maturation time after 80 days of storage. The error bars are SEMs of three replicates.

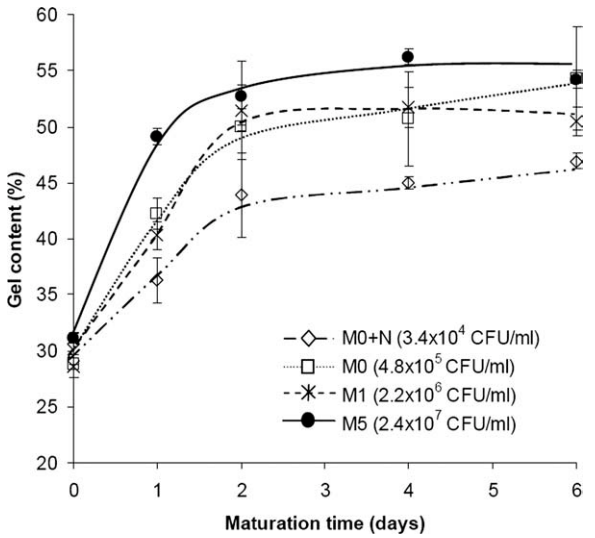

Figure 8 Evolution of the total gel content with the maturation time after 80 days of storage. The error bars are SEMs of three replicates.

maturation) displayed very similar initial gel contents with an average value of 30% (SEM = 0.64%). For all treatments, the gel quantity increased with the maturation time and reached a plateau after 2 days of maturation. The value associated with this plateau was proportional to the initial quantity of microorganisms; it ranged from 45% for antibiotic-treated rubber (M0+N) to 55% for the treatment with the highest initial microorganism content (M5). Nevertheless, for samples containing an antimicrobial agent (M0+N), an increase in the gel content was also observed to a lesser extent, and this means that gel formation may occur without microbial activity. This gel may be of a different nature as it did not affect P_0 of M0+N samples, which remained stable during maturation. This could have been be a thermoreversible physical gel that appeared during storage and disappeared during P_0 measurements at 100°C. Indeed, Voznyakovskii et al.[24] studied gel in NR by dynamic light scattering and showed that increasing the temperature of the solutions from 20 to 70°C led to a decrease in the mean size of the aggregates. The enzymatic activity of microorganisms may catalyze reactions involved in gel formation, such as the oxidation of polyunsaturated fatty acid leading to the formation of aldehyde groups and the release of amino acids or metallic ions. To better understand the mode of action of microorganisms, the study of *in situ* enzymatic activity is necessary.

CONCLUSIONS

The initial stages of the maturation of NR have been investigated. The obtained structure and properties of dried rubber have been further studied. It has been shown that phenomena occurring during the first stage of maturation of rubber are complex and clearly depend on the presence and amount of microorganisms in the initial latex. During the initial stage of maturation, microorganisms clearly enhance the sensitivity of rubber to thermal oxidation while concomitantly seeming to promote crosslinking between rubber chains. However, the exact mode of action of these microorganisms is not understood; hence, there is a need to focus on the role of the enzymes that they secrete.

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